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Lisianthus Flowers Emitted Volatile Components Including Iridoids and Actinidine Which Attract Cats

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Lisianthus (*Eustoma grandiflorum* [Raf.] Shinners) is one of the world's major cut flowers, characterized by its wide variety of flower colors, flower shapes, long stem, and long vase life. Lisianthus is said to be scentless, but there are cultivars that have a weak or faint scent. Cats exhibit a characteristic response to lisianthus flowers similar to their response to *Actinidia polygama* leaves, which have a very weak scent for humans. These observations suggested that the scent of lisianthus flowers may have a component that attracts cats. The volatile components of *Eustoma* 'New Lination White' flowers, which has a weakly sweet scent, and 12 lisianthus cultivars, which have a very faint scent, were analyzed. Thirty-six kinds of volatile components were detected in the flowers of 'New Lination White', including four iridoid compounds (nepetalactone, isodihydronepetalactone, iridomyrmecin, and isoiridomyrmecin) and actinidine, which have been recognized as attracting cats. The major volatile components are sesquiterpenes, and phenypropanoids such as eugenol were identified as components with a sweet scent. Iridoid compounds and actinidine were detected only in flowers, but not in leaves or stems. In addition, iridoid compounds were detected in all 12 cultivars analyzed. Lisianthus flowers were thought to be scentless but we identified many volatile components, including iridoid compounds and actinidine, that attracts cats. This research is the first report on the scent of lisianthus flowers.

Key Words: Eugenol, *Eustoma grandiflorum*, floral scent, matatabilactone.

Introduction

The genus *Eustoma* of the *Gentianaceae* family has only two species, *E. grandiflorum* (Raf.) Shinners with lavender-purple flowers that inhabits the eastern part of the Rocky Mountains of the United States and *E. exaltatum* with purple or white flowers that inhabits the southern United States, Mexico and Central America (Shinners, 1957; Turner, 2014). Lisianthus is characterized by various flower colors, long stems, and a long vase life, and has been bred mainly from *E. grandiflorum*. Since *E. grandiflorum* and *E. exaltatum* can easily be crossed, *E. exaltatum* was also used to breed small-flowered cultivars (Yashiro, 2004). Cultivation of lisianthus for cut flowers began in the 1970s and remained minor in the 1980s (Fujiwara and Kodama, 2008; Halevy and Kofranek, 1984; Roh and Lawson, 1984), but Japanese seed companies vigorously nurtured cultivars and seeds were supplied all over the world. By the end of the 20th century, it was ranked among the top 10 cut flowers in the world (Harbaugh et al., 2000).

Lisianthus has a variety of petal colors, including purple, white, pink, yellow, and greenish brown, but is thought to be completely scentless (Zaccai et al., 2001). Therefore, attempts have been made to enhance the scent of lisianthus by genetic recombination (Aranovich et al., 2007; Fang et al., 2021). However, we recognize that lisianthus flowers are not scentless, and most cultivars bred in Japan have a very faint, dry, grass-like odor, while some cultivars have a weak sweet scent.

Interestingly, the floral scents of lisianthus, which humans perceive as very weak, are attractive to cats. Domestic cats put their noses to the lisianthus flowers, sniff them, and gnaw and lick them (Fig. 1). These responses to lisianthus flowers resemble their characteristic responses to *Actinidia polygama* (silvervine, matatabi) and *Nepeta cataria* (catnip) (Bol et al., 2017; Todd, 1962; Tucker and Tucker, 1988) suggesting that there is a component that attracts cats in the floral scent of lisianthus.

In this study, we analyzed the volatile components

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emitted from flowers of 'New Lination White' with a weak sweet scent that attract cats, in order to clarify the components that humans perceive as scent and the components that attract cats. The weak sweet scent of 'New Lination White' could not be detected after harvest as a cut flower. Therefore, we compared the volatile components of potted flowers and cut flowers in a greenhouse or growth chamber. Furthermore, to investigate the presence of iridoids and actinidin in other lisianthus cultivars, we analyzed the volatile components emitted from flowers of 12 lisianthus cultivars with a very faint scent. This is the first report on the scent and volatile components emitted from lisianthus flowers that attract cats.

Materials and Methods

Plant materials

Seeds of *Eustoma* 'New Lination White' (Sakata Seed Corporation, Fig. 2A), a weakly scented flower, were sown in plastic germination trays with 288 cells on August 17, 2021. The seeded trays were maintained in a dark, cool-room at 10°C for 35 days (Tanigawa et al., 2002), before being transferred to an incubator (Nippon Medical & Chemical Instruments Co., Ltd., Tokyo, Japan) at 27°C, and a 20 h photoperiod (02:00–22:00) provided by a cool fluorescent lamp with 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) for 28 days (Fukushima et al., 2018, 2019). Seedlings were planted on plastic pots (10.5 cm diameter) and grown in an environmental chamber (Nippon Medical & Chemical Instruments Co., Ltd.) under a constant temperature of 25°C, a relative humidity (RH) of 70%, and a 12 h photoperiod (06:00–18:00) provided by metal halide lamp with 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD for 56 days. Then, seedlings were grown under natural conditions (day length, light intensity and temperature) in a greenhouse ranged between 15°C and 25°C. The maximum temperature was 26.2°C, the minimum temperature was 13.4°C, and the average temperature was 17.7°C. In January 2022, some of the flowering 'New Lination White' pots were transferred to a growth chamber (Nippon Medical & Chemical Instruments Co., Ltd.) under a constant temperature of 23°C, 50% RH, and a 12 h photoperiod (06:00–18:00) provided by a cool fluorescent lamp with 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. Twelve lisianthus cultivars with very faint scent flowers were grown under the same conditions as 'New Lination White'. Those cultivars were as follows; 'Candy Marine' (Miyoshi & Co., Ltd. Fig. 2B), 'Celebrity Pink' (Sumika Agrotech Co., Ltd., Fig. 2C), 'Celebrity Rich White' (Sumika Agrotech Co. Ltd., Fig. 2D), 'Dean' (Takii & Co., Ltd., Fig. 2E), 'Maquia Pink' (Sakata Seed Corporation, Fig. 2F), 'Orb Snow' (Sakata Seed Corporation, Fig. 2G), 'Prima Lavender' (Takii & Co., Ltd., Fig. 2H), 'Soiree Pink' (Miyoshi & Co., Ltd., Fig. 2I), 'Piccolosa Snow' (Sakata Seed Corporation, Fig. 2J), 'Reina White' (Sakata Seed Corporation, Fig. 2K), 'Rosina Type III Snow' (Sakata Seed Corporation, Fig. 2L) and 'Voyage Type II Blue' (Sakata Seed Corporation, Fig. 2M).

Sampling emitted floral volatiles

Sampling of emitted floral volatiles of 'New Lination White' was performed on intact flowers (potted flowers) in a greenhouse and on intact flowers (potted flowers) and harvested flowers (cut flowers) in a growth chamber from early-to-late January 2022. Conditions in the greenhouse and growth chamber were as indicated above. Cut flowers harvested at a length of 50 cm in the flowering stage on Day 0 of Figure 3, and the leaves that would likely be submerged in water were removed. They were put in tap water without antibacterial agents or sugars immediately after harvesting. Sampling of volatile components from 'New Lination White' potted flowers in the greenhouse in Table 1 was conducted in early January, and other samplings were conducted from mid to late January. An absorbent (Twister, a magnetic stir bar coated with a partitioning phase of 100% polydimethylsiloxane; Gerstel Inc., Mülheim, Germany) was used to collect volatile components. A Twister was clipped to the inside of a petal such that it did not touch the anthers or the petals. Then, the whole flower was covered with a transparent wrapper (Saran Wrap; Asahi KASEI, Tokyo, Japan) and the volatile components were collected over two hours between 9:00 and 12:00 on the flowering day (Day 0), Day 1, Day 3, and Day 5 after flowering (Fig. 3) in a greenhouse or a growth chamber (Oyama-Okubo and Tsuji, 2013). As a preliminary test, sensory evaluations were conducted at 9:00, 12:00, 15:00, and 18:00, and the smell was most noticeable at 9:00 and 12:00, so sampling was conducted from 9:00 to 12:00 in the main test. For the analysis of the volatile components of each floral organ, five flowers of 'New Lination White' on the day after flowering in a growth chamber were separated into petals, pistils, anthers, and filaments. The petals were placed in a 300 mL beaker and the other organs in a 30 mL beaker, covered with aluminum foil fitted with a Twister, and extracted for 2 h. As a control, leaf volatile components were sampled in the same manner as petals. The analysis of the results shown in Tables 1 and 2 was carried out on the flowers of 'New Lination White' on Day 1 after flowering, when the scent was perceived to be strongest. For the 12 lisianthus cultivars, the flowers that bloomed in the greenhouse were harvested from mid to late January, and the emitted volatile components of the cut flowers on Day 5 after flowering were collected using a twister in the same manner as above because the most volatile components were emitted from 'New Lination White' flowers on Day 5 after flowering, as shown in Figure 5. Collected samples were stored in a tightly closed container until analysis.

Analysis of emitted floral volatiles

Samples were directly introduced into a GC (capillary gas chromatography)-MS (mass spectrometry) using a thermal desorption unit (TDU, Gerstel Inc., Linthicum, MD, USA) and a cooling injection system (CIS4, Gerstel Inc., Linthicum, MD, USA). GC-MS was performed using an Agilent 7890B GC system coupled to an Agilent 5977A mass selective detector (Agilent Technologies, Santa Clara, CA, USA). The TDU conditions were heating from 30°C to 250°C at 60°C·min^{-1}, holding for 10 min at 250°C, and cryo-focusing at −50°C in the CIS4. Following tube desorption, the CIS4 was heated to 300°C at a rate of 12°C·s^{-1} in splitless mode to transfer the analytes to the GC column (DB-WAX capillary column, 30 m length, 0.25 mm i.d., and 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA). The temperature program of the column oven was set to 40°C for 2 min, 4°C·min^{-1} up to 180°C, held at 180°C for 5 min, 15°C·min^{-1} up to 250°C, and held at 250°C for 10 min. The injection, interface, and ion source temperatures were 250°C, 280°C, and 250°C, respectively. Helium was used as the carrier gas. The mass scan range was *m*/*z* 30–300 and the electron potential set to electron ionization was 70 eV. Compounds were identified with the Wiley 11th/NIST 2017 library search algorithm provided with the GC-MS software and/or comparison of mass spectra with standard compounds and retention

Fig. 1. A cat showing a characteristic reaction to a lisianthus flower.

Fig. 2. Plant materials. (A) 'New Lination White'. (B) 'Candy Marine'. (C) 'Celebrity Pink'. (D) 'Celebrity Rich White'. (E) 'Dean'. (F) 'Maquia Pink'. (G) 'Orb Snow'. (H) 'Prima Lavender'. (I) 'Soiree Pink'. (J) 'Piccolosa Snow'. (K) 'Reina White'. (L) 'Rosina Type III Snow'. (M) 'Voyage Type II Blue'. Scale bar = 1 cm.

Fig. 3. 'New Lination White' flowers sampled for volatile components.

Table 1. Volatile components of one 'New Lination White' flower (potted flower) on the first day after flowering in a greenhouse.

Compound	Retention index	Odor description[z]	Threshold values[z]	Composition ratio (%)
Monoterpenes				
β-Myrcene	1153	Spicy-herbal	n/a[y]	2.3±0.6[x]
1,8-Cineole	1197	Eucalyptus-herbal	1 to 64 ppb	3.1±0.8
β-Ocimene	1234	Floral-green	n/a	0.2±0.1
(E)-4,8-Dimethyl-1,3,7-nonatriene	1292	n/a	n/a	trace[w]
Iridoids				
Nepetalactone	1942	Earthy-spicy	n/a	0.6±0.1
Isodihydronepetalactone	2081	n/a	n/a	3.2±0.5
Iridomyrmecin	2128	n/a	n/a	0.9±0.1
Isoiridomyrmecin	2135	n/a	n/a	0.6±0.1
Sesquiterpenes				
α-Cubebene	1447	Herbal-waxy	n/a	0.2±0.1
β-Elemene	1572	Herbal-waxy-fresh	n/a	7.0±1.4
Caryophyllene	1579	Woody-spicy	64 to 90 ppb	2.1±1.1
(-)-Selina-5,11-diene	1601	n/a	n/a	0.7±0.2
Humulene	1648	Woody	n/a	trace
4,11-Selinadiene	1655	n/a	n/a	trace
Aristolochene	1659	n/a	n/a	0.5±0.2
α-Guaiene	1678	Sweet-woody	n/a	0.4±0.2
Eremophilene	1688	n/a	n/a	0.3±0.2
β-Selinene	1697	Dry-grassy	n/a	9.0±0.5
α-Selinene	1704	Herbal-grassy	n/a	28.0±3.3
Eudesma-2,4,11-triene	1714	n/a	n/a	2.4±0.8
δ-Cadinene	1734	Thyme-herbal	n/a	trace
7-*epi*-α-Selinene	1739	n/a	n/a	trace
Dihydro-β-agarofuran	1757	n/a	n/a	3.6±0.1
Unknowm (MW202)	1782	n/a	n/a	trace
Hinesol	2165	Spicy-woody	n/a	0.9±0.2
Agarospirol	2182	Spicy-woody	n/a	0.9±0.4
2(1H)Naphthalenone, 3,5,6,7,8,8α-hexahydro-4,8α-dimethyl-6-(1-methylethenyl)-(5β,7β,10β)-3,11-Eudesmadien-2-one	2239	n/a	n/a	4.4±0.9
	2435	n/a	n/a	7.7±0.9
Eudesma-3,11-dien-2-one	2493	n/a	n/a	9.7±1.5
Benzenoids/phenylpropanoids				
Veratrole	1688	Creamy-vanilla	n/a	0.1±0.1
Guaiacol	1815	Smoky-vanilla	3 to 31 ppb	0.7±0.5
Methyleugenol	1977	Sweet-spicy	68 ppb to 8.5 ppm	2.4±0.6
Eugenol	2120	Sweet-spicy	6 to 100 ppb	5.0±0.9
Others				
Actinidine	1847	n/a	n/a	0.5±0.1
Methyl dihydrojasmonate	2256	Sweet-floral	n/a	trace
Indole	2381	Floral (high dilution)	140 ppb	2.2±0.7
Peak area per flower				
Iridoids and actinidine				14,867,906
Total				254,587,433

[z] Excerpt from the Flavor Ingredient and/or The Good Scents Company Information System.
[y] Not applicable.
[x] ±standard error of mean (n=3).
[w] <0.1%.

index (Oyama-Okubo and Mikanagi, 2023). A standard compound of nepetalactone (*cis-trans* nepetalactone, Toronto Research Chemicals Inc., Toronto, Canada) was analyzed using the same method as above. Actinidin, isodihydronepetalactone, iridomyrmecin, and isoiridomyrmecin were tentatively identified with reference to the literature (Adachi et al., 2016; Bol et al., 2017; Uenoyama et al., 2021). Retention indices were

Table 2. Composition ratios of volatile components in floral organs of 'New Lination White' (potted flower) on the first day after flowering in a growth chamber (%).

	Petal	Pistil	Stamen	
			Filament	Anther
Monoterpenes				
β-Myrcene	7.2±0.4[z]	0.1±0.1	n.d.	n.d.
1,8-Cineole	7.1±0.4	3.1±0.1	15.5±0.6	n.d.
Others	3.3±0.1	n.d.	n.d.	n.d.
Iridoids				
Nepetalactone	0.1±0.0	n.d.	0.2±0.1	n.d.
Isodihydronepetalactone	0.4±0.0	0.2±0.0	2.7±0.3	2.0±0.2
Iridomyrmecin	0.2±0.0	0.2±0.0	2.1±0.2	1.0±0.1
Isoiridomyrmecin	0.2±0.1	0.2±0.0	2.1±0.3	0.6±0.2
Sesquiterpenes				
β-Elemene	11.6±4.9	2.5±0.1	1.8±0.4	7.1±0.7
Caryophyllene	0.3±0.1	48.6±0.7	0.7±0.0	4.1±0.3
β-Selinene	18.2±1.4	5.0±0.1	3.9±0.6	11.2±0.5
α-Selinene	27.2±2.0	26.9±0.3	14.2±1.5	27.2±1.9
Eremophilene	2.5±0.0	1.1±0.0	0.7±0.7	1.3±1.3
Dihydro-β-agarofuran	7.1±0.1	1.9±1.9	6.6±0.0	9.0±1.9
Others	8.6±0.4	5.2±0.5	0.3±0.3	0.5±0.5
Benzenoids/phenylpropanoids				
Methyleugenol	0.5±0.1	trace[x]	2.4±0.7	4.2±0.3
Eugenol	0.1±0.0	0.3±0.0	3.3±0.3	28.9±3.7
Others	n.d.[y]	0.2±0.1	1.7±0.1	1.8±0.4
Fatty acid derivatives				
cis-3-Hexenol	4.6±0.6	4.4±0.1	36.1±1.6	n.d.
Others	0.2±0.0	n.d.	5.2±0.3	n.d.
Others	0.6±0.1	trace	0.6±0.2	1.2±0.5
Peak area per flower				
Iridoids	1,638,971	877,538	2,291,693	504,281
Caryophyllene	489,807	82,016,041	211,892	577,121
Eugenol	188,387	540,023	1,088,799	4,048,255
Total	188,387,422	168,757,285	32,598,762	14,007,803

[z] ±standard error of mean (n=3).
[y] Not detected.
[x] <0.1%.

determined relative to a homogeneous series of n-alkanes (C10–C25) under the same operating conditions. The sensory features of major volatile compounds of each flower were derived by referring to Burdock (2010) and The Good Scents Company Information System (2021).

Sensory assessment of living flowers

The sensual features of three 'New Lination White' flowers (potted flowers) were evaluated by the authors in the greenhouse on Day 0, Day 1, Day 3 and Day 5 (Fig. 3). The sensual features of three flowers of 12 lisianthus cultivars were recorded by the authors before and after sampling emitted volatiles in a growth chamber.

Results and Discussion

Volatile components of Eustoma 'New Lination White'

GC-MS analyses of *Eustoma* 'New Lination White' one day after flowering revealed that one of the major volatile components was α-selinene, which has a grassy-like scent. Sesquiterpenes accounted for approximately 80% of the detected components (Table 1). Iridoid nepetalactone was also identified by matching the mass fragmentation pattern and retention index with the standard compound. In addition, isodihydronepetalactone (Bol et al., 2017; Uenoyama et al., 2021), iridomyrmecin (Adachi et al., 2016; Bol et al., 2017; Uenoyama et al., 2021), isoiridomyrmecin (Adachi et al., 2016; Bol et al., 2017; Uenoyama et al., 2021) and actinidin (Bol et al., 2017; Sakan et al., 1959), an alkaloid with a pyridine ring, were detected (Fig. 4).

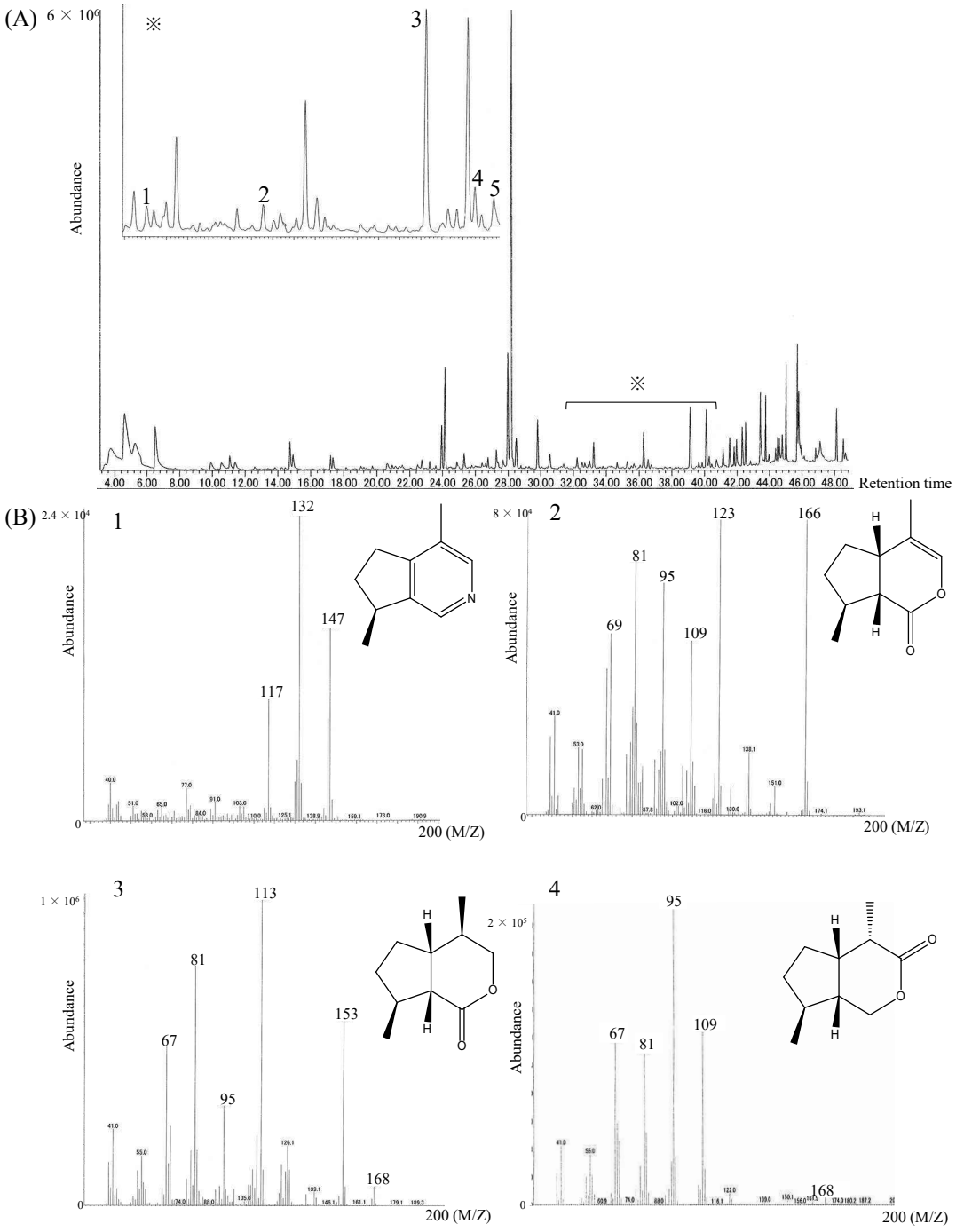

Fig. 4. Mass chromatogram (A) and mass spectrum (B) of volatile compounds of a 'New Lination White' flower. Iridoids and actinidin; 1, actinidin; 2, nepetalactone; 3, isodihydronepetalactone; 4, iridomyrmecin; 5, isoiridomyrmecin.

These iridoid compounds and actinidin are found in catnip and silvervine and are known to be compounds that trigger characteristic reactions in cats (Meinwald, 1954; Sakan et al., 1959; Uenoyama et al., 2021). About 90% of the volatile components of 'New Lination White' are terpenoids. Phenylpropanoids with a sweet and spicy odor, eugenol and methyl eugenol were also detected. The scent of 'New Lination White' perceived by humans is thought to be due to eugenol and methyl eugenol. On the other hand, cats are attracted to 'New Lination White' probably because it contains iridoid compounds and actinidin.

In addition, the analysis results of 'New Lination White' that bloomed in September in the preliminary test did not differ from the results shown in Table 1 using flowers that bloomed in January in terms of both the quantity and quality of volatile components (data not shown). Future tasks include time-course analysis

of the volatile components of lisianthus and research on the relationship between the cropping type and the amount of volatile components.

Comparison of volatile components of potted and cut flowers of Eustoma 'New Lination White' in a greenhouse or growth chamber

Volatile components were collected from potted plants flowers of 'New Lination White' in a greenhouse (outdoor), as well as cut flowers harvested from potted plants (intact plants) grown in a greenhouse first and then in a growth chamber (indoor). Volatile components were collected on flowering day (day 0), day 1, 3 and 5 after flowering (Fig. 3). The flowers of 'New Lination White' grown in a greenhouse had a faintly sweet scent on flowering day, a sweet scent on the day 1, a weak sweet scent on day 3, and a faintly sweet scent on day 5 (Table S). On the other hand, the floral scent of 'New Lination White' grown in a growth chamber was a weak sweet scent on day 1 for both potted flowers and cut flowers, but as the days passed, it became woody. The scent emitted by 'New Lination White' grown in a greenhouse and in a growth chamber differed greatly both in quantity and quality (Fig. 5A, B, C; Table S). The ratio of phenylpropanoids responsible for the sweet odor of 'New Lination White' was higher in greenhouse samples than in growth chamber samples at all stages. On the other hand, the amount of terpenoids, including iridoid compounds, was higher in potted flowers and cut flowers in a growth chamber than potted flowers in a greenhouse (Fig. 5A, B, C).

The production and emission of plant volatile compounds is greatly influenced by environmental factors such as light and temperature (Zhao et al., 2018). The plastid-localized 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway is primarily responsible for the production of monoterpenes and diterpenes, whereas the cytoplasmic mevalonate (MVA) pathway is primarily involved in the production of sesquiterpenes and triterpenes (Durbey, 2003). Light induces most genes in the MEP pathway and downregulates genes in the MVA pathway (Rodriguez-Concepción, 2006). The amount of sesquiterpenes was higher in lisianthus flowers in a low-light growth chamber than in a high-light greenhouse (Fig. 5A, B). Sesquiterpene biosynthesis in lisianthus may be inhibited by increasing light intensity in the greenhouse. On the other hand, more monoterpenes and iridoids, synthesized via the MEP pathway, were released from lisianthus flowers in the growth chamber than in the greenhouse. It has been suggested that monoterpenes such as geraniol found in tea scent are produced by the MVA pathway (Xu et al., 2018). It is possible that monoterpene and iridoid scent components in lisianthus are also produced by the MVA pathway, which is downregulated by light.

Greater amounts of phenylpropanoids responsible for the sweet scent of 'New Lination White' were emitted in the growth chamber on day 1, but more were emitted in the greenhouse as the days progressed (Fig. 5A, B). The expression of phenylpropanoid biosynthetic genes is induced by ultraviolet light contained in natural light (Wang et al., 2012). It is thought that the lisianthus grown in the greenhouse with natural light emitted more phenylpropanoid volatile components than the ones grown in the growth chamber with artificial light. Although the total amount of volatile components emitted by potted lisianthus flowers grown in the greenhouse (Fig. 5A; Table S) was not different from the results shown in Table 1, the amount of eugenol was significantly different between the two. The flowers in Table 1 bloomed in early January, and the flowers in Figure 5A bloomed in mid-January; the weather at the time of sampling was cloudy in the former and sunny in the latter. It is possible that the light intensity influenced the amount of eugenol emitted by lisianthus.

The volatile components emitted from potted flowers and cut flowers were similar in quality and quantity under the same environment in a growth camber (Fig. 5B, C). It has been reported that carnation cut flowers undergo a sharp decrease in the amount of

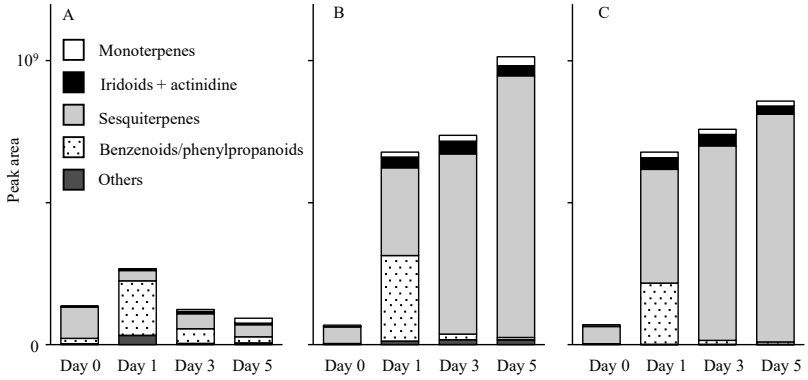

Fig. 5. Daily change in volatile components of potted and cut 'New Lination White' flowers placed in a greenhouse or a growth chamber (n = 3). Volatile composition, at different days after flowering, of potted flowers placed in a greenhouse (A), potted flowers placed in a growth chamber (B), cut flowers placed in a growth chamber (C).

volatile components emitted compared to intact plants (Kishimoto and Shibuya, 2021). In lisianthus flowers, stem damage does not seem to affect the emission of volatile components containing iridoid compounds.

Localization of volatile components in floral organs of Eustoma 'New Lination White'

α-Selinene, a major volatile component, was detected in all floral organs (petals, pistils, anthers, and filaments) of 'New Lination White' flowers, whereas the ratios of caryophyllene in pistils and eugenol in anthers were high (Table 2). Based on the total peak area value, the amount of caryophyllene in pistils was 143 to 359 times that of other organs, and the amount of eugenol in the anthers was 4 to 21 times that of other organs. It has been reported that the major volatile component of pistils and stamens (not separated) of *Dianthus superbus* was β-caryophyllene (Kishimoto et al., 2011), and the major volatile components of rose stamens (filaments and anthers not separated) were eugenol and its derivatives (Dubois et al., 2010). Similarly, in lisianthus, it is thought that the volatile components biosynthesized differ depending on the floral organ. A fatty acid derivative, *cis*-3-hexenol, was detected in petals, pistils, and filaments, but was not detected in intact flowers. *cis*-3-Hexenol, which is biosynthesized from linolenic acid when leaves are injured (Hatanaka et al., 1978), is thought to be produced in response to tissue damage when flowers are cut. Iridoid compounds were detected in all floral organs, and were particularly abundant in filaments and petals (Table 2). Actinidin was not detected in any floral organ, however, not all floral volatile components, including iridoid compounds, were detected in leaves and stems (data not shown).

These results are consistent with the behavior of cats that ignore the leaves and stems of lisianthus and lick and nibble only the flowers. Silvervine and catnip contain iridoid compounds in their plant bodies including the leaves and stems, but lisianthus is considered to produce iridoid compounds only in flowers. We plan to quantify the amount of iridoids and actinidin contained in flowers in the future.

Volatile components of 12 lisianthus cultivars

Flowers of the 12 lisianthus cultivars that were generally considered to be scentless had a very weak grassy or woody scent. As a result of analyzing the volatile components of cut flowers on the fifth day after flowering, the 12 cultivars were divided into three types according to their major volatile components: β-Myrcene, 'Candy Marine', 'Celebrity Rich White', 'Dean', 'Maquia Pink' and 'Prima Lavender'; sesquiterpenes such as α-selinene, 'Celebrity Pink', 'Orb Snow', 'Soiree Pink', 'Piccolosa Snow', 'Reina White' and 'Rosina Type III Snow'; caryophyllene and veratrole, 'Voyage Type II Blue' (Table 3). The main volatile component of 'Soiree Pink' was β-myrcene, but because the total proportion of sesquiterpenes was higher, it was classified as a sesquiterpene type. There was no correlation between the morphology of lisianthus flowers (petal number, petal weight, flower color) and the quality and quantity of volatile components (data not shown). 'Rosina Type III Snow' and 'Soiree Pink' had a higher amount of volatile components than other cultivars, but like 'Prima Lavender', which had the lowest amount of volatiles, they had a very weak scent. In addition, iridoid compounds were detected from all 12 cultivars. Iridoid compounds therefore be universally present in lisianthus flowers. Among the 12 cultivars, 'Rosina Type III Snow' flowers emitted the most iridoids and actinidine and emitted more of those compounds than 'New Lination White', which attracts cats. On the other hand, cats responded to 'Voyage Type II Blue', which has less than half the amount of iridoids as 'New Lination White' (data not shown). It is thought that cats will react if at least the amount of iridoid contained in 'Voyage Type II Blue' is present. In order to clarify the amounts of actinidin and iridoids in lisianthus flowers that attract cats and the threshold for cats, it is necessary to quantify these compounds in each cultivar and conduct experiments using cats.

The volatile component emitted by living lisianthus flowers ('Royal Pink') is reported to be benzyl alcohol (Aranovich et al., 2007). In addition, benzaldehyde, benzyl alcohol, phenylacetaldehyde and 2-phenylethanol have been reported as volatile components of lisianthus ('Excalibur Pink') flower extract (Fang et al., 2020). The reason for the difference between the results of this report and previous reports is thought to be due not only to the difference in the cultivar, but also to the difference in the method of collecting the volatile components. Aranovich et al. used the dynamic headspace method to sample emitted volatile components while flowing air at 1 $L \cdot min^{-1}$ for 12 h. We also conducted a preliminary experiment using the dynamic headspace method to sample emitted volatile components while flowing air at 300 $mL \cdot min^{-1}$ for 2 h, but were unable to collect volatile components as effectively as with the static headspace method we used this report. The static headspace method is not affected by background impurities caused by continuous airstream, making it suitable for collecting volatile components emitted from very weakly scented flowers such as lisianthus (Tholl and Rose, 2006). On the other hand, quantitative analysis is difficult with this method, so it is necessary to consider a suitable sampling method to quantify the volatile components emitted from living lisianthus flowers. We are currently investigating methods for quantifying volatile components from lisianthus flowers. Fang et al. used a solvent to extract scent components from frozen powdered flowers and samples using solid phase micro extraction (SPME) fibers. Because they did not analyze the components emitted from living flowers, their

Table 3. Composition ratios of volatile components of lisianthus cultivars (cut flower) on the fifth day after flowering placed in a growth chamber (%).

	'Candy Marin'	'Celebrity Pink'	'Celebrity Rich White'	'Dean'	'Maquia Pink'	'Orb Snow'	'Prima Lavender'	'Soiree Pink'	'Piccorosa Snow'	'Reina White'	'Rosina Type III Snow'	'Voyage Type II Blue'
Monoterpenes												
β-Myrcene	78.8±0.4[z]	0.3±0.2	79.1±1.1	74.8±0.2	68.3±4.2	0.6±0.2	72.3±2.2	28.4±2.6	5.2±1.2	0.9±0.2	1.5±0.3	n.d.
1,8-Cineole	n.d.[y]	0.6±0.2	n.d.	n.d.	n.d.	2.5±0.8	n.d.	0.3±0.0	n.d.	n.d.	3.8±0.4	0.7±0.3
β-Ocimene	4.5±0.3	0.2±0.1	11.0±0.2	4.8±0.2	6.9±0.4	0.6±0.2	7.6±0.8	2.0±0.1	1.0±0.2	2.2±0.5	2.4±1.0	1.3±0.4
Linalool	0.4±0.3	n.d.	0.3±0.0	1.4±0.0	0.2±0.1	n.d.	n.d.	2.0±0.3	n.d.	1.6±0.5	n.d.	n.d.
Ipsdienol	1.6±0.9	n.d.	1.3±0.1	7.8±0.0	1.5±0.2	n.d.	2.9±1.1	2.7±0.5	n.d.	n.d.	n.d.	n.d.
Others	1.6±0.5	0.3±0.2	0.9±0.2	n.d.	2.1±0.1	0.6±0.1	4.2±1.2	1.5±0.2	0.5±0.2	1.4±0.2	n.d.	n.d.
Iridoids												
Nepetalactone	trace[x]	0.5±0.2	trace	0.2±0.1	n.d.	0.4±0.3	0.2±0.1	trace	0.2±0.1	trace	0.6±0.2	n.d.
Isodihydronepetalactone	trace	0.6±0.2	0.2±0.1	0.1±0.0	trace	trace	0.6±0.1	trace	1.5±0.6	0.5±0.3	0.4±0.0	0.4±0.1
Iridomyrmecin	trace	0.5±0.2	n.d.	n.d.	trace	0.2±0.1	0.8±0.1	trace	0.6±0.1	0.4±0.1	0.6±0.4	0.2±0.1
Isoiridomyrmecin	trace	0.2±0.1	n.d.	n.d.	trace	n.d.	0.8±0.2	trace	0.2±0.1	0.1±0.0	0.4±0.3	n.d.
Sesquiterpenes												
β-Elemene	0.8±0.1	3.2±1.6	n.d.	n.d.	3.0±0.6	9.8±2.8	n.d.	4.4±0.3	1.9±0.1	12.9±1.7	9.9±2.9	n.d.
Caryophyllene	0.2±0.0	13.1±4.4	3.2±0.9	n.d.	n.d.	n.d.	5.7±1.6	8.7±2.2	31.6±7.8	n.d.	n.d.	32.4±15.8
Eremophilene	0.4±0.1	0.9±0.5	n.d.	n.d.	0.8±0.2	1.6±0.7	n.d.	1.1±0.1	1.1±0.2	2.1±0.2	2.1±1.2	n.d.
β-Selinene	1.2±0.2	3.4±0.4	n.d.	n.d.	2.5±0.5	5.0±1.6	n.d.	2.3±0.1	2.7±0.1	10.2±1.5	6.0±1.8	n.d.
α-Selinene	1.9±0.3	17.5±5.3	n.d.	n.d.	4.8±1.1	26.3±7.0	n.d.	18.7±3.0	11.7±1.5	13.7±2.3	31.7±3.6	n.d.
Dihydro-β-agarofuran	1.9±0.3	14.7±2.0	0.1±0.0	n.d.	0.9±0.2	23.3±3.0	n.d.	11.4±0.8	2.3±0.1	7.0±0.9	14.3±3.8	n.d.
Hinesol	n.d.	0.1±0.1	n.d.	n.d.	0.1±0.0	0.2±0.1	n.d.	0.5±0.1	n.d.	1.0±0.1	0.7±0.1	n.d.
Agarospirol	n.d.	0.3±0.3	n.d.	n.d.	trace	0.5±0.2	n.d.	0.6±0.2	n.d.	1.0±0.1	1.9±0.7	n.d.
Others	2.2±0.4	22.0±3.4	n.d.	n.d.	2.8±0.4	21.2±8.4	0.7±0.3	5.1±0.2	18.8±3.1	30.6±6.5	4.8±1.3	n.d.
Benzenoids/phenylpropanoids												
Veratrole	3.7±0.7	20.6±5.0	2.6±0.1	n.d.	6.2±1.0	9.1±1.1	1.9±0.5	9.2±1.5	11.1±2.7	6.8±0.5	7.8±2.9	55.6±11.8
Guaiacol	n.d.	1.0±0.4	n.d.	n.d.	n.d.	n.d.	1.7±0.4	0.2±0.0	1.3±0.2	n.d.	n.d.	n.d.
Eugenol	n.d.	n.d.	n.d.	n.d.	n.d.	0.3±0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	trace	n.d.	1.0±0.0	n.d.	n.d.	0.4±0.2	n.d.	n.d.	5.4±1.0	n.d.	2.7±1.0	n.d.
Others												
Actinidine	n.d.	0.2±0.1	n.d.	n.d.	n.d.	n.d.	trace	n.d.	0.2±0.0	n.d.	n.d.	n.d.
Methyl dihydrojasmonate	trace	trace	n.d.	n.d.	n.d.	n.d.	trace	trace	trace	1.7±0.1	n.d.	n.d.
Others	0.7±0.2	n.d.	0.3±0.0	10.3±0.5	0.2±0.1	1.5±0.4	0.6±0.0	n.d.	2.6±0.4	4.2±0.5	n.d.	9.5±2.5
Peak area per flower												
Iridoids and actinidine	1,177,022	14,554,016	1,602,380	2,441,125	577,001	4,646,313	8,590,145	2,495,785	10,165,490	4,181,743	39,926,433	5,891,506
Total	980,851,386	761,990,350	942,576,279	813,708,178	641,112,777	673,378,637	342,236,841	1,386,547,391	385,056,444	387,476,212	1,976,556,081	935,159,738
Sensual feature of flower	Faint-grassy	Faint-woody	Faint-grassy	Faint-grassy	Faint-grassy	Faint-woody	Faint-grassy	Faint-woody	Faint-woody	Faint-woody	Faint-woody	Faint-woody

[z] ±standard error of mean (n=3).
[y] Not detected.
[x] <0.1%.

results may have been significantly different from ours. If volatile components emitted from living flowers are collected using the static headspace method, terpenoids including the iridoid compounds may also be detected in 'Royal Pink' and 'Excalibur Pink'.

Biosynthesis of scent components in lisianthus flowers

The volatile components of lisianthus flowers were mainly divided into four groups of compounds: monoterpenes, iridoids, sesquiterpenes and benzenoids/phenylpropanoids (Fig. 6). Iridoids including nepetalactone, isodihydronepetalactone, iridomyrmecin and isoiridomyrmecin have been shown to be biosynthesized from geranyl pyrophosphate (GPP) via geraniol, 8-hydroxygeraniol, and 8-oxogeranial (Hallahan et al., 1998; Lichman, 2020; Miettinen, 2014). Actinidine, an alkaloid with a pyridine ring, has been shown to be biosynthesized from GPP-like iridoids (Auda, 1967). The major monoterpenes in the volatile components of lisianthus flowers are biosynthesized via linalyl pyrophosphate (LPP), such as β-myrcene. Since geraniol was not detected in lisianthus, it may be an intermediate in the biosynthesis of iridoids in lisianthus flowers.

Many sesquiterpenes were detected from the volatile components of lisianthus flowers; β-caryophyllene biosynthesized from farnesyl pyrophosphate (FPP) via humulyl cation, β-elemene biosynthesized from FPP via germacryl cation, compounds such as β-selinene with an eudesman skeleton formed from FPP via eudesmyl cation, dihydro-β-agarofuran with an agarofuran skeleton, and hinesol and agarospirole derived from dihydro-β-agarofuran (Fig. 6). The first detection of dihydro-β-agarofuran was from the essential oil of fungal-infected agarwood (*Aquilaria agallocha*) (Maheshwari et al., 1963). This compound has also been detected in essential oils of fragrant sandalwood, *Alpinia japonica* (Itokawa et al., 1985), and *Apium graveolens* (Foudah

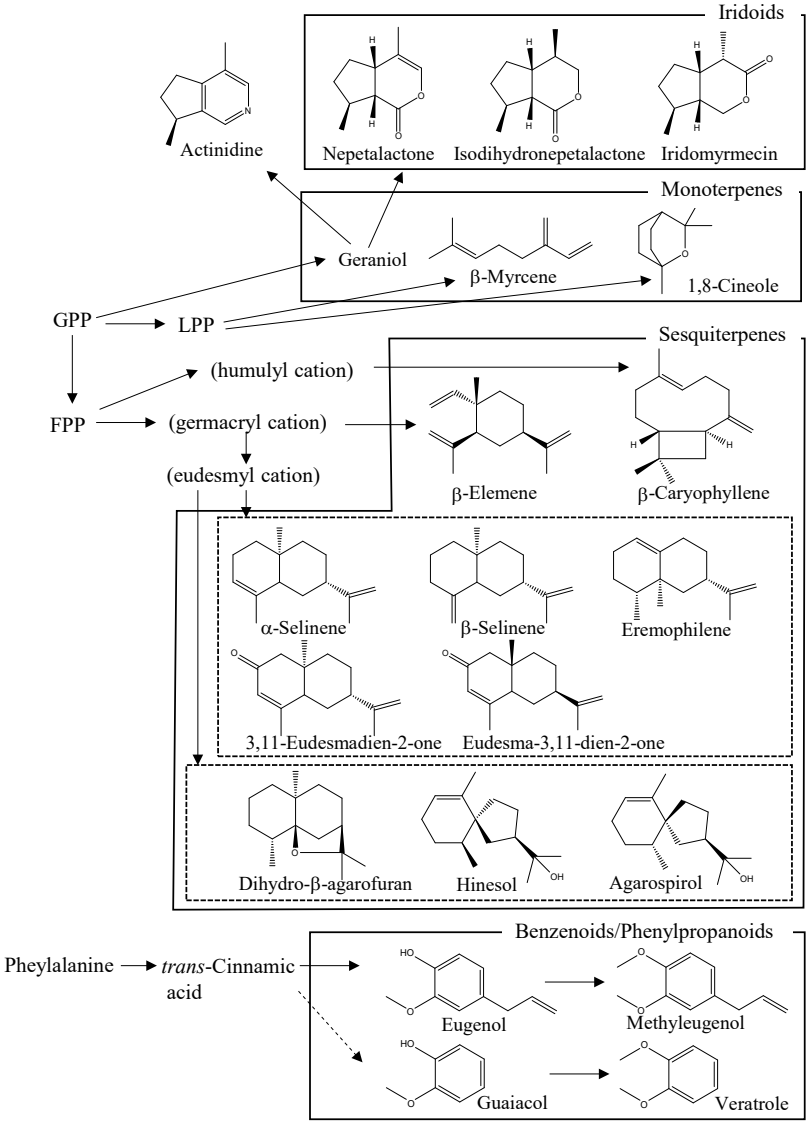

Fig. 6. Putative biosynthetic pathways of volatile components in lisianthus flowers. FPP, farnesyl pyrophosphate; GPP, geranyl pyrophosphate; LPP, linalyl pyrophosphate.

et al. 2021). There is no report that dihydro-β-agarofuran and its derivative compounds have been detected from floral volatiles. Sesquiterpenes with a dihydro-β-agarofuran skeleton are known to have anti-tumoral, immunosuppressive, antiviral and insecticidal activities (Gao et al., 2007). It is interesting that such a compound with medicinal properties was detected in the volatiles of lisianthus flowers.

Eugenol, methyl eugenol, guaiacol and veratrole were detected as benzenoids/phenylpropanoids in lisianthus flowers. It has been shown that eugenol is biosynthesized from phenylalanine via *trans*-cinnamic acid, *p*-coumaric acid, caffeic acid, ferulic acid, coniferyl alcohol, and coniferyl acetate in anise (Koeduka et. al., 2009), basil (Koeduka et al., 2006), clarkia (Koeduka et al., 2008), and petunia (Koeduka et al., 2006, 2008). It has been shown that guaiacol is biosynthesized from phenylalanine via *trans*-cinnamic acid, benzoic acid, salicylic acid, and catechol in white campion (Akhtar, 2013). Eugenol is one of the active compounds that attracts pollinators in *Gymnadenia* species (Huber et al., 2005; Schiestl et al., 2011). Veratrole is also one of the active compounds in white campion (Gupta et al., 2012). Although the pollinators of lisianthus are unknown, eugenol and veratrole, which are found in the scent of the flowers, may also be active compounds for pollinators.

Lisianthus was thought to be scentless, but this study revealed for the first time that various volatile components are emitted from lisianthus flowers. Furthermore, iridoid compounds and actinidine, which attract cats, were detected for the first time in the volatiles of flowers.

Lisianthus is a harmless plant for cats (Grota, 2020) and chewing and licking the flowers does not appear to adversely affect their health. Lisianthus flowers, which have volatiles that attract cats, can be attractive cut flowers to not only humans, but also to cats.

In the future, we plan to quantify the iridoids contained in the volatile components of the main lisianthus cultivars that are on the market, and identify cultivars that contain large amounts of these compounds. Additionally, we would like to investigate the effects of volatiles of lisianthus flowers on cats.

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Supplemental table. Composition ratios of daily change in volatile components of potted and cut 'New Lination White' flowers placed in a greenhouse or a growth chamber (%, Fig. 4).

	Greenhouse				Growth chamber							
	Potted flower				Potted flower				Cut flower			
	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5
Monoterpenes												
β-Myrcene	0.2 ± 0.2[z]	0.8 ± 0.5	4.7 ± 0.1	8.0 ± 2.1	1.0 ± 0.1	0.5 ± 0.0	1.1 ± 0.1	2.4 ± 0.7	1.2 ± 0.2	1.1 ± 0.4	0.8 ± 0.3	0.8 ± 0.6
1,8-Cineole	n.d.[y]	n.d.	1.2 ± 1.2	10.8 ± 1.1	n.d.	0.2 ± 0.2	0.7 ± 0.0	n.d.	n.d.	1.3 ± 0.3	1.0 ± 0.2	0.8 ± 0.0
Others	n.d.	n.d.	n.d.	0.3 ± 0.3	7.0 ± 0.6	1.8 ± 0.2	1.0 ± 0.0	0.8 ± 0.3	6.5 ± 1.4	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Iridoids and actinidine												
Actinidine	n.d.	0.3 ± 0.3	0.5 ± 0.2	0.3 ± 0.0	n.d.	1.0 ± 0.2	1.0 ± 0.0	0.2 ± 0.2	n.d.	0.6 ± 0.3	0.3 ± 0.1	trace
Nepetalactone	trace[x]	0.6 ± 0.4	0.8 ± 0.1	0.6 ± 0.0	n.d.	1.7 ± 0.7	0.6 ± 0.0	0.3 ± 0.1	n.d.	0.5 ± 0.3	0.4 ± 0.1	0.2 ± 0.0
Isodihydronepetalactone	0.6 ± 0.1	0.6 ± 0.0	2.6 ± 0.2	2.7 ± 0.5	0.5 ± 0.2	1.6 ± 0.2	3.2 ± 0.5	2.0 ± 0.8	0.6 ± 0.1	3.5 ± 1.9	3.2 ± 1.6	2.2 ± 0.7
Iridomyrmecin	0.3 ± 0.3	0.7 ± 0.7	1.7 ± 0.3	1.6 ± 0.3	n.d.	0.8 ± 0.0	0.8 ± 0.1	0.6 ± 0.1	n.d.	0.8 ± 0.4	0.7 ± 0.2	0.5 ± 0.3
Isoiridomyrmecin	0.2 ± 0.2	0.3 ± 0.2	0.8 ± 0.2	1.1 ± 0.1	n.d.	0.7 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	n.d.	0.7 ± 0.4	0.5 ± 0.2	0.5 ± 0.3
Sesquiterpenes												
β-Elemene	9.9 ± 0.1	2.0 ± 0.9	4.2 ± 0.0	4.1 ± 0.6	4.3 ± 0.7	6.0 ± 1.3	8.9 ± 1.5	8.9 ± 0.6	4.8 ± 0.3	3.8 ± 0.2	5.1 ± 2.4	5.4 ± 0.5
Caryophyllene	n.d.	0.2 ± 0.0	trace	n.d.	11.9 ± 1.4	2.8 ± 1.8	6.5 ± 4.5	1.9 ± 0.8	13.3 ± 0.5	4.3 ± 3.8	1.8 ± 1.4	2.0 ± 1.6
β-Selinene	15.9 ± 2.8	2.0 ± 1.0	6.6 ± 0.1	7.6 ± 2.0	14.6 ± 0.5	4.9 ± 0.6	8.1 ± 1.0	7.9 ± 0.1	16.4 ± 0.7	6.6 ± 1.0	8.1 ± 2.1	9.1 ± 0.9
α-Selinene	42.0 ± 4.5	5.3 ± 2.3	15.6 ± 0.4	21.0 ± 3.3	35.2 ± 0.5	11.6 ± 1.3	37.3 ± 4.1	43.7 ± 5.6	32.7 ± 3.2	12.1 ± 2.1	41.2 ± 5.1	48.8 ± 3.4
Dihydro-β-agarofuran	12.0 ± 1.5	2.7 ± 0.9	4.7 ± 0.7	4.5 ± 0.4	9.9 ± 0.6	3.7 ± 0.4	4.5 ± 0.4	3.8 ± 0.1	9.7 ± 1.2	4.2 ± 0.1	3.7 ± 0.2	4.1 ± 0.3
Others	6.2 ± 1.8	3.8 ± 0.5	11.0 ± 0.2	9.7 ± 0.9	9.9 ± 4.0	17.1 ± 2.1	20.8 ± 3.8	23.3 ± 3.0	11.3 ± 0.7	27.4 ± 1.5	28.8 ± 1.2	23.2 ± 0.7
Benzenoids/phenylpropanoids												
Methyleugenol	0.6 ± 0.0	9.2 ± 4.0	10.7 ± 0.3	3.2 ± 1.0	n.d.	7.3 ± 0.4	0.8 ± 0.4	0.5 ± 0.2	n.d.	4.9 ± 1.0	0.6 ± 0.3	0.6 ± 0.3
Eugenol	7.0 ± 3.7	53.5 ± 12.7	24.7 ± 5.3	14.0 ± 9.3	n.d.	35.8 ± 3.4	2.0 ± 0.9	0.3 ± 0.2	n.d.	26.4 ± 6.8	0.8 ± 0.4	0.5 ± 0.3
Others	2.7 ± 2.7	7.0 ± 2.8	7.2 ± 2.1	4.4 ± 0.1	2.9 ± 1.2	1.0 ± 1.0	n.d.	n.d.	1.2 ± 0.4	n.d.	0.4 ± 0.4	n.d.
Others	2.3 ± 0.1	11.2 ± 1.9	3.1 ± 1.0	6.2 ± 0.3	2.8 ± 0.2	1.7 ± 0.4	2.4 ± 0.1	1.6 ± 0.3	2.4 ± 0.6	1.3 ± 0.4	2.1 ± 0.6	0.9 ± 0.2
Peak area per flower												
Iridoids and actinidine	1,972,291	6,730,436	8,026,371	5,821,053	329,312	38,842,382	45,242,420	35,955,557	339,669	41,158,003	38,751,430	29,338,912
Total	134,726,760	267,080,786	123,672,900	92,691,927	68,497,527	677,877,514	736,847,237	1,030,245,189	58,563,703	678,056,067	768,877,576	857,862,923
Sensual feature of flower	Faint-sweet	Sweet	Weet-sweet	Faint-sweet	n.d.	Sweet	Faint-woody	Faint-woody	n.d.	Sweet	Faint-woody	Faint-woody

[z] ±standard error of mean (n = 3).

[y] Not detected.

[x] < 0.1%