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Research Article

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Composition and diurnal variation of floral scent emission in *Rosa rugosa* Thunb. and *Tulipa gesneriana* L.

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Abstract: This study was aimed to explore the composition and diurnal variation analyses of floral scent emission from *Rosa rugosa* Thunb. and *Tulipa gesneriana* L. The floral scent from the fresh flower were collected at different time points (9:00, 12:00, 15:00, 18:00, and 21:00) using dynamic headspace collection and were analyzed using autothermal desorber-gas chromatography/mass spectrometry (ATD-GC/MS). The results showed that a total of 62 volatile flavor compounds were detected from *Rosa rugosa* Thunb and a total of 70 volatile flavor compounds were detected from *Tulipa gesneriana* L. They were identified with eight functional categories: alcohols, fatty hydrocarbons, terpenes, aldehydes, ketones, esters, and other substances. The total release amount first decreased, and then increased with time, and arrived at the lowest at 15:00. The release amounts of different categories present distinct change patterns. Among the components, phenylethyl alcohol, citronellol, methylene chloride, hexane, and acetone showed relatively higher release amounts and were thought as the main components in floral scent of *Rosa rugosa* Thunb. Alpha-Farnesene, ethanol, pentadecane, beta-ocimene, longifolene, caryophyllene, and acetone showed relatively higher release amounts and were thought as the main components in floral scent of *Tulipa gesneriana* L. Research of roses and tulips in aromatic in the garden provides a theoretical basis and research and improvement of the aroma components of aroma.

Keywords: *Rosa rugosa* Thunb., *Tulipa gesneriana* L., floral scent, diurnal variation

1 Introduction

The release of flower fragrance is an important characteristic of flowering plants, and it is also the main ornamental character. It is an important factor to form and influence the ornamental value of flowers. Floral scent is widely used for perfumes, cosmetics, flavorings, and therapeutic applications [1,2]. Floral scent is a composite character determined by a complex mixture of low-molecular-weight volatile molecules [3–6] and is one of the main factors that make up and influence the ornamental value of flowers [7]. As an important part of plant volatile compounds, the floral scent plays a key role in plant ecophysiology and represents a decisive communication channel between plants and animals [8,9]. To achieve this, the floral scent is often developmentally and rhythmically regulated to be associated with the activity of corresponding pollinators. As insect pollinator exhibit rhythmic activities, the flowers emit scent on specific timing in a day. Light directly affects floral scent emission. Diurnal variations of floral scent emission have been observed in numerous plant species [10]. The release of flower scent of *Prunus davidiana* [11], *Gardenia jasminoides* from grandiflora [12], *Chrysanthemum*, and *Siberia kirilowii* [13] showed obvious diurnal variations.

With the development of society, aromatherapy has been gradually moving to people's lives, and the establishment of the aromatic botanical garden, night garden, and blind botanical garden and so on, with the characteristics of plant aroma, has the health care effect of green space. Rose and tulip with high ornamental

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value have attracted more and more attention because of their unique fragrance and curative effect widely used in landscaping. Experiments proved that aromatic plant volatiles can alleviate depression, anti-inflammatory and analgesic activities, and regulate the human nervous system, and it has a health function on the human body [14,15]. Roses are the most important crop in the floriculture industry and used as cut flowers, pot plants, and garden ornamentals. They are also a source of natural fragrances. Rose is used in producing rose water, attar of rose, and essential oils in the perfume industry. Tulip is world-famous flowers with bright colors, unique shape, and delightful scent. It is called "the queen of flowers". Tulip bulbs and roots can be used as a medicine, have a sedative effect, and can be used in the treatment of dirty mania.

The collection and adsorption of plant volatiles or flower fragrance are one of the key links in the identification of flower fragrance components. Automated thermal desorption (ATD), also known as thermal desorption, is based on the design principle of dynamic headspace. It completes the whole process of sampling, enrichment, concentration, and injection by thermal desorption instruments. It is a more comprehensive sample of pretreatment technology. Because the thermal desorption method is more direct and close to nature in the collection of volatile components, the biggest advantage of the thermal desorption method is that it can directly collect the volatiles of living plants and fully retain the original state of the samples. The sensitivity of this technology is high, and the detection limit can reach 10^{-9} levels [16–18]. ATD-gas chromatography/mass spectrometry (GC/MS) is a kind of experimental techniques that can be used to collect, absorb, and analyze the volatile components of living plants [19]. It can effectively eliminate the interference of external volatile components and reflect the components and their release amount of flower fragrance more truly. It is suitable for the qualitative and quantitative analyses of plant volatile components in the near-natural state [20]. Therefore, the study takes the living rose and tulip flowers as materials, collects, and analyzes the chemical components of the volatiles, summarizes the main aromatic characteristics of the rose and tulip, explores the significance of landscape value, and provides a clear reference for rational utilization.

Therefore, in this study, rose and tulip are used as experimental materials. These were collected using dynamic headspace collection method and ATD-GC/MS analysis technique combines the research and analysis of the diurnal variation of the aroma for further development and application research of roses and tulips in aromatic in the garden and provides a theoretical basis and research and improvement of the aroma components of aroma.

2 Experimental section

2.1 Materials

Selecting healthy, growing white tulip and red rose plants, used in this experiment was verified at the flowering stage as experimental materials cultivated in Xinjiang Academy of Forestry Shuixigou planting base in May 2018. The temperature range ranged from 19–31°C and relative humidity of 20–35%. The soil physical and chemical properties were weak alkaline (pH = 8.1) with organic matter of $36.06\,\mathrm{g\,kg^{-1}}$, and contained total N $2.7\,\mathrm{g\,kg^{-1}}$, CEC $15.49\,\mathrm{cmol\,kg^{-1}}$, and conductivity $2.24\,\mathrm{s\,mol^{-1}}$, soil mechanical composition: clay 15.24%, sand 21.34, and silt 63.42%. Sampling was done at different time points (9:00, 12:00, 15:00, 18:00, and 21:00), and each time point was done 3 times for the test.

2.2 Floral scent collection

A dynamic headspace method was used to collect the volatiles released from roses and tulip. An individual flower was put in a sampling bag (355 mm × 355 mm; Reynolds, USA), which releases and absorbs a few volatiles. A stainless-steel tube (0.25 × 3.5; USA) containing Tenax-TA (60–80 mesh) was used as the volatile trap, which avoided touching the flower. A Portable air sampler (QC 1; Beijing Municipal Institute of Labor Protection, China) served as the pump, and air filtered through a drying column filled with charcoal was pumped into the bag. In a short time, extract the air in the sampling bag, connect the adsorption tube, and then fill the bag with the air filtered by activated carbon. When the air in the bag reaches 3/4 full, stop filling and start to enter the headspace circulation sampling. The collected volatile substances are absorbed into the stainless steel adsorption tube containing Tenax-TA adsorption material. The volatiles were collected for 15 min at a flow rate of $300\,\mathrm{mL}$ to min^{-1} for each flower. Each variety was sampled 3 times during the same test session. Empty bags served as control samples. Afterward, the stainless steel tubes were sealed and placed in a refrigerator.

2.3 Floral scent analysis

2.3.1 Thermal desorption conditions

The ATD-GC/MS technique was used to analyze the floral scent. Install the adsorption tube with volatile components on the thermal desorption device. The floral scent collected in the stainless steel tube was desorbed by heating in an ATD (TurboMatrix 350; PerkinElmer Corp. Norwalk, CT, USA). Set the first desorption temperature as 250°C and the first desorption time as 10 min on the thermal desorption device, so that the volatile components absorbed in the adsorption tube can be resolved and brought into the cold well by carrier gas for condensation and concentration. The temperature of cold hydrazine is −30°C, the second desorption temperature is 300°C, and the second desorption time is 5 min, vaporize the concentrated volatiles in the cold trap and directly enter the gas chromatographic column head of GC-MS, the injection time is 1 min. Carrier gas (helium) pressure: 20 kPa, sample inlet temperature: 260°C.

2.3.2 Chromatographic conditions

The GC (7890 A Network GC System; Agilent Technologies, Santa Clara, CA, USA) was equipped with a capillary TR-5MS column (60 m × 0.25 mm, with a 0.25 µm film thickness). Carrier gas: high purity helium (purity $\geq$ 99.999%); programmed temperature rise: initial temperature 40°C, hold for 2 min, rise to 280°C at the rate of 6°C/min, hold for 3 min; inlet temperature: 250°C; split ratio: 1:1.

2.3.3 Mass spectrometry conditions

The MS (5975C Network MSD; Agilent Technologies, Santa Clara, CA, USA) was operated in EI source energy: 70 eV; MS interface temperature: 250°C; ion source temperature: 250°C; quadruple temperature: 150°C; sampling mode: full scan; EM voltage: 1576 V; mass scanning range: M/Z 40–500.

2.4 Data analysis

Data processing and mass spectrometry retrieval: using the NIST 11 and WIEIY Library in the TurboMass Version 5.4.2 software of GC-MS, we can automatically retrieve and analyze the mass spectrum data of the components, and search all the results and refer to the relevant standard maps for checking and supplemental retrieval. The composition of each volatile matter is qualitative, and the relative percentage content of the components in the total volatiles is calculated by the method of the total ion flow peak area normalization.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Analysis of the composition of floral scent

Figure 1 showed the chromatographic profiles of the floral scent emitted from rose and tulip at different time points, and a significant difference was found.

According to Table 2, 70 volatile compounds were identified in five time points. They were divided into eight kinds, including alcohols, fatty hydrocarbon, terpenes, aldehydes, ketones, esters, and other substances. The variety and release of volatile substances of flower fragrance at different time periods were different. Of all the ingredients, the volatilization of alpha-Farnesene was the largest, and at 21:00 it accounted for 30.7% of the total flower fragrance release. Followed by ethanol, pentadecane, beta-ocimene, longifolene, caryophyllene, and acetone, these may be the main sources of the smell of tulip. The composition of volatile substances in the diurnal variation showed a law that decreased first and then increased. At 15:00, the least volatile substances were detected (26 species), and the most volatile substances released at 9:00 were 44 species. Among the aroma components of the tulip, the number of fatty hydrocarbons (20 species) is the largest, and the number of terpenes is the least, only three kinds of compounds. The proportion of terpenes, arenes, and fatty hydrocarbons accounts for most of the results.

3.2 Analysis of diurnal variation of floral scent

There is a significant difference in the release of the aroma at different periods of the day in a rose (as shown in Table 1 and Figure 2). Among all volatile species, alcohols exhibited the highest release amount in volatile

Table 1: Analysis result of floral composition and relative content of rose

No.	Retention time	Main component	Relative content/%				
			9:00	12:00	15:00	18:00	21:00
Alcohol							
1	3.92	Ethanol, 2-(methylamino)-	0.294			1.434	1.237
2	4.104	Ethanol	0.281			0.786	1.482
3	10.593	3-Hexen-1-ol, (Z)-	0.746	1.111			
4	15.421	1-Hexanol, 2-ethyl-				1.12	
5	15.952	Benzyl alcohol	0.421	1.523	0.999		
6	18.139	Phenylethyl alcohol	29.432	59.474	49.44	12.831	1.623
7	20.699	Citronellol	17.976	13	18.301	0.725	0.623
8	21.245	Geraniol	16.147	5.321	2.808		
9	23.502	Propyl mercaptan				0.909	
Fatty hydrocarbon							
10	4.564	Methylene chloride	7.307	1.482	1.191	14.277	28.56
11	4.721	Pentane, 2-methyl-	1.197	0.567		1.363	1.891
12	4.856	Pentane, 3-methyl-	1.004			0.934	1.065
13	5.018	n-Hexane	7.82	3.893	1.047	8.956	16.69
14	5.489	Trichloromethane	1.923			2.104	5.607
15	5.922	Hexane, 2-methyl-	0.519				0.547
16	6.003	Cyclohexane	0.499				
17	6.074	Hexane, 3-methyl-	0.706				
18	9.127	Hexanal				1.23	0.634
19	17.132	Undecane	0.29			0.771	1.295
20	19.714	Dodecane					0.693
21	22.144	Tridecane		0.499		0.905	0.663
22	23.833	Dodecane, 2,6,10-trimethyl-					0.563
23	24.428	Tetradecane		0.962	0.597	2.754	1.737
24	25.678	Tetradecane, 4,11-dimethyl-	0.411		1.079	1.147	1.067
25	25.781	Heneicosane				0.751	0.582
26	26.577	Pentadecane		1.301		5.158	3.406
27	28.612	Hexadecane		0.892	0.639	3.669	1.03
28	28.607	Octadecane				3.669	
29	29.451	Hentriacontane				0.731	
30	30.528	Heptadecane				1.536	
Terpenes							
31	12.775	Alpha-pinene					0.561
32	15.546	D-Limonene	0.541				0.502
33	15.849	Beta-Ocimene	0.328				
34	23.427	2,6-Octadiene-2,6-dimethyl-		0.673	3.218		
35	25.381	Longifolene		2.382	1.431	2.33	0.785
36	26.847	Alpha-Farnesene	1.842		0.625		
Aldehydes							
37	6.653	Acetaldehyde					1.465
38	14.804	Octanal				0.882	
39	17.554	Nonanal		0.527		2.519	
40	20.168	Decanal				1.363	
41	21.088	2,6-Octadienal, 3,7-dimethyl-, (*Z*)-	0.433				
42	21.792	Citral	1.018				
Ketones							
43	4.277	Acetone	2.791	0.757	0.67	7.507	10.642
44	14.247	5-Hepten-2-one, 6-methyl-	0.861	0.514	0.501	2.566	0.64
45	16.953	Acetophenone				1.685	0.436
Esters							
46	4.472	Acetic acid, methyl ester	0.594				
47	5.278	Ethyl acetate	1.348	0.917		2.744	3.314
48	21.576	Acetic acid, 2-phenylethyl ester	0.716	1.281	2.274		

Table 1: continued

No.	Retention time	Main component	Relative content/%				
			9:00	12:00	15:00	18:00	21:00
49	24.119	Geranyl acetate	0.304	0.605	0.709		
Arenes							
50	6.058	Benzene				1.522	1.822
51	8.298	Toluene	0.567			1.171	1.577
52	10.821	Ethylbenzene	0.758			1.146	1.284
53	11.081	*p*-Xylene	1.105	0.646		1.75	2.174
54	11.768	Benzene, 1,3-dimethyl-	0.545			1.132	1.561
Others							
55	6.62	Mercaptamine				0.761	
56	14.047	Benzaldehyde				2.537	0.742
57	19.205	Benzoic acid		0.497		2.12	
58	20.228	Naphthalene					0.954
59	21.576						
60	22.956	Naphthalene, 2-methyl					0.55
61	23.946	Eugenol		1.74	9.696		

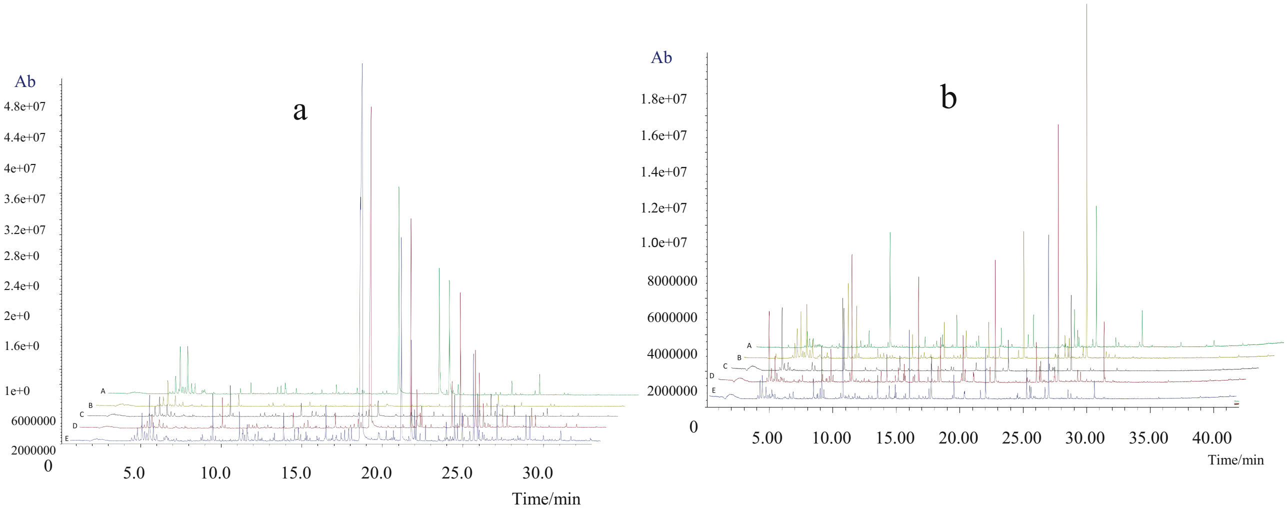

Figure 1: Total ion chromatography profiles of floral scent emitted from rose and tulip during the daytime ((a) Rose, (b) Tulip, (A) 9:00, (B) 12:00, (C) 15:00, (D) 18:00, (E) 21:00).

substances at 9:00, 12:00, and 15:00, and the highest release number of fatty hydrocarbons in volatile substances in 18:00 and 21:00 for two periods. At 15:00, the light is the strongest, but the aroma of the aroma is the least, which may be affected by transpiration. Terpenes, aldehydes, aromatic hydrocarbons, and other classes showed significant differences at 15:00, and aldehydes and aromatic hydrocarbons were released at 15:00 at zero. Fatty hydrocarbons, aldehydes, ketones, and aromatic hydrocarbons, and the total release rate of volatile substances is V type at 9:00, 12:00, 15:00, 18:00, and 21:00, that is to say, descend first and then rise.

Figure 3 shows the diurnal variation of the total release amount of aroma components of tulip. At different time points, the relative content of aroma components is distinct. The terpenes were at 12:00, 15:00, and 21:00 three time points, and the volatile substances showed the highest release amount. At 15:00, the light is the strongest, but the aroma of the aroma is the least, which may be affected by transpiration. The release of aldehydes at 15:00 is zero. At five time points, 9:00, 12:00, 15:00, 18:00, and 21:00, the release of alcohol substances first increased and then decreased, whereas in aldehydes, the amount of alcohol released first decreased and then increased.

Table 2: Analysis result of floral composition and relative content of tulip

No.	Retention time	Main component	Relative content/%				
			9:00	12:00	15:00	18:00	21:00
Alcohol							
1	3.909	Glycidol	0.387	0.925		1.67	2.16
2	4.077	Ethanol	2.335	3.661	4.075	11.356	6.46
3	4.71	1-Propanol, 3-ethoxy-	2.011	0.734		1.445	0.829
4	6.415	Silanediol, dimethyl-		0.904		1.284	
5	10.442	3-Hexen-1-ol, (*E*)-		0.659	1.054		
6	10.61	3-Hexen-1-ol	0.847	13.548	22.217	4.288	1.138
7	15.432	1-Hexanol, 2-ethyl-	0.388				
8	19.248	1-Nonanol				0.696	
Fattyhydrocarbon							
9	4.542	Methylene chloride	11.847	2.239	2.437	9.487	4.193
10	5.013	*n*-Hexane	10.425	1.485	0.858		5.985
11	5.468	Trichloromethane	3.544				
12	5.895	Hexane, 2-methyl-	0.599				
13	6.599	Propane			1.17		
14	8.634	1-Octene		0.716			
15	8.818	Octane	0.515	1.375			
16	11.568	Nonane	0.348	0.824			0.747
17	17.127	Undecane	0.352				
18	24.423	Tetradecane	0.349	0.654		1.17	
19	25.651	Hentriacontane				0.8	
20	25.765	Decane, 3,8-dimethyl-				0.649	
21	26.572	Pentadecane	1.063	1.656	1.337	3.056	1.058
22	28.612	Hexadecane	0.556	0.623	0.77	1.917	0.629
23	29.435	Heneicosane			1.623	0.585	
24	30.463	2-Hexene, 2,5,5-trimethyl-		2.528	7.101	7.287	3.343
25	30.512	Heptadecane				1.332	
26	33.511	Eicosane			1.187		
27	33.992	Heptadecane	0.933				
28	36.12	Nonadecane			1.534		
Terpenes							
29	15.546	Alpha-pinene		0.643			
30	15.86	Beta-ocimene	0.768	7.755	5.065	1.084	3.069
31	25.37	Longifolene	1.03	1.693	3.075	4.487	1.033
32	25.473	Caryophyllene	1.279	1.594	1.973	1.265	0.872
33	26.853	Alpha-Farnesene	12.008	20.056	23.752	12.985	30.734
Aldehydes							
34	4.84	Methacrolein	1.548	0.796			
35	6.675	Acetaldehyde	1.236			2.62	1.48
36	6.702	Pentanal		1.36			
37	9.116	Hexanal	0.65	1.103			1.029
38	14.025	Benzaldehyde	1.322	0.65		1.424	4.114
39	14.804	Octanal	0.446	0.7			0.661
40	16.293	Benzeneacetaldehyde	0.385				
41	20.168	Decanal	1.118	0.898		1.042	1.716
Ketones							
42	4.255	Acetone	7.674	5.135	2.363	5.411	5.298
43	5.089	2-Butanone		1.201	0.9	0.854	1.292
44	11.876	3-Nonanone	0.291				
45	14.274	5-Hepten-2-one, 6-methyl-	1.436	1.838	1.408	0.637	
46	16.921	Acetophenone	0.779			0.859	1.439
47	33.592	Megestrol acetate				0.572	
Esters							
48	5.257	Ethyl acetate	1.316	0.777			

Table 2: continued

No.	Retention time	Main component	Relative content/%				
			9:00	12:00	15:00	18:00	21:00
49	17.581	Benzoic acid, methyl ester	2.469	6.116	2.692	2.944	2.289
50	20.212	Methyl salicylate	0.984				
51	35.508	Dibutyl phthalate				0.971	
52	38.782	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester				0.83	1.45
53	41.105	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester					0.962
Arenes							
54	6.042	Benzene					0.833
55	8.288	Toluene	14.262	2.598	1.065		0.803
56	10.815	Ethylbenzene	0.59				
57	11.07	*p*-Xylene	1.202				
58	11.757	Benzene, 1,3-dimethyl-	0.48				
59	19.362	Benzene, 1,4-dimethoxy-	1.904	3.23	3.472	2.652	1.839
60	21.895	3,5-Dimethoxytoluene	7.38	6.833	5.888	8.912	6.159
Others							
61	4.932	Acetic acid			0.858	0.632	0.885
62	4.997	2-Nonenoic acid				0.85	
63	14.22	Phenol					1.263
64	14.734	3-Hexen-1-ol, acetate, (*E*)-		1.511	2.126		
65	16.629	5*H*-Naphtho[2,3-*c*]carbazole	0.488				
66	19.178	Benzoic acid				0.733	2.039
67	23.492	Phthalic anhydride				0.582	1.259
68	31.27	1-Methyl-1,3,3-triphenylindane	0.51				
69	34.366	2,6-Diphenylpyridine	0.933				
70	40.358	[1,1′:3′,1″-Terphenyl]-2′-ol					0.941

3.3 Discussion

Previous reports have concluded that the aroma of rose results from major volatiles with high relative contents. However, the composition of volatile organic compounds (VOCs) obtained from oils or plant organs is significantly different from those produced and emitted by living plant systems. The living plant approach better reveals the composition of volatile compounds, resulting in more accurate and reliable results [21]. The VOCs emitted from six commercial rose cultivars were collected in vivo by HS-SPME in green-houses and analyzed by gas chromatography coupled with mass spectrometry, which have abundant terpenes, aromatic hydrocarbons, and esters [22]. Linalool, a-pinene, b-pinene, and limonene, these volatile moieties were also detected by Knudsen, which is consistent with our analysis [23,24]. Used HS-SPME detected citronellol and the 2-phenylethyl alcohol, which are commonly found in the essential oil of Rosa, were detected in our study [22,25].

Oyama-Okubo [26] found two aromatics, two terpenoids and minor volatiles, which are consistent with our research. Benzyl alcohol and linalool were produced by Oriental hybrid lily flowers with a mild pleasant aroma. Although these two minor compounds were not detected in this study, the quantities of ethanol, beta-ocimene, longifolene, caryophyllene, and other compounds identified in *Tulipa gesneriana* L. make the fragrance for some consumers [27]. We found longifolene and caryophyllene are so-called green leaf volatiles (GLVs), which are de novo synthesized after herbivore damage as indicators of tissue disruption to the plant [28].

Our results confirmed that the emission of volatiles fluctuates in a diurnal rhythm before anthesis. This diurnal rhythm was also reported in *Rosa rugosa* Thunb. and *Tulipa gesneriana* L., producing high level of volatiles at night from flowers after anthesis. There is a significant difference in the release of the aroma at different periods of the day in rose and tulip in this study. Floral production is of great significance in plant biology

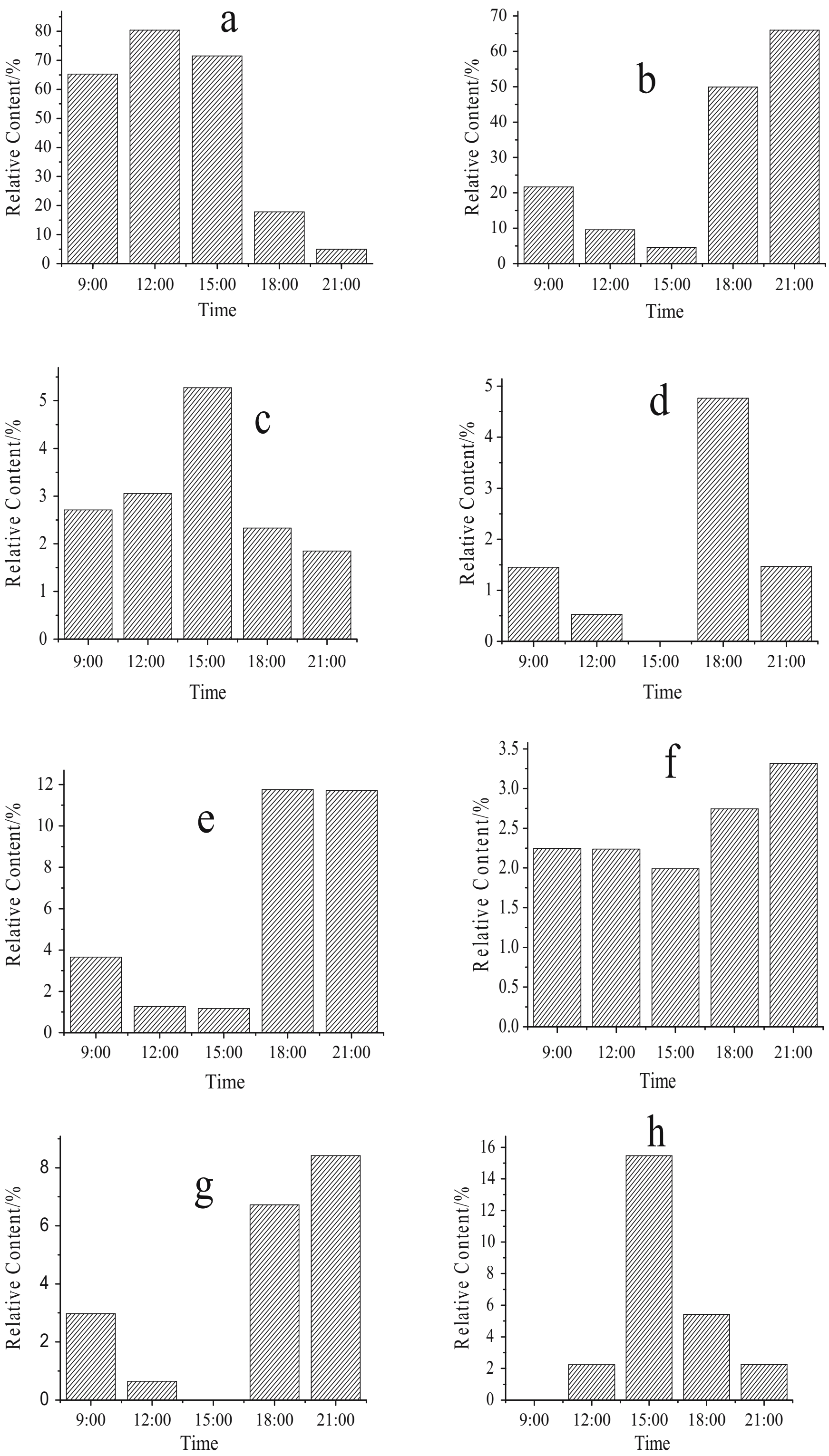

Figure 2: The diurnal variation of total release amount of floral scent from rose ((a) alcohol, (b) fatty hydrocarbon, (c) terpenes, (d) aldehydes, (e) ketones, (f) esters, (g) arenes, (h) others).

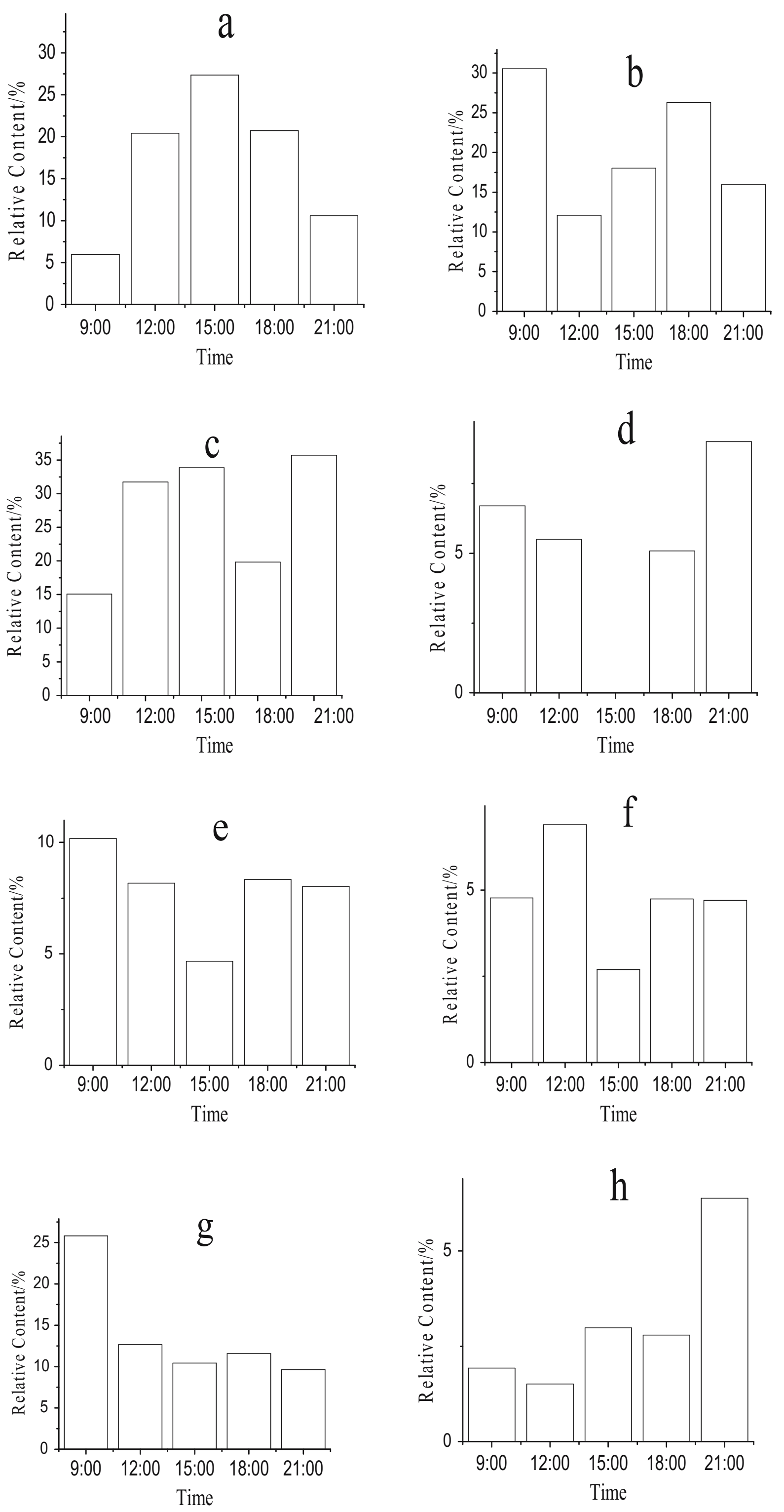

Figure 3: The diurnal variation of total release amount of floral scent from tulip ((a) alcohol, (b) fattyhydrocarbon, (c) terpenes, (d) aldehydes, (e) ketones, (f) esters, (g) arenes, (h) others).

and ecology. It promotes pollination through interaction with insects and other organisms, thus playing a role in reproduction, and it also provides value for many horticultural flowers with commercial value [29].

4 Conclusion

Limonene terpene compounds, α-pinene, β-pinene, and linalool alcohols can enhance the freshness of the air and can regulate the nervous system of the human body, can make people relax, has obvious sedative effect, with health effects on the human body. Roses and tulips are all aromatic plants used for landscaping. ATD-GC/MS analysis detected 62 and 70 compounds in the floral scent of rose and tulip, respectively. Volatile compounds were categorized as alcohols, fatty hydrocarbon, terpenes, aldehydes, ketones, esters, and other substances. There is a significant difference in the release of the aroma at different periods (9:00, 12:00, 15:00, 18:00, 21:00) of day in rose and tulip. Phenylethyl alcohol, citronellol, methylene chloride, hexane, and acetone may be the main source of the smell of roses. Ethanol, alpha-Farnesene, pentadecane, beta-ocimene, longifolene, caryophyllene, and acetone may be the main source of the smell of tulip. Research of roses and tulips in aromatic in the garden provides a theoretical basis and research and improvement of the aroma components of aroma.

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