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Characterization of VOCs Emitted by Foliage of Grapevine cv. Isabella for Prospecting Innovative Cropping Systems

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Abstract: Volatile organic compounds play an important role in communication within plants as well as with other organisms. In this work we identified the volatile organic compounds (VOCs) emitted from the foliage of the grapevine cv. Isabella, a largely known hybrid of *Vitis vinifera* × *Vitis labrusca*. Our data show 25 VOCs emitted by cv. Isabella. Different compound classes were found, including alcohols, hydrocarbons, esters, terpenes, ketones, and a green leaf volatile (GLV). The study highlighted differences between volatile profiles for diurnal and nocturnal treatments. The compounds: trans-3-dodecene, 5,5 dibutylnonane, ethyl 2-methylactate, 2-hexanol, 3-ethyl-2-heptanol, 3-nonanol, and 2-nonanol, have not been previously reported for *Vitis vinifera* foliage. Notably, eight compounds emitted by cv. Isabella, 1-heptanol, 1-octanol, 2-hexanol, 2-nonanone, β-pinene, camphene, cis-hexenyl acetate, and phenethyl alcohol, are of relevant interest for their role in plant defense. New knowledge on the emission of these compounds in cv. Isabella can help to understand the mechanisms of pathogen tolerance of this genotype and could be an important step in prospecting innovative cropping systems.

Keywords: VOCs emission; *Vitis labrusca* × *Vitis vinifera*; agroecological systems; phenethyl alcohol



Citation: Rodríguez-Declét, A.; Castro-Marín, A.; Moretti Conti, L.; Lombini, A.; Chinnici, F.; Rombolà, A.D. Characterization of VOCs Emitted by Foliage of Grapevine cv. Isabella for Prospecting Innovative Cropping Systems. *Agronomy* **2022**, *12*, 272. <https://doi.org/10.3390/agronomy12020272>

Academic Editor: Gerardo Fernández Barbero

Received: 21 December 2021

Accepted: 18 January 2022

Published: 21 January 2022

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1. Introduction

Volatile organic compounds (VOCs) represent a large and structurally diverse group of secondary metabolites produced by plants. VOCs are substances with low molecular weight [1] which are formed through many biochemical pathways, constitutively and/or after stress induction, with nearly all plant organs being able to secrete and emit a number of these compounds [2–5]. They can be emitted throughout the life cycle of the plant or, more commonly, at specific developmental stages such as leaf maturation, senescence, flowering, and fruit ripening [3]. The main classes of VOCs emitted by plants belong to the group of terpenoids, fatty-acid-derived C6-volatiles and derivatives, phenylpropanoid aromatic compounds as well as certain alkanes, alkenes, alcohols, esters, aldehydes, and ketones [6,7].

Plant emissions of VOCs can be influenced by both biotic and abiotic stresses and play an important role in communication within plants as well as with other organisms, such as the attraction of insects (herbivorous insects and pollinators) [4]. VOCs can repel herbivores or attract herbivore's parasitoids/predators [8,9]. In addition, VOCs can directly influence the development of pathogens [7] and prime plant defense mechanisms [10,11], and signal the activation of defense and control mechanisms against pests and pathogens [12]. Plant defense mechanisms involve the emission of well-known defense compounds (i.e., MeSA, MeJA, terpenoids, etc.), during pathogen and pest attack, highlighting the role of VOCs in plant–pathogen/pest interactions [13,14]. Examples show that defensive volatile chemicals can lead to an influence on multitrophic interactions, as a recent study showed that an infection of canola by the root pathogen *Plasmodiophora brassicae* Woronin reduced oviposition

by bertha army worm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae) [15]. Further, VOCs released from resistant plants can trigger specific defensive responses in other parts of the plant itself, and/or to neighboring plants of various species [12].

The *Vitis* genus is highly diverse with eighty species identified, and composed of several thousand varieties [16]. Consequently, considering that VOCs vary based on genotype, several profiles of different genotypes of *Vitis* spp. have been described, mostly focused on grape berries for oenological purposes [17–19]. Furthermore, a qualitative investigation of VOCs in cv. Moscato bianco (*Vitis vinifera*) clearly differentiated all vegetative organs confirming the specialization in volatile production among different organs [20]. Volatile emissions have also been described in *Vitis* root tissue [21]. In wild grapevine (*Vitis vinifera* subsp. *sylvestris*) sexual dimorphism was described in floral scent [22], with some VOCs involved in the attraction of well-known inflorescence visitors [23,24].

Differences in VOC profiles between downy mildew (*Plasmopara viticola*)-resistant and -susceptible grapevine genotypes have been reported [25–28]. Few studies have characterized the VOCs released by grape foliage, with some in vivo studies on cv. Marselan [29], cv. Chardonnay [30], cv. Pinot noir [31], and grapevine leaves of plants of cv. Sangiovese associated with arbuscular mycorrhizal fungi [32]. Fewer data are available on grapevine diurnal and nocturnal rhythms [31,33]. Diurnal and nocturnal rhythm could influence grapevine plant pathogens and insects. For example, the pests European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae) and European grape berry moth *Eupoecilia ambiguella* (Lepidoptera: Tortricidae) are crepuscular [34,35]. In addition, seasonal changes determine different susceptibility to common pests and diseases that attack grapevine, for example downy mildew symptoms mostly occur during springtime [36].

The Isabella cultivar is an interspecific spontaneous crossing of *Vitis vinifera* and *Vitis labrusca*, of American origin, which shows resistance to powdery mildew (*Erysiphe necator*), bunch rot (*Botrytis cinerea*), and tolerance to downy mildew (*Plasmopara viticola*), cultivated worldwide for juice, table grapes, and wine production [37]. Previous studies highlight the rich inherent antioxidant levels in the seed, stalk, and leaf components of cv. Isabella [38]. However, little is known about the VOCs emitted by the cultivar, in particular from epigeic plant tissues.

Grapevine management practices include fertilization, irrigation, soil and canopy management, and plant protection practices. Several pests and diseases affect grapevine production; thus, an intensive pesticide schedule is often required to meet production standards [39]. Nevertheless, it has been demonstrated that a continuous use of plant protection products has several negative implications for the environment and human health [40,41]. Consequently, there is a strong need to explore and re-valorize strategies to achieve sustainable production standards. VOCs can be a natural and agroecological solution to enhance crop defense mechanisms [42].

Thus, the main objective of the present study was to identify and characterize the VOCs emitted from foliage of the grapevine cv. Isabella during diurnal and nocturnal periods under controlled conditions. In addition, the experiments were performed in two different seasons (fall and spring).

The profiling of VOCs emitted by cv. Isabella can contribute to the understanding of plant defense mechanisms, as plants are able to produce chemical compounds that influence the growth and development of pathogens, pests, and weeds [3,7,13,42]. Knowledge on cv. Isabella VOC emissions could lead to the prospect of innovative sustainable systems based on intercropping.

2. Materials and Methods

2.1. Plant Material

The present study was carried out through two experiments performed under a controlled environment in a greenhouse located at the Department of Agriculture and Food Sciences at the University of Bologna, Italy.

Plants of the grapevine genotype Isabella (*Vitis vinifera* × *Vitis labrusca*), grafted on rootstock 225 Ruggeri in February 2017, were obtained from a nursery. Vines were maintained under refrigeration (4 °C) until being planted in the greenhouse at the end of June 2019. The first experiment was conducted in October 2019. Pruning was performed at the end of December 2019, as plants reached two years. In June 2020, the second experiment was performed. For the duration of the study, plants were watered to field capacity, and full-strength Hoagland solution was regularly provided to maintain satisfactory plant growth conditions. The plants sampled were similar in growth (shoot length and numbers of leaves) and nutritional status. No inflorescences or bunches were present. Only plants with a healthy appearance (visual inspection) were used for experiments.

2.2. Collection of VOCs

VOC sampling included two distinct collection times, a nocturnal and a diurnal period in fall and the following spring. The sampling was set up for a total of four hours for the diurnal collection time and twelve hours for the nocturnal collection time. Similar sampling durations have been employed in grapevine and other species [29–31,43]. While the nocturnal period extended to 12 h, the diurnal period was limited to 4 h to avoid possible interferences due to more intense gas exchanges.

The collection of the VOCs emitted by the cv. Isabella foliage was performed with the cartridge adsorbent Radiello™ BTEX/VOCs (RAD130, Sigma-Aldrich S.r.l., Milan, Italy), which acts as a diffusive sampler, used to quantify the VOCs emitted in the confined atmosphere surrounding the plant, as described in similar experiments [44].

The system for enclosing the plants (Figure 1) was a modified version of the system described by Kigathi et al. [43]. For the enclosure, a low-density polyethylene (PE.Ld) neutral food grade bag (20 L) (LDPE MOCA 2020, Cristianpack S.r.l., Osimo (AN), Italy) was used. The bag enclosed the foliage (shoots with approximately 40 and 27 leaves for the first and second experiment, respectively; spur and trunk) of each individual plant, with a respective cartridge placed inside the enclosed system. To avoid any interference the bag was supported from the outside and the rootstock and soil was separated from the upper part by closing the system from the scion up. After each collection period the cartridges were put in the provided volatile-free tube and immediately taken to the laboratory for analysis. Empty bags, without enclosing a plant, were also used to see any background noise, and traces of VOCs were detected (3-methyl-3-pentanol, 4-methyl tetradecane, 4-methyl-2-pentanol, methyl palmitate, octadecyldimethylsilvyl ether).

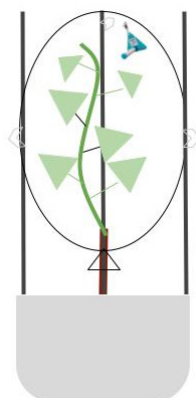


Figure 1. Conceptual sketch of the collection system by enclosed plant foliage and cartridge adsorbent Radiello™.

2.3. First Experiment (Fall 2019)

The treatment consisted of three biological replicates, each composed by one single plant. The same plants were used for both the diurnal and nocturnal collection times. Plants presented two shoots with an average length of 1.15 m and about 40 leaves. The nocturnal

sampling was initiated on 30 October 2019 (7:00 p.m.) and samples were collected the following day (7:00 a.m.) after 12 h of exposure; the diurnal collection (8:30–12:30 a.m.) period (4 h) immediately followed. Condensation moisture, associated with plant transpiration, was observed at the end of the diurnal collection time. During sampling, the temperatures were between 18.1 °C and 16.3 °C. Relative humidity (RH) ranged from 82% to 84% in the nocturnal period and from 84% to 88% in the diurnal period.

2.4. Second Experiment (Spring 2020)

On 4 June 2020, the second experiment was initiated. This experiment consisted of six biological replicates of one plant for every diurnal and nocturnal collection time. The sampling and collection system was consistent with the first experiment. The nocturnal period went from 7:00 p.m. to 7:00 a.m., and the diurnal period went from 6:30 to 10:30 a.m. (solar hour). Condensation moisture was observed at the end of the diurnal collection time. To achieve the twelve hours of darkness, as in the first experiment, for the nocturnal collection time, plant exposure to light was prevented by a dark cover. The diurnal and nocturnal collections were performed on different sets of plant. Plants presented three shoots with an average length of 38 cm and about 27 leaves. Temperature and relative humidity during the diurnal sampling period were on average 21.7 °C and 83% RH, and for the nocturnal sampling period an average of 20.1 °C with an average RH of 89%.

2.5. Analysis of Volatile Organic Compounds by GC-MS

Volatile organic compounds were extracted according to the manufacturer's protocol as modified by Joos et al. [45]. For the extraction, the cartridges were submitted to solvent desorption by using ultrapure analytical grade dichloromethane (Merck, Darmstadt, Germany). The use of this solvent avoided some peak tailing problems encountered in our specific chromatographic conditions when using CS₂, another solvent commonly used to desorb the cartridges.

The extraction was started by adding 2 mL of dichloromethane and 100 µL of a 2-octanol solution at 500 mg L⁻¹ as an internal standard directly in the cartridge glass tube without drawing out the cartridge. The tube, containing the cartridge and internal standard, was agitated from time to time for a total of 30 min. The cartridge was then discarded, and the solvent was transferred to a vial to be concentrated to a final volume of 200 µL under a stream of pure nitrogen (N₂), prior to GC-MS analysis.

The Trace GC ultra-apparatus coupled with a Trace DSQ mass selective detector (Thermo Fisher Scientific, Milan, Italy) was equipped with a fused silica capillary column Stabilwax DA (Restek, Bellefonte, PA, USA; 30 m, 0.25 mm i.d., and 0.25 µm film thickness). The carrier gas was He at a constant flow of 1.0 mL/min. In accordance with Castro Marin et al. [46], the GC programmed temperature was as follows: 45 °C (held for 3 min) to 100 °C (held for 1 min) at 3 °C/min, then to 240 °C (held for 10 min) at 5 °C/min. Injection was performed at 250 °C in splitless mode and the injection volume was 1 µL. Detection was carried out by electron ionization (EI) mass spectrometry in full scan mode, using ionization energy of 70 eV. The transfer line interface was set at 220 °C and ion source at 260 °C. The mass acquisition range was *m/z* 30–400 and the scanning rate 5.9 scan s⁻¹. Compounds were then identified by a triple criterion: (i) by comparing their mass spectra and retention time with those of authentic standards, (ii) compounds lacking standards were identified after matching their respective mass spectra with those present in the commercial libraries NIST 08 and Wiley 7, and (iii) matching their Linear Retention Index (LRI) obtained under our conditions with already published LRI on similar polar columns. VOCs were quantified from the total ion current according to the internal standard method. In Table 1, the compounds identified by comparison with pure standards have been highlighted.

Table 1. Rate of emission ($\mu\text{g plant}^{-1} \text{h}^{-1}$) of volatile organic compounds (VOCs) identified to be emitted by foliage of cv. Isabella. ¹ VOCs were collected during diurnal (4 h) and nocturnal (12 h) periods under controlled conditions in two different seasons (fall 2020 and spring 2020), by enclosed plant foliage and cartridge absorbent Radiello™.

No.	RI	Compound	Fall 2019		Spring 2020		
			Diurnal	Nocturnal	Diurnal	Nocturnal	
Terpenes							
1	1078	Camphene	0.0014 ± 0.0008	0.0012 ± 0.0005	0.0058 ± 0.0022	0.0011 ± 0.0006	*
2	1105	β -pinene ^a	0.0056 ± 0.0014	0.0050 ± 0.0011	0.0795 ± 0.0181	0.0034 ± 0.0010	**
Hydrocarbons							
3	1126	Ethylbenzene	nd	0.0014 ± 0.0008	nd	nd	*
4	1202	Dodecane ^a	nd	nd	0.5596 ± 0.2272	0.3431 ± 0.0352	*
5	1240	1-hexadecene	nd	nd	nd	0.0205 ± 0.0246	
6	1257	trans-3-dodecene	0.0018 ± 0.0014	0.0017 ± 0.0004	nd	nd	
7	1298	Tridecane ^a	nd	nd	0.3837 ± 0.2009	0.1641 ± 0.0514	*
8	1314	Butylbenzene	nd	0.0010 ± 0.0014	nd	nd	
9	1392	5,5 dibutylnonane	nd	nd	0.0748 ± 0.0773	0.0360 ± 0.0106	
10	1402	Tetradecane ^a	nd	nd	0.5657 ± 0.2668	0.2092 ± 0.1280	*
Green leaf volatiles (GLVs)							
11	1321	cis-3-hexenyl acetate ^a	0.0003 ± 0.0003	0.0016 ± 0.0024	nd	0.0132 ± 0.0209	
Esters							
12	1453	2-butoxyethyl acetate	nd	nd	0.0636 ± 0.0143	0.0417 ± 0.0147	
13	1495	Ethyl 2-methylactate	nd	0.0065 ± 0.0030	nd	nd	*
Ketones							
14	1364	4-hydroxy-4-methyl-2-pentanone ^a	nd	nd	0.2647 ± 0.2095	nd	**
15	1393	2-nonanone	nd	0.0004 ± 0.0006	nd	nd	
Alcohols							
16	1311	2-hexanol ^a	0.0167 ± 0.0289	0.0137 ± 0.0051	0.0120 ± 0.0123	nd	*
17	1450	1-heptanol ^a	0.0183 ± 0.0048	0.0074 ± 0.0026	0.0822 ± 0.0273	0.0227 ± 0.0184	*
18	1455	2-butoxyethanol ^a	nd	0.0079 ± 0.0029	nd	nd	**
19	1460	3-ethyl-2-heptanol	nd	0.0026 ± 0.0006	nd	nd	*
20	1479	2-ethylhexanol ^a	nd	0.0015 ± 0.0014	nd	nd	
21	1481	3-nonanol	nd	nd	0.0246 ± 0.0084	0.0067 ± 0.0084	**
22	1483	2-hexadecanol	0.0092 ± 0.0045	nd	nd	nd	*
23	1511	2-nonanol ^a	0.0133 ± 0.0050	0.0028 ± 0.0002	0.0112 ± 0.0058	nd	***
24	1557	1-octanol ^a	0.0019 ± 0.0020	0.0010 ± 0.0006	0.0132 ± 0.0111	nd	*
25	1939	Phenethyl alcohol ^a	0.0014 ± 0.0025	nd	0.0135 ± 0.0167	nd	
Total Sum			0.0698 ± 0.0325	0.0556 ± 0.0235	2.1542 ± 0.7873	0.8619 ± 0.1718	**

Identification of compounds by comparing with mass spectra, retention time with those of authentic and already published Linear Retention Index (LRI) on polar columns. Data represent the mean of 3 replicates (2019) and 6 replicates (2020) ± standard deviation. nd = not detected. Significant differences are referred to diurnal/nocturnal collection time within each experiment. Significant at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ (SNK-test). ^a Compound identified with pure standard.

For each volatile compound, the emission rate was calculated and expressed as micrograms per plant per hour ($\mu\text{g plant}^{-1} \text{h}^{-1}$) to account for the cartridge exposure time during sampling (Table 1). To calculate the emission rate of each volatile, the number of compounds adsorbed by the cartridge, obtained as described before, was referred to the pertinent exposure time (4 or 12 h) and were expressed as $\mu\text{g plant}^{-1} \text{h}^{-1}$.

2.6. Statistical Analysis

Data were submitted to statistical analysis using the MATLAB ver. R2021a (Boston, MA, USA). One-way analysis of variance (ANOVA) was carried out to compare diurnal/nocturnal collection time within each experiment. Means were compared by the Student–Newman–Keuls (SNK) test ($p \leq 0.05$).

3. Results

Our study identified a total of 25 volatile organic compounds emitted by the foliage of the cv. Isabella (Table 1). Representative chromatograms of the 4 different sampling (diurnal and nocturnal in seasons 2019 and 2020) are shown in Figure S1. Among the numerous types of VOCs described, a variety of compound classes were found: ten alcohols, eight hydrocarbons, two esters, two terpenes, two ketones, and one green leaf volatile (GLV) (Table 1).

3.1. First Experiment (Fall 2019)

A total of 17 volatile organic compounds were identified in the diurnal and nocturnal collection times performed in the first year trial (Table 1). Compounds 2-hexadecanol and phenethyl alcohol were present only in the diurnal sampling, and ethylbenzene, butylbenzene, 2-nonanone, 2-butoxyethanol, 3-ethyl-2-heptanol, 2-ethylhexanol, and ethyl 2-methylactate were found to be emitted by the plant exclusively during the night (Table 1).

3.2. Second Experiment (Spring 2020)

A total of 16 VOCs were identified to be emitted by the foliage of the cv. Isabella in the diurnal and nocturnal treatments performed in the second experiment (Table 1). Compounds 2-hexanol, 4-hydroxy-4-methyl-2-pentanone, 2-nonanol, 1-octanol, and phenethyl alcohol were identified only during the diurnal collection time and 1-hexadecene and cis-3-hexenyl acetate were found only during the nocturnal period (Table 1). In the second experiment, total VOC concentration increased up to 35% when compared with the first experiment.

4. Discussion

Terpenes, hydrocarbons, green leaf volatiles, esters, ketones, and alcohols were detected as VOCs emitted by foliage of cv. Isabella. Alcohols (10 compounds) and hydrocarbons (8 compounds) were the predominant classes in both diurnal and nocturnal conditions. Similar results were seen in Tasin et al. [30], with hydrocarbons as the most represented class emitted from leaves of cv. Chardonnay.

The predominant compounds detected in the first experiment (fall), for the diurnal and nocturnal periods, were alcohols: 1-heptanol, 2-hexanol, and 2-butoxyethanol (Table 1). As for the second experiment (spring), the predominant compounds identified for both diurnal and nocturnal collection times were tetradecane and dodecane, belonging to the hydrocarbons class (Table 1).

The second experiment, compared to the previous one, was characterized by a different photoperiod (longer light period, shorter dark period), higher light intensity and temperature, lower air relative humidity, and older plants which presumably modified the VOC emission. In *Vitis* spp., a comparison of the complete odor bouquet of cultivars Müller-Thurgau, Regent, and Pinot noir during the whole growing season revealed clear differences between the phenological stages and few between the cultivars [47]. The prevalence of hydrocarbons in the VOCs emitted by plants in the spring experiment may be mainly

attributed to leaf age and season. In grapevine, it has been reported that branches with young leaves collected in spring emit hydrocarbons, but not alcohols [30]. The prevalence of alcohols in the fall experiment, may be due to a predominant emission of oxygenated VOCs by old leaves near senescence, as reported in other species [48].

The most abundant volatiles emitted *in vivo* from leaf-bearing shoots of cv. Chardonnay was (E)-ocimene [30], (E,E)- α -farnesene in cv. Pinot Noir [31], and (E)-2-hexenal in leaf tissue of cv. Sangiovese [32]. In young and mature leaves of cv. Moscato bianco, geraniol and benzyl alcohol were the alcohols found in highest concentrations [20].

In our conditions, cv. Isabella released seven volatiles (5,5 dibutylnonane, ethyl 2-methyl lactate, 3-ethyl-2-heptanol, 2-hexanol, 2-nonanol, 3-nonanol, and trans-3-dodecene) which have not been previously reported in foliage (shoots and trunk) of grapevine.

Six of these compounds (5,5 dibutylnonane, 3-ethyl-2-heptanol, 2-hexanol, 2-nonanol, 3-nonanol, and trans-3-dodecene) have been found in other species [49–53]. Interestingly, trans-3-dodecene was found in rice cells elicited by rice blast (*Magnaporthe oryzae*) [54]. The compound 2-hexanol, known to elicit responses in several insects [55,56], has been recently found in grape berries [57]. The possible roles played by these compounds in cv. Isabella should be ascertained by further research.

VOCs identified in Isabella could play a role in various defense, physiological, and other functions, such as communication within and between plants and other organisms [4,12], attraction and repulsion of insects [58], interactions with pathogens [7], and plant defense mechanisms [10,11,58].

Data indicates that there are significant changes between diurnal and nocturnal emission times. In the 2019 experiment (fall), compounds 1-heptanol and 2-nonanol exhibited higher emission rates during daylight. Concerning the second experiment (spring 2020), camphene, β -pinene, dodecane, tridecane, 4-hydroxy-4-methyl-2-pentanone, tetradecane, 1-heptanol, 3-nonanol, 2-nonanol, and 1-octanol emission rates were found higher during the diurnal collection time (Table 1).

In the nocturnal collection times, considering both years, eight compounds (ethylbenzene, 1-hexadecene, butylbenzene, ethyl 2-methyl lactate, 2-nonanone, 2-butoxyethanol, 3-ethyl-2-heptanol, 2-ethylhexanol) were found that were not identified in the diurnal collection times (Table 1).

Some compounds showed distinct day/night emission behaviors. In the spring experiment, cis-3-hexenyl acetate was observed only during nighttime. In previous studies cis-3-hexenyl acetate showed distinct emission bursts, in apple and grapevine, following the daily light switch-off [31]. Cis-3-hexenyl acetate shows an influence on certain insects with crepuscular behavior. For instance, when emitted by grapevine, it served as an attractant for the crepuscular insect *L. botrana* [30]. Another example of an important Dipteran insect attracted to cis-3-hexenyl acetate is the wheat midge, *Sitodiplosis mosellana* (Diptera: Cecidomyiidae), a twilight-active wheat pest [59,60]. In contrast to cis-3-hexenyl acetate, in the spring experiment, 2-hexanol, 4-hydroxy-4-methyl-2-pentanone, 2-nonanol, and 1-octanol were detected only in the diurnal period. In the fall experiment, 2-hexadecanol was seen only in the diurnal period. Phenethyl alcohol was found only during the diurnal collection time in both experiments (Table 1).

VOCs released by grape foliage were reported to follow diurnal and nocturnal rhythms [31,33], which is a typical occurrence in plants [61]. In young leaves of plantlets of *Vitis vinifera* cv. Marselan, monoterpenes showed an overall day/night emission rhythm, with lower emission rates observed during the night phase; similarly, sesquiterpenes displayed a diurnal course with maximum rates close to the end of the light phase [29].

Our data suggests that distinct collection periods (which differed in terms of light intensity, temperature, and relative humidity) may influence the emission of the VOCs in cv. Isabella. In addition, differences between years were recorded (Table 1), which could be due in part to the different climatic conditions between the seasons (late spring and fall) and the phenological stage of the vines.

The emission of certain compounds was found to vary with light intensity [62]. Nonetheless, light seems to be correlated with temperature; for instance, the emission rates from beech (*Fagus sylvatica* L.) and sunflower (*Helianthus annuus* L.) plants were seen to be affected by light intensity as well as by temperature, and it was stated that these two factors should be considered simultaneously [63]. With terpenes, the emission occurs from storage structures and is generally separate from photosynthesis as it may occur at night [64]. This may explain why camphene and β -pinene, the only terpenes found in cv. Isabella, were emitted at a similar rate in both diurnal and nocturnal collection times in the first experiment (Table 1). Notably, Isabella leaves are characterized by the presence of dense trichomes [65] and cuticular waxes [66], which might have contributed as storage structures for terpene emissions, as reported in other species [67,68]. Terpenes play multiple roles in mediating antagonistic and beneficial interactions among organisms [69,70] and are involved in plant-to-plant communication and signaling [71].

Temperature can also contribute to explaining the differences among the diurnal and nocturnal collection times. VOCs are normally emitted as temperature-dependent behavior; therefore, higher temperatures lead to a faster change from liquid/solid phases to the gas phase which implies an increase in emissions [72], as noted during the diurnal period (2020) with significantly higher emissions during the diurnal collection time (Table 1) when higher temperatures were registered. Additionally, volatiles are also released from intact storage structures by diffusion in a temperature-dependent way, so higher emissions are liberated from storage under warmer conditions [73].

Terpenes, as discussed before, are known to be released by storage structures; in spring 2020 (warmer temperatures than fall 2019), they were found to be much more emitted during diurnal than nocturnal sampling. Increased temperature can be considered one of the reasons for higher rates in the majority of compounds found during the diurnal collection time in both the first and second experiment (Table 1). Humidity has been reported to have significant effects on volatile emissions as well. For instance, factors of significance during a trial for apple tree emissions under field conditions were temperature and relative humidity [74]. Furthermore, high humidity may also lead to a swelling and to the consequent explosion of the structures containing storage pools while temporarily opening the stomata. This would allow the release of large pools of VOCs produced by leaf cells [3]. Nonetheless, emission patterns can be induced by light or darkness, and besides light-induced emission cycling, the emission of some volatiles can be modulated by the endogenous biological clock [61].

During the second experiment (2020), higher emission rates were recorded compared to the first experiment (2019), during both diurnal and nocturnal collection times (Table 1), as a consequence of the different environmental conditions (temperature, humidity, etc.) and plant canopy features (canopy structure, leaf age, etc.). Compounds 1-heptanol, 1-octanol, 2-hexanol, 2-nonanone, β -pinene, camphene, cis-3-hexenyl acetate, and phenethyl alcohol, identified in our study (Table 1), have been reported as of relevant interest for their plant protection roles in *Vitis* spp. (phenethyl alcohol [27]; 2-nonanone [75]) and in other species [76–81]. Interestingly, phenethyl alcohol was found to be emitted by the resistant *Vitis* genotypes Kober 5BB and Solaris and showed promising inhibitory effects against *P. viticola* [27]. In that investigation, phenethyl alcohol was present in the leaves at significant concentrations before inoculation, implying its possible contribution to disease reduction in systemic parts of locally attacked plants and in neighboring plant receivers [27]. It is a compound also found in the profile VOCs of the yeast *Aureobasidium pullulans* which was active against postharvest fruit pathogens [82].

Plants that express resistance emit VOCs that can trigger resistance responses in undamaged neighbors by different mechanisms, such as the priming or induction expression of resistance genes in the receiver or direct inhibitory effects on microbial pathogens [83]. In our experiments, the foliage consisted of intact shoots bearing leaves with no signs of pathogen inoculation or wounds, which suggests that VOCs were constitutively emitted. Our findings open the door for prospective innovative agronomic strategies. It has

been demonstrated that volatiles emitted by intact plants can be adsorbed by neighboring species, with their subsequent release observed in both laboratory and field conditions [82]. Many intercropping examples have shown effective benefits in controlling and/or reducing pests and diseases [84–88].

Notably, volatile compounds of intact Isabella grapes revealed their inhibitory action on the formation and pathogenicity of downy mildew (*Botrytis cinerea*) in vitro and in situ, as when clusters of the cv. Isabella (resistant) were placed next to clusters of the *Vitis vinifera* cv. Roditis (susceptible) [89] or fruits of kiwifruit of cv. Hayward [90], inoculated with *B. cinerea*, pathogen growth and/or incidence was suppressed. The uniqueness of the compounds present in cv. Isabella suggests they play a role in its well-known tolerance to main fungal pathogens, such as downy mildew (biotroph), powdery mildew (biotroph) and bunch rot (necrotrophy) [37]. These pathogens are relevant during the springtime, and bunch rot also constitutes a main concern during berry ripening. They are affected by morphological (i.e., trichomes, bunch compactness) [65,91], anatomical (i.e., cuticle wax) [66], and physiological (i.e., degree of stomatal opening) [92] traits, secondary metabolites including VOCs [1,6,7,27].

VOCs can be a natural and agroecological solution to enhance crop defense via both priming and induction mechanisms [42], and the possible benefits of VOCs in intercropped systems, with cultivars tolerant to main fungal pathogens, such as Isabella, could be considered for future research.

5. Conclusions

Volatile organic compounds emitted by the foliage of the cv. Isabella (*Vitis vinifera* × *Vitis labrusca*) were characterized. Seven VOCs were not reported before as volatiles emitted by *Vitis vinifera* foliage. The study demonstrated distinct volatile profiles for diurnal and nocturnal collection times.

Eight compounds emitted by cv. Isabella, 1-heptanol, 1-octanol, 2-hexanol, 2-nonanone, β -pinene, camphene, cis-3-hexenyl acetate, and phenethyl alcohol, have been reported as of relevant interest for their possible involvement in plant defense. The uniqueness of the compounds present in cv. Isabella suggests they play a role in its well-known tolerance to main fungal pathogens.

The characterization of VOCs emitted by cv. Isabella can be a useful contribution to understanding plant defense mechanisms of pathogen tolerance and could be an important step in the perspective of designing innovative agroecological cropping systems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12020272/s1>, Figure S1: Representative Total Ion Chromatograms of VOCs emitted by foliage of grapevine cv. Isabella following diurnal (2019 D and 2020 D) and nocturnal (2019 N and 2020 N) sampling in 2019 and 2020. At 17.00 min the peak of 2-octanol (Internal Standard).

Author Contributions: Conceptualization, A.D.R.; methodology, A.D.R. and F.C.; investigation, A.R.-D., A.C.-M. and L.M.C.; writing—original draft preparation, A.R.-D., A.C.-M. and L.M.C.; writing—review and editing, A.R.-D., A.C.-M., A.L., F.C. and A.D.R.; supervision, F.C. and A.D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The authors declare that the data supporting this study are available from the corresponding author on reasonable request.

Acknowledgments: The authors would like to thank the nursery Vivai Maiorana for providing the cv. Isabella plants.

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

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