Temperature and Different Organs Create Volatile Profile Differences of Edible *Gynura* [*Gynura bicolor* (Roxb. ex Willd.) DC]

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Abstract. The volatile profile of the edible vegetable *Gynura bicolor* [*Gynura bicolor* (Roxb. ex Willd.) DC] was analyzed using gas chromatography-mass spectrometry (GC-MS). Isocaryophyllene (23.2%), α -pinene (16.8%), α -humulene (9.1%), β -pinene (7.3%), and copaene (7.0%) were identified as the major compounds in the leaves. In the stems, α -pinene (27.1%), β -pinene (13.0%), isocaryophyllene (7.8%), β -myrceneb (7.8%), 1-undecene (5.7%), and copaene (5.3%) were the main components. *G. bicolor* grows best at 25°C. When cultivated at different temperatures (20 to 35°C in increments of 5°C), the volatile profiles shifted. The proportion of isocaryophyllene was lower at 20°C than at the other temperatures. The relative amounts of α -pinene and α -humulene were highest at 20°C, whereas copaene was highest at 35°C. Principal component analysis (PCA) was used to explore the correlation between volatile compounds identified from the vegetative tissues and temperature treatments. It reveals the same trend with the previous statements and the first principal component (PC1) and the second principal component (PC2) explains up to 90% of the variance. Experimental results revealed that both temperature and vegetative organ correlate with the volatile emission profile of *G. bicolor*.

G. bicolor is in the Asteraceae family and cultivated as a traditional vegetable (Hóng Fēng Cài) in various locations, including Taiwan, China, and Japan. Its leaves and stems are edible. The leaves of *G. bicolor* are characteristically green on the adaxial side and reddish-purple on the abaxial side. In Taiwan, there are large-leaved varieties with reddish stems and small-leaved varieties with greenish stems. *G. bicolor* is aromatic and has a high nutritional value because it is rich in iron, flavonoids, and anthocyanins. It is

also used as a medicinal plant (Chao et al., 2015; Shimizu et al., 2009; Yoshitama et al., 1994).

A recent study identified 108 volatile components from the essential oil of *G. bicolor* (Miyazawa et al., 2016). Plant essential oils are complex mixtures of volatile constituents, and their major components are terpenoids, like isoprenoids (C5), monoterpenoids (C10), and sesquiterpenoids (C15), which are naturally occurring organic products (Holopainen and Gershenzon, 2010). Volatile compounds can protect plants from biotic and abiotic stresses by quenching stress-generated reactive oxygen species (ROS) and stabilizing cell membranes (Brilli et al., 2019; Loreto and Velikova, 2001; Velikova et al., 2011). Volatiles are stored in specialized tissues, such as resin ducts or glandular trichomes, and the stored volatiles may act as necessary signals for herbivores when selecting host plants (Mita et al., 2002). As plant defense mechanisms, volatile emissions can reduce herbivore feeding activity and inhibit the protease activities of pathogens (Baier et al.,

2002; Niinemets et al., 2013). Volatile compounds also impart flavor and health benefits to humans (Ayseli and İpek Ayseli, 2016).

Plant volatile profiles and emissions are dependent on species, organs, developmental stages, and environmental conditions. Previous studies indicated that temperature affects both the synthesis and emission of volatile compounds (Copolovici and Niinemets, 2016; Grote et al., 2013; Guenther et al., 2012). Temperature can determine the gas-liquid phase partition of volatiles within the plant. A temperature increase converts more volatiles into the gas phase; thereafter, they can be emitted from the plant (Harley, 2013). For basil, temperature has significant effects on plant growth parameters that also affect the volatile oils of the fresh leaves (Chang et al., 2005). In young corn plants, the emission of terpenoids and indoles were high at 22 and 27°C, respectively (Gouinguéné and Turlings, 2002). In addition, the production and emission of volatiles can be detected at specific developmental stages and from different parts of the plant, such as leaves, roots, and flowers (Chiu et al., 2017; Possell and Loreto, 2013; Ren et al., 2014).

G. bicolor is an aromatic plant in the Asteraceae family and is cultivated as a vegetable in Taiwan. When compared with leafy vegetables grown in the same agricultural systems, *G. bicolor* is generally less affected by pests and diseases. In the field, high temperature (summer season) is a significant factor that limits plant growth and development of *G. bicolor*. We were curious to know whether its ability to produce certain volatile compounds suppresses pest and disease attacks. Therefore, this study aimed to determine the optimum temperature conditions for the growing season and to clarify the effects of temperature and vegetative parts on the volatile compound profiles and quality of *G. bicolor*.

Using a solid-phase microextraction (SPME) method coupled with GC-MS, we analyzed the volatile compound profiles of the leaves and stems of *G. bicolor* after growth at 25 ± 1°C and the volatiles emitted by the leaves of *G. bicolor* grown under different temperatures in otherwise controlled environmental conditions. The released volatile compounds were grouped by different temperatures and the harvested organ by PCA.

Materials and Methods

Plant materials. Apical shoots (≈20 cm) of the *G. bicolor* commercial cultivar Hóng Fēng Cài were transplanted to pots and grown for 14 d on an outdoor balcony (28 ± 3°C) under shade. After 14 d, the plants were transferred to a growth chamber. The shoots were cultivated in the growth chamber for 50 d at 25 ± 1°C with a 13-h light/11-h dark photoperiod and a light intensity of 129.0 ± 7.5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to obtain the material for the experiments.

Volatile compounds in vegetative parts of *G. bicolor*. The two aerial parts of *G. bicolor* (leaves and stems) were separated at the petiole from the main stem and cut into pieces

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with scissors. The volatile compounds from 4.0 g of leaves/stems were placed into 20-mL headspace vials (20 mm outside diameter $\times$ 83 mm height $\times$ 15 mm inside diameter). The volatiles were extracted by headspace solid-phase microextraction (HS-SPME) for 25 min at $25 \pm 3^\circ\text{C}$ with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber purchased from Supelco (Bellefonte, PA). The fiber was injected in a GC-MS injection unit. The sample extractions were conducted in triplicate.

Volatile profiles of plants grown at different temperatures. Apical shoots of *G. bicolor* were transplanted to pots and grown for 14 d outdoors on a balcony under a shade canopy. The plants were transferred to different daytime temperature treatments (20, 25, 30, and 35°C) in the growth chamber for 50 d. At the end of each temperature treatment, the following growth parameters were measured: plant height, plant fresh weight and dry weight, leaf area, number of nodes, and leaf thickness. Eight replicates were measured for the growth parameters. Fresh leaves (4.0 g) were collected and cut into pieces with scissors. These samples were placed in 20-mL headspace vials and extracted for 25 min to adsorb volatiles to the headspace SPME fiber. The fiber was injected in the injection unit of the GC-MS system. Four replicates were analyzed for each sample.

Analysis of GC-MS. The GC-MS analyses were performed using an Agilent 7890B GC Plus coupled to a quadrupole mass spectrometry device (5977B MSD; Agilent, Palo Alto, CA) equipped with an HP-5MS column (30 m $\times$ 0.25 mm inside diameter; film thickness, 0.25 μm). The temperature was maintained at 250°C in a splitless GC injector and detector. The oven temperature was maintained at 40°C for 1 min, increase to 150°C at $5^\circ\text{C}\cdot\text{min}^{-1}$, maintained for 1 min, increased to 200°C at $10^\circ\text{C}\cdot\text{min}^{-1}$, and maintained for 11 min. The helium carrier gas flow rate was $1\text{ mL}\cdot\text{min}^{-1}$ (Huang et al., 2010). Kovats indices were calculated for separate components relative to a series of C5-C25 *n*-alkanes (Schomburg and Dielmann, 1973). The constituents were identified by matching their spectra with those recorded in the Wiley 11/NIST 2018 MS library.

Statistical analysis. The mean values of the experimental data were subject to an analysis of variance with the least significant difference test at the 1% level of significance using SAS (SAS Enterprise v 7.1; SAS, Cary, NC). The PCA was performed using all the volatile compounds identified from the vegetative tissues and temperature treatments. The PCA results were obtained using the *prcomp* command of the R package statistical software (Jolliffe and Cadima, 2016).

Results and Discussion

Volatile compounds in vegetative parts of *G. bicolor*. Shoots of *G. bicolor* were grown at 25°C and harvested separately as stems and leaves for analysis using GC-MS (Fig. 1). A total of 33 volatile compounds were

identified, but had differing leaves and stems (Table 1). The main constituents in the leaves were isocaryophyllene (23.2%), α -pinene (16.8%), α -humulene (9.1%), β -pinene (7.3%), and copaene (7.0%); however, the major constituents in the stems were α -pinene (27.1%), β -pinene (13.0%), isocaryophyllene (7.8%), β -myrcene (7.8%), 1-undecene (5.7%), and copaene (5.3%). The identified volatile compounds of the stems and the leaves accounted for 94.4% and 85.9% of the total volatile content, respectively. The remaining volatile compounds in the stems (5.6%) and leaves (14.1%) were unknown (Table 1). According to the total ion chromatogram (TIC) of the volatiles, sesquiterpenes (isocaryophyllene) were the most

abundant components in the leaves of *G. bicolor*. In the stems, monoterpene compounds (α -pinene) comprised the higher percentage (Fig. 1).

We performed a PCA to group the volatile compounds according to which part of the plant released them (Fig. 2). The two principal components (PCs) accounted for 99.7% of the observed variance, with PC1 explaining $\approx 99.1\%$ of the variation. According to the PCA, isocaryophyllene and α -humulene were more highly emitted from the leaves, whereas α -pinene, β -pinene, and 1-undecene were more highly emitted from the stems. Miyazawa et al. (2016) reported that isocaryophyllene, α -pinene, and α -humulene were the three main compounds in the leaves of *G.*

Fig. 1. Total ion chromatogram (TIC) of the volatile compounds from the leaves (upper) and stems (lower) of *G. bicolor* grown at 25°C in a growth chamber (peak labeled per numbering in Table 1).

Table 1. The mean percent peak area based on the TIC (TIC area %) of volatile compounds in two vegetative parts of *G. bicolor* as identified by GC-MS.

No. ^z	Compounds	RI ^y	RI ^x	Peak area (%)	
				Leaves	Stems
1	<i>trans</i> -3-hexenol	874	894	2.5 ± 0.4 ^w	—
2	α-pinene	913	901	16.8 ± 1.2 b	27.1 ± 1.0 a
3	camphene	928	921	—	0.3 ± 0.0
4	β-pinene	957	955	7.3 ± 0.7 b	13.0 ± 0.4 a
5	β-myrcene	975	974	4.1 ± 0.2 b	7.8 ± 0.2 a
6	α-phellandrene	996	990	—	0.5 ± 0.0
7	δ-3-carene	1007	995	0.6 ± 0.1 b	1.8 ± 0.3 a
8	α-terpinene	1003	1002	0.3 ± 0.0 b	0.5 ± 0.0 a
9	limonene	1019	1016	1.3 ± 0.1 b	3.8 ± 0.2 a
10	α-ocimene	1052	1037	1.6 ± 0.4	—
11	<i>trans</i> -β-ocimene	1033	1039	—	4.4 ± 0.2
12	γ-terpinene	1042	1048	0.4 ± 0.0 b	1.0 ± 0.1 a
13	1-undecene	1091	1079	1.1 ± 0.0 b	5.7 ± 0.8 a
14	1-octen-3-yl acetate	1112	1098	—	0.2 ± 0.0
15	(<i>E</i>)-4,8-dimethylnona-1,3,7-triene	1117	1103	—	0.5 ± 0.1
16	<i>allo</i> -ocimene	1127	1113	0.2 ± 0.0 b	0.4 ± 0.0 a
17	1-tridecene	1290	1269	0.3 ± 0.0 b	2.5 ± 0.1 a
18	α-cubebene	1323	1297	0.4 ± 0.0 a	0.3 ± 0.0 a
19	ylangene	1342	1324	0.8 ± 0.1	—
20	copaene	1353	1339	7.0 ± 0.7 a	5.3 ± 0.4 a
21	β-elemen	1366	1358	1.3 ± 0.0 b	2.4 ± 0.2 a
22	α-cedrene	1386	1385	—	0.2 ± 0.0
23	isocaryophyllene	1398	1407	23.2 ± 0.4 a	7.8 ± 0.7 b
24	aromadendrene	1440	1423	3.4 ± 0.2	—
25	α-humulene	1448	1444	9.1 ± 0.5 a	2.3 ± 0.2 b
26	germacrene D	1476	1471	0.6 ± 0.1 a	0.9 ± 0.1 a
27	β-selinene	1480	1478	0.6 ± 0.1 a	1.0 ± 0.2 a
28	α-farnesene	1503	1500	0.4 ± 0.1	—
29	γ-cadinene	1510	1511	0.3 ± 0.0 a	0.2 ± 0.0 a
30	α-selinene	1505	1516	0.1 ± 0.0 b	3.2 ± 0.2 a
31	δ-cadinene	1521	1522	2.1 ± 0.3 a	1.5 ± 0.1 a
32	α-cadinene	1538	1537	0.1 ± 0.0	—
33	α-calacorene	1542	1543	—	0.1 ± 0.0
	Total			85.9 ± 5.7	94.4 ± 5.7

^zNumber marks are according to the peak numbers in Fig. 1.

^yRI: retention indices from the literature were checked using the HP-5MS column.

^xRI: retention indices were determined using the HP-5MS column with *n*-alkanes (C5-C25) as reference materials.

^wData are mean ± SD of three replicates. Different letters indicate significant differences at *P* < 0.01 according to the least significant difference test.

GC-MS = gas chromatography-mass spectrometry; TIC = total ion chromatogram.

bicolor, whereas α-pinene, β-pinene, and myrcene were the three major components in the stems. These results were similar to the findings of our study. Isocaryophyllene is a major sesquiterpene in various essential oils from vegetable species and tends to accumulate in plant leaves (Vila et al., 1997; Zoghbi et al., 1995).

The emission of plant volatiles from vegetative parts is known to be involved in biotic and abiotic stresses. Isocaryophyllene and α-humulene (α-caryophyllene) are the isomers of β-caryophyllene; their biological effects include antimicrobial, antioxidative, and anticancer activities (Dahham et al., 2015). Previous experiments showed that β-caryophyllene from plant flowers serves as a defense against bacterial pathogens (Huang et al., 2012). The monoterpene pinene exists as two structural isomers (α-pinene and β-pinene) and are common in plant essential oils. Previous studies indicated that α-pinene, β-pinene, myrcene, limonene, and 3-carene have phytotoxic effects that inhibit insect feeding (López et al., 2011; Shi and Sun, 2010; Xu et al., 2014). These volatiles

have several functions, such as signaling, resistance to oxidative stress, and defense against herbivores and pathogens (Dong et al., 2016).

Changes in the volatile profile with changes in temperature. The plants of *G. bicolor* were grown under different temperature treatments in the growth chamber (Fig. 3). Plants grown at 25 °C showed the best values for growth indices such as plant height, leaf areas, node number, fresh weight, and dry weight (Table 2). Plants grown at 35 °C had smaller leaves, fewer nodes, and lighter fresh and dry weights, but the leaves were thicker (Table 2). The GC-MS analysis showed that the volatile compounds released from fresh leaves of *G. bicolor* changed with the ambient temperature (Table 3). The mean percent peak area was based on TIC (TIC area %) of volatile compounds. The total amounts of volatile compounds were not significantly different at 20 °C (86.0%), 25 °C (86.8%), 30 °C (85.1%), or 35 °C (85.6%), but other compounds in the leaves (13% to 15%) were unknown (Table 3). These results were similar to the effects of temperature on the

emission of induced volatiles from corn plants (Gouinguéné and Turlings, 2002). However, different temperatures could affect the number of monoterpenoids and sesquiterpenoids arising from the plant. Total monoterpenoids were significantly higher at moderate temperatures (20 and 25 °C) than at high temperatures (30 and 35 °C), whereas total sesquiterpenoid amounts were significantly increased at 30 and 35 °C (Table 3).

Several factors, such as environmental and genetic factors and agricultural practices, can affect the volatile composition (Copolovici and Niinemets, 2016; Nurzyńska-Wierdak, 2012). Temperature is the key factor that influences the growth and development of plants as well as their volatiles content (Chang et al., 2005). Previous studies have shown that temperature affects the enzymes that synthesize volatile compounds. The duration of the temperature shift also influences the content of plant volatiles, with short-term changes enhancing the synthesis of volatiles and long-term changes reducing it (Kleist et al., 2012; Pazouki et al., 2016). In addition, temperature can induce changes in leaf structure and biomass, which can alter the physiological characteristics per unit area (Rasulov et al., 2015). Chang et al. (2005) reported that sweet basil grown at 25 °C had larger leaf areas, higher leaf fresh weights, and increased volatile oil content per unit weight than basil grown at other temperatures (15 and 30 °C). This study found that *G. bicolor* plants grown at 25 °C had larger leaf areas and higher leaf fresh weights than those grown at a higher temperature (35 °C) (Table 2). Additionally, plants growth at 35 °C had small and clustered leaves; this change might affect the generation of volatile components (Fig. 3).

The volatile compounds identified in the leaves of *G. bicolor* were mainly terpene hydrocarbons, such as monoterpenes and sesquiterpenes. The main volatiles were isocaryophyllene (16.0% to 23.3%), α-pinene (17.1% to 21.2%), copaene (6.5% to 13.4%), α-humulene (6.2% to 9.2%), and β-pinene (7.6% to 8.3%), as reported in previous studies (Miyazawa et al., 2016; Shimizu et al., 2009). Temperature did affect the relative content of these main volatiles. The proportion of isocaryophyllene was significantly lower at 20 °C (*P* < 0.01) than at other temperatures. In contrast, the relative amounts of α-pinene and α-humulene were highest at 20 °C, and that of copaene was highest at 35 °C (Table 3). The PCA was used to group the volatiles emitted by temperature treatments. The two main PCs explained ≈92.1% of the total variance in volatile emissions among the different temperatures. PC1 explained ≈60.6% of the variation and PC2 explained ≈31.5% of the variation. According to the PCA results, *trans*-3-hexenol and α-pinene were most influenced at 20 °C, whereas β-myrcene and aromadendrene were most influenced at 25 °C. The major volatiles were isocaryophyllene at 30 °C and copaene and δ-cadinene at 35 °C (Fig. 4). Gouinguéné and Turlings (2002) reported that the relative β-caryophyllene emission quantity from corn plants was highest at 37 °C.

Fig. 2. A principal component analysis of the correlation between volatile compounds and aerial vegetative tissues of *G. bicolor* ($n = 3$). 25CL indicates volatile compounds identified from the leaves grown at 25 °C in a growth chamber. 25CS indicates volatile compounds identified from the stems grown at 25 °C in a growth chamber. (See Supplemental Fig. 1. for a zoom into the volatile compounds with small loading values for the first and second principal components.)

Fig. 3. Potted *G. bicolor* plants were grown in growth chambers with different temperatures (20, 25, 30, and 35 °C, bar = 2.54 cm).

Table 2. Comparison of plant growth indices under different temperatures in a growth chamber.

	Plant ht (cm)	Fresh wt of stems (g)	Fresh wt of leaves (g)	Leaf area (cm ²)	Nodes (no.)	Leaf thickness (mm)	Dry wt of stems (g)	Dry wt of leaves (g)
20 °C	28.2 ± 2.4 b ^c	18.6 ± 2.6 a	24.2 ± 4.1 ab	660.0 ± 120.9 b	28 ± 3 a	0.35 ± 0.02 c	1.6 ± 0.4 a	1.6 ± 0.3 a
25 °C	38.1 ± 3.2 a	19.6 ± 5.5 a	27.6 ± 7.5 a	870.3 ± 210.4 a	31 ± 5 a	0.33 ± 0.03 c	1.6 ± 0.5 a	1.7 ± 0.5 a
30 °C	19.4 ± 1.9 d	12.6 ± 2.5 b	23.1 ± 6.3 ab	613.6 ± 150.1 b	24 ± 2 b	0.43 ± 0.04 b	1.0 ± 0.2 b	1.5 ± 0.4 a
35 °C	25.3 ± 1.2 c	11.5 ± 2.6 b	20.9 ± 3.1 b	433.6 ± 71.4 c	18 ± 2 c	0.60 ± 0.03 a	1.0 ± 0.3 b	1.4 ± 0.2 a

^cData are mean ± SD of eight replicates. Different letters indicate significant differences at $P < 0.05$ according to the least significant difference test.

Previous studies showed that temperature determined the gas–liquid phase partition of the volatile compounds within plants according to Henry's law constant (Harley, 2013; Niinemets et al., 2004). Additionally, temperature could change the stomatal conductance, which controls the emission of volatiles by diffusion resistance (Possell and Loreto, 2013). Recent studies showed that heat stress led to the release of sesquiterpenes and had selective effects on different monoterpenes in *Solanum lycopersicum* (Copolovici and Niinemets, 2016; Copolovici et al., 2012). For pine, emissions of sesquiterpenes were temperature-dependent and probably triggered as a direct stress response to high temperatures (>30 °C), whereas monoterpenes were more abundant at lower temperatures (Helmig et al., 2006, 2007). These results were similar to those of our study (Table 3). The terpenoids have a role in stabilizing membranes at high temperatures, which may protect plants from high temperatures (Singsaas, 2000).

The PCA is a type of linear transformation of a given data set with many variables to achieve lower dimensional projection of the given data set to preserve the maximum variance. The process of the PCA involves performing singular value decomposition (SVD) of the matrix to obtain eigenvalues and eigenvectors. Every eigenvector has a corresponding eigenvalue. PCs are new variables that are calculated by linear combinations of the given variables. PC1 has the maximum variance of all linear combinations and PC2 has the second highest variance. The eigenvector with the highest eigenvalue is the eigenvector of PC1, and the eigenvector with the second highest eigenvalue is the eigenvector of PC2. Figures 2 and 4 show a visualization of the variations in PC1 and PC2. The two PCs accounted for 99.7% and 92.1% of the variance of the observed datasets in Figs. 2 and 4, respectively.

G. bicolor is an aromatic leaf green that has a high nutraceutical value, partly because of its flavonoids and anthocyanins, which have various biological activities (Li et al., 2009; Wu et al., 2013). Furthermore, the volatile compounds of *G. bicolor* are mainly terpenes, which have functions such as antibacterial, cytoprotective, and pesticidal activities (Salehi et al., 2019). These components of *G. bicolor* may offer opportunities for developing biopesticides to reduce pests and disease damage in the field or for medicinal uses. This study of the volatile compounds of *G. bicolor* provides information that can help maximize the production of useful volatiles by regulating the cultivation temperature and

Table 3. The mean percent peak area was based on the TIC (TIC area %) of volatile compounds from fresh leaves of *G. bicolor* grown at different temperatures as identified by GC-MS.

Compounds	RI ^z	RI ^y	Peak area (%)			
			20 °C	25 °C	30 °C	35 °C
<i>trans</i> -2-octene	810	791	—	0.6 ± 0.2	—	—
Hexanal	806	822	—	—	1.2 ± 0.2	0.4 ± 0.0
<i>trans</i> -3-hexenol	874	895	4.3 ± 0.4 a ^x	2.2 ± 0.2 b	1.9 ± 0.1 b	0.8 ± 0.1c
1-nonene	892	880	—	0.5 ± 0.1	—	—
α-pinene	913	901	21.2 ± 0.8 a	19.2 ± 1.9 ab	17.4 ± 1.0 b	17.1 ± 0.7 b
β-pinene	957	955	8.0 ± 0.3 a	8.3 ± 0.6 a	7.8 ± 0.9 a	7.6 ± 1.0 a
β-myrcene	975	974	4.3 ± 0.2 b	5.4 ± 0.2 a	4.0 ± 0.4 b	4.0 ± 0.5 b
α-phellandrene	996	988	—	—	0.2 ± 0.0	0.2 ± 0.0
δ-3-carene	1007	994	0.7 ± 0.1 b	0.9 ± 0.1 a	0.7 ± 0.1 ab	0.8 ± 0.1 ab
α-terpinene	1003	1002	0.3 ± 0.0 a	0.3 ± 0.0 a	0.2 ± 0.0 a	0.3 ± 0.0 a
<i>p</i> -cymene	1016	1011	—	—	0.4 ± 0.0	—
limonene	1019	1016	1.5 ± 0.1 ab	1.7 ± 0.1 a	1.2 ± 0.2 b	1.6 ± 0.2 ab
<i>trans</i> -β-ocimene	1027	1027	—	0.1 ± 0.0	0.6 ± 0.1	—
α-ocimene	1052	1037	1.2 ± 0.1	2.0 ± 0.5	—	0.3 ± 0.1
γ-terpinene	1042	1048	0.5 ± 0.1 b	0.5 ± 0.0 ab	0.6 ± 0.0 a	0.5 ± 0.1 ab
(<i>E</i>)-4,8-dimethylnona-1,3,7-triene	1117	1103	0.2 ± 0.0	—	—	—
<i>allo</i> -ocimene	1127	1113	0.1 ± 0.0	0.2 ± 0.0	—	—
1-tridecene	1290	1269	0.3 ± 0.0 b	0.5 ± 0.0 a	0.2 ± 0.0 c	0.1 ± 0.0 c
α-cubebene	1323	1296	0.4 ± 0.0 c	0.4 ± 0.0 c	0.7 ± 0.1 b	1.3 ± 0.2 a
ylangene	1342	1323	1.0 ± 0.1	—	—	—
copaene	1353	1338	8.3 ± 0.3 b	6.5 ± 0.5 c	8.6 ± 0.4 b	13.4 ± 0.6 a
β-elemene	1366	1357	0.9 ± 0.0	1.2 ± 0.2	1.2 ± 0.2	—
isocaryophyllene	1398	1402	16.0 ± 0.9 b	21.6 ± 0.6 a	23.3 ± 1.7 a	21.2 ± 0.2 a
aromadendrene	1440	1421	2.8 ± 0.2 b	3.3 ± 0.2 a	1.8 ± 0.1 c	0.7 ± 0.1 d
α-humulene	1434	1442	9.2 ± 0.4 a	8.0 ± 0.3 b	7.7 ± 0.8 b	6.2 ± 0.2 c
germacrene D	1476	1470	0.7 ± 0.1 c	0.5 ± 0.1 c	1.0 ± 0.1 b	1.9 ± 0.2 a
β-selinene	1480	1477	0.7 ± 0.1	0.7 ± 0.1	0.3 ± 0.0	—
α-murolene	1514	1494	—	—	—	1.2 ± 0.2
α-farnesene	1503	1500	0.4 ± 0.1	0.2 ± 0.1	—	—
<i>cis</i> -γ-bisabolene	1506	1501	—	—	0.5 ± 0.1	—
γ-cadinene	1510	1510	0.4 ± 0.0 bc	0.4 ± 0.0 c	0.5 ± 0.0 b	0.8 ± 0.1 a
α-selinene	1505	1516	—	0.1 ± 0.0	—	—
δ-cadinene	1521	1522	2.6 ± 0.1 b	1.6 ± 0.1 c	2.9 ± 0.2 b	5.0 ± 0.6 a
α-cadinene	1538	1537	0.1 ± 0.0 bc	0.1 ± 0.0 c	0.2 ± 0.0 b	0.3 ± 0.1 a
α-calacorene	1542	1543	0.1 ± 0.0	—	—	—
Total monoterpenes			37.8 ± 1.1 a	38.6 ± 2.4 a	33.2 ± 2.1 b	32.3 ± 2.3 b
Total sesquiterpenes			43.9 ± 1.8 b	44.5 ± 1.5 b	48.7 ± 3.2 a	52.0 ± 1.9 a
Total volatiles			86.0 ± 4.5	86.8 ± 6.3	85.1 ± 6.9	85.6 ± 5.5

^zRI: retention indices from the literature were checked using the HP-5 MS column.

^yRI: retention indices were determined using the HP-5 MS column with *n*-alkanes (C5-C25) as reference materials.

^xData are mean ± SD of four replicates. Different letters indicate significant differences at *P* < 0.01 according to the least significant difference test for the same compound at different temperatures.

GC-MS = gas chromatography-mass spectrometry; TIC = total ion chromatogram.

carefully harvesting different vegetative organs for different volatile profiles.

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Fig. 4. A principal components analysis of the correlation between volatile compounds from fresh leaves of *G. bicolor* and temperature treatment ($n = 4$). (See Supplemental Fig. 2. for a zoom into the volatile compounds with small loading values for the first and second principal components.)

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Supplemental Fig. 1. The volatile compounds with small loading values of the PC1 and PC2 in two vegetative parts of *G. bicolor*.

Supplemental Fig. 2. The volatile compounds with small loading values of PC1 and PC2 at different temperature conditions from fresh leaves of *G. bicolor*.