Analysis of Floral Fragrance Compounds of *Chimonanthus praecox* with Different Floral Colors in Yunnan, China

Liubei Meng 1,†, Rui Shi 2,†, Qiong Wang 1,* and Shu Wang 1,2,*

1 College of Landscape Architecture and Horticulture, Southwest Forestry University, Kunming 650224, China; 18877548059@163.com
2 Southwest Landscape Architecture Engineering Research Center of National Forestry and Grassland Administration, Kunming 650224, China; shirui@swfu.edu.cn
* Correspondence: millmeng@swfu.edu.cn (Q.W.); wangshu@swfu.edu.cn (S.W.)
† These authors contributed equally to this work and should be considered as co-first authors.

Abstract: In order to better understand the floral fragrance compounds of *Chimonanthus praecox* belonging to genus *Chimonanthus of Chimonanaceae* in Yunnan, headspace solid-phase microextraction combined with gas chromatography-mass spectrometry was used to analyze these compounds from four *C. praecox* plants with different floral colors. Thirty-one types of floral fragrance compounds were identified, among which terpenes, alcohols, esters, phenols, and heterocyclic compounds were the main compounds. Interestingly, the floral fragrance compounds identified in the flowers of *C. praecox* var. *concolor* included benzyl acetate, α-ocimene, eugenol, indole, and benzyl alcohol. By contrast, the floral fragrance compounds α-ocimene, α-ocimene, and trans-β-ocimene were detected in *C. praecox* var. *patens*. Cluster analysis showed that *C. praecox* var. *concolor* H1, H2, and *C. praecox* var. *patens* H4 were clustered in one group, but *C. praecox* var. *patens* H3 was individually clustered in the other group. Additionally, principal component analysis showed that α-ocimene, benzyl alcohol, benzyl acetate, cinnamyl acetate, eugenol, and indole were the main floral fragrance compounds that could distinguish the four *C. praecox* with different floral colors in Yunnan. This study provides a theoretical basis for further elucidating the mechanism and pathway of the floral fragrance release of *C. praecox*.

Keywords: floral fragrance compounds; *Chimonanthus praecox*; different floral colors; HS-SPME-GC-MS

1. Introduction

*Chimonanthus praecox* (L.) Link, belonging to the genus *Chimonanthus of Chimonanaceae*, is primarily distributed in the south, central, east, southwest, and northwest of China [1]. Due to its fragrance, *C. praecox* has been widely used in landscaping and as cut flowers and bonsai materials [2,3]. Furthermore, it also has important economic value in the tea kiln, essential oil, and cosmetics industries [4]. In addition, *C. praecox* is rich in numerous volatile components and has great potential for use in drug research and development [5–7].

Floral fragrance, derived from volatile compounds in plants, is an important characteristic of *C. praecox*. Flower fragrance is useful for driving away herbivores [8,9], resisting pathogens [10], protecting flowers from harmful insects [11], attracting pollinators [12], communicating with other plants [13,14], treating diseases [15], improving esthetic value, attracting tourists [16], etc. Floral fragrances have important scientific and economic significance. Therefore, cultivating ornamental plants with floral fragrance has always been the goal of scientists.

Previous studies have shown that terpenes, esters, and alcohols are the main floral fragrance compounds of *C. praecox* var. *concolor* and var. *patens* [17–20]; however, a marked difference was observed in the diversity of floral fragrance compounds in *C. praecox* with different floral colors. Different studies have shown that the main floral fragrance compounds of *C. praecox* var. *concolor* were different, including trans-β-ocimene,
linalool, and β-myrcene [17]; alloocimene, benzyl acetate, and methyl salicylate [18]; linalool, benzyl acetate, and methyl salicylate [19]; linalool, benzyl alcohol, and methyl salicylate [20]; and z-muurolene, z-elemene, and L-bornyl acetate [21], respectively. For C. praecox var. patens, the main floral fragrance compounds vary in different studies, such as benzyl acetate, linalool, and caryophyllene [17]; benzyl acetate, alloocimene, and methyl salicylate [19]; β-ocimene, linalool, and benzyl acetate [20]; elemol, z-elemene, and β-cubebene [21]; and trans-β-ocimene, 2,6-dimethyl-1,3,5,7-octatetracene, and (1S, 2S, 3R, 5S) -(-)-2,3-pinaneol [22]. Hence, it is necessary to analyze the diversity of floral fragrance compounds in C. praecox with different floral colors.

There are also considerable differences in the composition of floral fragrance compounds in Chimonanthus plants from different geographical regions. The floral fragrance compounds of C. praecox are primarily terpenes, benzene derivatives, alkanes, esters, acids, and other types of compounds [23]. Marked differences were observed in the floral fragrance compounds of C. praecox, with different floral colors from the same region. For example, the main floral fragrance compounds of C. nitens, C. zhejiangensis, and C. salicifolius from Zhejiang were hedycaryol and α-myrcene [24]. However, linalool, benzyl acetate, trans-β-ocimene, and β-ocimene were found in different genotypes of C. praecox (H93, H36, SW001, and H29) from Wuhan, and the contents of these floral fragrance compounds were different [19,20]. At the same time, the floral fragrance compounds of the same variety of C. praecox also differed in different planting areas. For example, the main floral fragrance compounds of C. nitens in Zhejiang and Jiangxi are hedycaryol and α-myrcene [24] and dehydroaromadendrene and (-)-spathulenol [25], respectively. The C. praecox var. concolor main floral fragrance compounds in Hubei and Sichuan were alloocimene and benzyl acetate [18] and z-muurolene and z-elemene [21], respectively. Benzyl acetate and alloocimene [18] and elemol and z-elemene [21] were the main floral fragrance compounds of C. praecox var. patens in Hubei and Chongqing, respectively. In addition, similar research results showed that there were considerable differences in the floral fragrance compounds of C. praecox var. grandiflorus in Jinhong and Nanjing, and ocimene, a mixture of ocimene isomers, and linalool were the main floral fragrance compounds [26]. As mentioned above, different geographical regions have an important influence on the floral fragrance compounds of C. praecox with different floral colors. However, the diversity of floral fragrance compounds among different floral colors of C. praecox in Yunnan has not been reported.

In this study, headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME-GC-MS) was used to analyze the diversity of floral fragrance compounds of four C. praecox plants with different floral colors from Heilongtan Park, Yunnan. The present study aimed to explore the diversity of floral fragrance compounds in C. praecox with different floral colors and aimed to identify the typical floral fragrance compounds of C. praecox in Yunnan. This study also aims to provide a theoretical basis for the further exploration of the metabolic pathways and changing trends of floral fragrance compounds in C. praecox and to lay a foundation for the development and utilization of the plant resources of C. praecox from Yunnan.

2. Materials and Methods
2.1. Plant Materials

Four C. praecox plants with different floral colors in Heilongtan Park (Kunming, Yunnan) were collected, including two species of C. praecox var. concolor (H1 and H2) and two species of C. praecox var. patens (H3 and H4) (Figure 1). The morphological characteristics of C. praecox were as follows: (I) H1, light yellow flowers, white heart with rolled outer perianth, medium flowers, and sweet fragrance; (II) H2, dark yellow flower, white heart with slightly wrinkled perianth edge, large flower, and sweet fragrance; (III) H3, yellow and white flowers, cover with deep purple stripes, small flowers, and strong fragrance; (IV) H4, dark yellow flowers with light purplish red halo and stripes on the inner surface, wrinkled edge, large flowers, and strong fragrance.
2.2. Plant Collection

In sunny weather without wind, at 10:00 a.m. on 21 December 2020, different floral colors of *C. praecox* flowers were collected on the same branch and then placed in headspace bottles. This was repeated three times, and the total number of flowers was 24 (2 flowers/bottle). After the sample was collected, it was quickly placed in a 20 mL SPME bottle (clear glass, flat bottom; 20 mm open seal with PTFE/silicone septa, aluminium crimp cap, Thermo Fisher Scientific, Waltham, MA, USA) and immediately sealed with a sealing clamp for aroma collection.

2.3. HS-SPME Analysis

HS-SPME analysis was performed using an automatic SPME device (Supelco, Bellefonte, PA, USA) equipped with a 100 µm polydimethylsiloxane SPME fiber. At room temperature, all samples were equilibrated for 30 min and prepared for analysis. The SPME fiber was conditioned at the GC injection port for 40 min at 260 °C before volatile collection. The fiber was then inserted into a capped SPME vial with an automatic SPME device (fiber conditioning temp 250 °C, 20 min, TriPlus autosampler, Thermo Fisher Scientific, Waltham, MA, USA) to absorb floral fragrance compounds for 40 min at room temperature.

2.4. GC-MS Analysis

When the adsorption was complete, the SPME fibers were withdrawn, inserted into the GC-MS injection port (Trace GC Ultra/ITQ900, Thermo Fisher Scientific, Waltham, MA, USA), and desorbed for 1 min at 250 °C. GC-MS was then used to collect the data. GC conditions were as follows: An DB-624UI (60 m × 0.32 mm × 1.80 µm, Agilent J&W, Santa Clara, CA, USA) capillary column was used, and helium (99.999%) was used as the carrier gas with a flow rate of 1.0 mL/min. The sample volume was 1 µL, without splitting. Temperature rising procedure was conducted as follows: The injection port temperature was 250 °C, and the initial column temperature was 60 °C. The temperature was increased by 10 °C/min for 2 min to 90 °C, and then the temperature was raised by 3 °C/min to 220 °C and maintained for 12 min. MS conditions were proceeded follows: An EI ion source with an ionization energy of 70 eV was used. The temperature of the ion source and the transfer line was 200 °C and 260 °C, and the scan mass range was 50–650 amu.

2.5. Data Analysis

The data for aroma chemical compounds were identified and confirmed by the National Institute of Standards and Technology mass spectrometry database (NIST 14). The relative content of each compound was determined by using a peak normalization procedure based on the total ion flow chromatogram. The statistics of all data were obtained by using Microsoft Excel 2019. GraphPad Prism 8 software was used for the PC stacking diagram. In addition, hierarchical cluster analysis was performed by using TBtools software and PCA using IBM SPSS 22.0 software to calculate the eigenvector load values.

3. Results

3.1. Analysis on Types of Floral Fragrance Compounds of *C. praecox* with Different Floral Colors

The floral fragrance compounds of *C. praecox* with different floral colors are summarized in Table 1. A total of eight types of floral fragrance compounds were detected in

![Figure 1. The flower morphological characteristics of *C. praecox* with different floral colors.](image-url)
the four *C. praecox* with different floral colors from Yunnan (H1, H2, H3, and H4 with seven, seven, three, and six types compounds, respectively). In terms of floral fragrance compounds, terpenes were the most abundant (7–12 compounds), whereas the amount of alcohols was relatively small (≤2).

**Table 1.** Classification statistics of floral fragrance compounds and contents of *C. praecox* with different floral colors.

| Compound Category | Relative Content (%) ± SD and Type Number |
|-------------------|------------------------------------------|
|                   | H1           | H2           | H3           | H4           |
| Terpenes          | 28.48 ± 0.37 (9) | 26.04 ± 1.18 (7) | 96.65 ± 2.98 (9) | 88.15 ± 2.28 (12) |
| Alcohols          | 4.23 ± 0.46 (2)  | 11.85 ± 0.65 (2)  | 0.84 ± 0.03 (1)  | 3.12 ± 0.14 (2)  |
| Esters            | 39.88 ± 1.27 (3) | 31.90 ± 1.46 (5)  | -              | 6.89 ± 0.54 (2)  |
| Phenols           | 10.63 ± 0.38 (1) | 16.44 ± 0.30 (1) | -              | 0.06 ± 0.02 (1)  |
| Aldehydes         | 0.42 ± 0.03 (2)  | 0.64 ± 0.13 (1)  | -              | -              |
| Aromatic hydrocarbons | -            | -              | 0.43 ± 0.09 (1) | 0.56 ± 0.10 (2) |
| Heterocyclic      | 9.88 ± 0.46 (1)  | 4.86 ± 0.63 (1)  | -              | -              |
| Others            | 1.22 ± 0.08 (1)  | 0.87 ± 0.20 (1)  | -              | 0.02 ± 0.01 (1)  |
| Total             | 94.74 ± 3.05 (19)  | 92.60 ± 4.55 (18)  | 97.92 ± 3.10 (11) | 98.8 ± 3.09 (20) |

1 The number of compounds types. 2 Not detected or did not exist.

Terpenes, alcohols, esters, phenols, and heterocyclic compounds contents were more abundant in the main floral fragrance compounds. The relative abundances of terpenes and alcohols detected in *C. praecox* were as follows: H1, 28.48% and 4.23%; H2, 26.04% and 11.85%; H3, 96.65% and 0.84%; and H4, 88.15% and 3.12%, respectively. Except for *C. praecox* var. *patens*, esters and phenols accounted for the highest percentage in the *C. praecox* var. *concolor* and varied from 31.90% to 39.88% and 10.63% to 16.44%, respectively. Additionally, heterocyclic compounds were detected only in *C. praecox* var. *concolor* (H1 and H2), and the relative amounts were 9.88% and 4.86%, respectively.

### 3.2. Analysis of Floral Fragrance Compounds of *C. praecox* with Different Floral Colors

In total, 31 different floral fragrance compounds were identified from four *C. praecox* plants with different floral colors. As shown in Table 2, four *C. praecox* plants with different floral colors emitted higher amounts of β-ocimene, α-ocimene, benzyl alcohol, benzyl acetate, eugenol, indole, and trans-β-ocimene.

In *C. praecox* var. *concolor* (H1 and H2), the most abundant relative floral fragrance compound (top 5) was benzyl acetate, followed by α-ocimene and eugenol. Indole or benzyl alcohol had the lowest abundance. In *C. praecox* var. *patens*, the floral fragrance compounds of H3 had a higher relative content of α-ocimene (45.09%), trans-β-ocimene (18.75%), germacrene D (9.70%), β-caryophyllene (6.25%), and α-phellandrene (4.45%). Different results showed that the main floral fragrance compounds, including β-ocimene (71.51%), α-ocimene (11.92%), benzyl acetate (4.49%), benzyl alcohol (2.95%), and methyl salicylate (2.40%), were detected in H4.

### 3.3. Principal Component (PC) Stacking Diagram of Floral Fragrance Compounds of *C. praecox* with Different Floral Colors

Eighteen compounds represented more than 1% of the total emission in *C. praecox* with different floral colors, among which α-ocimene (12.23% to 46.25%) and benzyl alcohol (0.86% to 9.71%) accounted for a large percentage of the total (Figure 2). Except for H3, the relative amounts of benzyl acetate (4.61–39.26%) and eugenol (0.06–17.96%) were higher in *C. praecox* var. *concolor* (H1 and H2) than in *C. praecox* var. *patens* (H4). Moreover, valencene, β-caryophyllene, and α-phellandrene were not detected in H1, H2, and H4, respectively. Cinnamyl acetate was detected only in *C. praecox* var. *concolor* (H1 and H2), whereas isolecine and alloocimene were only detected in *C. praecox* var. *patens* (H3 and H4). In addition, indole was only emitted from *C. praecox* var. *concolor*, which changed from 5.31% (H2) to 10.53% (H1). The relative amounts of β-ocimene accounted for the
highest proportion (77.37%) in H4, indicating that β-ocimene contributed the most to the floral fragrance of C. praecox var. patens (H4). As mentioned above, β-ocimene, α-ocimene, benzyl alcohol, benzyl acetate, eugenol, indole, and trans-β-ocimene were the main floral fragrance compounds of C. praecox in Yunnan.

| Classification | Compound Name | RI ¹ | Relative Content (% ± SD) |
|---------------|---------------|------|---------------------------|
|               |               |      | H1           | H2            | H3            | H4            |
| Terpenes      | Cyclooctatetraene | 850  | 0.07 ± 0.02 | -             | 0.10 ± 0.02   |
|               | α-Thujene     | 929  | 0.19 ± 0.04 | -             | -             |
|               | α-Pinene      | 937  | 0.78 ± 0.10 | 0.80 ± 0.16   | 3.60 ± 0.27   | 1.02 ± 0.16   |
|               | Sabine n e    | 974  | -            | -             | -             | 0.33 ± 0.06   |
|               | α-Phellandrene| 1005 | 0.04 ± 0.01 | 0.12 ± 0.02   | 4.45 ± 0.38   |
|               | β-Occimene    | 1037 | -            | -             | -             | 71.51 ± 0.60  |
|               | α-Ocimene     | 1047 | 24.59 ± 0.05 | 24.52 ± 0.88  | 45.09 ± 0.87  | 11.92 ± 0.94  |
|               | trans-β-Ocimene | 1049 | 0.85 ± 0.00 | 0.25 ± 0.01   | 18.75 ± 0.23  | 1.09 ± 0.14   |
|               | γ-Terpine n e | 1060 | 0.04 ± 0.00 | -             | -             | 0.11 ± 0.03   |
|               | Terpinolene   | 1088 | 0.15 ± 0.00 | -             | -             | -             |
|               | Alloocimene   | 1131 | -            | 3.57 ± 0.18   | 0.63 ± 0.10   |
|               | Isoledene     | 1375 | -            | 1.61 ± 0.38   |
|               | β-Caryophyllene| 1419 | 1.30 ± 0.13 | 6.25 ± 0.12   | 0.71 ± 0.08   |
|               | Aromandendrene| 1440 | -            | -             | 0.24 ± 0.04   |
| Alcohols      | Germacrene D  | 1481 | 0.54 ± 0.04 | 0.15 ± 0.06   | 9.70 ± 0.28   | 0.25 ± 0.07   |
|               | Valencene     | 1492 | -            | 0.13 ± 0.03   | 3.63 ± 0.27   | 0.24 ± 0.04   |
| Ethers        | Benzyl alcohol| 1036 | 3.62 ± 0.33 | 8.89 ± 0.64   | 0.84 ± 0.03   |
|               | Cinnamyl alcohol| 1313 | 0.61 ± 0.13 | 2.96 ± 0.01   |
|               | Benzyl acetate| 1164 | 36.85 ± 0.97 | 28.97 ± 0.97  | 4.49 ± 0.24   |
|               | Methyl salicylate| 1192 | 1.72 ± 0.14 | 0.96 ± 0.14   | 2.40 ± 0.30   |
|               | Bornyl acetate| 1285 | -            | 0.28 ± 0.07   | -             |
|               | Methyl cinnamate| 1379 | -            | 0.05 ± 0.01   | -             |
|               | Cinnamyl acetate| 1445 | 1.31 ± 0.16 | 1.64 ± 0.27   |
| Phenols       | Eugenol       | 1357 | 10.63 ± 0.38 | 16.44 ± 0.30  | 0.06 ± 0.02   |
|               | Benzyaldehyde | 962  | 0.24 ± 0.00 | -             | -             |
|               | Cinnamaldehyde| 1274 | 0.18 ± 0.03 | 0.64 ± 0.13   | -             |
| Aromatic      | p-Xylene      | 865  | -            | -             | 0.40 ± 0.07   |
| hydrocarbons  | o-Cymene      | 1022 | -            | -             | 0.16 ± 0.03   |
|               | α-Cymene      | 1023 | -            | -             | 0.43 ± 0.09   |
| Heterocyclic  | Others        | 1295 | 9.88 ± 0.46 | 4.86 ± 0.63   | -             |
| Others        | Phenyl-pentamethyl-disiloxane| 1157 | 1.22 ± 0.08 | 0.87 ± 0.20   | 0.02 ± 0.01   |
| Total         |               | 31   | 19           | 19            | 11            |

¹ The values of the Kovats retention index. ² Not detected or did not exist.

Figure 2. PC stacking diagram of floral fragrance compounds of C. praecox with different floral colors (>1%).
3.4. Hierarchical Cluster Analysis of Floral Fragrance Compounds of C. praecox with Different Floral Colors

In order to explore the relationship between C. praecox and different floral colors in Yunnan, hierarchical cluster analysis was conducted on the main floral fragrance compounds. As shown in Figure 3, the four C. praecox plants with different floral colors were clustered into two groups. C. praecox var. concolor H1, H2 and C. praecox var. patens H4 were clustered into one group because of their similar contents of α-ocimene in which C. praecox var. concolor H1 and H2 were grouped into one subgroup because of the near contents of benzyl acetate, eugenol, indole, and benzyl alcohol. However, C. praecox var. patens H3 was individual clustered in the other group as it contained high contents of trans-β-ocimene, β-caryophyllene, and germacrene D.

![Figure 3. Heatmap and hierarchical cluster analysis of the main floral fragrance compounds of C. praecox with different floral colors.](image)

3.5. PC Analysis (PCA) of Floral Fragrance Compounds of C. praecox with Different Floral Colors

PCA was performed in order to simplify the multidimensional dataset based on floral fragrance compound profiles (Table 2). The variances of PC1, PC2, and PC3 were 68.409%, 24.314%, and 7.277%, respectively. In total, the cumulative contribution rate was 98.187%, which indicated that the three PCs covered most of the floral fragrance compounds of C. praecox in Yunnan. Moreover, we created a 3D loading plot to further explore the influence of each floral fragrance compound on the differentiation of C. praecox with different floral colors (Figure 4). As a result, the three compounds with the highest loading values in each of the three PCs were selected as the main factors, which showed that α-ocimene, eugenol, and indole contributed the most to the floral fragrance compounds. Additionally, other compounds, including benzyl acetate, benzyl alcohol, cinnamyl acetate, trans-β-ocimene, isoledene, germacrene D, α-phellandrene, and β-caryophyllene were also representative floral fragrance compounds of C. praecox with different floral colors.
Figure 4. A 3D loading plot of eigenvector loading values for 18 floral fragrance compounds from PC1, PC2, and PC3 of C. praecox with different floral colors.

4. Discussion

4.1. Comparison of Floral Fragrance Compounds of C. praecox with Different Floral Colors

Floral scents vary among C. praecox of different species or cultivars [17–21]. The difference in floral scents resulting from the specific flower fragrance was determined by the composition and amount of volatiles [27]. In our study, 23 floral fragrance compounds were identified in C. praecox var. concolor (H1, H2), which is not in accordance with the results of previous studies [17–21]. These studies have indicated that the types of floral fragrance compounds identified in C. praecox var. concolor ranged from 26 to 38. Moreover, the main floral fragrance compounds of C. praecox var. concolor are esters, terpenes, phenols, alcohols, and heterocyclic compounds, whereas previous studies have found that terpenes, esters, and alcohols are the main compounds [17–21]. Additionally, the floral fragrance compounds with relatively high contents in C. praecox var. concolor were benzyl acetate, β-Ocimene, eugenol, benzyl alcohol, and indole. It has been reported that benzyl acetate and benzyl alcohol are the main fragrance compounds in C. praecox var. concolor [17–20]. Interestingly, high levels of α-Ocimene, eugenol, and indole were found in C. praecox var. concolor flowers, which differ from the reported results of other studies [17–21]. Therefore, it was speculated that α-Ocimene, eugenol, and indole were the typical characteristic compounds responsible for the floral aroma of C. praecox var. concolor in Yunnan.

In total, 23 compounds were identified in C. praecox var. patens (H3 and H4), which is lower than the number identified in other studies (27 [17], 30 [19], 36 [20], 42 [21], and 34 [22]). The floral fragrance compounds of C. praecox var. patens are primarily terpenes, esters, alcohols, and phenols [20]. Except for phenols, the other main floral fragrance compounds identified in our study were similar to those reported in previous studies. β-Ocimene was the main floral fragrance compound of C. praecox var. patens, which is similar to the results of Li et al. [20] but different from the results of Yu et al. [19]. Feng et al. found that although β-oicimene is a floral fragrance compound of C. praecox var. patens, it is not the main floral fragrance compound [22]. In addition, trans-β-oicimene was identified as the main floral fragrance compound in some C. praecox var. patens, which is consistent with our results [22]. α-Ocimene, one of the main floral fragrance compounds, was only
found in Yanling C. praecox [28]. Surprisingly, α-ocimene was discovered for the first time in C. praecox var. patens.

Phenol and ester contents in C. praecox var. concolor were higher than in C. praecox var. patens. By contrast, the content of terpenes in C. praecox var. concolor was lower than that of C. praecox var. patens. Moreover, aldehydes and heterocyclic compounds were detected only in C. praecox var. concolor, whereas aromatic hydrocarbons were only detected in C. praecox var. patens. PCA showed that α-ocimene, benzyl alcohol, benzyl acetate, cinnamyl acetate, eugenol, and indole were the main factors distinguishing C. praecox in Yunnan. β-Ocimene is the main floral fragrance compound in C. praecox [19]. However, in the present study, α-ocimene was found to be the main floral fragrance compound in C. praecox var. concolor and var. patens. The results showed that a high content of benzyl alcohol was detected in C. praecox with different floral colors, which is consistent with the results of Zhou et al. [18].

Previous studies have shown that linalool is the main floral fragrance compound emitted from different species, such as C. praecox var. concolor (H64) [20], C. praecox var. patens [22], Yanling C. praecox [28], and C. praecox var. intermedius [29]. However, the main alcohol identified in this study was benzyl alcohol rather than linalool. In addition, higher contents of benzyl acetate and indole and a lower content of cinnamyl acetate were found in C. praecox var. concolor, and the results agree with those of Azuma et al. [30] and Li et al. [31], respectively. Other studies have found that benzyl acetate is the main floral fragrance compound of C. praecox [18]. Furthermore, a higher content of eugenol (10.63–16.44%) was found only in C. praecox var. concolor, whereas only trace eugenol (0.03–2.2%) was detected in different genotypes of C. praecox [19]. Therefore, we speculated that eugenol might be a typical floral fragrance compound emitted from C. praecox var. concolor in Yunnan.

### 4.2. Comparison of Floral Fragrance Compounds of C. praecox in Different Geographical Areas

The geographical environment results in variations in floral fragrance compounds in different plants [32,33]. In this study, 31 types of floral fragrance compounds of C. praecox with different floral colors were identified, whereas 86, 48, 72, 31, 15, 31, 71, and 65 compounds were identified in C. praecox from Hubei [20], Chongqing [21], Jiangxi [25], Henan [28], Japan [30], Shanghai [34], Zhejiang [35], and Shandong [36], respectively. The typical floral fragrance compounds emitted from C. praecox in Yunnan consist of β-ocimene, α-ocimene, benzyl alcohol, benzyl acetate, eugenol, trans-β-ocimene, and indole. As a result, floral fragrance compounds from C. praecox in Yunnan are similar to those of C. praecox in Hubei [20] but are different from those of C. praecox in Jiangxi [25], Shandong [36], and Zhejiang [37].

For C. praecox var. concolor, benzyl acetate, α-ocimene, eugenol, indole, and benzyl alcohol were observed to be the main floral fragrance compounds, which was in disagreement with the studies in Sichuan and Japan [21,30]. Moreover, β-ocimene, α-ocimene, and trans-β-ocimene were the main floral fragrance compounds of C. praecox var. patens, which was different from the floral fragrance compounds of C. praecox var. patens in Henan and Chongqing [21,22]. In summary, the main floral fragrance compounds emitted from the same variety of C. praecox in different regions were significantly different; this may be due to the influence of the growth environment, which changes the main floral fragrance compounds of C. praecox. Furthermore, we found that C. praecox with different floral colors in Yunnan had a shorter flowering period than those in Henan and Sichuan [38,39]. In the future, comparative transcriptome and metabolomics analyses are necessary in order to better understand the fragrance mechanism and pathway in C. praecox with different floral colors in Yunnan.

### 5. Conclusions

In total, 31 floral fragrance compounds were identified in different C. praecox with different floral colors in Yunnan, among which terpenes, alcohols, esters, phenols, and heterocyclic compounds were the main floral fragrance compounds. Moreover, benzyl acetate, α-ocimene, eugenol, indole, and benzyl alcohol were identified as the main floral fragrance
1. Chen, L.Q. Research Advances on Calycanthaceae. Chin. Landsc. Archit. 2012, 28, 49–53. [CrossRef]

2. Lin, S.Q.; Chen, J.D. Landscape Application and Industry Progress of Wintersweet. Chin. Landsc. Archit. 2020, 36, 104–108. [CrossRef]

3. Tang, G.L.; Wang, M.B. On Aesthetic Value and Landscape Application of Wintersweet. J. Nanjing For. Univ. Humanit. Soc. Sci. Ed. 2017, 14, 156–162. [CrossRef]

4. Lu, A.X.; Zhou, X.R.; Ye, Y.L.; Li, X.L.; Xie, G.H.; Wang, B.; Tong, H.R. Changes of Sensory Characteristic and Volatiles of Harvested Flowers of Chimonanthus praecox During Spreading Process. Acta Hortic. Sin. 2020, 47, 73–84. [CrossRef]

5. Shen, Z.G.; Sun, M.; Yuan, D.Y.; Cheng, J.M.; Ding, X.; Shang, Z.H. HS-SPME-GC-MS Analysis of Volatile Components in Tender Shoots from Six Plants of Calycanthaceae. Acta Hortic. Sin. 2020, 47, 2349–2361. [CrossRef]

6. Li, S.L.; Zhou, Z.R. Research Progress on Flavonoids and Coumarins from Chimonanthus praecox. Chin. Tradit. Herb. Drugs 2018, 49, 3425–3431.

7. Xu, J.B.; Pan, J.J.; Lv, Q.D.; Cheng, K.J. Research Advances on Chemical Constituents from Chimonanthus praecox and its Pharmacological Activities. Chin. Tradit. Herb. Drugs 2018, 49, 3425–3431.

8. Pichersky, E.; Gershenzon, J. The Formation and Function of Plant Volatiles: Perfumes for Pollinator Attraction and Defense. Physiol. Metab. 2002, 5, 237–243. [CrossRef]

9. Unsicker, S.B.; Kunert, G.; Gershenzon, J. Protective Perfumes: The Role of Vegetative Volatiles in Plant Defense Against Herbivores. Plant Biol. 2009, 12, 479–485. [CrossRef] [PubMed]

10. Arimura, G.I.; Ozawa, R.; Kugimaya, S.; Takabayashi, J.; Bohlmann, J. Herbivore-Induced Defense Response in a Model Legume. Two-Spedt Spider Mites Induce Emission of (E)-β-Ocimene and Transcript Accumulation of (E)-β-Ocimene Synthase in Lotus japonicas. Plant Physiol. 2004, 135, 1976–1983. [CrossRef] [PubMed]

11. Li, Y.Y.; Wan, Y.M.; Sun, Z.H.; Li, T.Q.; Liu, X.F.; Ma, H.; Liu, X.X.; He, R.; Ma, Y.; Li, Z. Floral Scent Chemistry of Chimonanthus praecox var. concolor, whereas the main floral fragrance compounds of C. praecox var. patens were β-ocimene, α-ocimene, and trans-β-ocimene. In addition, cluster analysis showed that C. praecox var. concolor H1, H2, and C. praecox var. patens H4 were clustered in one group, but C. praecox var. patens H3 was individual clustered in the other group. PCA showed that α-ocimene, benzyl alcohol, benzyl acetate, cinnamyl acetate, eugenol, and indole are the typical characteristic floral fragrance compounds that can be used to distinguish C. praecox with different floral colors in Yunnan.

Author Contributions: Conceived and designed the experiments: S.W.; performed the experiments: Q.W. and L.M.; analyzed the data: R.S. and L.M.; wrote the paper: S.W. and L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (31860229) and Yunnan ten thousand people plan youth top talent project (80201435).

Conflicts of Interest: The authors declare no conflict of interest.
22. Feng, N. Determination of Floral Volatile Components and Preliminary Study on Function of Two Terpene Synthases in Wintersweet. Master’s Thesis, Huazhong Agricultural University, Wuhan, China, 2017.

23. Lin, X. Research Progress on Volatile Components of Chimonanthus Lindl. Fujian Agric. Sci. Technol. 2019, 7, 57–64. [CrossRef]

24. Xu, M.; Zhang, J.W.; Wu, L.S.; Liu, J.J.; Si, J.P.; Zhang, X.F. Determination of Volatile Components from Chimonanthus Flowers by HS-SPME-GC-MS. Sci. Silvae Sin. 2016, 52, 58–65. [CrossRef]

25. Xu, N.J.; Bai, H.B.; Yan, X.J.; Xu, J.L. Analysis of Volatile Components in Essential Oil of Chimonanthus nitens by Capillary Gas Chromatography-Mass Spectrometry. Instrum. Anal. 2006, 25, 90–93. [CrossRef]

26. Pan, C.F. Studies on the Chemical Constituents from Chimonanthus praecox Flower and Its Extractions Obtained by Supercritical CO2. Master’s Thesis, Southwest University, Chongqing, China, 2017.

27. Pichersky, E.; Dudareva, N. Scent Engineering: Toward the Goal of Controlling How Flowers Smell. Trends Biotechnol. 2007, 25, 105–110. [CrossRef] [PubMed]

28. Zheng, Y.Q.; Zhu, Y.; Zhang, R.Y.; Sun, Y.L.; Wu, Z.P.; Liu, M.X. Study on the Aroma Components of Chimonanthus praecox. Acta Sci. Nat. Univ. Pekin. 1990, 36, 667–673. [CrossRef]

29. Li, C.; Zhang, X.H.; Huang, Y.; Si, H.Q. Identification and Analysis on Aroma Components of Chimonanthus praecox. Acta Phytotax. Geobot. 2005, 56, 197–201. [CrossRef]

30. Azuma, H.; Toyota, M.; Asakawa, Y. Floral Scent Chemistry and Stamens Movement of Chimonanthus praecox (L.) Link (Calycanthaceae). Acta Phytotax. Geobot. 2005, 56, 197–201. [CrossRef]

31. Cai, B.G.; Jiang, X.W.; Chen, Y. Analysis of Perfumed Constituent in Chimonanthus praecox by SPME-GC-MS. J. Technol. 2016, 16, 257–261. [CrossRef]

32. Azuma, H.; Thien, L.B.; Kawano, S. Molecular Phylogeny of Magnolia (Magnoliaceae) Inferred from cpDNA Sequences and Evolutionary Divergence of the Floral Scents. J. Plant Res. 1999, 112, 291–306. [CrossRef]

33. Raguso, R.A.; Schlumberger, B.O.; Kaczorowski, R.L.; Hollisford, T.P. Phylogenetic fragrance patterns in Nicotiana sections Alatae and Suaveolentes. Phytochemistry 2006, 67, 1932–1942. [CrossRef]

34. Deng, C.H.; Song, G.X.; Hu, Y.M. Rapid Determination of Volatile Compounds Emitted from Chimonanthus praecox Flowers by HS-SPME-GC-MS. Z. Für Nat. C 2004, 59, 636–640. [CrossRef] [PubMed]

35. Li, Z.G.; Cao, H.; Lee, M.R.; Shen, D.L. Analysis of Volatile Compounds Emitted from Chimonanthus praecox (L.) Link in Different Florescence and QSRR Study of GC Retention Indices. Chromatographia 2009, 70, 1153–1162. [CrossRef]

36. Jiang, T.; Yuan, J.P.; Cheng, C.G.; Li, S.E.; Wang, X.; Chen, L.Z. Analysis of the Essential Oil from Chimonanthus praecox. Chin. J. Spectrosc. Lab. 2005, 22, 211–214. [CrossRef]

37. Wang, Y.G.; Huang, Y.H.; Zhang, C.; Fu, J.X.; Zhao, H.B. Analysis of Aroma Compounds from Calycanthus floridus var. glaucus Flowers. In Proceedings of the Ornamental Horticulture Symposium in China 2015, Xiamen, China, 18 August 2015.

38. Ding, X.; Shen, X.H.; Guo, W.; Tang, Z.H. Breeding of a New Cultivar Chimonanthus praecox ‘Yuqiao No.1’ and Its Key Cultivation Techniques. North. Hortic. 2020, 13, 178–180. [CrossRef]

39. Song, X.R.; Yuan, P.Y.; He, X.D. A New Cultivar of Chimonanthus praecox ‘Juanbei Jinpan’. Acta Hortic. Sin. 2020, 47, 3108–3109. [CrossRef]