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Deciphering the chemical origin of the semen-like floral scents in three angiosperm plants

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ABSTRACT

The chemical origin and biological role of distinct semen-like odor occasionally found in some flowers are very curious but remain scarcely studied. Here, we used direct ambient corona discharge ionization mass spectrometry (MS) to study the volatile chemical composition behind the semen-like odor emitted by the fresh flowers of *Photinia serrulata*, *Castanopsis sclerophylla* and *Stemona japonica* without any chemical pretreatment. Chemical identification was performed using high-resolution MS analysis in combination with tandem MS analysis and whenever possible was confirmed by the analysis of standard reference compounds. A total of 19 compounds, mostly belonging to nitrogenous volatiles, were identified in *P. serrulata*, *C. sclerophylla*, and *S. japonica* flowers, 1-pyrroline, 1-piperideine, 2-pyrrolidone, and phenethylamine being common in all the three studied species. Several lines of evidence indicate that the major component responsible for the semen-like odor is most likely 1-pyrroline. 1-Pyrroline is most probably formed *via* the oxidative deamination of putrescine, as indicated by the observation of signal from 4-amino-butanal intermediate. Flower visitation observations suggest that the released volatiles serve to attract dipterans, including Syrphidae, Calliphoridae, and Muscidae. On the analytical side, the comparison of our results to earlier studies also indicate that compared to the traditional GC-MS approach the direct corona discharge ionization mass spectrometry provides more sensitive detection of VOCs with high proton affinity, in particular volatile amines, and therefore can be used to complement traditional GC-MS approach for the highest chemical coverage of VOC analysis.

1. Introduction

Plants synthesize a multitude of volatile organic compounds (VOCs) that mediate their interaction with the environment, such as communication with other plants, protection from harmful insects or pollinator attraction (Lucas-Barbosa et al., 2011; Schwab et al., 2008). The majority of known flowers produce delightful sweet scents to attract pollinators searching for nectar, such as bees (Twidle et al., 2015). Sweet flower scents are typically composed by terpenoids, typical representative VOCs including terpinolene, α-terpinene, linalool, etc (Knudsen et al., 2006). In contrast to the flowers that emit sweet odors, a smaller group of flowers relies on fetid scents to attract pollinators, typically flies or other saprophagous insects (Jürgens et al., 2006, 2013; Zito et al., 2015). For example, the flowers of sapromyiophilous plants emit strong and fetid scents reminiscent of decaying organic matter in order to lure saprophagous flies (Jürgens et al., 2006, 2013; Urru et al., 2011; Zito et al., 2015, 2013, 2014). Sapromyiophilous flowers often present adaptations to their special method of pollinator attraction. Well-known examples for pollination strategies of sapromyiophilous plants often include oviposition site deceit, food source deceit, sexual deceit, mating location deceit, etc (Jürgens et al., 2013; Jersáková et al., 2006; Raguso, 2004; Urru et al., 2011). Recent studies have suggested that the VOCs emitted by sapromyophilous flowers play an important role in attracting saprophagous flies by mimicking different types of decomposing substrates (Chen et al., 2015; Jürgens et al., 2006, 2013; Urru et al., 2011; Zito et al., 2013,

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2015). The foul flower odors of sapromyiophilous plants are usually composed by sulphides (e.g., dimethyl disulphide) and/or nitrogenous compounds (e.g., indole), etc (Jürgens et al., 2006, 2013; Urru et al., 2011; Zito et al., 2013, 2015).

Apart from the flowers emitting sweet or fetid scents, there occasionally encounter flowers that emit very specific scent strongly reminiscent of the human semen odor (Chen et al., 2015, 2017; Kaiser, 2006; Naef et al., 2002; Shuttleworth, 2016). Recent studies suggest that the semen-like odor may have a biological function in plants (Chen et al., 2015; Shuttleworth, 2016), and other organisms (Hu et al., 2016; Robacker et al., 1997). Chen et al. (2015) discovered strong semen-like odor emitted by the flowers of *Stemona japonica*. Solid-phase micro-extraction coupled to gas chromatography mass spectrometry (SPME-GC-MS) analysis of *S. japonica* vapor identified only five volatile compounds: 1-pyrroline, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methylbutanal, and 3-methylbutanal, among which 1-pyrroline was solely responsible for the semen-like odor. *S. japonica* does not produce nectar and in natural habitats is mainly pollinated by the shoot flies of the genus *Atherigona* (Muscidae). The authors found that a synthetic mixture of the five identified compounds was attractive to *Atherigona* flies in natural habitats, whereas single 1-pyrroline alone was not attractive. The existence of synergistic effect between 1-pyrroline and the other four scent components from the flowers of *S. japonica* to attract flies has been proposed, however the exact mechanism of fly attraction, e.g., whether by mimicking oviposition site, food source or sexual deceit, remains unclear. The possibility of sexual deceit pollination is particularly curious as it is hinted by the earlier identification of 1-pyrroline as a pheromone component produced by the male Mediterranean fruit fly *Ceratitis capitata* (Robacker et al., 1997). Chen et al. (2015) also reported that 1-pyrroline was responsible for the semen-like odor emitted from *S. japonica* flowers. In another study, Kaiser (2006) reported that the semen-like odor component frequently encountered in *Berberis vulgaris* flower scents is due to a minor amount of 1-pyrroline and 2-aceyl-1-pyrroline. Shuttleworth (2016) reported that the semen-like odor emitted by *Xysmalobium parviflorum* flowers contained large amount of 1-pyrroline identified by GC-MS. These observations suggest that 1-pyrroline may be a common source for the semen-like odor in flowers.

Photinia serrulata, which belongs to the family of Rosaceae, is a well-known herb in Chinese Traditional Medicine and is widely cultivated in South East Asia for decorative purpose (Cheng et al., 2013; Hou et al., 2007; Song et al., 2008). This plant is also popular for the treatment of nephropathy, rheumatism and spermatorrhea (Cheng et al., 2013; Hou et al., 2007; Kokubun et al., 1995; Song et al., 2008; Wei et al., 2013). It was noticed that the flowers of *Photinia serrulata* emit a strong odor reminiscent of human semen (confirmed by three independent human observers). Interestingly, no 1-pyrroline or other compounds that could be responsible for the semen-like odor have been identified in the scent of *P. serrulata* by SPME-GC-MS in earlier studies (Wei et al., 2013). Therefore, the chemical origin of semen-like odor emitted by the flowers of *P. serrulata* remains unknown. *Castanopsis sclerophylla*, which belongs to the family of Fagaceae, is another widely distributed plant in subtropical Eastern Asia well known for the pronounced semen-like odor of its flowers (Shi et al., 2011). The chemical origin of semen-like odor emitted by *C. sclerophylla* flowers also remains unknown due to the lack of VOCs studies.

We incidentally discovered many *P. serrulata* and *C. sclerophylla* plants in our campus during the blooming season (April). We questioned: (1) What is the chemical origin of the semen-like odor of *P. serrulata* flowers and *C. sclerophylla* flowers? (2) What is the biological significance of the semen-like odor released by these species? To answer these questions, the volatiles released by *P. serrulata* flowers and *C. sclerophylla* flowers were analyzed using direct ambient corona discharge ionization mass spectrometry (MS). The method is a variation of the classical atmospheric pressure chemical ionization (APCI) in which flower VOCs are transported to the tip of the discharge needle using room temperature nitrogen gas without carrier solvent and accessory heating, which has been reported in our previous studies (Chingin et al., 2015; Hu et al., 2016; Liang et al., 2014). The advantage of this approach is that the analysis is done directly on the freshly collected flowers, absolutely no VOCs collection being required. APCI is particularly sensitive to volatile amines, which are the most probable candidates responsible for the semen-like odor. Along with the *P. serrulata* flowers and *C. sclerophylla* flowers, we also analyzed the volatiles released by *S. japonica* flowers (Stemonaceae) in order to compare our results with the results from SPME-GC-MS analysis reported by Chen et al. (2015). Based on the VOCs composition we discuss the chemical profile and biological role of semen-like odor in flowers.

2. Results

2.1. Flower visitor observations

We observed that the visitors of *P. serrulata* flowers were mainly dipterans from four species: *Eristalis tenax* (Syrphidae family), *Phytomia zonata* (Syrphidae family), *Chrysomya megacephala* (Calliphoridae family), and *Musca domestica* (Muscidae family), as depicted in Fig. 1. We did not observe visitation by honeybees. Interestingly, syrphid flies, which were observed to visit *P. serrulata* flowers in this study, resemble honeybees in appearance and have previously been considered to be Batesian mimics (Howarth et al., 2004). Recent research has shown that syrphid flies mimics honeybee behavior so as to gain greater protection through mimicry (Golding and Edmunds, 2000).

Similar to *P. serrulata* flowers, *C. sclerophylla* flowers were also observed to be mainly visited by dipterans from the families of Muscidae, Syrphidae, Calliphoridae, Atherigona, etc. Species of these families were also observed as widespread flower visitors on fetid stapeliads (Jürgens et al., 2006).

Similar to *P. serrulata* flowers and *C. sclerophylla* flowers, *S. japonica* flowers were also observed to be mostly visited by dipterans. No visitation by honeybees has been detected. The visitation of *S. japonica* flowers in natural habitats by various saprophilous flies in the families Muscidae, Sarcophagidae, Anthomyiidae, Lauxaniidae, and Tachinidae has been documented in detail by Chen et al. (2015) in earlier study.

2.2. Chemical profiling of VOCs

The entire list of VOC signals from the studied three flowers is listed in Table 1. Chemical assignment of MS signals was done based on the high resolution mass measurement of parent ions in full MS mode and fragment ions in MS/MS mode. Comparison with reference standard compounds was performed whenever possible as indicated in Table 1. In total, 19 VOCs were identified. Most of the identified VOCs belong to nitrogen-containing compounds. Some of the reported VOCs have not been identified in the corresponding plants by previous GC-MS studies (Chen et al., 2015; Wei et al., 2013), as indicated in Table 1. Below we discuss the identified VOCs for each of the studied plants.

2.2.1. *P. serrulata* flowers

Fig. 2 shows a fingerprint mass spectrum of VOCs emitted by *P. serrulata* flowers recorded using ambient corona discharge ionization MS ca. 10 min after the flower collection. The spectrum

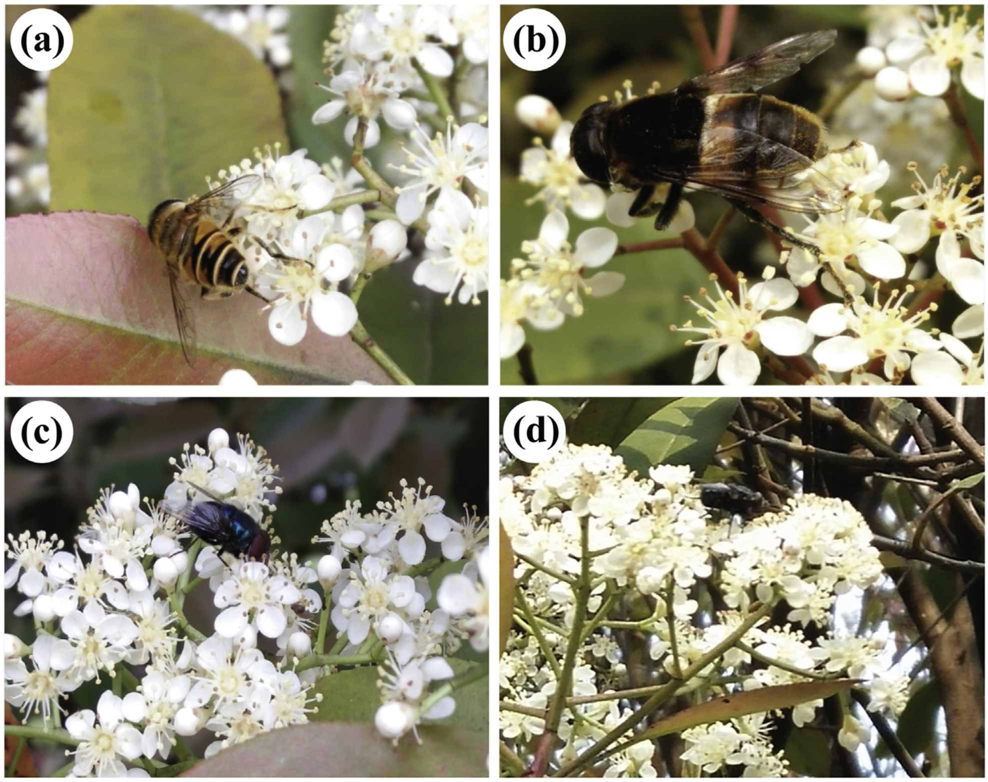

Fig. 1. Flowers of *P. serrulata* visited by different insects: (a) *Eristalis tenax* (Syrphidae), (b) *Phytomia zonata* (Syrphidae), (c) *Chrysomya megacephala* (Calliphoridae), (d) *Musca domestica* (Muscidae).

Table 1
Major VOCs detected in *P. serrulata*, *C. sclerophylla*, and *S. japonica* flowers by ambient corona discharge ionization HR-MS.

No.	Measured (*m/z*)	Formula	Theoretical (*m/z*)	Error (ppm)	Fragment ions (*m/z*)[c]	Identification	*Photinia serrulata* flowers	*Castanopsis sclerophylla* flowers	*Stemona japonica* flowers
1	60.08107	$C_3H_{10}N^+$	60.08078	4.9	N/A	trimethyl-amine[a,e]	+ (6.6%)[d]	–	–
2	70.06544	$C_4H_8N^+$	70.06513	4.5	43,42,28	1-pyrroline[b,e]	+ (5.2%)	+ (100%)	+ (100%)
3	84.08109	$C_5H_{10}N^+$	84.08078	3.7	67,56,42	1-piperideine[a,e]	+ (0.3%)	+ (2.2%)	+ (0.1%)
4	86.06038	$C_4H_8NO^+$	86.06004	3.9	58,57,68	2-pyrrolidone[b,e]	+ (0.5%)	+ (0.2%)	+ (0.8%)
5	88.07598	$C_4H_{10}NO^+$	88.07569	3.3	71,70,60	4-amino-butanal[a,e]	+ (100%)	–	–
6	95.04905	$C_6H_7O^+$	95.04914	4.3	77,67,63	2-vinyl-furan[a]	+ (4.2%)	–	–
7	99.08087	$C_6H_{11}O^+$	99.08044	4.3	81,71	hex-1-en-3-one[a]	–	–	+ (1.1%)
8	103.07585	$C_5H_{11}O_2^+$	103.07536	4.8	85,71,59	tetrahydrofurfuryl alcohol[a,e]	+ (2.3%)	–	–
9	105.07008	$C_8H_9^+$	105.06988	2.8	79	styrene[a,e]	+ (2.0%)	+ (0.5%)	–
10	107.04959	$C_7H_7O^+$	107.04914	4.2	79	benzaldehyde[a]	+ (1.1%)	–	–
11	108.08102	$C_7H_{10}N^+$	108.08078	2.3	93,91,81,80,79	2-ethylpyridine[a,e]	+ (34.2%)	–	–
12	110.06033	$C_6H_8NO^+$	110.06004	2.6	95, 82, 68,67	2-acetylpyrrole[b,e]	+ (8.1%)	–	–
13	113.05996	$C_6H_9O_2^+$	113.05971	2.2	95,85,71,67,57	dehydromevalonic lactone[a]	+ (2.9%)	–	–
14	122.09661	$C_8H_{12}N^+$	122.09643	1.5	105,94,93	phenethylamine[a,e]	+ (5.2%)	+ (3.9%)	+ (0.2%)
15	128.10717	$C_7H_{14}NO^+$	128.10699	1.4	84	2-acetylpiperidine[a,e]	+ (2.6%)	+ (16.3%)	–
16	132.10211	$C_6H_{14}NO_2^+$	132.10191	1.6	117,114,104,89,72	valine methyl ester[b,e]	+ (3.9%)	–	–
17	146.11781	$C_7H_{16}NO_2^+$	146.11756	1.7	128,86	leucine methyl ester[b,e]	+ (16.9%)	–	–
18	153.12752	$C_{10}H_{17}O^+$	153.12739	0.8	135,125,121,111,109,107,97,95	hotrienol[a,e]	+ (0.8%)	–	–
19	155.14319	$C_{10}H_{19}O^+$	155.14304	0.9	137,127,119,109,113,111,99,97	linalool[b]	+ (1.4%)	–	–

[a] The identification was based on high-resolution MS, CID and published MS data.
[b] The identification was based on high-resolution, CID and reference analysis of standard compound.
[c] Underlined value indicates the base peak fragment ion.
[d] The number in parentheses indicates the relative intensity.
[e] According to our literature search, these VOCs were identified in the corresponding species for the first time.

showed the obvious presence of intense signals at *m/z* 88.07598 and *m/z* 70.06544. The only possible chemical formulas that fit the values within 5 ppm tolerance are $C_4H_{10}ON^+$ and $C_4H_8N^+$, respectively. All other possible chemical formulas differ from *m/z* 88.07598 and *m/z* 70.06544 by > 150 ppm. Structurally, the $C_4H_8N^+$ ion was unambiguously identified as protonated 1-pyrroline (Fig. 3)

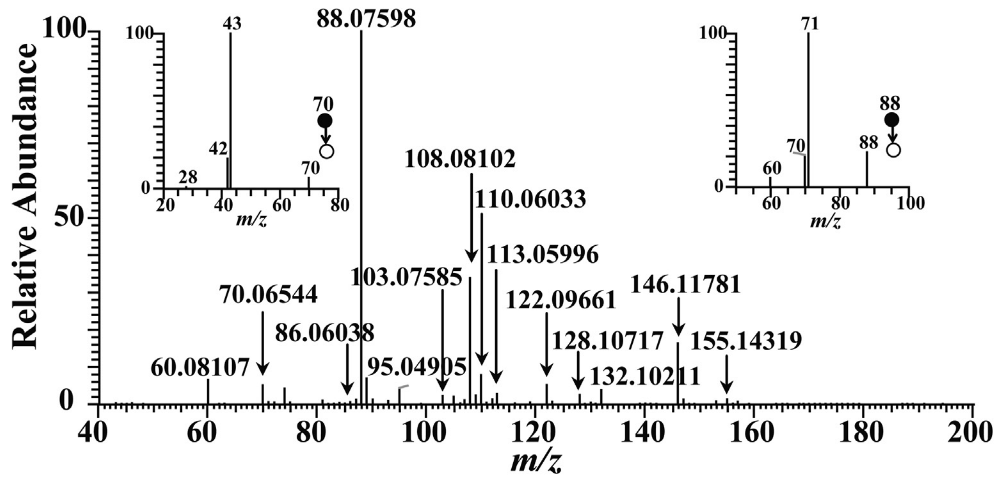

Fig. 2. Ambient corona discharge ionization mass spectra of VOCs from *P. serrulata* flowers (inserts are CID-MS spectra of *m/z* 70 and *m/z* 88).

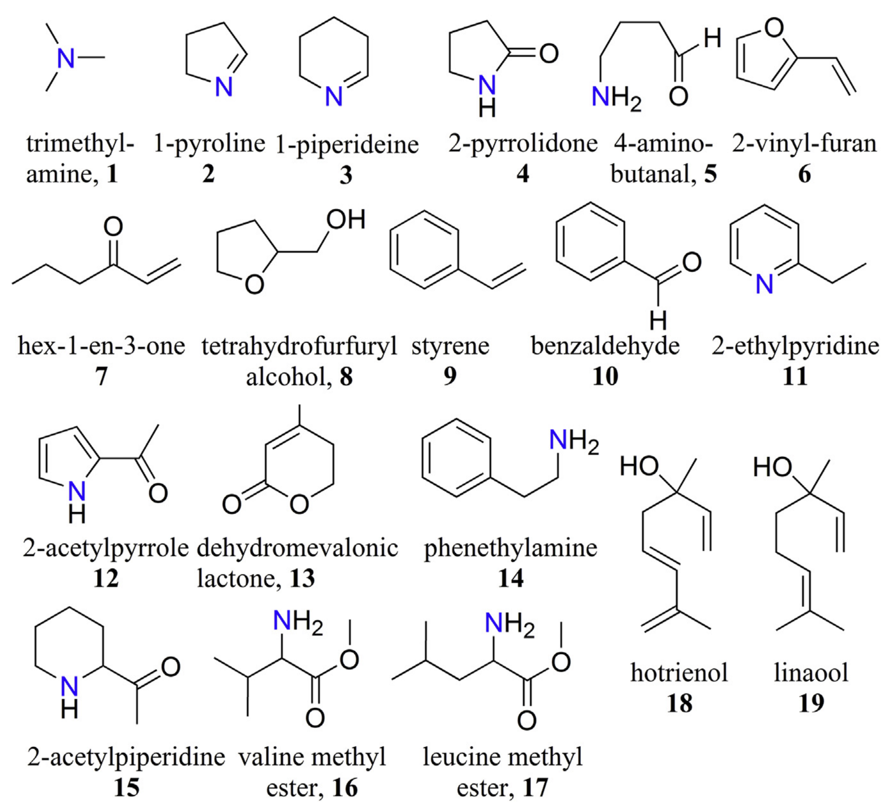

Fig. 3. Structures of VOCs identified in *P. serrulata*, *C. sclerophylla*, and *S. japonica* flowers.

based on the exact match of its collision-induced dissociation (CID) pattern (insert of Fig. 2) compared to that of the synthetic 1-pyrroline (Fig. S1). Upon CID, protonated 1-pyrroline mainly generates three product ions at *m/z* 43, *m/z* 42 and *m/z* 28, by loss of HCN, $CH_2{=}CH_2$, and $CH_3CH{=}CH_2$, respectively (insert of Fig. 2). Note that 1-pyrroline is yet not listed in NIST library, and this can be the reason for the lack of 1-pyrroline observation in the earlier study of *P. serrulata* VOCs by SPME-GC-MS. The $C_4H_{10}ON^+$ ion (base peak) was tentatively assigned to protonated 4-amino-butanal (Fig. 3), which is the key short-lived intermediate during the oxidation of putrescine (Agostinelli et al., 2004; Massa et al., 2010). This is the first time that these two compounds have been identified in *P. serrulata*. Earlier plant studies revealed that 1-pyrroline can be formed by the spontaneous cyclization of 4-amino-butanal. In some plants 1-pyrroline and 4-amino-butanal are found in enzymatically-controlled equilibrium (Agostinelli et al., 2004; Massa et al., 2010). The simultaneous observation of 1-pyrroline and 4-amino-butanal in *P. serrulata* vapor indicates that these two compounds in the plant are probably present in enzymatically-controlled equilibrium. Upon collision energy dissociation (CID), protonated 4-amino-butanal (*m/z* 88) mainly generates three product ions at *m/z* 71, *m/z* 70 and *m/z* 60, by loss of NH_3, H_2O, and CO, respectively (insert of Fig. 2). The detailed formation mechanism is depicted in Fig. 4.

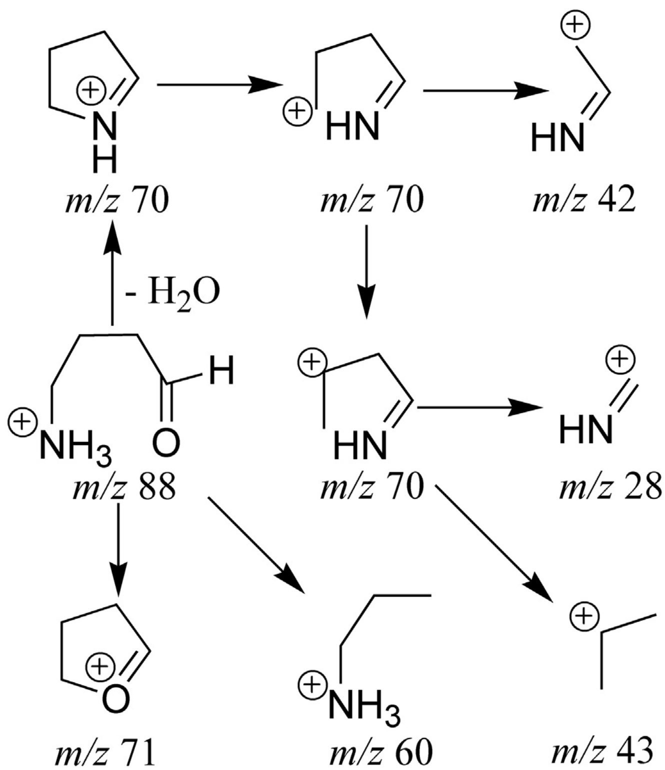

Fig. 4. The formation and possible fragmentation mechanism of 1-pyrroline and 4-amino-butanal.

The minor peaks at *m/z* 95.04905 ($C_6H_7O^+$), *m/z* 107.04959 ($C_7H_7O^+$), *m/z* 113.05996 ($C_6H_9O_2^+$) and *m/z* 155.14319 ($C_{10}H_{19}O^+$) were assigned to protonated 2-vinyl-furan, benzaldehyde, dehydromevalonic lactone, and linalool, respectively, based on their CID spectrum (Figs. S2 and S3-a), which have also been reported in the *P. serrulata* flowers by Wei et al. (2013). Upon CID, protonated linalool generated major fragments at *m/z* 137, 127, 119, 111, 109, 99, 97, and 95 (Fig. S3-a). These fragments are also detected from the authentic linalool, as shown in Fig. S3-b. The minor peak at *m/z* 153.12752 ($C_{10}H_{17}O^+$) was 2 Da lower than protonated linalool. The both signals shared similar fragment patterns with a mass shift 2 Da (Fig. S4). Therefore, the compound observed at *m/z* 153.12752 was tentatively identified as hotrienol. Hotrienol has also been reported as characteristic constituent in *Ailantus glandulose* (Joulain, 1987), *Ligustrum ovaligolium* (Joulain, 1987), and *Citrus* flowers (Alissandrakis et al., 2003). The flavor of hotrienol has been described as sweet and flowery (Alissandrakis et al., 2003).

Interestingly, the methyl esters of valine and leucine identified in *P. serrulata* flower vapor were earlier reported as characteristic constituents in the unusual odor of bird cherry flowers (Surburg et al., 1990). The peaks at *m/z* 132.10211 ($C_6H_{14}NO_2^+$) and 146.11781 ($C_7H_{16}NO_2^+$) were assigned to protonated valine methyl ester and leucine methyl ester, respectively. Upon CID, protonated leucine methyl ester generated major fragment at *m/z* 86, and minor fragments at *m/z* 128, and 118, owing to the elimination of acetic acid, H_2O, and CO, respectively (Fig. S5-a). These fragments are in accordance with those from authentic protonated leucine methyl ester (Fig. S5-b). The fragmentation patterns of protonated valine methyl ester were very similar to those of leucine methyl ester (Fig. S6).

The minor peaks at *m/z* 84.08109 ($C_5H_{10}N^+$) and *m/z* 86.06038 ($C_4H_8NO^+$) were tentatively assigned to 1-piperideine and 2-pyrrolidone. To the best of our knowledge, these two compounds have not been identified in flower scents previously. The molecular structures (Fig. 3) of 1-piperideine and 2-pyrrolidone were tentatively assigned based on high resolution MS and tandem MS analysis. The fragment ions are listed in Table 1. The fragmentation pattern of *m/z* 86 was very similar to that of protonated 2-pyrrolidone standard (Fig. S7). Upon CID, protonated 1-piperideine generated fragment ions at *m/z* 67, 56, and 42, owing to the elimination of NH_3, $CH_2{=}CH_2$, and $CH_3CH{=}CH_2$, respectively (Fig. 5-a). The proposed fragmentation mechanism is depicted in Fig. 6 at below. Tabor (1951) suggested that 1-piperideine could be the product of the oxidation of 1,5-diaminopentane, and the ring-compound formation is due to the spontaneous cyclization of the aldehyde group and the second amine group with elimination of water. 1-Piperideine is a cyclic homolog of 1-pyrroline.

According to the previous reports (Frattini et al., 1977), the peak at *m/z* 110.06033 ($C_6H_8NO^+$) was assigned to 2-acetylpyrrole. Upon CID, protonated ion at *m/z* 110 generated fragment ions at *m/z* 95, 82, 68, and 67 owing to the elimination of CH_3, CO, $CH_2{=}C{=}O$, and [CH_3+CO], respectively (Fig. S8-a). The fragmentation pattern of *m/z* 110 was in accordance with that of protonated 2-acetylpyrrole standard (Fig. S8). As shown in Fig. 5-b, during the CID process, the precursor ion at *m/z* 128 generated a base peak at *m/z* 84 by the loss of CH_3CHO. Note that the MS^2 spectrum of *m/z* 84 (Fig. 5-a) has a fragmentation pattern similar to the MS^3 spectrum of *m/z* 128 (Fig. 5-b, insert). Therefore, the peak at *m/z* 128.10717 ($C_7H_{14}NO^+$) is assigned to 2-acetylpiperidine. The proposed fragmentation mechanism of protonated 2-acetylpiperidine is depicted in Fig. 6 (Tressl et al., 1985).

Based on the high resolution experiments and on the results of earlier literature reports, several other compounds, such as protonated trimethyl-amine, *m/z* 60.08107 (Fukami et al., 2002; Kite and Hetterscheid, 2017), tetrahydrofurfuryl alcohol, *m/z* 103.07585 (Yamaguchi and Shibamoto, 1980), styrene, *m/z* 105.07008 (Yamaguchi and Shibamoto, 1980), 2-ethylpyridine, *m/z* 108.08078 (Fukami et al., 2002) and phenethylamine, *m/z* 122.09661 (Ough et al., 1981) were identified by analogous procedures from *P. serrulata* flowers.

2.2.2. *C. sclerophylla* flowers

The direct ambient corona discharge ionization MS fingerprints of VOCs emitted by *C. sclerophylla* flowers are shown in Fig. 7-a. In the spectrum of *C. sclerophylla* flowers, the detected five peaks are *m/z* 70.06544 ($C_4H_8N^+$), *m/z* 84.08109 ($C_5H_{10}N^+$), *m/z* 86.06038 ($C_4H_8NO^+$), *m/z* 105.07013 ($C_8H_9^+$), *m/z* 122.09611 ($C_8H_{12}N^+$), and *m/z* 128.10717 ($C_7H_{14}NO^+$), in which *m/z* 70.06544 is the base peak (Fig. 7-a). Note that the signals detected in the VOCs spectrum of *C. sclerophylla* flowers were also entirely present in the VOCs spectrum of *P. serrulata* flowers. The CID mass spectra of these signals were found to match those of corresponding compounds identified in *P. serrulata* flowers. Thus, the peaks at *m/z* 70.06544, *m/z* 84.08109, *m/z* 86.06038, *m/z* 105.07013, *m/z* 122.09611, and *m/z* 128.10717 were assigned to protonated 1-pyrroline, 1-piperideine, 2-pyrrolidone, styrene, phenethylamine, and 2-acetylpiperidine, respectively.

2.2.3. *S. japonica* flowers

The direct ambient corona discharge ionization MS fingerprints of VOCs emitted by *S. japonica* flowers are shown in Fig. 7-b. Obviously, the spectra of *C. sclerophylla* flowers and *S. japonica* flowers displayed high similarity in the chemical fingerprints. For instance, the peaks at *m/z* 70.06544, *m/z* 84.08109, *m/z* 86.06038, and *m/z* 122.09661 were shared in all the studied species. 1-Pyrroline (*m/z* 70.06544) is also a base peak in *S. japonica* flowers. This is also similar to earlier work on floral VOCs from *S. japonica* flowers by Chen et al. found that 1-pyrroline was the most

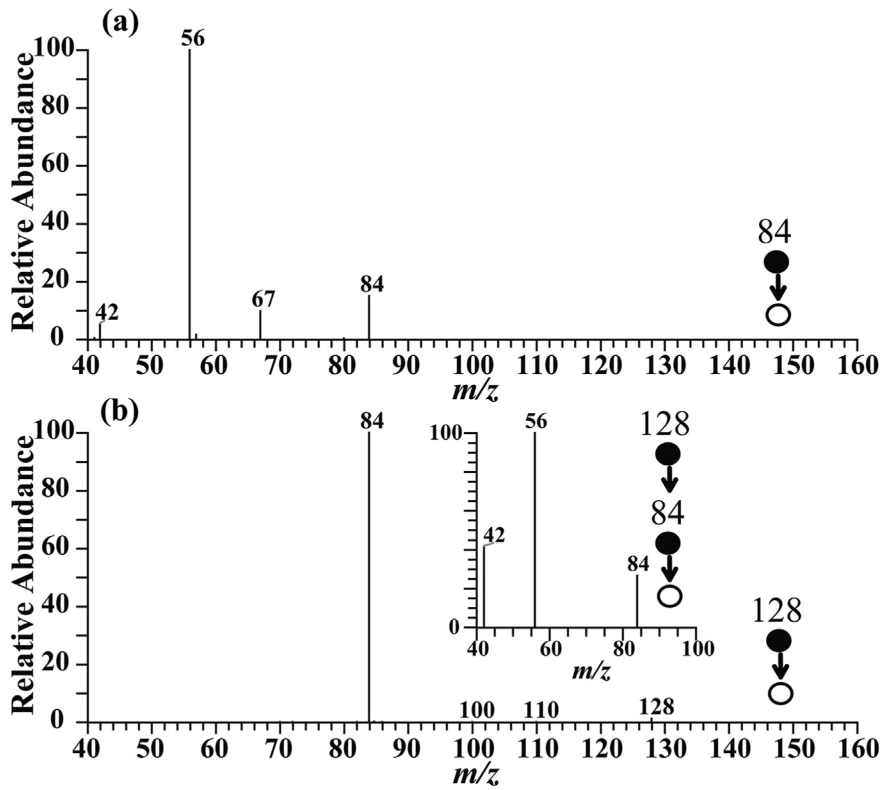

Fig. 5. CID-MS spectra of protonated ion at *m/z* 84 (a) and protonated ion at *m/z* 128 (b) in *P. serrulata*, *C. sclerophylla*, and *S. japonica* flowers. Insert is MS^3 spectrum of *m/z* 128.

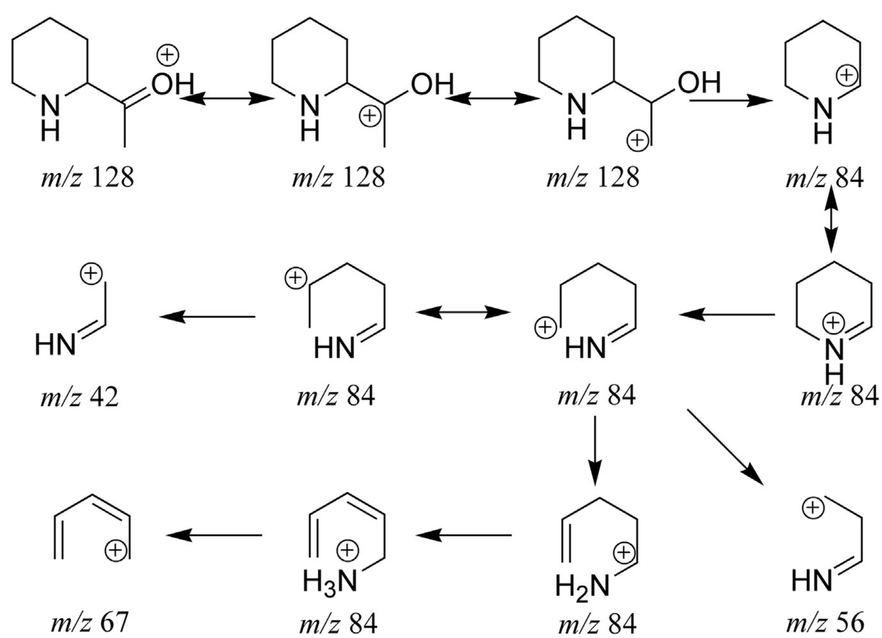

Fig. 6. The possible fragmentation mechanism of 2-acetylpiperidine and 1-piperideine.

abundant (Chen et al., 2015). Interestingly, the volatile profile for *S. japonica* flowers by ambient corona discharge ionization MS revealed new VOCs that have not been observed in *S. japonica* flowers previously by GC-MS (Chen et al., 2015). The peaks at *m/z* 84.08109 ($C_5H_{10}N^+$), *m/z* 86.06038 ($C_4H_8NO^+$), *m/z* 99.08087 ($C_6H_{11}O^+$), *m/z* 122.09661 ($C_8H_{12}N^+$) were assigned to protonated 1-piperideine, 2-pyrrolidone, hex-1-en-3-one, and phenethylamine, respectively. The results of structural analysis are presented in Fig. 3 and Table 1. The minor peak at *m/z* 99.08087 corresponds to protonated hex-1-en-3-one. Upon CID, it produced major fragments of *m/z* 81, 71, and 57, by the loss of H_2O, CO and $CH_2{=}C{=}O$, respectively.

Overall, our results indicate the abundant release of 1-pyrroline, which is apparently the major cause of the semen-like odor emitted

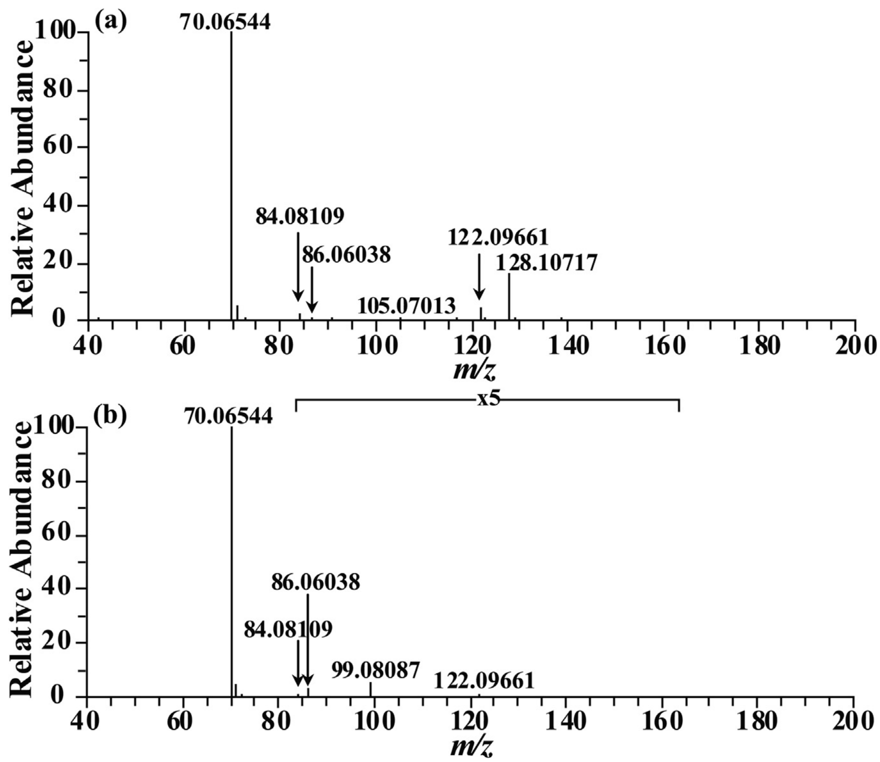

Fig. 7. Ambient corona discharge ionization mass spectra of VOCs emitted by *C. sclerophylla* flowers (a) and *S. japonica* flowers (b).

by *P. serrulata* flowers, *C. sclerophylla* flowers, and *S. japonica* flowers. Also, many volatile amines such as 1-pyrroline, 1-piperideine, 2-pyrrolidone, and phenethylamine have been identified in *P. serrulata* flowers and *C. sclerophylla* flowers for the first time. The most probable explanation is the high sensitivity of APCI detection to high proton-affinity VOCs, in particular volatile amines.

3. Discussion

Ambient VOCs analysis of the freshly collected flowers of *C. sclerophylla*, *S. japonica* and *P. serrulata* revealed a number of compounds, in particular volatile amines (Table 1), which have not been reported in the corresponding species by earlier GC-MS studies (Chen et al., 2015; Wei et al., 2013). This is most likely due to the high sensitivity of our approach to volatiles with high proton affinity (PA). Ambient ionization of VOCs by corona discharge occurs in a ladder-like fashion in which protons are gradually transferred from compounds with lower PA values to compounds with higher PA values. This greatly favors the observation of high-PA VOCs, such as volatile amines, even when at a trace level invisible to GC-MS. In contrast to the high sensitivity toward the detection of high-PA VOCs, ambient corona discharge ionization of low-PA VOCs, such as acids, alcohols, hydrocarbons, etc., is much less efficient. As a result, a few VOCs discovered by GC/MS are not visible by using ambient ionization by corona discharge. Apparently, ambient corona discharge ionization MS and SPME-GC-MS well complement each other for the deeper VOCs profiling.

The detection of abundant nitrogenous VOCs emitted by the flowers of *C. sclerophylla*, *S. japonica* and *P. serrulata* is consistent with the observed visitation behavior of these species. According to earlier reports, saprophilous flies are typically lured by specific nitrogen-containing compounds emitted from plants (Jürgens et al., 2006, 2013; Zito et al., 2015). The nitrogen-containing floral scent compounds are derived almost exclusively from amino acids metabolism or polyamine metabolism. For example, the most likely pathway for the synthesis of phenethylamine in plants is *via* decarboxylation of phenylalanine by an aromatic amino acid decarboxylase (Tieman et al., 2006). The precursor of 1-pyrroline and 4-amino-butanal in plants is putrescine, and the precursor of 1-piperidine is cadaverine, respectively. Putrescine and cadaverine are oxidatively deaminated by diamine oxidases (Edreva, 1996; Šebela et al., 2001). The miscellaneous cyclic compounds can be of diverse biosynthetic origin, but some are undoubtedly derivatives of fatty acids or amino acids.

As only 1-pyrroline, 1-piperideine, 2-pyrrolidone, and phenethylamine are shared in the MS fingerprints of these three semen-like odor flowers, it can be concluded that the semen odor is most probably composed by one (or the combination) of these compounds. Several lines of evidence indicate that the major component responsible for the semen-like odor is most likely 1-pyrroline. The floral scents from *P. serrulata*, *C. sclerophylla* and *S. japonica* are nearly identical to the odor of 1-pyrroline, at least as far as the human nose can tell. Our experiments indicated that 20 male volunteers failed to differentiate between 1-pyrroline and the scents from these flowers. Human nose is extremely sensitive to 1-pyrroline (Amoore et al., 1975). Also, the semen-like odors emitted by *C. sclerophylla* and *S. japonica* flowers are stronger than the semen-like odor from *P. serrulata* flowers (confirmed by 10 male observers). Consistently, the intensity of 1-pyrroline signal recorded from *C. sclerophylla* and *S. japonica* flowers was significantly higher than the intensity of 1-pyrroline signal from *P. serrulata* flowers (Fig. 7). This is another indication that the semen-like flower odor in all the studied species is mainly contributed by 1-pyrroline. However, the role of 1-piperideine, 2-pyrrolidone, and phenethylamine in shaping the semen-like floral odor should be rigorously tested in future bioassay experiments.

Despite the fact that the occurrence of semen-like odor in flowers is well known, very few studies identified 1-pyrroline as a major cause of this odor (Chen et al., 2015; Shuttleworth, 2016). Generally, 1-pyrroline remains very poorly studied. For example, 1-pyrroline is not mentioned in the comprehensive review of floral volatiles written by Knudsen et al. (2006). The major reason may be due to the difficulty of 1-pyrroline identification. The compound has poor chemical stability (Baker et al., 1992; Zhang et al., 2017). There is no commercially available 1-pyrroline standard for reference measurements. For the reliable identification 1-pyrroline should be synthesized in-house, such as in the present study (Chen et al., 2015). Further VOCs studies as well as revisiting earlier data are necessary to reliably evaluate the ubiquity and understand the functional significance of 1-pyrroline in floral scents.

Of particular interest is the observation that the release of 1-pyrroline in each of the three studied species was also accompanied by nitrogenous 1-piperideine, 2-pyrrolidone, and phenethylamine (Table 1). This observation suggests that a combination of 1-pyrroline, 1-piperideine, 2-pyrrolidone, and phenethylamine rather than single 1-pyrroline may be essential to attract flies. This hypothesis is indirectly supported by the earlier studies indicating that the attractiveness of 1-pyrroline to insects is high only when 1-pyrroline is present in mixture with other VOCs. Thus, it has been reported that 1-pyrroline is an essential constituent of a sex pheromone mixture released by the male Mediterranean fruit fly, whereas pure 1-pyrroline has no pheromone activity (Robacker et al., 1997). Furthermore, Chen et al. (2015) also found that a synthetic mixture of 1-pyrroline and other four C5-branched floral VOCs released by *S. japonica* flowers was attractive to *Atherigona* flies in natural habitats, whereas single 1-pyrroline alone was not attractive. Evidence has also been published that under certain conditions flies are very sensitive to VOCs containing protonizable nitrogen (such as 1-piperideine, 2-pyrrolidone, and phenethylamine) even when present at minor amounts (Robacker and Bartelt, 1997). Apart from attracting flies, evidence for the defensive function of some floral odors has also been proposed (Lucas-Barbosa et al., 2011). Thus, Lev-Yadun et al. (2009) reported that flowers emitting carrion or dung odors not only can attract the flies and beetles for pollination but may also provide defense against mammalian herbivores. Therefore, the nitrogen-containing volatiles emitted by *P. serrulata*, *C. sclerophylla*, and *S. japonica* flowers, apart from attracting flies, might also additionally protect these plants against certain herbivores. More research is needed on this side. It is therefore of particular interest for further research to test the possible synergy effect between 1-pyrroline and the discovered volatile amines for the insect attraction.

4. Conclusion

High-resolution ambient corona discharge ionization MS in combination with tandem MS analysis revealed pronounced similarities in the chemical composition of semen-like flower odor produced by the plant species (*P. serrulata*, *C. sclerophylla*, and *S. japonica*) belonging to three different families (Rosaceae, Fagaceae, and Stemonaceae). The shared chemicals included nitrogen-containing volatiles 1-pyrroline, 1-piperideine, 2-pyrrolidone, and phenethylamine. The semen-like odor as it is perceived by humans is merely contributed by 1-pyrroline, which is produced *via* oxidative deamination of putrescine. Our observation that the semen-like odor of flowers from different plant families is produced by 1-pyrroline indicates that 1-pyrroline may be a universal source for the semen-like odor in plants. Insect visitor observations indicate that the major role of the semen-like odor in plants is probably to attract saprophagous insects. We suggest that the attraction of saprophagous insects by the studied flowers is mainly mediated by the combination of shared four chemicals, 1-pyrroline, 1-piperideine, 2-pyrrolidone, and phenethylamine. The synergy effect between nitrogen-containing volatiles for the insect attraction has been also indicated in earlier studies. As 1-pyrroline has been earlier found to be an ingredient of sex pheromone released by some flies, it is of particular interest to deeper explore the effect of 1-pyrroline odor on insect behavior and its role in pollination in future studies. The exact mechanism of pollination, e.g. *via* oviposition mimicry, food source mimicry or sex attraction, as well as the exact delineation of chemicals essential for the pollinator attraction remain the subject for further research.

5. Experimental

5.1. Chemicals and reagents

2-Acetylpyrrole, valine methyl ester, leucine methyl ester, linalool, and 2-pyrrolidone were purchased from Sun Chemical Technology (Shanghai, China) Co, Ltd, with a purity > 99%. Ultra-purity nitrogen (>99.99%) was obtained from Jiangxi Guoteng Gas Co. Ltd (Nanchang, China). 1-Pyrroline was synthesized in our lab as described previously (Zhang et al., 2017). The synthesis protocol was adapted from the earlier described protocol by (Mores et al., 2008) The purified compound was used as a standard compound for the identification of 1-pyrroline in flower odor by reference MS analysis.

5.2. Materials

Fresh flowers of *P. serrulata* and *C. sclerophylla* were collected at the campus of East China University of Technology during the time of highest floral scent emission (12:00–14:00), on April 2017. Fresh flowers of *S. japonica* were collected at School of Life Sciences in Nanchang University, on May 2017. The flower chemotypes have been identified prior to sample collection by Professor Luo Liping (Nanchang University, Jiangxi, China). The flower were placed separately in a 5 mL centrifuge tube (5 cm long, 1 cm diam, Solarbio, Beijing, China) and analyzed ca. 0.5 h after collection on commercial mass spectrometry. Three replicates of total fresh flowers were sampled, and an empty conical flask with un-open flower as control.

5.3. Flower visitor observations

Flower visitors were observed at the campus of East China University of Technology during the top flowering season of the *P. serrulata* and *C. sclerophylla* in April and May 2017. The flower visitors of *P. serrulata* were recorded continuously during the flower opening time from 09:00 to 17:00 for 6 days between April 9th and 14th. The flower visitors of *C. sclerophylla* and *S. japonica* were recorded during the flower opening time from 09:00 to 17:00 for 3 days from May 4th to 6th 2017 and from May 15th to 17th 2017, respectively. Some flower visitors were caught and preserved for later identification by an insect expert.

5.4. Instruments and working conditions

All experiments were carried out on a commercial Orbitrap-XL mass spectrometer (Thermo Scientific, San Jose, CA, USA) and ion trap mass spectrometer (LTQ-XL, Thermo Scientific, San Jose, CA, USA) using home-made corona discharge ionization as detailed in our earlier studies (Chingin et al., 2015; Hu et al., 2016; Liang et al., 2014). The headspace volatiles of a flower in centrifuge tube were continuously transferred into ionization region *via* plastic tubing (ID 0.5 mm) assisted by nitrogen gas flow (0.1 MPa). Ambient corona discharge was created by applying +4.5 kV to a stainless steel needle (OD 150 μm) with a sharp end (curvature radius ~7.5 μm). The angle between the discharge needle and the outlet of the sample tubing was 30°. The distance from the tip of the ion probe to the inlet of the MS capillary was 6 mm. Mass spectrum was averaged in the *m/z* 15–200 range from the 30 scans obtained over the sampling period of 10 s. The instrument was operated at a high-resolution up to 100 000. In MS/MS experiments, mass width of the selected ion was 1 Da, and the normalized collision energy for the selected ion was set at the range of 20%–30% with helium being used as the collision gas.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.phytochem.2017.11.001.

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