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Floral scents of typical *Buddleja* species with different pollination syndromes

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ABSTRACT

Floral scents from *Buddleja lindleyana*, *Buddleja loricata*, *Buddleja cordata* and *Buddleja tubiflora* were evaluated using the dynamic headspace adsorption method and identified with coupled gas chromatography and mass spectrometry. In total, 13 compounds were identified from the flowers of *B. cordata*, 19 from *B. lindleyana* and 29 from *B. loricata*, representing 95.5%, 93.1% and 96.2% of the total floral scents in the three species, respectively. No floral scents were recorded from *B. tubiflora*. Main volatile compounds in *B. cordata* were *trans*-β-ocimene (53.4%), lilac alcohol (9.6%) and lilac aldehyde (5.6%). Main volatile compounds in *B. lindleyana* were β-caryophyllene (17.6%), α-farnesene (16.0%) and 3-octanone (9.8%). Main volatile compounds in *B. loricata* were 4-oxoisophorone (27.1%), α-farnesene (17.9%) and linalool (10.6%). The study suggested that the floral scents may have evolved in conjunction with the sensory capabilities of different visitors as a specific group of pollinators in representative *Buddleja* species.

1. Introduction

Floral characters of butterfly-adapted plants include vividly colored flowers and a narrow corolla tube (Faegri and van der Pijl, 1979; Jennersten, 1988). Amongst the floral traits in pollination, floral scents were thought to have evolved to adapt to the olfactory requirements of efficient pollinators (Faegri and van der Pijl, 1979). Studies indicate that floral scents played crucial roles as attractants for bees or lepidopteran insects (Honda et al., 1998; Pellmyr and Thien, 1986; Pham-Delegue et al., 1993; Schiestl et al., 1997). Andersson and Dobson (2003) found that linalool from *Warszewiczia coccinea* (Vahl) Klotzsch, 4-oxoisophorone from *Buddleja davidii* Franch., phenylacetaldehyde and linalool oxide from *Cirsium arvense* (L.) Scop., and the synthetic floral scent 1-octen-3-ol can elicit strong antennal responses in the butterfly *Heliconius melpomen* L.. Andersson (2003) indicated that 4-oxoisophorone from *B. davidii* could elicit the strongest antennal responses in the butterflies *Inachis io* L., *Aglais urticae* L. and *Gonepteryx rhamni* L. The study of Guédot et al. (2008) suggested α-farnesene, 4-oxoisophorone and benzaldehyde from *B. davidii* could trigger consistent antennal responses in some moth species. In this study, we will explore whether the floral scents composition of an Asian *Buddleja lindleyana* Fortune, an African *Buddleja loricata* Leeuwenberg and two American *Buddleja cordata* Kunth and *Buddleja tubiflora* Benth. are similar to *B. davidii*, which is a species where the floral scent has been fully studied in *Buddleja*.

B. lindleyana Fortune is a perennial, semi-deciduous shrub, which is native to central China (Li and Leeuwenberg, 1996; Chen et al., 2007). The species has abundant, colorful flowers that occur in 4–20 cm long inflorescences that have a sweet

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fragrance. *B. lindleyana* has been introduced as an ornamental to the Americas and Europe because of its fragrant and colorful flowers (Stuart, 2006). Flower color of *B. lindleyana* is blue or purple, which is attractive to butterflies and is commonly used in horticultural plantings (Stuart, 2006; Fig. 1 A).

B. cordata Kunth is a large deciduous shrub or tree, which is endemic to Mexico, growing along forest edges and water courses at elevations of 1000–3000 m (Norman, 2000). Inflorescence of *B. cordata* is paniculate, with 2–4 orders of branches, flowers in groups of 5–10 cymes, 0.5–1 cm in diameter. Corolla of *B. cordata* is white, cream, yellow or turning orange at maturity (Fig. 1B). Acting as a local honey plant, the flower of *B. cordata* smells sweetly and attracts many small bees and flies and to a lesser extent butterflies (Norman, 2000).

B. tubiflora Benth. is a small shrub, which is distributed from central Paraguay to Cordoba, Argentina, at edge of woodlands, thickets and in old fields at low elevations (Norman, 2000). Inflorescence of *B. tubiflora* is 5–20 cm long with 6–12 pairs of axillary cymes. Each cyme has 6–13 sessile flowers. Unscented flowers are chiefly distinguished by its striking orange corolla with 13–16 mm long (Norman, 2000; Fig. 1C). Pollination of *B. tubiflora* is by hummingbirds which feed on the sweet nectar at the base of the corolla (Norman, 2000).

B. loricata Leeuwenb. is a perennial shrub, which is native to Lesotho and Transkei in Africa at elevation from 1600 to 2700 m (Leeuwenberg, 1979). Flowers of *B. loricata* are arranged in terminal, congested 2–12 cm long paniculate, inflorescences. Flowers are shortly pedicellate with white, creamy, or pale yellow color (Fig. 1D). Flowers are highly aromatic, which some people think the flowers smell sweet like honey (Stuart, 2006). The species is attractive to butterflies and flies in its introduced place, Kunming Botanical Garden, Yunnan, China.

Though taxa from *Buddleja* are known as fragrant ornamental plants, research about why their floral scents can attract all kinds of pollinators is limited. As far as we know, only three species, *B. davidii*, *B. yunnanensis* and *B. officinalis* have had floral scents studied (Andersson et al., 2002; Andersson, 2003; Andersson and Dobson, 2003; Guédot et al., 2008; Chen and Sun, 2011; Chen et al., 2011). In this study, through comparison of floral scents to four *Buddleja* species, the objective of this research was to chemically characterize the floral scents of the taxa and to explore whether or not there were convergent pattern in floral scent composition in *Buddleja* species.

2. Materials and methods

2.1. Plant materials

Floral scents of *B. lindleyana* were collected from wild population in Zhijiang county, Hunan province, China (26°42′36″N, 110°35′05″ E; 364m; voucher GJ2010-09). Floral scents of *B. loricata*, *B. cordata* and *B. tubiflora* were collected from plants in Kunming Botanical Garden, Yunnan province, China (25°08′49″N, 102°44′41″E; 1788 m; voucher CHEN1001, 1002, 1003). Floral scents were collected using the dynamic headspace adsorption method during the sunniest time of the day between 12:00 and 15:00, which coincided with the time for butterfly, bee, or fly feeding. Because the inflorescences size was different, eight inflorescences of *B. lindleyana*, 10 of *B. tubiflora*, 30 of *B. cordata*, and 40 of *B. loricata* were collected in this study (with 3 replicates for each species combined to create one sample, respectively).

2.2. Floral scent collection

Newly blooming inflorescences of *B. lindleyana*, *B. loricata*, *B. cordata* and *B. tubiflora* were enclosed in Tedlar bags (Dupont, USA) and volatiles were drawn from the enclosures into cartridges containing the adsorbent Porapak Q (150 mg, mesh 60/80, Waters Associates, Inc.) for 3 h using a pump with an inlet flow rate of 300 ml/min. Prior to use, the adsorbent cartridges were cleaned with 2 ml of diethyl ether, and dried with nitrogen gas (60 min). Control samples were collected from air without inflorescence volatiles. Trapped volatiles were eluted with 300 μl dichloromethane and concentrated to one-fifth the original

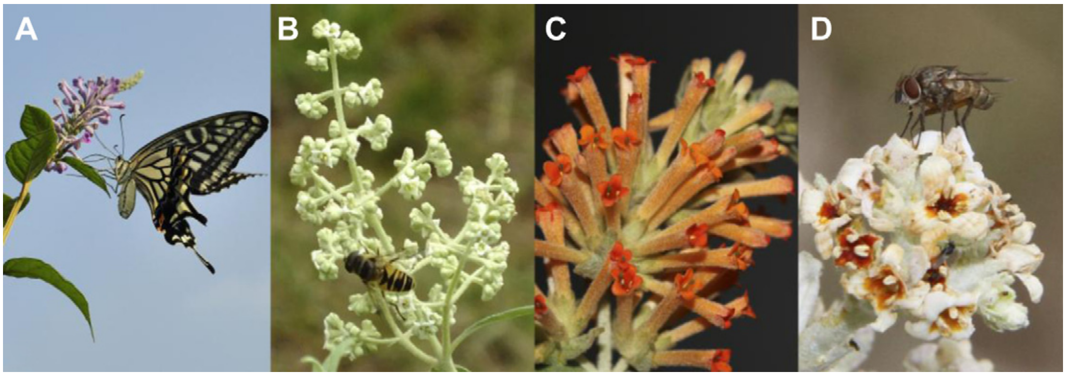

Fig. 1. Floral scent collection from different *Buddleja* species in this study. (A) *B. lindleyana*; (B) *B. cordata*; (C) *B. tubiflora* and (D) *B. loricata*.

volume by a gentle stream of nitrogen (200 ml/min). The extracts were stored at −20 °C in refrigerator for subsequent analysis.

2.3. GC–MS analysis

Extracts from inflorescences were analyzed using an HP 6890 gas chromatograph (Agilent Technologies, USA), equipped with a HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness), and linked to an HP 5973 mass spectrometer (Agilent Technologies, USA). Helium was used as a carrier gas at a flow of 1 ml/min, and injector temperature was set to 250 °C. Column temperature was 40 °C and after injection, was increased to 250 °C at a rate of 3 °C/min. Compounds were identified by comparing their gas chromatography retention times and mass spectrometry spectra with those of the authentic compounds, or tentatively identified by MS spectra in the Wiley 7n.1 mass spectral library and retention indexes reported elsewhere (such as: NIST Chemistry WebBook (http://webbook.nist.gov) and RI database (Adams, 2001: Adams's RI database).).

Kovats retention indices were calculated using the formula $I_x = 100n + 100\ (t_x - t_n)/(t_{n+1} - t_n)$, where I_x is the retention index of the compound of interest, t_x is the retention time of the compound of interest, t_n and t_{n+1} are the retention times of the *n*-alkanes (Sigma–Aldrich Company, USA) eluting immediately before and after the compound of interest, and n is the number of carbon atoms in the *n*-alkane eluting immediately before the compound of interest (Van Den Dool and Kratz, 1963).

3. Results

Gas chromatography–mass spectrometry analysis revealed 50 compounds from inflorescences of four *Buddleja* species (Table 1). Of the compounds, 13 compounds were identified from the flowers of *B. cordata*, 19 from *B. lindleyana* and 29 from *B. loricata*, representing 95.5%, 93.1% and 96.2% of the total floral scents in the three species, respectively. However, *B. tubiflora* did not emit any volatiles at our limit of detection. The species had no scents discernible to the authors' noses. Only two of the compounds (α-farnesene and linalool) occurred in all three species. Main volatile compounds in *B. cordata* were *trans*-β-ocimene (53.4%), lilac alcohol (9.6%) and lilac aldehyde (5.6%). Main volatile compounds in *B. lindleyana* were β-caryophyllene (17.6%), α-farnesene (16.0%) and 3-octanone (9.8%). Main volatile compounds in *B. loricata* were 4-oxoisophorone (27.1%), α-farnesene (17.9%) and linalool (10.6%). When listed in terms of relative amounts of the compound classes in the three species (Table 1), the order was as follows: sesquiterpenoids (40.6%), fatty acid derivatives (35.8%), monoterpenoids (10.4%), and benzenoids (6.3%) in *B. lindleyana*; monoterpenoids (32.7%), irregular terpenes (30.6%), sesquiterpenoids (17.9%), fatty acid derivatives (9.7%) and benzenoids (5.3%) in *B. loricata*; monoterpenoids (78.0%), fatty acid derivatives (14.9%) and sesquiterpenoids (2.6%) in *B. cordata*. Notably, irregular terpenes and benzenoids were absent from inflorescences of *B. cordata*. Irregular terpenes were absent from inflorescences of *B. lindleyana*. As far as alcohols and ketones were concerned, the fatty acid derivatives were absent from inflorescences of *B. cordata* (Table 1). Some siloxanes from adsorbent Porapak Q were identified in samples and control.

4. Discussion

Differences in floral traits among related species have been explained as adaptations to different pollinators, whereas floral convergence in uncorrelated species has been explained to parallel adaptation to the same pollinators, yet the phenomenon has been generally accepted as pollination syndromes (Schemske, 1981; Schemske and Bradshaw, 1999; Muchhala, 2003; Patterson and Givnish, 2004; Whittall et al., 2006; Streisfeld and Kohn, 2007). Previous studies also indicated that pollinators have been shown to exert selection pressure on flower color, corolla shape, and nectar reward. (Galen, 1989; Cresswell and Galen, 1991; Melendez-Ackerman et al., 1997; Smith et al., 2008).

Amongst the floral traits of "pollination syndromes", floral scents were thought to have evolved to adapt the olfactory requirements of pollinators (Faegri and van der Pijl, 1979). Some studies indicated floral scent played crucial roles to attract bees, flies, or lepidopteran visitors (Honda et al., 1998; Pellmyr and Thien, 1986; Pham-Delegue et al., 1993; Schiestl et al., 1997; Dobson, 2006; Schiestl and Dötterl, 2012). In this study, we estimated correlations between several groups of pollinators and floral scent using four species of *Buddleja* from different continents, which exhibits dramatic diversity in flower form and color (Fig. 1). Visitors to the *Buddleja* species were classed into four functional groups: hummingbirds, Hymenoptera (bees), Lepidoptera (moths or butterflies), and Diptera (flies). The pollination syndromes of these species were inferred from previous data (Norman, 2000; Stuart, 2006) and our field investigation.

Andersson (2003) found that the most abundant floral scent from *B. davidii* was irregular terpene 4-oxoisophorone, which can elicit the strongest antennal responses in butterflies, while sesquiterpenoid α-farnesene emitted in relatively high amounts can also elicit relatively high responses in some other butterflies. Borg-Karlson et al. (1996) and Nilsson (1992) found that linalool was emitted from *Daphne mezereumand* L. and *Anacamptis pyramidalis* L. in relative large amount to attract butterfly pollinators. In this study, the floral scent compounds 4-oxoisophorone (27.1%), α-farnesene (17.9%) and linalool (10.6%) were found to be the main compounds in the African *Buddleja* sp., *B. loricata*. Butterflies visited the highly aromatic inflorescences of *B. loricata* at the Kunming Botanical Garden, Yunnan, China. So, we support the view of Andersson et al. (2002) that these compounds may serve as signals to attract pollinating butterflies to locate flowers as nectar sources.

Table 1
Average relative amounts (%) of floral scent compounds from *B. lindleyana* (Bli), *B. loricata* (Blo) and *B. cordata* (Bco) using GC–MS analysis.

Compound	RI		CAS #	Bli	Blo	Bco
	RI[a]	Ri[b]				
Total number of compounds				19	29	13
Fatty acid derivatives						
Alcohols				26.0	5.7	0
2-Methyl-1-butanol	729	736	137-32-6	–	0.4	–
2,3-Butanediol	773	782	513-85-9	–	0.4	–
3-Hexanol	790	802	623-37-0	0.5	–	–
3-Hexen-1-ol	850	855	928-96-1	3.4	–	–
2-Hexanol	864	868	626-93-7	5.2	–	–
1-Octen-3-ol	981	986	3391-86-4	8.8	3.1	–
3-Octanol	999	996	589-98-0	4.8	0.8	–
(*Z*)-3-hexen-1-ol	1008	1005	928-96-1	3.3	1.0	–
Ketones				9.8	4.0	0
4-Methyl-3-penten-2-one	791	801	141-79-7	–	0.7	–
3-Octanone	987	989	106-68-3	9.8	3.3	–
Aldehydes				0	0	7.7
Nonanal	1107	1102	124-19-6	–	–	3.6
Decanal	1192	1200	112-31-2	–	–	4.1
Hydrocarbons				0	0	7.2
Tetradecane	1401	1400	629-59-4	–	–	1.9
Pentadecane	1499	1500	629-62-9	–	–	3.6
2-Methylpentadecane	1528	1533	1560-93-6	–	–	1.7
Benzenoids						
Benzaldehyde	958	966	100-52-7	–	2.1	–
Benzyl alcohol	1039	1037	100-51-6	–	1.0	–
Phenylacetaldehyde	1046	1044	122-78-1	–	1.4	–
3-Ethylbenzaldehyde	1167	1168	34246-54-3	3.2	–	–
4-Ethylbenzaldehyde	1182	1194	53951-50-1	1.1	–	–
3-Hydroxy-4-phenyl-2-butanone	1354	1348	5355-63-5	–	0.8	–
2,6-Di-tert-butyl-4-methylphenol	1522	1514	128-37-0	2.0	–	–
Monoterpenoids						
α-Thujene	921	925	2867/5/2	–	0.4	5.1
Sabinene	970	980	3387-41-5	–	2.6	–
β-Pinene	972	969	127-91-3	–	0.4	–
β-Myrcene	990	992	123-35-3	–	3.1	–
1-Limonene	1029	1027	5989-27-5	–	1.5	–
1,8-Cineole	1032	1030	470-82-6	–	0.7	–
cis-β-Ocimene	1042	1041	3338-55-4	–	1.3	1.6
trans-β-Ocimene	1051	1052	502-99-8	–	1.6	53.4
cis-Linalool oxide	1075	1075	5989-33-3	–	9.7	–
trans-Linalool oxide	1089	1091	34995-77-2	–	0.2	–
Linalool	1102	1101	78-70-6	3.2	10.6	0.6
Lilac aldehyde	1127	1133	53447-46-4	–	–	5.6
Ocimene oxirane	1146	1114	69103-20-4	3.5	–	–
Thujol	1153	1146	513-23-5	–	–	2.1
Menthol	1178	1183	491-02-1	2.0	–	–
Lilac alcohol A	1201	1211	33081-34-4	–	–	9.6
α-Limonene	1411	1032	5989-27-5	1.7	–	–
2-Ethanol-6,6-dimethyl-2-norpinene	1430	1400	128-51-8	–	0.6	–
Irregular terpenes						
3,5,5-Trimethyl-3-cyclohexen-1-one	1043	1118	78-59-1	–	0.5	–
(*E*)-4,8-dimethyl-1,3,7-nonatriene	1117	1110	51911-82-1	–	1.5	–
4-Oxoisophorone	1149	1152	1125-21-9	–	27.1	–
Safranal	1200	1201	116-26-7	–	0.5	–
β-Cyclocitral	1222	1222	432-25-7	–	1.0	–
Sesquiterpenoids						
α-Cedrene	1422	1416	79120-98-2	2.0	–	–
β-Caryophyllene	1427	1419	87-44-5	17.6	–	–
α-Caryophyllene	1429	1428	87-44-5	2.6	–	–
α-Farnesene	1512	1509	502-61-4	16.0	17.9	2.6
Caryophyllene oxide	1596	1573	1139-30-6	2.4	–	–
Fatty acid derivatives				*35.8*	*9.7*	*14.9*

Table 1 (*continued*)

Compound	RI		CAS #	Bli	Blo	Bco
	RI[a]	Ri[b]				
Benzenoids				*6.3*	*5.3*	*0*
Monoterpenoids				*10.4*	*32.7*	*78.0*
Irregular terpenes				*0*	*30.6*	*0*
Sesquiterpenoids				*40.6*	*17.9*	*2.6*
Total (%)				93.1	96.2	95.5

"–", Not detected.

[a] Retention index (RI) on HP-5MS column for this study.

[b] Retention index (RI) reference according to HP-5MS column data in NIST Chemistry WebBook (http://webbook.nist.gov) and published RI values in: R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Illinois, USA, 2001.

However, we also found the flower of *B. loricata* attracted to a lesser extent flies. The importance of each group needed to be investigated in the future.

The study of Guédot et al. (2008) suggested that α-farnesene from *B. davidii* could trigger consistent antennal responses in some moth species. Notably, synthetic or natural floral scent 1-octen-3-ol can elicit strong antennal responses in the butterfly *H. melpomen* (Andersson and Dobson, 2003). In this study, we found β-caryophyllene (17.6%), α-farnesene (16.0%) and 1-octen-3-ol (8.8%) were the main compounds in the Asian *Buddleja* sp., *B. lindleyana*. Butterflies and moths visited inflorescences of *B. lindleyana* in its native habitat (Wei-chang Gong et al., unpublished data). So, we suggest that these compounds may have evolved in conjunction with the sensory capabilities of butterflies as a specific group of pollinators.

Interestingly, the scent blends of *B. lindleyana* were dominated by sesquiterpenoids (40.6%) and fatty acid derivatives (35.8%). However, the monoterpenoids (32.7%) and irregular terpenes (30.6%) were the main compounds of scent blends in *B. loricata* (Table 1). The most abundant floral scent emitting from inflorescences of the African *Buddleja* sp., *B. loricata*, was irregular terpene 4-oxoisophorone, which was similar to Asian *B. davidii*, while the compound was absent from the Asian *Buddleja* sp., *B. lindleyana*. Our study suggested that the flower of *B. lindleyana* and *B. loricata* could emit typical compounds to attract moths or butterflies. However, the two species may take different tactics. There were not convergent pattern in floral scent composition between the Asian *Buddleja* sp., *B. lindleyana*, and the African *Buddleja* sp., *B. loricata*.

Aromatic, terpenoid, and non-ketone aliphatic compounds were the main volatiles in most insect-pollinated plants (Dobson, 2006). *B. cordata* is a local honey plant, the flower of *B. cordata* smells sweetly and attracts many small bees and flies (Norman, 2000). Our present study found monoterpenoids (78.0%) and fatty acid derivatives (14.9%) were the main compounds in this species including *trans*-β-ocimene (53.4%). The *trans*-β-ocimene is ubiquitous components of floral scents that are attractive to pollinating species, and foraging bees can detect this compound (Downs et al., 2000; Wright et al., 2005). Maisonnasse et al. (2010) found *trans*-β-ocimene could be the signal for the transition of middle-aged bees to foragers. In this study, we found bees (*Apis cerana* and *A. mellifera*) visited flowers of *B. cordata* in the Kunming Botanical Garden, Yunnan, China suggesting that these compounds may serve as signals to attract pollinating bees to locate flowers as nectar sources.

In the classical description of pollination syndromes, bird-pollinated flowers reflect longer wavelengths (especially red) than most insect-pollinated flowers, and the flowers are generally described as being scentless (Stiles, 1976; Faegri and van der Pijl, 1979; Vogel, 1990; Harrison et al., 1999; Altshuler, 2003; Dressler et al., 2004; Knudsen et al., 2004). The data presented here about red and unscented inflorescence of *B. tubiflora* support the widely held opinion that hummingbird-pollinated flowers are scentless.

Our recent study indicated that butyl acetate (81.6%) was the most abundant floral volatile from inflorescences of *Buddleja officinalis* Maxim., which was visited by some ants and butterflies (Chen and Sun, 2011). It would be worthwhile to compare the "pollination syndromes" among more *Buddleja* species and to study if the flowers of these taxa produce similar or different floral scents and how that relates to entomophily. Considering *Buddleja* species are important landscape ornamentals around the world, we think the results revealed for the first time the natural floral scents of four *Buddleja* species would provide the scientific data for germplasm innovation, such as creation of new odor type varieties by hybridization in *Buddleja* in future.

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