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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