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Characteristic Aroma-Active Compounds of Floral Scent in Situ from *Barringtonia racemosa* and Their Dynamic Emission Rates

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ABSTRACT: *Barringtonia racemosa* is a nocturnal flowering plant. Information on its floral volatiles and the dynamic emission profiles was very limited. In this study, the floral volatiles of *B. racemosa* were monitored hourly during its florescence via detached and in situ collection for the first time. The dynamic odor activity value (OAV) was calculated to elucidate the active aroma components of floral scent. Results of compositional analyses showed that the predominant floral volatiles were linalool and phenylacetaldehyde. Their emission started around 8:00 p.m., and the peak emissions were 20541 and 18234 ng h^{-1} $flower^{-1}$, respectively, during 10:00 p.m.−2:00 a.m. Results from dynamic OAV profiling revealed that linalool (409 min^{-1}) and phenylacetaldehyde (547 min^{-1}) had higher OAVs than other components (<10 min^{-1}), indicating that linalool and phenylacetaldehyde contributed mainly to the floral scent with a strong, sweet, and pleasant aroma.

KEYWORDS: *aromatherapy, Barringtonia racemosa, floral volatiles, emission rate, odor activity value*

INTRODUCTION

Flowers play an important role for many flowering plants in the ecological system. They emit a variety of chemical compounds, including terpenoids, benzenoids, phenylpropanoids, and nitrogen- and sulfur-containing compounds. These compounds are responsible for attraction of butterflies, moths, and bats for pollination. They also act as semiochemicals for communication with neighboring plants.

Some detached flowers possess commercial value due to their pleasant scent during their florescence. Flowers with scent are usually distillated to obtain essential oils for further applications such as aromatherapy, perfume, flavoring, and food additives. Aromatherapy is one kind of complementary and alternative medicine therapy.[1] Inhalation of the volatile compounds from essential oils has a sedative effect. It was also reported to be a safe and effective treatment for dementia.[2] The term "forest bathing" was proposed by the Japanese Ministry of Agriculture, Forestry, and Fisheries in 1982.[3] Forest bathing was defined as taking a walk in the forest and inhaling the volatiles emitted from trees. It was believed that forest bathing could reduce stress and provide relaxation. Recent scientific findings provide evidence for its physiological and psychological effects on humans.[4]

For full utilization of floral scent or essential oil, it is important to know their chemical compositions and scent characteristics. Floral volatiles are commonly analyzed using several techniques such as solid-phase microextraction (SPME) and Tenax TA adsorbent. However, the flowers analyzed were usually detached from plants, thus limiting our understanding of the variations and profiling of floral volatiles during their florescence. Collection of floral volatiles from living flowers (in situ) is therefore imperative and can shed light on the relationship of plants with other plants, insects, and mammals during their florescence.

To understand the aroma-active compounds in floral scent or essential oil, the odor threshold value (OTV) and the odor activity value (OAV) of each component in a sample were examined. The OTV denotes the lowest concentration of the tested compound that can be sensed by humans, whereas the OAV represents the contribution of the compound to the aroma of a sample. These values are widely and popularly used in the study of the scent characteristics of fruits, wines, and foods.[5,6]

Barringtonia racemosa (L.) Bl. ex DC. is a native tree in Taiwan. It belongs to the family Lecythidaceae and is distributed over East Asia and Australia. Although many components of *B. racemosa* have been reported to exhibit a number of bioactivities, including antioxidation[7] and anti-inflammation effects,[7] little is known about its flowers. *B. racemosa* is a nocturnal plant, which usually starts blossoming around 6:00 p.m. The flowers bloom at night between 7:00 p.m. and 8:00 p.m. and remain in full blossom till the following morning (6:00−8:00 a.m.) before falling on the ground. During the florescence of *B. racemosa*, the strong, pleasant, and distinctive floral scent in the air in its vicinity would draw people's attention even at a distance. However, neither the functions of floral scent in *B. racemosa* nor the effect of its floral scent on human health is much known. To make up for such deficiency, this study examined the volatile composition of *B. racemosa* flower during the florescence.

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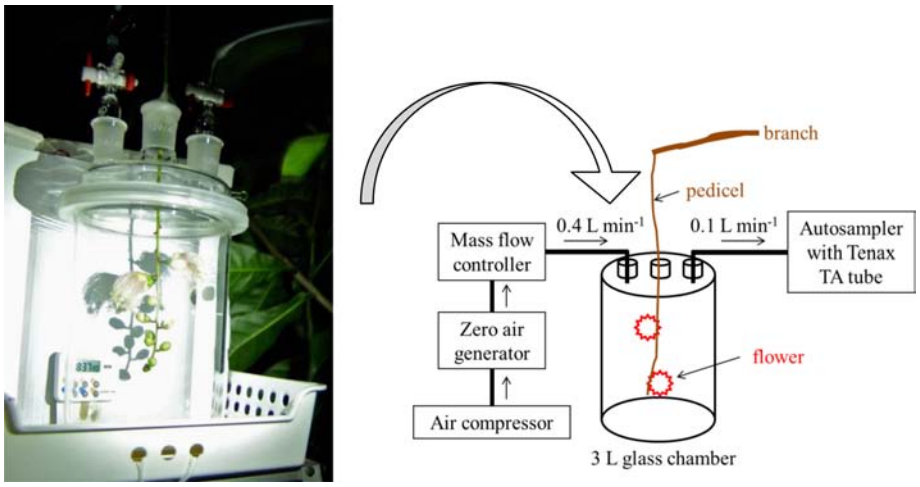

Figure 1. Diagram of enclosure system for in situ collection of floral scent from *Barringtonia racemosa*.

To the best of our knowledge, the floral volatiles emitted from *B. racemosa* have not been reported. This study analyzed the compositions of floral volatiles and monitored their emission kinetics by means of in laboratory (detached flowers) and in situ (living flowers) sampling for the first time. OAV profiling was also performed to examine the changes in aroma of the floral scent. The results obtained may provide useful information that can be applied to further studies on tree physiology and the interaction of trees with abiotic and biotic factors in the ecosystem.

MATERIALS AND METHODS

Plant. *B. racemosa* (species code N 61-70_0034) was located in the campus of National Taiwan University (Taipei City, Taiwan) (25.016902° N, 121.539339° E).

Chemicals. Chemical compounds used for identification and quantification included benzaldehyde (98%, Acros), *trans*-β-caryophyllene (90%, TCI), α-copaene (95%, Sigma), *n*-decanal (96%, Alfa-Alsar), α-humulene (96%, Aldrich), phenylacetaldehyde (95%, Alfa Alser), indole (99%, Acros), linalool (97%, Acros), *trans*-linalool oxide (97%, Fluka), methyl benzoate (99%, Alfa-Alsar), methyl salicylate (TCI), β-myrcene (90%, Sigma), naphthalene (98%, Fluka), β-ocimene (90%, SAFC), and phenyl ethyl alcohol (99%, Aldrich).

Sampling Device. For in situ sampling, a sampling device was set up near the *B. racemosa* tree. A pedicel with buds ready to blossom was carefully enclosed in the 3 L glass chamber (Figure 1) around 3:30 p.m. For sampling of volatiles from detached flowers, another pedicel with buds ready to blossom was cut and immediately enclosed in the 3 L glass chamber around 3:30 p.m. Air from an air compressor was filtered by the zero air generator (75-83NA, Parker-Balston, Haverhill, MA, USA). Fresh and clear air was pumped into the glass chamber at 0.4 L min^{-1} controlled by a mass flow controller (Type 8711, Burkert Co., Baden-Württemberg, Germany). An autosampler (Nutech 2602, GD Environmental Supplies Inc., Richardson, TX, USA) equipped with sampling tubes (Tenax TA mesh 60/80, Markes International Ltd., Wales, UK) was employed to collect the floral volatiles. Sampling period was from 6:30 p.m. to 9:45 a.m. Each sampling tube collected the floral volatiles at the flow rate of 0.1 L min^{-1} for 1 h. To avoid sampling the contaminants from tubing, Teflon tubing (i.d. $^1/_4$ in.) was used for transportation of gas in this study. After sampling, all flowers were detached and dried in a 100 °C oven for 2 days to obtain their dry weight. The emission rate (ER) was calculated by ER (ng h^{-1} $flower^{-1}$) = CQ/F, where C is the concentration (ng L^{-1}) of the compound of interest, Q is the flow rate (L min^{-1}) of pumping air, and F is the number of flowers enclosed in the glass chamber.

Thermal Desorption (TD). Floral volatiles adsorbed in the sampling tube were thermally desorbed using thermal desorption (Thermal Desorber Turbo Matrix 150, Perkin-Elmer Inc., Shelton, CT, USA). The sampling tube was first purged for 3 min to remove the water adsorbed on Tenax TA adsorbent, then followed by primary desorption at 220 °C for 5 min and secondary desorption at 230 °C for 8 min. The split ratios during primary and secondary desorption were 4.8:1 and 21.5:1, respectively. The desorbed samples were finally flowed through the transfer line at 1 mL min^{-1} to the gas chromatograph for further analysis.

Qualitative Analysis of Biogenic Volatile Organic Compounds (BVOCs). Both gas chromatography (Clarus 600 gas chromatograph, Perkin-Elmer Inc.) and mass spectrometry (Clarus 600S mass spectrometer, Perkin-Elmer Inc.) were performed to identify the compositions of volatiles obtained by TD. The temperature of the ion source was 200 °C. Electron impact mass spectra were acquired over the mass range of 50−450 amu at an ionization energy of 70 eV. The temperature of the transfer line was 230 °C. The gas chromatograph was equipped with a DB-5 ms column of 30 m × 0.25 mm × 0.25 μm (J&W Scientific, Folsom, CA, USA). The initial oven temperature was held at 40 °C for 1 min, then heated at 3 °C min^{-1} to 55 °C, again at 5 °C min^{-1} to 175 °C, finally at 30 °C min^{-1} to 250 °C, and held for 5 min. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The Kovats indices (KI) were calculated for all volatile compounds using a homologous series of *n*-alkanes (C_9−C_{16}) on the DB-5 ms column. Data analyses were conducted using TurboMass software (v. 5.4.2. 1617). Individual components were identified using the Wiley/NBS Registry of Mass Spectral Database (version 7) and NIST MS Search (version 2), published literature, and several authentic reference compounds.

Quantitative Analysis of BVOCs. GC−flame ionization detection (GC-FID) was employed for quantitative analyses using a gas chromatograph (GC 7890A, Agilent Technologies, Santa Clara, CA, USA) with a FID (Agilent Technologies) equipped with a 30 m × 0.25 mm × 0.25 μm DB-5 column (J&W Scientific). The temperatures of the injection port and the detector were 250 and 270 °C, respectively. Samples were desorbed in the split mode (split ratio 10:1). The oven temperature program was from 60 to 80 °C at 3 °C min^{-1}, to 120 °C at 8 °C min^{-1}, to 140 °C at 1 °C min^{-1}, and finally to 220 °C at 10 °C min^{-1}. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The peak areas of the target compounds were used to quantify the absolute contents compared to that of calibration samples with known concentrations.

RESULTS AND DISCUSSION

Compositions of Volatiles from Detached *B. racemosa* Flowers. To understand the compositions of floral volatiles of *B. racemosa*, the pedicel with buds ready to blossom was detached from the tree and enclosed immediately in the glass chamber. The floral volatiles were collected every 1.5 h starting

in the early evening (6:30 p.m.−3:30 a.m.) because the flowers of *B. racemosa* blossomed in the night. The volatile compounds collected were analyzed by TD-GC/MS. Figure 2 illustrates the

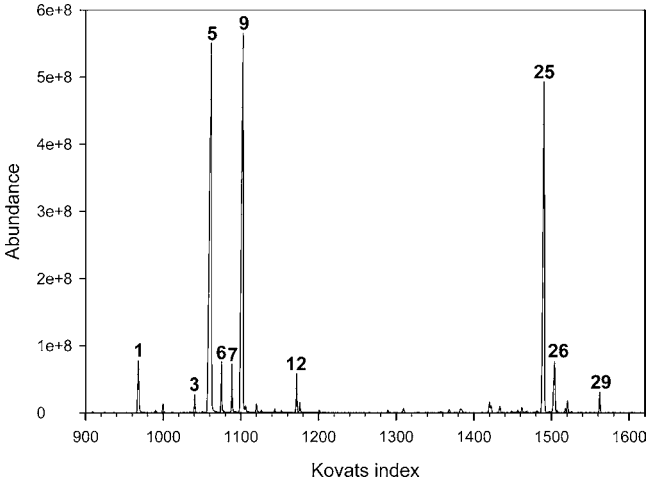

Figure 2. TD-GC/MS chromatogram of floral volatiles from *Barringtonia racemosa*: benzaldehyde (**1**); *cis*-β-ocimene (**3**); phenylacetaldehyde (**5**); *cis*-linalool oxide (furanoid) (**6**); *trans*-linalool oxide (furanoid) (**7**); linalool (**9**); *cis*-linalool oxide (pyranoid) (**12**); germacrene D (**25**); (*E*,*E*)-α-farnesene (**26**); dendrolasin (**29**).

representative chromatogram of floral volatiles analyzed in this study. As can be seen, the volatiles comprised mainly benzaldehyde (**1**), *cis*-β-ocimene (**3**), phenylacetaldehyde (**5**), *cis*-linalool oxide (furanoid) (**6**), *trans*-linalool oxide (furanoid) (**7**), linalool (**9**), *cis*-linalool oxide (pyranoid) (**12**), germacrene D (**25**), (*E*,*E*)-α-farnesene (**26**), and dendrolasin (**29**). Apart from dendrolasin, the other compounds are widely distributed in many families of seed plants.[8] Among the floral volatiles analyzed, benzaldehyde, *cis*-β-ocimene, and linalool are present in more than half of the families of seed plants.

In view of the abundant emission of floral scent between 12:30 and 1:30 a.m., the compositions of floral volatiles from detached flowers of *B. racemosa* were first analyzed. Table 1 shows the relative contents (%) of 29 volatile compounds emitted from *B. racemosa* flowers. Commonly found in many plants, these 29 volatile compounds were divided into three classes (monoterpenoids, sesquiterpenoids, and benzenoids). The contents of monoterpenoids, sesquiterpenoids, and benzenoids were 52.18, 18.40, and 27.07%, respectively. Knudsen and Mori[9] have reported 12 volatile compounds (2-methylpropyl acetate, 3-methylpropyl acetate, methyl benzoate, methyl 2-hydroxybenzoate, 2-phenylethanol, β-pinene, *trans*-β-ocimene, 1,8-cineol, linalool, lavandulyl aceate, nerol, α-copaene, caryophyllene, germacrene B, (*E*,*E*)-α-farnesene, and indole) from species in Lecythidaceae; however, few references related to volatiles emitted from the genus *Barringtonia* have been published. In the present study, the compositions of floral volatiles from *B. racemosa* were quite different from that of other species in Lecythidaceae.[9]

To investigate the emission pattern of volatile compounds from flowers, the emission rates of six compounds, benzaldehyde (**1**), *cis*-β-ocimene (**3**), phenylacetaldehyde (**5**), *cis*-linalool oxide (furanoid) (**6**), *trans*-linalool oxide (furanoid) (**7**), and linalool (**9**), were measured. Figure 3 illustrates the emission rates of two major components and the total emission rate of floral scent. The emission rate of linalool (**9**) was lower than 2000 ng h^{-1} $flower^{-1}$ before 9:30 p.m. Flowers emitted a large amount of linalool (4930 ng h^{-1} $flower^{-1}$) from 11:00 p.m. and reached the steady phase (~7000 ng h^{-1} $flower^{-1}$) after 12:30 a.m. The emission rate of phenylacetaldehyde (**5**) increased with time and reached the peak (3828 ng h^{-1} $flower^{-1}$) at 11:00 p.m. In comparison with the two major compounds, lower amounts of benzaldehyde (**1**), *cis*-β-ocimene (**3**), *cis*-linalool oxide (furanoid) (**6**), and *trans*-linalool oxide (furanoid) (**7**) were emitted (data not shown). Their highest emission rates ranged from 100 to 600 ng h^{-1} $flower^{-1}$ at 11:00 p.m.

Plants produce a variety of phytochemicals with a few specific bioactivities for self-protection. For instance, phenylacetaldehyde and linalool are commonly found in fruits, leaves, and flowers of plants.[10−12] Phenylacetaldehyde possessed allelopathic effects on cowpea (*Vigna unguiculata* (L.) Walp).[13] Several plants, such as Brazilian medicinal plants (*Mikania glomerata* Spreng.)[14] and coniferous tree (*Chamaecyparis formosensis* Matsum.),[15] were reported to contain germacrene D. Both linalool and germacrene D possessed antimicrobial activity against some bacteria and fungi.[14,16]

Compositions of Floral Scent in Situ. Studies related to emission rate of floral volatiles from living flowers are relatively scarce, and research on *B. racemosa* has not been reported so far. In this study, floral volatiles from *B. racemosa* flowers in situ were collected every 1 h from 3:30 p.m. to 9:30 a.m. on three different days. Table 1 shows the relative contents (%) of components collected from TD-GC/MS analysis in view of maximum amounts of floral volatiles emitted during 12:30−1:30 a.m. The floral volatiles collected in situ were monoterpenoids (48.74%), sesquiterpenoids (10.03%), and benzenoids (39.12%). These compositions were similar to those collected from detached flowers. The relative contents of linalool (**9**), phenylacetaldehyde (**5**), and germacrene D (**25**) were 44.73, 36.33, and 8.12%, respectively.

To examine the emission pattern of floral volatiles, seven compounds emitted from living flowers were quantified in this study. Figure 4 illustrates the individual and total emission rates of the seven components studied. Flowers emitted a small amount (<1000 ng h^{-1} $flower^{-1}$) of linalool and phenylacetaldehyde before 8:00 p.m. (Figure 4a,b). At 9:00 p.m., the emission rates of linalool and phenylacetaldehyde were 2842 and 3114 ng h^{-1} $flower^{-1}$, respectively. Thereafter, their emission rates kept increasing with time. Linalool reached the peak emission rate (20541 ng h^{-1} $flower^{-1}$) during 1:00−2:00 a.m. The emission of phenylacetaldehyde was at its maximum rate of 18234 ng h^{-1} $flower^{-1}$ at 11:00 p.m., whereas that of benzaldehyde (Figure 4c) increased slowly and peaked (9057 ng h^{-1} $flower^{-1}$) at 4:00 a.m. Panels d−g of Figure 4 show the emission rates of other components (methyl benzoate, *cis*-linalool oxide, *trans*-linalool oxide, and β-myrcene). As can be seen, they were emitted in lesser amounts, and their peak emission rates were all lower than 2000 ng h^{-1} $flower^{-1}$. The total emission rate (Figure 4h) of floral volatiles showed a trend similar to that of linalool. The total emission rates exceeded 40000 ng h^{-1} $flower^{-1}$ during 12:00−3:00 a.m. (peak rate of 44291 ng h^{-1} $flower^{-1}$ at 1:00 a.m.). Monoterpenoids (β-myrcene, linalool, and *cis*-/*trans*-linalool oxide) and benzenoids (phenylacetaldehyde, benzaldehyde, and methyl benzoate) were biosynthesized from the methylerythritol phosphate (MEP) pathway and the shikimate pathway, respectively (Figure 5). It was found that both linalool and phenylacetaldehyde were produced predominantly from 11:00 p.m. to

Table 1. Compositions of Floral Volatiles from *Barringtonia racemosa* during 12:30−1:30 a.m.

no.	KI[a]	rKI[b]	compound	relative content[c] detached	relative content[c] in situ	identification[d]
			monoterpenoids (%)	**52.18**	**48.74**	
2	992	991	*β*-myrcene	0.12		MS, KI, ST
3	1041	1037	*cis*-*β*-ocimene	0.32		MS, KI, ST
4	1051	1050	*trans*-*β*-ocimene	0.07		MS, KI, ST
6	1073	1072	*cis*-linalool oxide (furanoid)	1.21	1.13	MS, KI, ST
7	1088	1087	*trans*-linalool oxide (furanoid)	1.22	1.37	MS, KI, ST
9	1102	1097	linalool	48.13	44.73	MS, KI, ST
12	1171	1174	*cis*-linalool oxide (pyranoid)	0.88	1.22	MS, KI
13	1176	1176	*trans*-linalool oxide (pyranoid)	0.23	0.29	MS, KI
			sesquiterpenoids (%)	**18.40**	**10.03**	
18	1329	1333	bicycloelemene	0.13	0.17	MS, KI
19	1374	1377	*α*-copaene	0.13	0.21	MS, KI, ST
20	1383	1391	unknown + *β*-elemene	0.15	0.18	MS, KI
21	1417	1419	*β*-ylangene + *trans*-*β*-caryophyllene	0.58	0.06	MS, KI, ST
22	1429	1432	*β*-copaene	0.21	0.23	MS, KI
23	1454	1454	*α*-humulene	0.17	0.12	MS, KI, ST
24	1475	1479	*γ*-muurolene	0.08		MS, KI
25	1479	1485	germacrene D	13.90	8.12	MS, KI
26	1499	1505	(*E*,*E*)-*α*-farnesene	2.59	0.55	MS, KI
27	1510	1513	*γ*-cadinene	0.14	0.11	MS, KI
28	1515	1523	*δ*-cadinene	0.31	0.25	MS, KI
30	1609	1600	cedrol	0.01	0.03	MS, KI
			benzenoids (%)	**27.07**	**39.12**	
1	965	960	benzaldehyde	2.34	2.09	MS, KI, ST
5	1048	1042	phenylacetaldehyde	24.05	36.33	MS, KI, ST
8	1095	1090	methyl benzoate	0.01	0.14	MS, KI, ST
10	1112	1107	phenyl ethyl alcohol	0.47		MS, KI, ST
14	1183	1183	naphthalene	0.01	0.13	MS, KI, ST
15	1194	1181	methyl salicylate	0.06		MS, KI, ST
17	1288	1290	indole	0.13	0.43	MS, KI, ST
			others (%)	**0.91**	**0.87**	
11	1143	1152	lilac aldehyde A	0.17	0.16	MS, KI
16	1206	1201	*n*-decanal	0.02	0.26	MS, KI, ST
29	1572	1576	dendrolasin	0.72	0.45	MS, KI
			identified (%)	**98.56**	**98.76**	

[a]Kovats index determined relative to *n*-alkanes (C_9−C_{16}) on the DB-5 ms column. [b]Reference Kovats index based on Adams.[35] [c]Replications of detached and in situ were 1 and 3, respectively. [d]Identification based on comparison of the mass spectrum (MS), Kovats index (KI), and co-injection with standard compounds (ST).

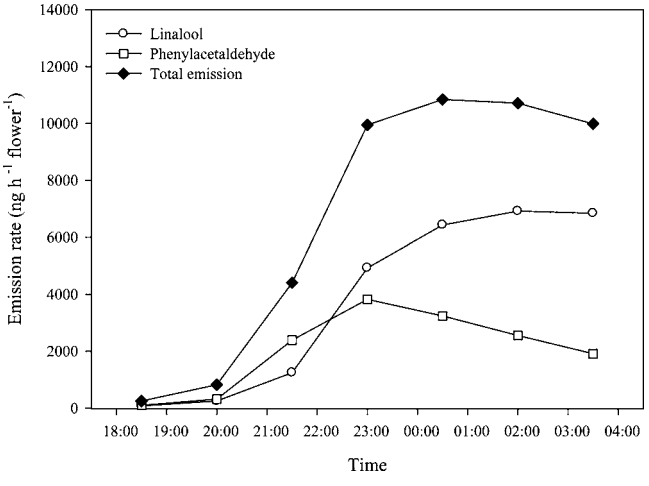

Figure 3. Emission rate of volatile compounds from detached flowers of *Barringtonia racemosa* (n = 1).

1:00 a.m. Some abiotic (temperature, humidity, light) or biotic (insects, animals) factors may have positive or negative effects on the enzymes involved in the biosynthetic pathways and eventually resulted in constituent variations of floral compounds.

Several studies related to the emission rate of floral volatiles were reported. For example, Majetic et al.[17] collected floral scent from different populations of *Hesperis matronalis* by in situ headspace extraction and indicated that floral scent comprised mainly aromatics and terpenoids. Among the five populations, the highest emission rates of aromatics, terpenoids, and total scent were around 3, 10, and 13 μg h^{-1} flower^{-1}, respectively. Kolosova et al.[18] reported that snapdragon (*Antirrhinum majus*) flowers emitted methyl benzoate at a rate of 1−5 μg h^{-1} flower^{-1}. Raguso et al.[19] reported the floral emission rates of nine species of *Nicotiana*. Among them, *Nicotiana alata* emitted total scent compounds at 81 ng h^{-1} flower^{-1}. This emission rate was 2-fold larger than that of the remaining species. Zhuang et al.[20] reported that floral volatiles

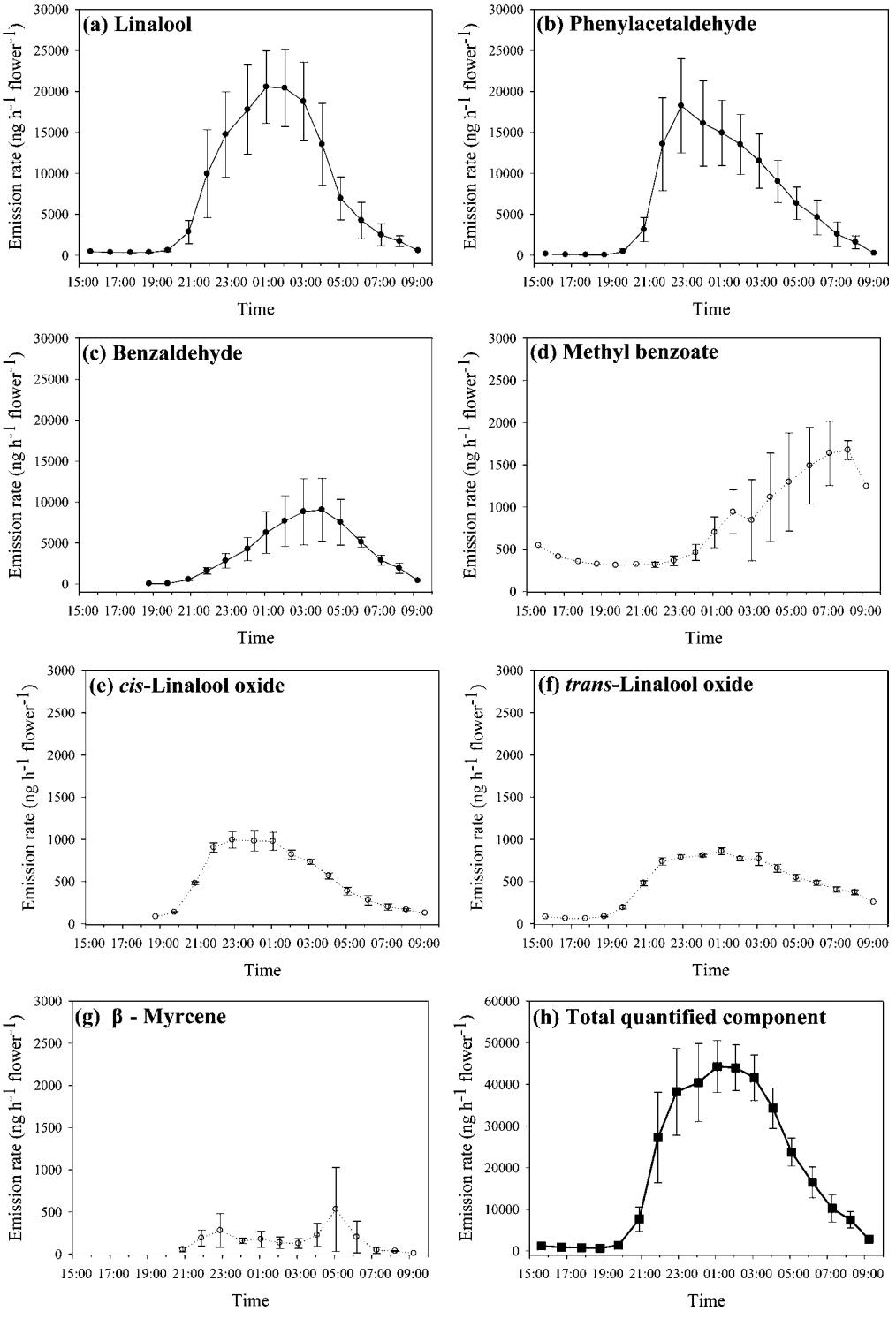

Figure 4. Emission rate of volatile compounds from living flowers of *Barringtonia racemosa* (n = 3).

of dogwood (*Cornus florida* L.) were composed of four major components including 3-formylpyridine, linalool, ketoisophorone, and decanal. Among them, the emission rate of linalool in six floral units ranged from 2 to 14 ng h^{-1}. It is surprising that *B. racemosa* flower emitted considerable quantities of floral volatiles at night compared with those reported in the literature.

There were 80−120 pedicels in one tree of *B. racemosa* during its florescence. Each pedicel had 25−35 buds, and 10−15 of them blossomed at the same time. In other words, around 800−1800 flowers of one *B. racemosa* tree blossomed in a night. According to the findings on emission rates of floral volatiles (Figure 4), around midnight, linalool and phenylacetaldehyde were emitted from a *B. racemosa* tree at considerable rates of 16−32 and 14−28 mg h^{-1}, respectively. The total emission rate of a tree was 32−72 mg h^{-1} $tree^{-1}$. This may be why the scent of *B. racemosa* flowers during florescence can always be sensed effortlessly by humans and attracts some specific insects for pollination.

During the experimental period and on an ordinary day in the field, honey bees (*Apis* spp.) were found flying around the

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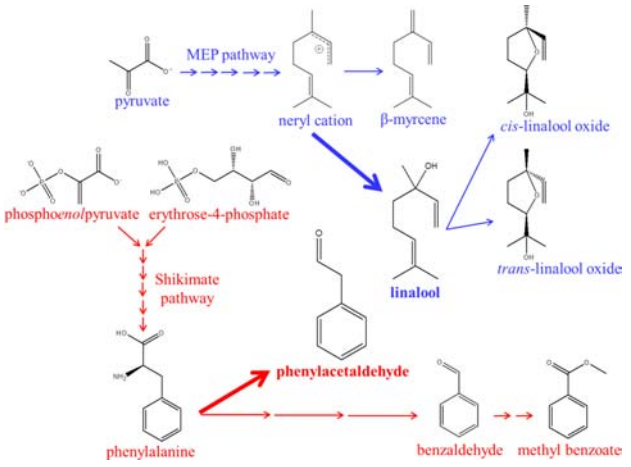

Figure 5. Biosynthetic pathways of monoterpenoids and benzenoids emitted from living flowers of *Barringtonia racemosa*.

B. racemosa flowers. Bee-pollinated plants usually emit a high portion of terpenoids.[21] It is likely that the honey bees are attracted by the terpenoids of the floral scent, such as linalool, *cis*-β-ocimene, germacrene D, and (*E*,*E*)-α-farnesene. According to the literature,[22] moths belonging to several families (Crambidae, Geometridae, Noctuidae, Sphingidae, and Zygaenidae) in Lepidoptera visited the flowers of *B. racemosa*. Moths are attracted by phenylacetaldehyde and benzaldehyde. It was reported that flowers visited by these moths had floral scent that contained a high content of benzenoids. In addition, a few bats hovered around the trees of genus *Barringtonia* in the field. Results obtained from the analysis by GC-FID with a chiral column revealed that the enantiomeric purity of linalool in the floral scent of *B. racemosa* was (*S*)-linalool. It is reasonably assumed that *B. racemosa* flowers emitted this compound to attract bats for pollination.[21]

Many studies demonstrated that plants released a variety of semiochemicals to interact with herbivores, pollinators, seed disseminators, mammals, and so forth. For example, plants released some volatiles to defend themselves against herbivores. Linalool has been proved to reduce the survival of eggs of moth (*Manduca sexta*) and decrease the oviposition rates of *M. sexta* on damaged leaves.[23] While plants were fed on by insects, they emitted certain volatiles, such as germacrene D and α-farnesene. These two compounds not only reduce the oviposition behavior of moths but also repel the larvae.[24] In addition, apple seedlings, infested with moth larvae, emitted germacrene D to attract a parasitoid.[25] Eby et al.[26] reported that inflorescences of showy milkweed (*Asclepias speciosa*) emitted large amounts of phenylacetaldehyde (4500 ng h^{-1} inflorescence^{-1}). Large amounts of the components emitted attracted both male and female clearwing moths (*Synanthedon myopaeformis*), which are major pests in apple orchards. According to the aforementioned results, large amounts of components (linalool, phenylacetaldehyde, and germacrene D) emitted from living flowers of *B. racemosa* may play an important role in their reproduction.

Odor Activity of Volatiles from *B. racemosa* Flowers. In this study, the floral volatiles emitted from *B. racemosa* comprised at least 29 different compounds. Some of them, such as linalool, phenylacetaldehyde, benzaldehyde, *cis*-/*trans*-linalool oxide, and methyl benzoate, had fragrant odor.[5,27,28] However, which compound plays the most important role in floral scent was still unclear. Many studies employed the OAV to elucidate the contribution of volatile compounds from food or plant to the aroma.[29] The OAV of a compound is calculated by dividing its concentration in a sample by its odor threshold value. Because this study used fresh flowers in situ as samples, emission rates of floral scent changed when measurements were made. Therefore, the unit of OAV (min^{-1}) of the floral volatiles used in this study was slightly different from that in other studies.

Figure 6 shows the OAV profiling of six volatile compounds, namely, phenylacetaldehyde, linalool, benzaldehyde, *cis*-linalool oxide, β-myrcene, and methyl benzoate, released from *B. racemosa* flowers during the sampling period (from 3:30 p.m. to 9:30 a.m.). In the beginning of the experiment (3:30−8:00 p.m.), the OAVs of both phenylacetaldehyde and linalool were <10 min^{-1}. Phenylacetaldehyde increased rapidly to the maximum OAV (547 min^{-1}) during 10:00−11:00 p.m. and decreased steadily to the original level (2 min^{-1}). Linalool exhibited a tendency similar to that of phenylacetaldehyde, for which the OAV increased at 9:00 p.m., reached the maximum OAV (409 min^{-1}) at 1:00 a.m., and lasted until 2:00 a.m. (407 min^{-1}). The OAVs of both phenylacetaldehyde and linalool exceeded 10 min^{-1} either in initial blossom or in full blossom, indicating that these two compounds with extremely high OAVs could be taken as active odorants.[29] Linalool has a typical pleasant floral and citrus-like odor.[30] Phenylacetaldehyde was proved to contribute to the aroma of Spanish honeydew honeys with high OAV (572−1343).[31] It has an unpleasant, pungent, and bitter flavor, but at low concentration, it has a sweet and fruit-like aroma.[30] The other four compounds, benzaldehyde, *cis*-linalool oxide, β-myrcene, and methyl benzoate, exhibited high OAVs (>1 min^{-1}) in some periods during the experiment. Nevertheless, they contributed mildly to the floral scent because their OAVs were 100-fold weaker than those of phenylacetaldehyde and linalool. In addition, germacrene D had a characteristic odor with warm-spicy-wood notes,[32] but its OAV was not measured because its odor threshold value was not available. Germacrene D contributed slightly to the floral scent because the smell contained a mild woody odor. The

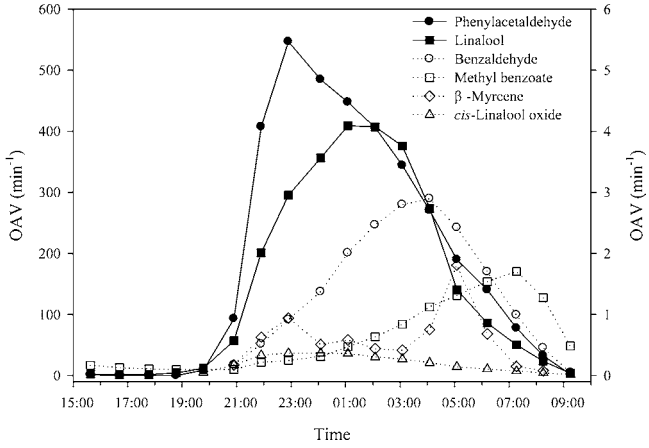

Figure 6. Profiling of odor activity value (min^{-1}) of six floral volatiles from *Barringtonia racemosa*. (Values of solid symbols are read in the left axis, whereas those of open symbols are read in the right axis.) Emission rate (ng min^{-1} g^{-1} (dry weight flower)) of floral volatile was divided by its odor threshold value (ppb): phenylacetaldehyde (4 ppb);[30] *cis*-linalool oxide (320 ppb);[30] *trans*-linalool oxide (320 ppb);[30] methyl benzoate (110 ppb);[30] linalool (6 ppb);[30] benzaldehyde (350 ppb);[36] β-myrcene (36 ppb).[11]

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floral scent of *B. racemosa* can be described as green, sweet, fruit-like, and floral with a pleasant odor. An artificial mixture of phenylacetaldehyde spiked with linalool using the ratio from Table 1 was quite similar to the actual floral scent. In addition, it was observed that the floral scent can always be smelled effortlessly from 10:00 p.m. to 2:00 a.m. in the field. This characteristic correlated well with the OAV profiling. Taken together, these results suggest that linalool and phenylacetaldehyde predominantly contribute to the floral scent of *B. racemosa*.

The OAV profiling in this study not only provides useful information for future research (ecology, genetic, or metabolic engineering) but also increases the economic or practical value of *B. racemosa*. In addition, linalool was proved to have a positive effect on autonomic nerve activity in mammals. Rats exposed to linalool can also increase gastric vagal nerve activity and decrease adrenal sympathetic nerve activity.[33] Recent studies showed that inhalation of linalool not only had a sedative effect but also released stress and extended sleeping duration.[34] According to the results obtained in this study, taking a stroll in the vicinity of *B. racemosa* in the night could be regarded as a free and natural aromatherapy.

In conclusion, results from compositional analyses of 29 compounds emitted from *B. racemosa* living flowers collected by in situ headspace extraction showed that linalool, phenylacetaldehyde, and germacrene D were the predominant floral volatiles. Dynamic emission rates of seven volatile components were investigated. Among them, linalool and phenylacetaldehyde showed similar trends of a remarkably considerable emission rate (>20000 ng h^{-1} $flower^{-1}$) during midnight (10:00 p.m.−3:00 a.m.). The emission rates of remaining components during florescence were all <10000 ng h^{-1} $flower^{-1}$. The total emission rate of floral volatiles from fresh flowers reached the highest level (44291 ng h^{-1} $flower^{-1}$) around 1:00 a.m. In addition, results of dynamic OAV profiling showed that both linalool and phenylacetaldehyde possessed extremely high OAVs (>100 min^{-1}) during 9:00 p.m.−5:00 a.m., whereas those of the other components were <5 min^{-1}. According to the results of emission rate and OAV profiling, linalool and phenylacetaldehyde contribute principally to the floral scent of *B. racemosa*.

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Notes

The authors declare no competing financial interest.

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