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Effect of Combined Sulfur and Nitrogen Foliar Supply on Olive Oil Volatile Compounds and Sensory Attributes

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Abstract: Up to date, there are no reports on the effects of combined sulfur (S) and nitrogen (N) foliar application in olive orchards on volatile compounds and sensory characteristics of virgin olive oil (VOO). In this work, the effects of increasing the fertilizer S and N concentration on volatile compound composition and odor and taste attributes of monovarietal VOOs of Istarska bjelica and Leccino cultivars were investigated. The volatile compounds were analyzed by gas chromatography with flame ionization and mass spectrometric detection after isolation by solid-phase microextraction, while sensory attributes were assessed by a professional panel. In all the investigated VOO samples, aldehydes were the most represented group of compounds, with (*E*)-2-hexenal as the most abundant, followed by (*Z*)-3-hexenal and 1-penten-3-one. Significant differences in the volatile profiles and sensory attributes were observed between VOOs from different treatments, mainly attributable to the interactions between all the factors that were investigated: treatment, cultivar, and year. Although significant interactions were noted for sensory attributes as well, the score for overall quality was generally lower for Leccino VOOs and for VOOs from both cultivars that were obtained after the highest SN dose treatment. All the samples were graded by overall sensory scores that were higher than eight and were characterized by well-pronounced fruitiness and the absence of sensory defects. The results of this study showed that foliar application of S and N significantly affects the quality of VOO, confirming that such a practice can modulate the characteristic olive oil odor and taste attributes and thus possibly influence its acceptability and preference among consumers.

Keywords: virgin olive oil; volatiles; sensory analysis; SPME; cv. Istarska bjelica; cv. Leccino; SN foliar fertilization; leaves

1. Introduction

Besides its health benefits, VOO became very popular among customers due to its pleasant taste and aroma [1]. The unique, delicate, and distinct aroma of olive oil is related to the presence of volatile compounds (VOCs) mainly deriving from the degradation and

cleavage of fatty acids through a cascade of enzymatic reactions in the so-called lipoxygenase (LOX) pathway [2]. VOCs are low molecular weight molecules which vaporize at room temperature and belong to different chemical classes, such as aldehydes, alcohols, esters, ketones, hydrocarbons, etc. It is well-known that the major VOCs that are present in olive oil are aldehydes, alcohols, and esters with six carbon atoms in a molecule (i.e., C6 compounds) followed by the corresponding C5 compounds. Although they are present in relatively low concentrations, VOCs are extremely important since the sensory attributes they produce, together with other quality parameters such as free fatty acids, peroxide value, and extinction coefficients, are the base for the classification of olive oils into different commercial quality categories [2–4]. The concentration of VOCs is affected by numerous factors including cultivar, environmental conditions, fertilization practices, fruit maturity, processing methods, and oil storage conditions [2,5–8].

The accumulation of VOCs starts at the moment of cell disruption during oil extraction, when the LOX pathway is initiated and the enzymes that are involved are released [9]. The quantity and activity of the enzymes are cultivar-dependent, which leads to different volatile profiles of olive oils that are obtained from different cultivars even if they are grown under the same conditions and processed in the same way [10]. Both cultivars that were selected for this study, Istarska bjelica and Leccino, are commonly grown in Croatia, while Leccino is also extensively cultivated in other countries. Leccino VOO was previously found to contain a higher content of VOCs, especially C6 aldehydes and alcohols, and a lower content of C5 compounds in comparison to VOO of autochthonous Istrian cultivar Istarska bjelica [11].

In addition to genotype, different chemical environments can cause differences in the enzymatic activity. The levels of available nutrients and their translocation in the plant can significantly affect olive oil quality, so the application of foliar fertilizers can be used to optimize the chemical composition of olive oil and enrich it with beneficial phytochemicals [12]. Nitrogen (N) is a widely used fertilizer. Its deficiency can affect sugar metabolism and reduce the number of flowers and yield [13–15]. On the other side, N over-fertilization may favor the synthesis of proteins over that of phenylpropanoids and catalyze oxidation of *o*-diphenols into quinones, resulting in lower phenol concentrations [16,17]. Another important element that can affect olive oil quality is sulfur (S). It plays a crucial role in the biosynthesis of S-containing amino acids and can increase the lipid content in some seeds [18–20]. It is recommended to apply S together with N due to their synergistic effects and the fact that a deficiency in S or N reduces the uptake and assimilation of the other element [21]. Thus, it is important to maintain the optimal S and N levels in olives.

Many papers investigating the effects of genotype, processing, and storage conditions etc., on olive oil quality have been published to date [11,22–27], but only a few have studied how the application of foliar fertilizers influences the volatile composition and sensory attributes of the obtained olive oil. Dabbaghi et al. [7] investigated the modification of olive oil volatile profile as a response to two foliar fertilizers of which the first was based on N, phosphorus (P), and potassium (K), and the second on calcium (Ca). Both resulted in a higher concentration of volatile compounds in general. Atta et al. [12] reported that foliar application of magnesium (Mg) and K sulfate resulted in higher intensity of olive fruity attribute, but also positively affecting other desirable sensory attributes. Regni et al. [28] found that N foliar fertilization did not cause any change in the sensory attributes of oil. Considering the general lack of information on this regard and contradictory results that were obtained in different studies, a need emerged for more detailed studies. To the best of our knowledge, there are no available reports on the effect of combined SN foliar application on the volatile and sensory profiles of olive oil. Thus, the aim of this work was to investigate how increasing fertilizer S and N concentration affects the volatile compound composition and odor and taste attributes of monovarietal VOOs of Istarska bjelica and Leccino cultivars. The repercussions on the concentration of other elements in olive leaves were also assessed in order to gain a more detailed insight into the interrelationship between the available nutrients and olive oil quality.

2. Materials and Methods

2.1. Experimental Conditions, Treatments, Sampling, Minerals Concentration Measurement, and Oil Extraction

All the experimental conditions, treatments, sampling, leaves' minerals concentration measurement, and oil extraction were described in detail in our previously published paper [29]. Briefly, a two-year field experiment was set up as a completely randomized design with two olive cultivars (Istarska Bjelica and Leccino) and four foliar fertilization treatments which were applied two times during the summer period in each experimental year. The foliar treatments consisted of the control treatment (0 mL SN) and three SN treatments (7.5 mL SN, 15 mL SN, and 22.5 mL SN) in which variable concentrations of Thiotrac fertilizer [Yara International ASA, Oslo, Norway] were used. Thiotrac fertilizer was composed of 750 g/L SO_3 and 200 g/L N. Over the experimental years, the average air temperature was 16.3 °C in 2019 and 16.2 °C in 2020, respectively. The total rainfall was 881.5 mm in 2019 and 702.7 mm in 2020, respectively. The meteorological conditions are described in detail in our previous paper [29].

During 2019 and 2020 harvest period, olive leaves were sampled and dried until a constant mass at 75 °C in an oven, then were milled to a fine powder (0.2 mm sieves, Ultra Centrifugal mill ZM 200; Retsch Maschinen GmbH, Setzingen, Germany) and used for analysis.

The analysis of minerals in the olive leaves was performed as described by Vidović et al. [30]. Briefly, dried and milled olive leaf samples (0.2 g) were accurately weighted and after the addition of a mixture of nitric acid (6 mL) and hydrogen peroxide (2 mL), microwave-assisted digestion was performed. After dilution with deionized water, the samples were analyzed by an inductively coupled plasma atomic emission (ICP-AE) spectrometer. The measurement accuracy was validated by the use of certified reference material (WEPAL, Wageningen, The Netherlands).

For olive oil production, two kg of fruits of both cultivars were hand-harvested in their technical maturity during mid-October (2019 and 2020) and processed into oil by Abencor laboratory oil mill. The olives were ground with a metal hammer mill, the paste was malaxed at 25 ± 1 °C in vertical mixers for 40 min, and the oil was separated by centrifugation. The oil was separated from the water, centrifuged for 1 min at 4000 rpm, decanted, and used for further analysis.

2.2. HS-SPME GC-FID Analysis of Volatile Compounds

Analysis of volatile compounds was performed according to the modified method that was proposed by Brkić-Bubola et al. [31] after their isolation by headspace solid-phase microextraction (HS-SPME) with divinylbenzene/Carboxen/polydimethylsiloxane fiber (1 cm length, 50/30 μm film thickness, Supelco, Bellefonte, PA, USA). The oil (4.00 g) was placed into a 10 mL glass vial which was then sealed with a septum. Prior to the extraction, equilibration at 40 °C for 15 min under stirring at 800 rpm was performed to stabilize the headspace. The extraction was carried out at 40 °C for 40 min. GC injection was performed at 245 °C for 3 min in splitless mode. GC analyses were done using a Varian 3350 gas chromatograph (Varian Inc., Harbor City, CA) equipped with a split/ splitless injector, a flame ionization detector (FID) operating at 248 °C, and a capillary column Rtx-WAX (60 m × 0.25 mm × 0.25 μm, Restek, Bellefonte, PA). The initial oven temperature was set at 40 °C, then increased to 170 °C at 2.5 °C/min, further increased to 245 °C at 15 °C/min, and kept stable for 15 min. The carrier gas was helium with the constant pressure of 138 kPa at the column head. Identification was performed using a Varian 3900 GC (Varian Inc., Middelburg, Netherlands, EU) coupled to a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc., Walnut Creek, CA, USA), with the same column and oven parameters. Mass spectra were acquired in EI mode (70 eV) at 1 s/scan with a range of 30–450 m/z. Helium was used as a carrier gas with a constant flow of 1.2 mL/min. Identification was performed by comparing the retention times and mass spectra of the compounds of interest with those of pure standards, and with mass spectra from the NIST05 library. Linear retention indices (relative to C7–C24 *n*-alkanes) were calculated and compared to those from the

literature. Quantification was carried out using calibration curves that were constructed after analysis of standard solutions in fresh refined sunflower oil containing the following pure standards in different concentrations: 3-pentanone, 1-penten-3-one, (*E*)-2-penten-1-ol, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, hexyl acetate, (*Z*)-2-penten-1-ol + (*Z*)-3-hexenyl acetate, 3-methylbutanal, octanal, ethyl 2-methylbutanoate, isoamyl acetate, and (*E*)-2-octenal. For other compounds, semi-quantitative analysis was carried out, and their concentrations (mg/kg) were expressed as equivalents of compounds with similar chemical structure for which standards were available, assuming a response factor equal to one. The total concentration was calculated as the sum of all the identified volatile compounds.

2.3. Sensory Analysis

Quantitative descriptive sensory analysis of Istarska bjelica and Leccino VOO samples was performed by the Panel for sensory assessment of VOO of the Institute of Agriculture and Tourism in Poreč (Croatia), comprised of eight assessors (4 females, 4 males, average age 39), according to the International Olive Council method described in the European Commission Regulation [4]. The panel is accredited according to the EN ISO/IEC 17025:2017 standard and recognized by IOC in continuation since 2014. Positive and negative sensory attributes were quantified using a 10-cm unstructured intensity ordinal rating scale from 0 (no perception) to 10 (the highest intensity). Differently from the standard method, a modified profile sheet expanded with additional odor (olive fruitiness, green grass/leaves, apple, tomato, green almond, aromatic herbs, chicory, green banana, green coffee beans) and taste (astringency, sweet) attributes was used. Moreover, the hedonic quality attributes (complexity, harmony, persistency) were assessed using a 10-point overall structured rating scale from 0 (the lowest quality) to 10 (the highest quality). Overall VOO sensory quality was graded using an evaluation scale ranging from 1 (the lowest quality) to 9 (the highest quality).

2.4. Statistical Analysis

A three-way analysis of variance (ANOVA) was performed using all the data, with treatment, cultivar, and year as the main factors. Multiple comparisons of means were based on a Tukey's test at $p \leq 0.05$. Statistical analysis was performed using the Statistica 13.2 software (StatSoft®, Palo Alto, CA, USA).

3. Results

3.1. Concentration of Minerals in Olive Leaves

Concentration of macro- and micro-elements in Istarska bjelica and Leccino leaves, after foliar treatment with fertilizer containing S and N (0 mL SN, 7.5 mL SN, 15 mL SN, and 22.5 mL SN) that were collected in two consecutive years are reported in Table 1. As demonstrated in our previous report, the concentration of S was significantly higher in the leaves that were treated with 15 mL SN and 22.5 mL SN when compared to other two treatments, while no effect of the applied fertilizer on the N concentration was observed [29]. A higher concentration of S was found in Leccino leaves in 2020 when compared to all other combinations. The concentration of S remained unchanged in Istarska bjelica leaves in both years. The Leccino leaves in 0 mL SN and 7.5 mL SN treatments were more abundant in N than Istarska bjelica leaves [29]. Treatment significantly affected the concentrations of Mg and manganese (Mn) with the lowest concentrations found at 22.5 mL SN when compared to all the other treatments. Istarska bjelica contained more K, Cu, and Si than Leccino, while Leccino had more Mn and Mo than Istarska bjelica. The effect of year was significant for most of the studied elements. While the concentration of P, Ca, Iron (Fe), Mn, Cu, Mo, and Si was higher in 2020 than in 2019, K was found to be the only element that was more abundant in leaves in 2019 than in 2020.

Table 1. The effect of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water), cultivar (cv.) (Istarska bjelica, Leccino), and collection year (Y) (2019, 2020) on the concentrations of macro- and micro-elements in olive leaves.

Source of Variation	g/kg					mg/kg					
	Phosphorus	Potassium	Calcium	Magnesium	Sodium	Iron	Manganese	Copper	Boron	Molybdenum	Silicon
Treatment (T)											
0 mL SN	2.74 ± 0.17	11.12 ± 0.51	12.69 ± 0.79	0.88 ± 0.03 [a]	58.09 ± 5.17	28.63 ± 2.01	43.84 ± 2.26 [a]	18.84 ± 1.75	15.41 ± 0.61 [a]	1.14 ± 0.10	141.93 ± 10.02
7.5 mL SN	2.41 ± 0.14	11.02 ± 0.33	13.43 ± 1.09	0.88 ± 0.04 [a]	52.70 ± 4.85	28.35 ± 2.55	38.40 ± 2.52 [b]	16.85 ± 0.91	14.88 ± 0.60 [a]	1.04 ± 0.09	139.43 ± 12.82
15 mL SN	2.78 ± 0.15	11.79 ± 0.52	12.36 ± 0.78	0.88 ± 0.02 [a]	52.98 ± 4.40	28.17 ± 2.11	39.15 ± 1.96 [a,b]	18.29 ± 1.15	14.88 ± 0.46 [a]	1.11 ± 0.11	145.87 ± 11.37
22.5 mL SN	2.67 ± 0.18	11.70 ± 0.49	12.92 ± 0.70	0.75 ± 0.04 [b]	52.17 ± 4.72	26.59 ± 1.65	38.48 ± 2.69 [b]	18.13 ± 0.98	13.59 ± 0.28 [b]	1.04 ± 0.09	137.79 ± 10.23
p-value	n.s.	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	**	n.s.	n.s.
Cultivar (Cv.)											
Istarska bjelica	2.71 ± 0.11	10.51 ± 0.26 [a]	10.62 ± 0.34 [b]	0.83 ± 0.03	64.60 ± 2.68 [a]	29.34 ± 1.61	33.43 ± 1.05 [b]	20.96 ± 0.82 [a]	15.68 ± 0.42 [a]	0.96 ± 0.06 [b]	152.26 ± 6.47 [a]
Leccino	2.59 ± 0.12	12.30 ± 0.31 [b]	15.08 ± 0.53 [a]	0.86 ± 0.02	43.37 ± 2.86 [b]	26.53 ± 1.27	16.50 ± 1.39 [a]	15.09 ± 0.54 [b]	13.70 ± 0.21 [b]	1.20 ± 0.07 [a]	130.25 ± 8.46 [b]
p-value	n.s.	***	***	n.s.	***	n.s.	***	***	***	**	**
Year (Y)											
2019	2.38 ± 0.11 [b]	12.66 ± 0.25 [a]	11.64 ± 0.56 [b]	0.86 ± 0.03	43.78 ± 3.09 [b]	22.66 ± 0.93 [b]	35.91 ± 1.46 [b]	16.79 ± 1.05 [b]	14.46 ± 0.28	0.88 ± 0.06 [b]	111.93 ± 6.97 [b]
2020	2.92 ± 0.09 [a]	10.15 ± 0.24 [b]	14.07 ± 0.55 [a]	0.83 ± 0.02	64.19 ± 2.52 [a]	33.21 ± 1.29 [a]	44.03 ± 1.60 [a]	19.26 ± 0.57 [a]	14.92 ± 0.45	1.28 ± 0.05 [a]	170.58 ± 4.15 [a]
p-value	***	***	***	n.s.	***	***	***	**	n.s.	***	***
T × Cv.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.
T × Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Cv. × Y	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	***	n.s.	n.s.
T × Cv. × Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.

[1] The results are expressed as means ± standard errors (*n* = 4). Different superscript lowercase letters in a column represent statistically significant differences between the mean values for each main effect at $p \leq 0.05$ that were obtained by a three-way ANOVA and Tukey's test. First- (T × Cv., T × Y, Cv. × Y) and second-order interactions (T × Cv. × Y) are presented. Significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s.—not significant.

First-order interactions between the treatment and cultivar was significant only for Ca (Figure 1a). A lower leaf Ca concentration was found in Istarska bjelica than in Leccino leaves in all the treatments except at 22.5 mL SN, while no difference was observed between the treatments within each cultivar. The interaction between cultivar and year affected only the concentration of sodium (Na, Figure 1b). In both years, the leaves of Istarska bjelica were more abundant in Na, while in the leaves of both cultivars, a higher concentration was found in 2020 compared to 2019. The second-order interaction was significant for the concentration of boron (B, Figure 1c). The treatments 7.5 mL SN and 15 mL SN in 2020 showed a higher concentration of B in Istarska bjelica leaves compared to all the other combinations. No difference in the concentration of B in Leccino leaves was observed between the treatments and between years.
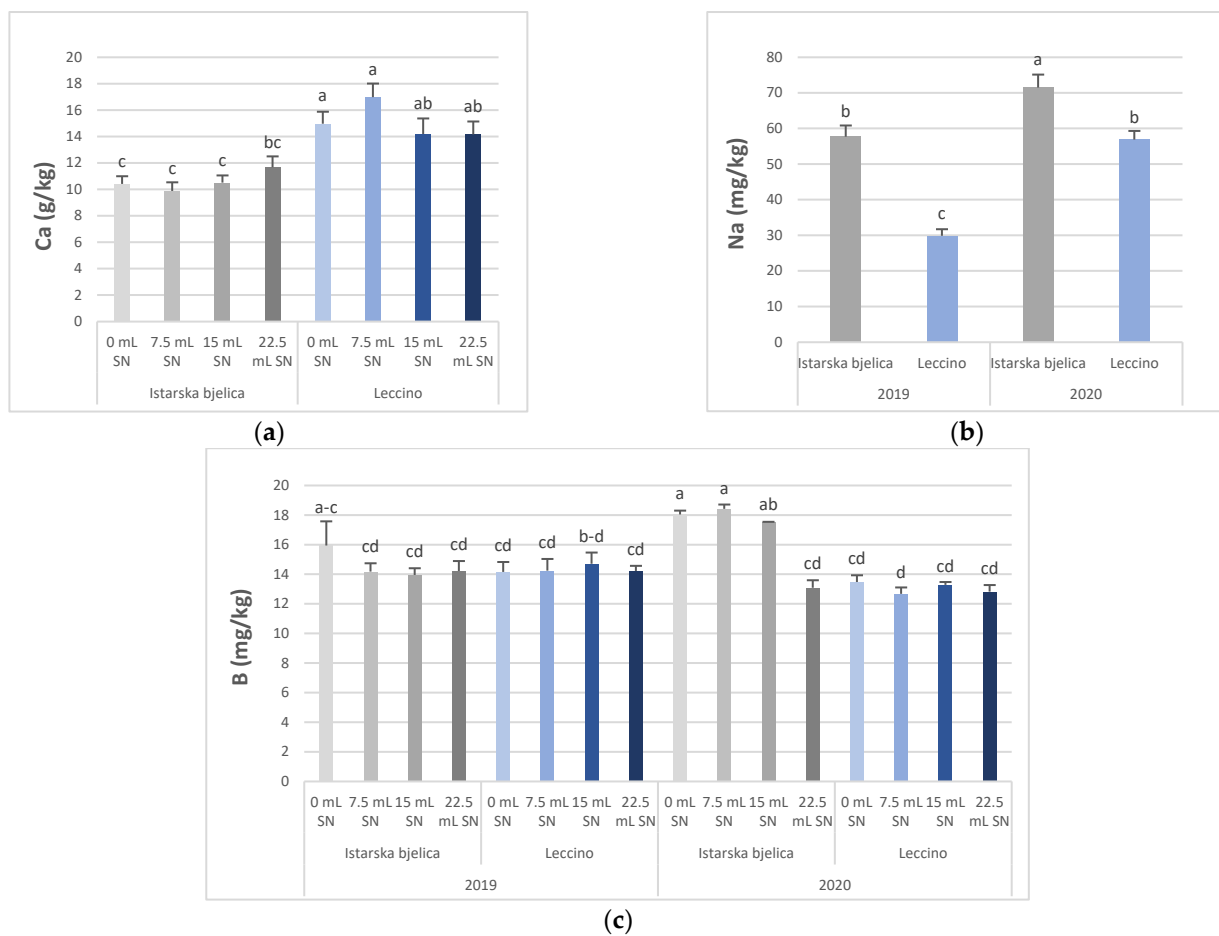

(a)

(b)

(c)

Figure 1. Multiple comparisons of the effects of (**a**) treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water) and cultivar (Cv.) (Istarska bjelica, Leccino) on the concentration of calcium (Ca); (**b**) cultivar, and year (Y) (2019, 2020) on the concentration of sodium (Na); and (**c**) treatment, cultivar, and year on the concentration of boron (B) in olive leaves. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test.

3.2. VOO Volatile Compounds

The concentrations of VOO volatile compounds are shown in Table 2 and typical chromatogram of volatile aroma compounds that are found in monovarietal oil by HS-SPME-GC-MS is shown in Figure S1 Istarska bjelica VOO contained a higher amount of 1-penten-3-one when compared to Leccino VOO, while opposite results were obtained for 3,7-decadiene I. The first-order interaction between treatment and cultivar had a significant impact on the concentration of (*E*)-2-hexenal (Figure 2a), (*Z*)-3-hexenal (Figure 2b),

3-pentanone (Figure S2a), 3,7-decadiene II (Figure S2b), and (*E*)-3-hexenal (Figure S2c). Generally, the concentration of the first three above-mentioned compounds was higher in Istarska bjelica than in Leccino VOOs. The concentration of 3-pentanone was higher in Istarska bjelica VOOs of 0 mL SN, 7.5 mL SN, and 15 mL SN treatments, while no difference between the cultivars was observed at 22.5 mL SN treatment. The concentration of this compound was higher in 15 mL SN treatment Istarska bjelica VOO in relation to 7.5 mL SN and 22.5 mL SN treatments of the same cultivar. The concentrations of (*E*)-3-hexenal and (*Z*)-3-hexenal were significantly higher in all the samples of Istarska bjelica VOO than in those of the Leccino cultivar. The concentration of (*E*)-3-hexenal was higher at 0 mL SN than at 7.5 mL SN treatment, while the concentration of (*Z*)-3-hexenal was higher at 0 mL SN than at 15 mL SN and 22.5 mL SN treatments (Figure 2b). The concentration of none of these three compounds varied between the treatments in the Leccino VOO samples. The concentration of 3,7-decadiene II did not vary among the treatments in any of the cultivars, but it was significantly higher in the 0 mL SN and 7.5 mL SN treatments for Leccino VOOs than in the corresponding Istarska bjelica samples. The concentration of (*E*)-2-hexenal was higher in Istarska bjelica VOO of 22.5 mL SN treatment than in those of 0 mL SN and 7.5 mL SN treatments of this cultivar, while for the Leccino cultivar, a higher concentration was found at 15 mL SN when compared to the 22.5 mL SN treatment (Figure 2a). In all the treatments, the concentration of this volatile was higher in the Leccino samples. When we summed-up the concentrations of all the identified VOCs, the values in all the treatments were higher in Leccino VOOs then in Istarska bjelica samples (data not shown).

The first-order interaction between treatment and year significantly affected the concentrations of the majority of the studied volatile compounds (Table 2). The concentration of all the volatiles that are shown in Figure 3 and Figure S2, except (*E*)-3-hexenal and (*Z*)-2-hexenal, did not vary in 2019, being at the same time lower than in 2020. Specifically, the concentration of 3-pentanone in the Leccino samples was higher at 0 mL SN and 15 mL SN treatments compared to the remaining two (Figure S3a). The concentrations of 3-ethyl-1,5-octadiene and 3,7-decadiene II followed the same pattern and were higher in the VOOs of the 22.5 mL SN treatments in 2020 when compared to 0 mL SN and 7.5 mL SN treatments of the same year (Figures S2c and S3b). The concentrations of 1-penten-3-one (Figure 3b), (*E*)-2–pentenal (Figure S3d), and (*E*)-2-hexenal (Figure 3a) reached their maximum in VOOs of 15 mL SN and 22.5 mL SN treatments in 2020, while the concentration of 3,7-decadiene I (Figure S3e) was higher only in the 22.5 mL SN treatment VOO when compared to the 0 mL SN one. The concentration of (*E*)-3-hexenal was higher in 15 mL SN and 22.5 mL SN treatment VOOs of 2020 when compared to the same treatments from 2019, but no significant difference between the years was noted for the other two treatments (Figure S3f). In 2019 a higher concentration of this volatile compound was found in 0 mL SN than in 22.5 mL SN treatment VOO, while in 2020 a lower concentration was found in the 7.5 mL SN treatment than in 15 mL SN and 22.5 mL SN treatment VOOs. The concentration of (*Z*)-2-hexenal was higher after all the treatments in 2020 when compared to 2019. Its concentration in 22.5 mL SN treatment in 2019 was lower in relation to the 0 mL SN treatment VOO, and in 2020 higher values were reached in the 15 mL SN treatment than in the 0 mL SN and 7.5 mL SN treatment VOOs (Figure S3g). The total VOCs concentration, obtained by summing up the concentrations of all the identified VOCs, in all the treatments was higher in 2020 than in 2019. In 2020, the total VOCs concentration was higher in 15 mL SN and 22.5 mL SN treatments when compared to the remaining two, while in 2019, the values were higher in VOOs of 0 mL SN treatment with respect to the 15 mL SN and 22.5 mL SN treatments (data not shown).

Table 2. The effect of treatment (T) (0, 7.5, 15, and 22.5 mL of the applied SN foliar fertilizer per 1 L of water), cultivar (cv.) (Istarska bjelica, Leccino), and collection year (Y) (2019, 2020) on the concentrations of volatile compounds in olive oil.

Source of Variation	Aldehydes (mg/kg)											Alcohols (mg/kg)					
	3MB	HAL	Z2PAL *	E2PAL	E3HAL *	Z3HAL *	Z2HAL *	E2HAL	OAL	E,EHAL *	E,ZHAL *	E2POL	HOL	E3HOL	Z3HOL	E2HOL	Z2HOL
Treatment (T)																	
0 mL SN	0.05 ab	0.25 b	0.03 a	0.04 b	0.09 a	1.00 a	0.23 a,b	21.12 b	0.04 a	0.04	0.05 a	0.03 c	0.02	<LOD	0.30 a	0.18	<LOD b
7.5 mL SN	0.04 b	0.25 b	0.02 b	0.05 b	0.07 b	0.82 a,b	0.19 c	19.81 b	0.03 b	0.04	0.04 ab	0.04 bc	0.09	<LOD	0.23 ab	0.40	<LOD b
15 mL SN	0.06 a	0.42 a	0.02 b	0.06 a	0.09 a,b	0.72 b	0.25 a	24.03 a	<LOD c	0.04	0.04 ab	0.05 b	0.03	<LOD	0.34 a	0.23	0.06 a
22.5 mL SN	0.05 a,b	0.42 a	0.03 a	0.06 a	0.08 a,b	0.73 b	0.21 b,c	21.78 a,b	<LOD c	0.04	0.03 b	0.06 a	0.03	<LOD	0.13 b	0.14	0.04 a
p-value	**	***	***	***	***	***	***	***	***	n.s.	**	***	n.s.	*	***	n.s.	***
Cultivar (Cv.)																	
Istarska bjelica	0.02 b	0.35	0.03 a	0.05	0.11 a	1.60 a	0.24 a	11.23 b	0.04 a	0.08 a	0.07 a	0.04 b	0.03	<LOD	0.42 a	0.22	0.05 a
Leccino	0.08 a	0.32	0.02 b	0.05	0.05 b	0.04 b	0.19 b	32.14 a	<LOD b	<LOD b	0.01 b	0.05 a	0.06	<LOD	0.08 b	0.25	<LOD b
p-value	***	n.s.	***	n.s.	***	***	***	***	***	***	***	*	n.s.	***	***	n.s.	***
Year (Y)																	
2019	0.04 b	0.12 b	0.04 a	0.05	0.07 b	0.68 b	0.16 b	15.20 b	0.04 a	0.04	0.08 a	0.04 b	0.06	<LOD	0.14 b	0.23	<LOD b
2020	0.06 a	0.55 a	0.01 b	0.05	0.09 a	0.96 a	0.28 a	28.17 a	0.00 b	0.04	<LOD b	0.05 a	0.04	<LOD	0.36 a	0.25	0.05 a
p-value	***	***	***	n.s.	***	***	***	***	***	n.s.	***	***	n.s.	*	***	n.s.	***
T×Cv.	*	***	**	n.s.	*	***	n.s.	***	***	n.s.	*	***	n.s.	n.s.	***	n.s.	***
T×Y	***	***	*	***	**	n.s.	***	***	***	**	**	***	n.s.	n.s.	**	n.s.	***
Cv.×Y	***	*	n.s.	***	n.s.	***	***	n.s.	***	*	***	***	n.s.	***	***	*	***
T × Cv.×Y	***	**	*	n.s.	n.s.	n.s.	n.s.	n.s.	***	**	*	***	n.s.	*	*	n.s.	***

Source of Variation	Carboxylic Acid (mg/kg)	Ketones (mg/kg)		Unsaturated Hydrocarbons (mg/kg)				Other (mg/kg)
	AA *	3PO	PEN3O	3E 1,5 OEN *	DECAI *	DECAII *	DECAIII *	Z2P + Z3H
Treatment (T)								
0 mL SN	0.15	0.08 a	0.80 b	0.60	0.28	0.37	0.04 b	0.75 b
7.5 mL SN	0.15	0.06 b,c	0.81 b	0.58	0.26	0.33	0.13 a	0.75 b
15 mL SN	0.14	0.08 a,b	1.04 a	0.65	0.28	0.38	0.03 b,c	1.37 a
22.5 mL SN	0.13	0.06 c	1.01 a	0.66	0.30	0.39	0.01 c	1.26 a
p-value	n.s.	***	***	n.s.	n.s.	n.s.	***	***
Cultivar (Cv.)								
Istarska bjelica	0.14	0.09 a	1.04 a	0.59 b	0.26 b	0.31 b	0.03 b	1.38 a
Leccino	0.14	0.05 b	0.79 b	0.65 a	0.30 a	0.43 a	0.08 a	0.69 b
p-value	n.s.	***	***	*	**	***	***	***

Table 2. *Cont.*

Source of Variation	Aldehydes (mg/kg)											Alcohols (mg/kg)					
	3MB	HAL	Z2PAL *	E2PAL	E3HAL *	Z3HAL *	Z2HAL *	E2HAL	OAL	E,EHAL *	E,ZHAL *	E2POL	HOL	E3HOL	Z3HOL	E2HOL	Z2HOL
Year (Y)																	
2019			0.14	0.04 b		0.83 b		0.53 b		0.26 b		0.32 b		0.02 b			0.99 b
2020			0.14	0.10 a		1.00 a		0.71 a		0.30 a		0.41 a		0.09 a			1.08 a
p-value			n.s.	***		***		***		**		***		***			*
T×Cv.			*	**		n.s.		n.s.		n.s.		*		***			***
T×Y			***	***		***		***		***		***		***			***
Cv.×Y			n.s.	***		n.s.		*		n.s.		n.s.		***			n.s.
T×Cv.×Y			***	n.s.		n.s.		n.s.		n.s.		n.s.		***			*

[1] Different superscript lowercase letters in a column represent statistically significant differences between the mean values for each main effect at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test. First- (T × Cv., T × Y, Cv. × Y) and second-order interactions (T × Cv. × Y) are presented. Significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s.—not significant. Identification of volatile compounds, 3MB—3-methylbutanal; HAL—hexenal; Z2PAL—(*Z*)-2-pentenal; E2PAL—(*E*)-2-pentenal; E3HAL—(*E*)-3-hexenal; Z3HAL—(*Z*)-3-hexenal; Z2HAL—(*Z*)-2-hexenal; E2HAL—(*E*)-2-hexenal; OAL—octanal; E,EHAL—(*E,E*)-2,4-hexadienal; E,ZHAL—(*E,Z*)-2,4-hexadienal; E2POL—(*E*)-2-penten-1-ol; HOL—1-hexanol; E3HOL—(*E*)-3-hexen-1-ol; Z3HOL—(*Z*)-3-hexen-1-ol; E2HOL—(*E*)-2-hexen-1-ol; Z2HOL—(*Z*)-2-hexen-1-ol; AA—acetic acid; 3PO—3-pentanone; PEN3O—1-penten-3-one; 3E1,5OEN —3-ethyl-1,5-octadiene; DECAI—3,7-decadiene I; DECAII—3,7-decadiene II; DECAIII—3,7-decadiene III; Z2P + Z3H—(*Z*)-2-penten-1-ol + (*Z*)-3-hexenyl acetate. LOD—limit of detection [30]. * Volatile compounds for which pure standards were not available were quantified semi-quantitatively, and their concentrations (mg/kg) were expressed as equivalents of compounds with similar chemical structure for which standards were available, assuming a response factor = 1.
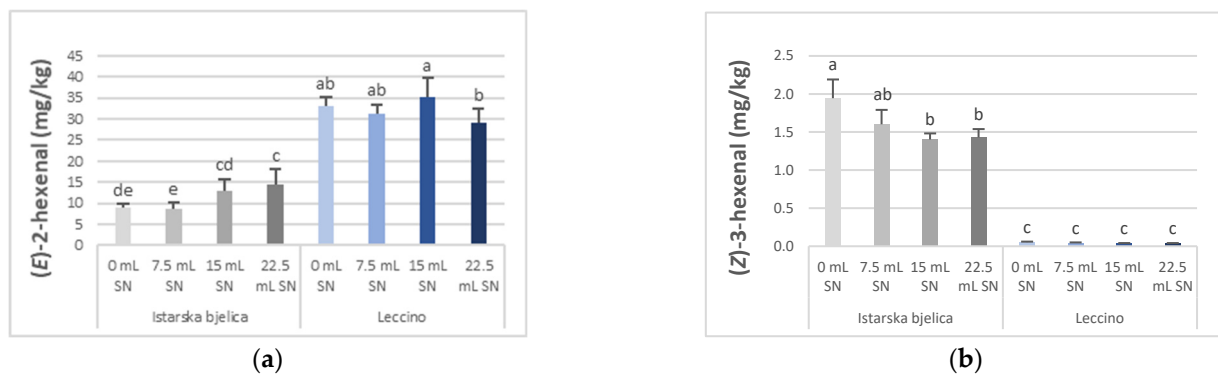
(**a**)

(**b**)

Figure 2. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of the applied SN foliar fertilizer per 1 L of water) and cultivar (Cv.) (Istarska bjelica, Leccino) on the concentration of (**a**) (*E*)-2-hexenal, and (**b**) (*Z*)-3-hexenal. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test.
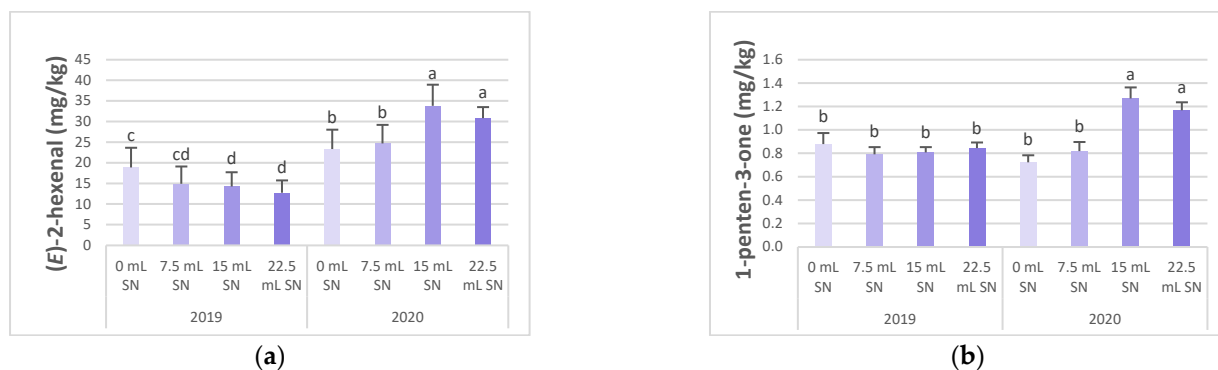

(**a**)

(**b**)

Figure 3. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water) and year (Y) (2019, 2020) on the concentration of (**a**) (*E*)-2-hexenal, and (**b**) 1-penten-3-one. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test.

The first order interaction between cultivar and year resulted in a significant change in the concentrations of (*Z*)-3-hexenal (Figure 4) and other five compounds shown in Figure S4. In particular, concentration of 3-pentanone and (*Z*)-2-hexanal did not vary between the cultivars in 2019 and was lower than the corresponding values in 2020 (Figure S3a,b). The concentration of both of these compounds in 2020 was higher in Istarska bjelica VOOs. In addition, the concentration of 3-ethyl-1,5-octadiene was stable in 2019, but in 2020 higher values were obtained in Leccino VOOs (Figure S4c). The concentration of (*E*)-2-pentenal was higher in the Istarska bjelica cultivar VOO in 2019 and did not vary between the cultivars in 2020 (Figure S4d). The concentration of (*Z*)-3-hexenal was higher in Istarska bjelica than in Leccino VOO samples in both years. Its concentration in Leccino VOOs did not vary between the years and its concentration in Istarska bjelica VOOs was higher in 2020 than in 2019.

The second-order interaction significantly affected the majority of the studied volatile compounds (Figure S5). The concentration of 3–methylbutanal was generally higher in the oils of the Leccino cultivar. The concentration of this compound did not significantly differ between the years in any of the treatments of the Istarska bjelica cultivar, while for Leccino higher values were found in 15 mL SN and 22.5 mL SN treatment VOOs in 2020 when compared to the same treatments in 2019 (Figure S5a). The concentration of hexanal was stable for both cultivars in all the treatments in 2019, while in 2020 its concentration

significantly differed only at 7.5 mL SN, being higher in Istarska bjelica VOOs. For both cultivars in 2020, a higher concentration was found in 15 mL SN and 22.5 mL SN treatment VOOs compared to the remaining two treatments (Figure S5b). The concentration of 3,7-decadiene III was even in VOOs of all the treatments on Istarska bjelica cultivar in both years and for both cultivars in 2019. The concentration of this compound was higher at 0 mL SN and 7.5 mL SN treatments of the Leccino VOO samples in 2020 than in 2019 (Figure S5c). The concentration of (*Z*)-2-pentenal was generally higher in 2019 and in Istarska bjelica VOOs. The presence of octanal was detected only in 0 mL SN and 7.5 mL SN VOOs of Istarska bjelica cultivar in 2019, being higher in 0 mL SN treatment than in the 7.5 mL SN treatment VOOs. The concentration of (*E*)-2-penten-1-ol in 2019 differed between the cultivars only at 22.5 mL SN, with a higher value determined in Leccino VOO. In 2020, a significant difference was not found between the cultivars nor between the treatments within each cultivar. Istarska bjelica VOOs from 15 mL SN and 22.5 mL SN treatments from 2020 contained a higher amount of this compound than the corresponding samples from 2019. Similarly, Leccino 0 mL SN treatment showed higher values in 2020, but VOOs from 22.5 mL SN treatment in 2019 contained more (*E*)-2-penten-1-ol than those from 2020 (Figure S5d). The Istarska bjelica cultivar VOOs generally contained a higher amount of (*Z*)-2-penten-1-ol + (*Z*)-3-hexen-1-yl acetate. Specifically, for this cultivar there was no difference in the concentration of the above-mentioned sum of compounds between the years after treatment with 0 mL SN and 7.5 mL SN, while in 2020 higher amounts were found at 15 mL SN and 22.5 mL SN treatments. The concentration of (*Z*)-2-penten-1-ol + (*Z*)-3-hexen-1-yl in the Leccino samples did not vary between the years (Figure S5e). The concentration of (*Z*)-3-hexen-1–ol was even in all the treatments and for both cultivars in 2019 and additionally in the Leccino cultivar VOOs from 2020. Istarska bjelica VOOs from 0 mL SN and 15 mL SN treatments contained a higher concentration of this compound in 2020 than in the corresponding samples from 2019 (Figure S5f). The presence of (*E*,*Z*)-2,4–hexadienal was detected only in samples from 2019. (*Z*)-2-hexen-1-ol was found only in VOOs deriving from 15 mL SN and 22.5 mL SN treatments of the Istarska bjelica cultivar from 2020. The concentration of acetic acid in Istarska bjelica oils was higher in the 0 mL SN treatment in 2019 than in 2020, but in 15 mL SN and 22.5 mL SN treatment VOOs it was higher in 2020 than in 2019. The Leccino cultivar 0 mL SN treatment VOO contained more acetic acid in 2019 and the 22.5 mL SN treatment VOO was more abundant in this compound in 2020 than in 2019. No difference for the other two treatments was noticed between the years. In 2020, no difference in acetic acid concentration was found between the cultivars, while in 2019 Istarska bjelica VOOs from 15 mL SN and 22.5 mL SN treatments contained less acetic acid than Leccino VOOs, while the remaining two treatments were evenly abundant in this carboxylic acid (Figure S5g).
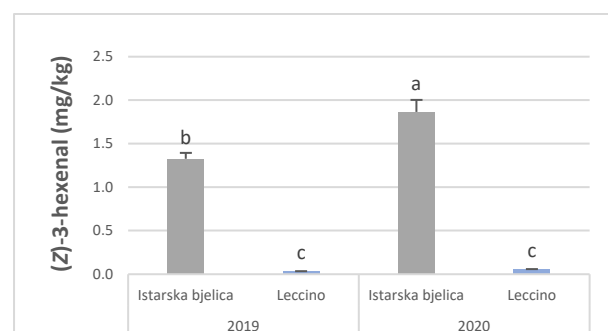

Figure 4. Multiple comparisons of the effects of cultivar (Cv.) (Istarska bjelica, Leccino) and year (Y) (2019, 2020) on the concentration of (*Z*)-3-hexenal. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test.

3.3. Sensory Analysis

The results of sensory analysis of the investigated VOOs are shown in Table 3. All first-order interactions were significant only for the overall sensory quality (Figure 5) and green banana odor (Figure S5a–c). The interaction T x Cv. significantly affected the overall sensory quality as follows: Istarska bjelica VOOs obtained from 22.5 mL SN treatment had a lower overall sensory score when compared to the 15 mL SN treatment ones, while Leccino VOOs that were obtained from the 22.5 mL SN treatment had lower overall sensory score when compared to all the other treatments. Generally, Istarska bjelica VOOs were of higher sensory quality compared to Leccino VOOs. Regarding T × Y interaction, 0 mL SN treatments in 2019 produced VOOs of higher sensory quality compared to 7.5 mL SN treatments, while in 2020 VOOs from the 22.5 mL SN treatment had the lowest sensory quality score when compared to the other treatments. Only VOOs that were produced at 22.5 mL SN treatment differed in quality between the years, with those from 2020 being of lower sensory quality, while no difference between the years was found for other treatments. The Cv. × Y interaction resulted in a higher overall sensory score for Istarska bjelica samples in both years revealing no difference between them. Leccino VOOs were of higher sensory quality in 2019. For green banana odor, the first-order interaction between cultivar and treatment revealed that this odor was more intense in VOOs of the Istarska bjelica cultivar. No difference in the intensity of green banana odor was noticed between the treatments in VOOs of this cultivar, while it was more intense in 7.5 mL SN treatment compared to 0 mL SN and 22.5 mL SN treatments for Leccino VOOs. Green banana odor was more pronounced in 2020 at 0 mL SN, 7.5 mL SN, and 15 mL SN treatments VOOs, while no difference between the treatments was noted in 2019. The interaction of cultivar and year revealed that in VOO of Istarska bjelica from 2020 the green banana odor was more intense than in 2019, while for Leccino VOO no difference was found between the years.

No perception of sweet taste was noticed in oils of Istarska bjelica cultivar (data not shown). The odor of green coffee beans was significantly affected by T × Y interaction (Figure S6d). VOOs from 0 mL SN, 7.5 mL SN, and 15 mL SN treatments from 2020 had a more intense green coffee bean aroma, while no difference for VOOs deriving from 22.5 mL SN treatment was found between the years. In 2020, VOOs deriving from different treatments did not differ in green coffee bean odor intensity, while in 2019 this odor was more pronounced in 22.5 mL SN compared to 0 mL SN and 15 mL SN treated samples. From the Cv. x Y interaction, it was clear that Istarska bjelica VOOs, especially those from 2020, contributed the most to the value of the intensity of this odor in this multiple comparison (Figure S6e).

The second-order interactions were significant for all other odor and taste attributes (Figure 6 and Figure S7). The odor of olive fruits was more pronounced in Istarska bjelica VOOs, especially in 2020 (Figure 6a). Similar results were obtained for green grass/leaves, aromatic herbs, and chicory odors (Figure S7a–c); bitterness (Figure 6b), pungency, and astringency; as well as for complexity and persistency of the oils (Figure S7d–g). The Istarska bjelica samples from 2019 were not characterized by apple odor. In 2020, only the 22.5 mL SN treatment Leccino VOOs was characterized by green tomato aroma. Green almond odor was not present in Istarska bjelica VOOs from 2019. In 2019, more harmonious VOOs were obtained by 22.5 mL SN Istarska bjelica treatments than by the corresponding Leccino ones, while in 2020, in addition to 22.5 mL SN VOOs, a greater sensation of harmony was also observed for the Istarska bjelica cultivar VOOs of 0 mL SN treatment (Figure S7h).

Table 3. The effect of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water), cultivar (cv.) (Istarska bjelica, Leccino), and collection year (Y) (2019, 2020) on the intensities of sensory attributes and the overall sensory scores of olive oil.

Source of Variation	Olive Fruity	Green Grass/ Leaves	Apple	Tomato	Green Almond	Aromatic Herbs	Chicory	Green Banana	Green Coffee Beans	Bitter	Pungent	Astring	Complex	Harmon	Persist	Overall
Treatment (T)																
0 mL SN	6.26 a	4.10 a	1.69 a	0.83 b	2.39 a	2.23 b	2.48 c	2.91 a	1.43	5.95 c	6.43 c	1.71 c	8.63 a,b	8.72 a	8.84 b,c	8.46 a
7.5 mL SN	6.14 b	4.02 a,c	1.54 b	0.72 b	2.22 b	2.52 a	2.98 a	3.06 a	1.43	6.30 a	6.78 a	2.24 a,b	8.69 a,b	8.41 b,c	9.09 a	8.43 a
15 mL SN	6.13 b	3.80 b	1.37 c	0.69 b	2.18 b	2.38 a	2.69 b	2.94 a	1.34	6.06 b,c	6.57 b	2.14 b	8.75 a	8.53 b	8.94 a,b	8.46 a
22.5 mL SN	5.87 c	3.31 c	1.54 b	1.25 a	1.92 c	2.18 b	2.38 c	2.57 b	1.58	6.19 a,b	6.68 a	2.33 a	8.56 b	8.28 c	8.72 c	8.26 b
p-value	***	***	***	***	***	***	***	***	n.s.	***	***	***	*	***	***	***
Cultivar (Cv.)																
Istarska bjelica	6.78 a	4.57 a	0.97 b	0.96 a	1.53 b	2.92 a	3.55 a	3.19 a	2.77 a	7.19 a	7.41 a	2.64 a	9.20 a	8.66 a	9.55 a	8.71 a
Leccino	5.42 b	3.04 b	2.10 a	0.79 b	2.83 a	1.73 b	1.72 b	2.54 b	0.12 b	5.06 b	5.81 b	1.57 b	8.11 b	8.31 b	8.25 b	8.09 b
p-value	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Year (Y)																
2019	5.84 b	3.38 b	1.04 b	1.53 a	1.43 b	1.69 b	1.92 b	2.45 b	1.09 b	6.21 a	6.78 a	2.05 b	8.45 b	8.48	8.84 b	8.43 a
2020	6.36 a	4.24 a	2.03 a	0.22 b	2.92 a	2.96 a	3.35 a	3.28 a	1.79 a	6.04 b	6.44 b	2.16 a	8.86 a	8.48	8.95 a	8.38 b
p-value	***	***	***	***	***	***	***	***	***	***	***	**	***	n.s.	*	*
T × Cv.	***	***	***	***	***.	***	***	***	n.s.	***	***	n.s.	***	***	*	***
T × Y	***	***	***	***	***	***	***	***	***	***	***	n.s.	***	**	*	***
Cv.× Y	***	***	***	***	***	***	n.s.	***	***	***	***	***	***	**	***	***
T × Cv. × Y	***	**	***	***	***	***	***	n.s.	n.s.	*	***	***	**	*	*	n.s.

[1] Different superscript lowercase letters in a column represent statistically significant differences between the mean values for each main effect at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test. First- (T × Cv., T × Y, Cv. × Y) and second-order interactions (T × Cv. × Y) are presented. Significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s.—not significant. Identification of sensory attributes and scores, odor attributes: green—green grass and leaves; taste attributes: astring—astringency; complex—complexity; harmon—harmony; persist - persistency; overall—overall sensory score.
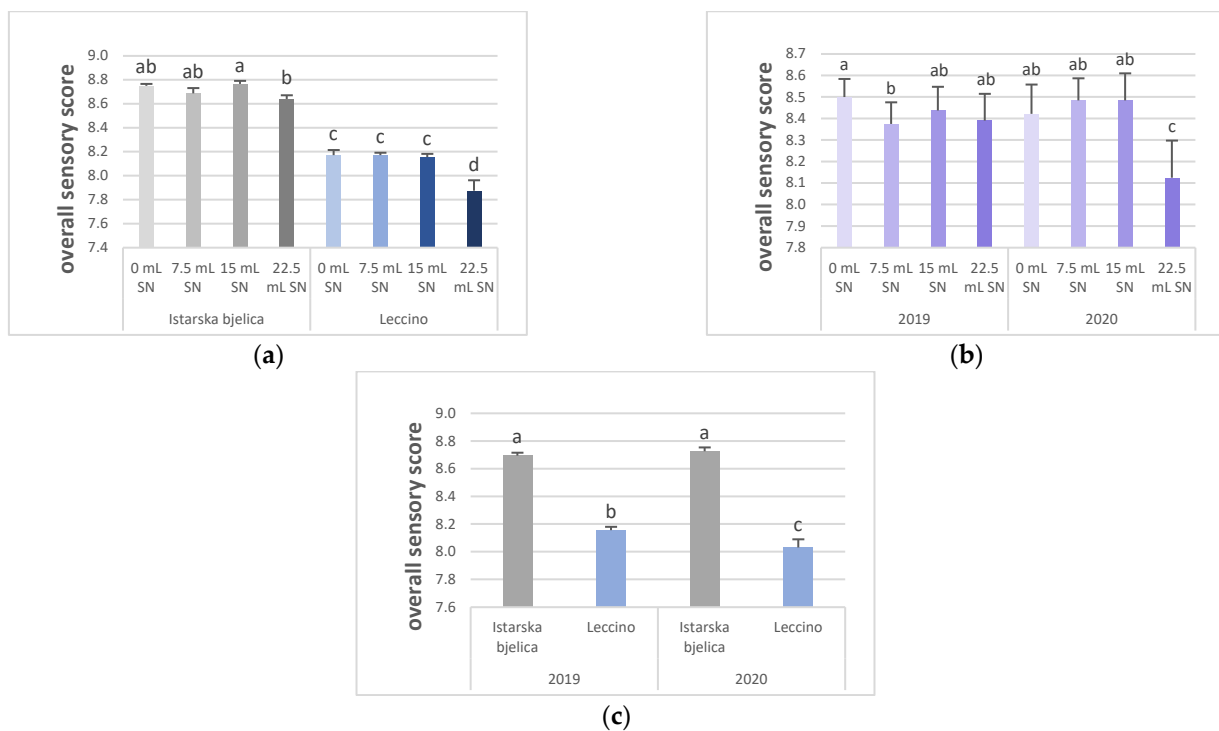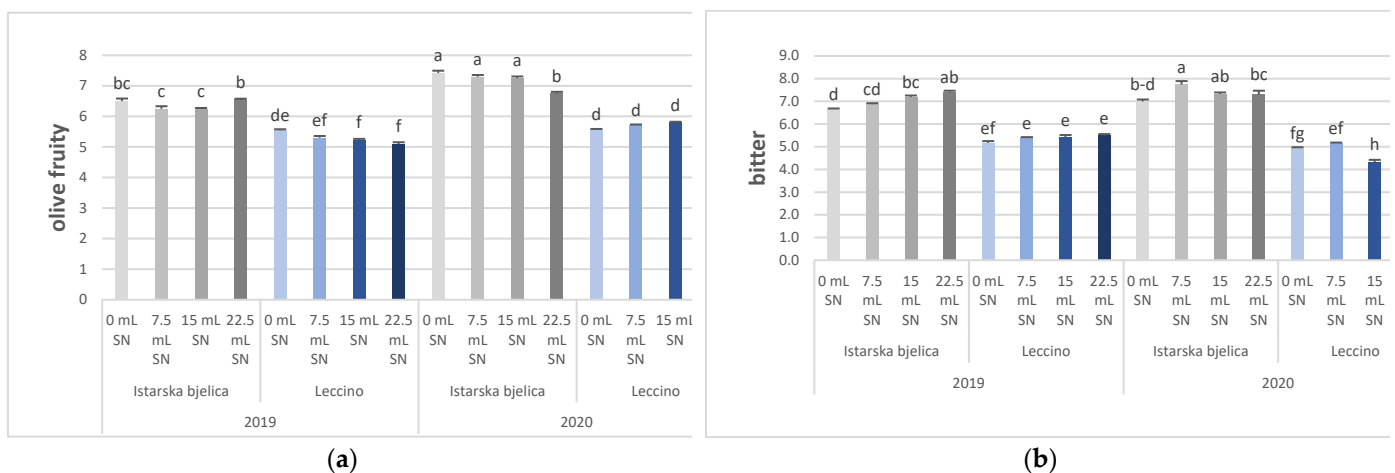
(a)

(b)

(c)

Figure 5. Multiple comparisons of the effects of (**a**) treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water) and cultivar (Cv.) (Istarska bjelica, Leccino), (**b**) treatment and year (Y) (2019, 2020), and (**c**) cultivar and year on the overall sensory score of olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test.

(a)

(b)

Figure 6. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water), cultivar (Cv.) (Istarska bjelica, Leccino), and year (Y) (2019, 2020) on the intensity of (**a**) olive fruity and (**b**) bitter attributes of olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test.

4. Discussion

4.1. Minerals in Olive Leaves

As it is well-known, the determination of leaf-nutrient status is the best method for predicting tree nutritional requirements, and consequently planning future fertilization [32]. Although the concentration of N and S applied by foliar fertilization in our experiment

was variable, analysis of the leaf mineral status showed optimal concentrations of both nutrients in all the samples [33]. In addition, the N concentration remained even in all the treatments while the S concentration increased under SN nutrition, confirming that the key role of N is to improve S assimilation [29]. Furthermore, the concentration of all the other elements in olive leaves was adequate after all the treatments, with the exception of Mg and B whose concentrations were at the limit of deficiency and Na whose concentration was excessive (Table 1) [32]. The concentration of S that was determined in leaves of the Leccino olive cultivar was positively correlated with that of 3-methylbutanal (r = 0.71, $p < 0.001$, data not shown) and with the intensity of the sensory perception of sweet taste attribute (r = 0.78, $p < 0.001$, data not shown) determined in the investigated VOOs. A possibility that the relationship between the concentrations of S and 3-methylbutanal and the intensity of sweet attribute is causal should not be a priori rejected, especially knowing that in a previous study, a correlation between the latter two parameters was also determined [27].

Mn and Ca were positively correlated with the concentration of (*E*)-2-hexenal (r = 0.75, $p < 0.001$ and r = 0.76, $p < 0.001$, respectively, data not shown) showing at the same time a positive correlation with the sweet attribute perception. Although in the literature the contribution of (*E*)-2-hexenal is mostly related to odors that are reminiscent of almonds, green, green apple-like, and cut grass, as well as the perception of bitterness [27], our results indicate this compound might be also indirectly related to VOOs sweet taste. The level of Mn in leaves had a negative correlation with taste attributes such as bitter and pungent, while K correlated negatively with the intensity of green grass/leaves odor (data not shown).

4.2. Volatile Compounds in Virgin Olive Oils

The mineral nutrition of higher plants is the most important factor affecting the nutritional value and quality of fruits and consequently of the obtained oils. Despite this, to the best of our knowledge, this is the first study reporting the effect of combined S and N foliar fertilization on the volatile composition and sensory attributes of VOO. Zheljazkov et al. [34] conducted S and N fertilization on sweet basil (each nutrient separately), while Wang et al. [35] conducted an experiment in which they applied N alone or in a combination with S on sweet corn. Both research groups found a general increase in the volatile compound contents as a response to N fertilization, but S seemed to have no effect on the concentration of volatiles. Significant differences in the generation of volatiles in relation to S fertilization was determined by Bloem et al. [36] for oil radish, chamomile, field beans, and peas, but not for white mustard. From these results, no unequivocal conclusion could be made because obviously SN nutrition affected different plants in different ways. However, there is clearly a potential of SN nutrition for enhancing oil volatile content. In this study, in all the examined samples, the most abundant volatile compound was (*E*)-2-hexenal, followed by (*Z*)-3-hexenal and 1-penten-3-one (Table 2). (*E*)-2-hexenal is formed mainly through the lipoxygenase (LOX) pathway by the oxidation of linolenic acid [37], its contribution is described as reminiscent of green grass and other positive odors, and its quantitative dominance in the volatile profile is typical for European virgin olive oils [38]. Comparing the VOO samples deriving from different treatments, a significant difference in their volatile profiles was observed; however, for all the determined volatile compounds except (*E*)-3-hexenol, the first- and/or second-order interactions were also significant (Figures 2–4 and Figures S1–S4). Regni et al. [28] found that the quality of olive oil was not influenced by foliar treatment with N and the content of polyphenols in the oil in their trial remained unaltered. Dabbaghi et al. [7] reported that foliar fertilization with N, together with K and P, negatively affected the concentrations of (*E*)-2-hexenal, (*E,Z*)-2,4-heptadienal, 4,8-dimethyl-1,3,7-nonatriene, and (*E,Z*)-2,4-decadienal. In our study, opposite results were obtained for (*E*)-2-hexenal, while concentrations of (*E,Z*)-2,4-hexadienal and 3,7-decadiene isomer III decreased in 22.5 mL SN treatment when compared to 0 mL SN treatment VOOs, confirming, on the other hand, the results of Dabbaghi et al. [7]. The same group of authors observed an increase in the concentration of saturated aldehydes in samples that were treated with N, which is a result that is opposite to ours, possibly due to differences in the composition

of the fertilizer that was used (P and K vs. S). Ancín-Azpilicueta et al. [39] reported that the application of N in the form of urea in the vineyard increased the concentrations of some esters in wine, while the total concentration of alcohols decreased, which is in line with the results of this study. The concentration of (*E*)-2-hexenal in our experiment was higher in VOOs of 15 mL SN treatment than in those of 0 mL SN and 7.5 mL SN treatments, the concentration of (*Z*)-3-hexen-1-ol decreased at 22.5 mL SN treatment when compared to 0 mL SN and 15 mL SN VOOs, while no effect was observed for the concentration of hexanol (Table 2). Abdeljelil et al. [40] reported that foliar fertilization with a S-containing fertilizer drastically increased the concentration of (*E*)-2-hexenal in olive leaves. In our case, the highest dose of S did not cause any effect on the concentration of this unsaturated aldehyde in oil, but its concentration was significantly higher after 15 mL SN treatment then after 7.5 mL SN and 0 mL SN treatments. 1-Penten-3-one, that according to some authors [27] contributes to the bitter taste of olive oil, was confirmed to be the fundamental ketone in all the samples and its concentration was enhanced in 15 mL SN and 22.5 mL SN treatment VOOs compared to the remaining two. In brief, by summing up the concentrations of individual compounds belonging to the same chemical group, we observed that an increase in the contents of saturated aldehydes can be noticed in VOOs of 15 mL SN and 22.5 mL SN treatments compared to those of the 0 mL SN treatment. The VOO of 15 mL SN treatment was characterized by a far higher concentration of ketones compared to the VOOs of the other treatments, somewhat higher concentration of aldehydes and, together with VOO of the 7.5 mL SN treatment, by higher concentration of alcohols. The concentration of total unsaturated hydrocarbons and acetic acid did not change significantly among the treatments. By summing up the concentrations of all detected volatiles, we observed that their concentration was higher in VOOs of 15 mL SN treatment (31,08 mg/kg) when compared to 0 mL SN (27.18 mg/kg) and 7.5 mL SN treatments (25.79 mg/kg, data not shown).

Besides fertilization, the composition of VOO can be influenced by other factors such as cultivar, growing and environmental conditions, harvest time, fruit storage, olive oil production conditions, etc. [2,8,22,41,42]. With the aim to minimize the influence of other factors that were not relevant for this study, the experiment was performed in the same way during two consecutive years, and the collected fruits were processed under the same conditions [31]. A significant difference in the volatile profiles between the two cultivars was observed (Table 2). The concentration of many of the studied volatile compounds varied as a consequence of the first and/or the second order interactions between cultivar and the remaining two factors, treatment and year (Figure 2, Figure 4, Figures S1, S3 and S4). Only the concentrations of 1-penten-3-one and 3,7-decadiene I were dependent exclusively on the cultivar and no interactions with other factors were found, while the concentration of hexanol remained stable in all combinations (Table 2). The concentration of 1-penten-3-one in Istarska bjelica VOOs was 1.04 mg/kg and in Leccino VOOs was 0.79 mg/kg (Table 2). Koprivnjak et al. [11] reported a lower concentration of this ketone in VOO of Istarska bjelica and Leccino cultivars, Servilli et al. [43,44] obtained a concentration that was similar to ours in Leccino, while Brkić-Bubola et al. [31] reported by far lower concentration of this compound in oils from other Croatian cultivars, such as Buža, Črna and Rosinjola. The concentration of the most abundant volatile compound, (*E*)-2-hexenal, was much higher in the Leccino samples than in those from Istarska bjelica (Table 2) and this could be among the reasons why the intensity of green almond odor was more pronounced in Leccino VOOs. The concentration of the most abundant volatile compound, (E)-2-hexenal, was much higher in the Leccino samples than in those from Istarska bjelica. This difference was more pronounced in samples from 0 mL SN and 7.5 mL SN treatment (around four-times higher concentration in the Leccino samples) than in 15 mL SN and 22.5 mL SN treatments (three- and two-times higher in the Leccino samples, respectively, Figure 3a). The concentration of this compound was reported to be lower in VOOs from Istarska bjelica and other Croatian cultivars, while in the Leccino samples, Koprivnjak et al. [11] found higher a concentration. Servilli et al. [43] reported the concentration of this compound to be very similar to those that were determined in this study. Runcio et al. [45] found that the concentration of (*Z*)-3-hexenol in Leccino oil was 0.44 mg/kg.

Similar results were also obtained by Koprivnjak et al. [11]. Servilli found 0.22 mg/kg [43], while Leccino VOOs in this study contained only 0.08 mg/kg of this compound on average (Table 2). Taking into account that the first- and the second-order interactions were mainly due to the differences in the concentration of (Z)-3-hexenol in VOOs of Istarska bjelica, it can be concluded that these two results are in a disagreement. The concentration of (E)-2-hexenol in VOOs that were extracted from Istarska bjelica olives in this study was higher than that which was reported by Koprivnjak et al. [11]. The concentration of the same compound observed in Leccino VOOs was much lower when compared to that which was obtained by Koprivnjak et al. [11], but was in excellent agreement with the results of Servilli et al. [43] for this cultivar. The concentration of hexanal in this study was as twice as high as that which was reported by Koprivnjak et al. [11], but still much lower than that which was reported by Servilli et al. [43] for Leccino oils. However, it seemed that the concentration of this C6 aldehyde, a precursor of hexanol, another important VOO aroma volatile [46], was not cultivar-dependent, since a significant effect of cultivar was not determined (Table 2). In brief, by summing up the concentrations of individual compounds belonging to the same chemical group, we observed that the Leccino cultivar VOO was characterized by a much higher concentration of aldehydes, a somewhat higher content of unsaturated hydrocarbons, and a lower amount of alcohols and ketones when compared to Istarska bjelica EVOO, while the concentration of acetic acid was approximately equal in the samples that were obtained from both cultivars on average. When we summed up the concentrations of all the identified VOCs, their concentration was far higher in oils of the Leccino cultivar (37.18 mg/kg) than in those of the Istarska bjelica cultivar (19.00 mg/kg, data not shown).

Besides fertilization practices and genotype, environmental conditions may affect the activity of enzymes and, subsequently, the chemical composition of olive oil in general, including also their volatile profiles [22]. Most of the examined volatiles had a greater concentration in 2020 compared to 2019 (Table 2). Some authors reported that the concentration of hexanal, (E)-2-hexenal, and (E)-2-hexen-1-ol can be affected by high temperatures [38,47]. In this experiment, the year 2020 was generally characterized by higher temperatures, but in 2019 the heat stress during fruit development was very severe and could have influenced the obtained results. The concentrations of hexanal and (E)-2-hexenal were much higher in 2020, while year did not affect the concentration of the latter one. The concentration of the first two compounds was affected by higher order interactions (Figure 3 and Figure S4b). From the second-order interaction, it was clear that the differences in hexanal concentrations were mainly due to the effect of year, which is in accordance to the results that were published by Kaya et al. [48], but in disagreement with those that were obtained by Runcio et al. [45]. The latter authors reported that harvest year did not have any effect on the concentration of this aldehyde but found a large difference among the cultivars. The concentration of (E)-2-hexenal was mostly influenced by the interaction between treatment and year (Figure 3a). Gomez-Rico et al. [49] reported an increase in (E)-2-hexenal, (Z)-3-hexen-1-ol and hexanol concentration with the increase of the available water amount. Generally, 2020 was characterized by much higher precipitation than 2019, specifically during the summer months and October, which could have contributed to the higher concentrations of (E)-2-hexenal and (Z)-3-hexen-1-ol that were found in 2020, while the concentration of hexanol did not differ significantly among the years. Kaya et al. [48] found that year did not have any effect on 3-ethyl-1,5-octadiene, unlike cultivar that significantly affected its concentration. In our study, the concentration of 3-ethyl-1,5-octadiene was significantly influenced by interactions of year with both treatment and cultivar (Figures S2b and S3c). From the Cv. x Y interaction, it was clear that this variability was mainly due to the differences between cultivars in 2020. According to some authors [50,51], the effects of temperature and humidity significantly depend on the interaction with cultivar. In this study a great influence of the interaction between year and treatment was also confirmed for most of the studied volatiles. Tura et al. [52] reported no influence of temperature on the volatile composition of Leccino cultivar VOO. In brief, after we summed up the concentrations of individual compounds belonging to the same chemical group,

we observed that VOOs from 2020 contained a higher concentration of all the examined classes of compounds than those from 2019, except acetic acid. By summing up the concentrations of all the identified VOCs, we found far higher values in 2020 (35.57 mg/kg) when compared to 2019 (20.62 mg/kg, data not shown).

4.3. Sensory Attributes

From a sensory point of view, all the analyzed VOO samples belonged to the class of extra VOO due to the presence of fruitiness and the absence of any sensory defects [53]. Generally, Istarska bjelica VOOs were mainly characterized by high intensities of bitter, pungent, and olive fruity odor, while Leccino VOOs had medium intensities of the mentioned attributes (Table 3). Sensory attributes that are connected to the odor of VOO are closely related to the volatile composition, and certain attributes or sensations are usually a result of complex combinations and interactions of various volatile compounds. However, differences in the relative concentrations of certain volatiles in VOO provide useful information on its sensory quality, supporting the work of sensory panels. Bitter is the primary taste attribute and derives mainly from secoiridoid and other phenols and, to a much lesser degree, volatile compounds [2]. The characteristic olive fruity odor of VOO is characteristic for VOOs deriving from healthy and fresh olive fruits and it is a basic positive attribute [2]. Pungent taste is characteristic for oils that are produced from olives that are unripe [2]. All the three attributes (olive fruity, bitter, and pungent) were significantly affected by interaction between treatment, cultivar, and year. Olive fruity notes were most pronounced in Istarska bjelica VOOs from 2020 in all treatments except in that with the highest dose of S and N, where a slightly lower intensity was perceived (Figure 6a). A bitter taste was mainly associated to Istarska bjelica VOOs from both years, with VOOs from 7.5 mL SN treatment being more bitter in 2020 than in 2019 (Figure 6b). Istarska bjelica VOOs had higher pungent intensities then Leccino VOOs, especially at 22.5 mL SN treatment in 2020 (Figure S7d). In addition, other sensory attributes such as odors that are reminiscent of cut grass, green almond, aromatic herbs, and green banana are also considered quite important for the overall VOO aroma. The presence of hexanal is often associated with the perception of green apple and cut-grass notes, while (*E*)-2-hexenal is a carrier of, among other things, bitter almond nuances. These two compounds are often accounted among principal contributors to the green odor notes of VOO [2,54,55]. (*Z*)-3-hexen-1-ol is also related to green grass/leaves and green banana flavor [10]. While C5 and C6 volatiles are in general associated with positive attributes of VOO, particular C7-C10 volatiles are often associated with odor defects [55]. The concentration of C7-C10 volatiles in this experiment remained unchanged under the applied treatments or was inversely proportional to the applied concentration of S and N. The presence of short-chain carboxylic acids in VOO is also often linked to the occurrence of sensory defects [2]. Acetic acid was the only investigated compound belonging to this class and its concentration remained even under all the applied treatments. The overall sensory score was lower for VOOs of both cultivars after 22.5 mL SN treatment compared to all the others (Figure 5a), but it is important to underline that all the investigated VOOs, independent of the treatment, were graded by overall sensory scores that were higher than eight which classified them as extra virgin from the sensory quality point of view.

The Leccino cultivar VOOs had less pronounced olive fruity, bitter, and pungent sensory attributes and were, therefore, sweeter in comparison to Istarska bjelica VOOs (Table 3). This result could be partially attributed to the probably higher content of phenols that are ordinarily found in Istarska bjelica oils [29], which were not analyzed in this study. Despite being described as having more pronounced apple and green almond odors (Table 3), Leccino VOOs were graded by overall sensory scores that were slightly lower than those that were attributed to Istarska bjelica (Table 3). Such results support the thesis that fruits from different cultivars grown under the same environmental conditions produce oils with a different volatile composition, and consequently have different sensory attributes. These differences are mostly due to the enzymatic heritage that is linked to genotype. On

the other hand, several authors underlined the importance of the influence of environment and harvest year on the composition of volatiles and thus the sensory attributes of olive oil [56–58], which was confirmed in this study. However, the quality of all the investigated samples strongly depended on the interactions between all the three investigated factors.

5. Conclusions

Foliar application of S and N significantly affected the composition of volatile compounds and sensory quality of VOO, confirming that such a practice can modulate characteristic olive oil odor and taste attributes and thus possibly influence its acceptability and preference among consumers. In all the investigated samples, the most abundant volatile compound was (*E*)-2-hexenal, followed by (*Z*)-3-hexenal and 1-penten-3-one. From the sensory point of view, all the analyzed VOOs complied with the requirements of the extra virgin olive oil quality category, having pronounced fruitiness and being free of sensory defects. The overall sensory score was highly dependent on the first-order interactions and was lower for samples that were obtained from the treatment with the highest SN dose for Leccino VOOs and VOOs that were produced by this treatment in 2020. Istarska bjelica VOOs were graded by higher overall sensory score, probably due to more pronounced green grass/leaves, aromatic herbs, bitter, pungent, and astringent sensory attributes that were noted in these samples. The results of this study could help to better understand the link between specific fertilization practices and the sensory attributes of olive oil, which can be of economic importance. Still, mostly due to multiple complex interactive effects of the three investigated factors, many issues remained unresolved and further studies need to be conducted to elucidate, in more detail, the possible causal relationships between SN fertilization and the resulting olive oil sensory quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8100912/s1, Figure S1. Sample of chromatogram of volatile aroma compounds found in monovarietal oil by headspace solid-phase microextraction combined with gas chromatography/ mass spectrometry (HS-SPME-GC-MS); Figure S2. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water) and cultivar (Cv.) (Istarska bjelica, Leccino) on the concentration of (a) 3-pentanone, (b) 3,7-decadiene II, and (c) (*E*)-3-hexenal in olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test; Figure S3. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water) and year (Y) (2019, 2020) on the concentration of (a) 3-pentanone, (b) 3-ethyl-1,5-octadiene, (c) 3,7-decadiene II, (d) (*E*)-2-pentenal, (e) 3,7-decadiene I, (f) (*E*)-3-hexenal, and (g) (*Z*)-2-hexenal in olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test; Figure S4. Multiple comparisons of the effects of cultivar (Cv.) (Istarska bjelica, Leccino) and year (Y) (2019, 2020) on the concentration of (a) 3-pentanone, (b) (*Z*)-2-hexenal, (c) 3-ethyl-1,5-octadiene, and (d) (*E*)-2-pentenal in olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test. Figure S5. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water), cultivar (Cv.) (Istarska bjelica, Leccino), and year (Y) (2019, 2020) on the concentration of (a) 3-methylbutanal, (b) hexanal, (c) 3,7-decadiene III, (d) (*Z*)-2-pentenal, (e) (*Z*)-2-penten-1-ol + (*Z*)-3-hexen-1-yl acetate, (f) (*Z*)-3-hexen-1-ol, and (g) acetic acid in olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test; Figure S6. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of applied SN foliar fertilizer per 1 L of water) and cultivar (Cv.) (Istarska bjelica, Leccino) (a) on the intensity of banana flavor; treatment and year (Y) (2019, 2020) (b) on the intensity of banana flavor; cultivar and year (c) on the intensity of banana flavor; treatment and year (d) on the intensity of coffee flavor; cultivar and year (e) on the intensity of coffee flavor of olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test. Figure S7. Multiple comparisons of the effects of treatment (T) (0, 7.5, 15, and 22.5 mL of

applied SN foliar fertilizer per 1 L of water), cultivar (Cv.) (Istarska bjelica, Leccino), and year (Y) (2019, 2020) on the intensity of (a) grass, (b) herbs, and (c) chicory flavors and on the intensity of (d) spicy, (e) astringency, (f) complexity, (g) persistency, and (h) harmony attributes of olive oil. Different superscript lowercase letters represent statistically significant differences between the mean values at $p < 0.05$ that were obtained by a three-way ANOVA and Tukey's test.

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