

1 **SUPPLEMENTARY MATERIAL**

2 **Comparative study of volatile components from male and female**
3 **flower buds of *Populus × tomentosa* by HS-SPME-GC-MS**

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28 **ABSTRACT**

29 The differences of volatile components in male (MFB) and female flower buds (FFB) of
30 *Populus × tomentosa* were analysed and compared by HS-SPME with GC-MS for the first
31 time. A total of 34 compounds were identified. Two clusters were clearly divided into male
32 and female by hierarchical clustering analysis. Both the male and female flower buds showed
33 methyl salicylate (22.83% and 24.09%, respectively) and 2-hydroxy-benzaldehyde (10.05%
34 and 12.41%, respectively) as the main volatile constituents. The content of
35 2-cyclohexen-1-one, benzyl benzoate, and methyl benzoate in FFB was remarkably higher
36 than in MFB. In contrast, the content of ethyl benzoate in MFB was greater than that in FFB.
37 The phenomena showed the characteristic differences between MFB and FFB of *P. ×*
38 *tomentosa*, which enriched the basic studies on dioecious plant.

39 **KEYWORDS:** *Populus × tomentosa* Carrière; dioecious plants; flower buds; volatile
40 components; HS-SPME-GC-MS.

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46 **Experimental**

47 *Plant materials and chemicals*

48 The fresh mature MFB and FFB samples (**Figure S1**) of *P. × tomentosa* were
49 collected on March 14, 2017 near the Sanyuan bridge which is located at 39°57'37.31"
50 north latitude, 116°27'28.53" east longitude, and an altitude of 44 m (Beijing, China).
51 The spieces were identified by Prof. Zhimao Chao (Institute of Chinese Materia
52 Medica, China Academy of Chinese Medical Science). Six voucher specimens
53 (labeled M2, M7, M13, F9, F10, and F13) were deposited at the 1016 room of
54 Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences,
55 Beijing, China. The sexuality of the samples had already been clearly marked in April,
56 2015, and it could be further distinguished by the sepals on the buds on March 14,
57 2017.

58 ***General Experimental Procedures***

59 Headspace sampling was done using AOC-5000 solid-phase micro-extraction (SPME)
60 device coated with 75 μm /carboxen/PDMS fibre (Supelco, USA). Each sample was
61 weighed, placed in screw-type glass bottle (15 mL), and sealed by
62 polytetrafluoroethylene (PTFE) gasket. The temperature increased from 25°C to 80°C
63 within 2 min. The fiber by aging treatment was inserted into the bottle, extracted for
64 35 min at 80°C, pulled out, transferred to the injection port of the GC/MS systems
65 immediately, and desorbed for 1 min.

66 GC/MS was performed with a Shimadzu QP 2010 gas chromatography-mass
67 spectrometry (GC-MS) (Shimadzu, Japan) equipped with a VF-WAX ms elastic
68 quartz capillary column (30 m \times 0.25 mm I.D., 0.25 μm film thickness). The oven
69 temperature programmed from 40°C for 5 min, 40°C to 180°C at 4°C/min, 180°C to
70 270°C at 40°C/min, and 270°C for 2 min. The injector temperature was 250°C. The
71 flow rate of carrier gas (Helium) was 1.0 mL/min. The injection volume was 0.5 μL
72 (10% hexane solution) and the split ratio maintained 1:20. The analysis was
73 performed in EI mode (70 eV). The ion source and transfer line temperature were set
74 at 200°C and 220°C, respectively. The mass range was set at m/z 32~400.

75 Identification of the chemical constituents was confirmed by comparison of the
76 mass spectral data with published data, computer-supported mass spectral library
77 (NIST 08, NIST 08s, NIST 147, NIST 27, and WILEY 9), and professional analysis.
78 Moreover, the relative content of each compound was determined by peak area
79 normalization according to the total ion chromatogram by GC-MS.

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88 Table S1. Chemical composition of volatiles from MFB and FFB of *P. × tomentosa*

No	t/min*	compound	molecular formula	Relative mass fraction/%							
				MFB				FFB			
				M2	M7	M13	M	F9	F10	F13	F
1	11.80	benzaldehyde	C ₇ H ₆ O	1.95	0.91	1.56	1.47	2.96	2.47	3.49	2.97
2	14.40	2-cyclohexen-1-one	C ₆ H ₈ O	2.77	2.79	2.09	2.55	7.83	8.66	14.36	10.28
3	15.38	2-hydroxy-benzaldehyde	C ₇ H ₆ O ₂	9.10	8.50	12.54	10.05	13.37	12.46	11.41	12.41
4	16.33	α -methyl- α -[4-methyl-3-pentenyl]- oxiranemethanol	C ₁₀ H ₁₈ O ₂	1.25	0.28	1.73	1.09	0.60	0.55	0.67	0.61
5	16.60	1,2-cyclohexanediol	C ₆ H ₁₂ O ₂	1.41	1.75	2.56	1.91	2.50	0.63	0.82	1.32
6	17.42	methyl benzoate	C ₈ H ₈ O ₂	0.21	0.20	0.20	0.20	0.69	1.49	0.83	1.00
7	17.80	nonanal	C ₉ H ₁₈ O	5.36	4.58	2.36	4.10	5.82	4.28	5.79	5.30
8	18.32	phenylethyl alcohol	C ₈ H ₁₀ O	1.52	0.55	0.77	0.95	0.66	0.64	0.41	0.57
9	20.06	(E)-2-nonenal	C ₉ H ₁₆ O	0.22	0.27	0.32	0.27	0.17	0.15	0.15	0.16
10	20.47	ethyl benzoate	C ₉ H ₁₀ O ₂	4.48	2.84	2.38	3.23	0.41	0.53	0.56	0.50
11	20.67	1-nonanol	C ₉ H ₂₀ O	-	-	-	-	0.68	0.53	0.66	0.62
12	20.98	naphthalene	C ₁₀ H ₈	0.33	0.41	0.44	0.39	0.13	0.09	0.08	0.10
13	21.41	methyl salicylate	C ₈ H ₈ O ₃	18.91	27.97	21.60	22.83	24.85	25.04	22.38	24.09
14	21.82	decanal	C ₁₀ H ₂₀ O	1.37	1.06	0.74	1.06	1.00	0.55	0.79	0.78
15	23.96	(E)-2-decenal	C ₁₁ H ₂₀ O	0.91	0.41	0.78	0.70	0.36	0.67	0.48	0.50
16	24.20	ethyl 2-hydroxy-benzoate	C ₉ H ₁₀ O ₃	0.83	1.22	1.06	1.04	0.26	0.32	0.34	0.31
17	24.89	1-dodecene	C ₁₂ H ₂₄	0.37	0.39	0.28	0.35	0.14	0.17	0.22	0.18
18	25.17	tetradecane	C ₁₄ H ₃₀	1.48	1.27	0.93	1.23	0.86	0.50	0.87	0.74
19	25.55	undecanal	C ₁₁ H ₂₂ O	0.48	0.38	0.38	0.41	0.39	0.22	0.38	0.33
20	27.26	2-methoxy-3-(2-propenyl)-phenol	C ₁₀ H ₁₂ O ₂	0.04	0.07	0.09	0.07	1.31	1.23	1.05	1.20
21	27.83	copaene	C ₁₅ H ₂₄	2.42	4.42	3.43	3.42	1.83	1.91	1.50	1.75
22	28.39	1-tetradecene	C ₁₄ H ₂₈	2.37	1.02	1.24	1.54	0.89	0.96	1.23	1.03
23	29.10	β -caryophyllene	C ₁₅ H ₂₄	4.65	2.33	3.91	3.63	3.27	2.19	3.06	2.84
24	29.76	2,6-dimethyl-6-(4-methyl-3-pentenyl)-bicycl	C ₁₅ H ₂₄	7.66	2.86	6.64	5.72	6.38	4.90	6.14	5.81

o[3.1.1]hept-2-ene											
25	3132	1-dodecanol	C ₁₂ H ₂₆ O	-	-	6.20	2.07	1.28	0.72	1.09	1.03
26	3193	heptadecane	C ₁₇ H ₃₆	2.66	1.74	2.75	2.38	0.72	0.96	0.91	0.86
27	3433	3(Z)-hexen-1-yl benzoate	C ₁₃ H ₁₆ O ₂	0.67	0.77	0.55	0.66	0.66	1.15	1.10	0.97
28	3458	hexyl benzoate	C ₁₃ H ₁₈ O ₂	1.34	1.46	1.22	1.34	1.03	1.91	1.88	1.61
29	3553	tetradecanal	C ₁₄ H ₂₈ O	1.47	1.89	1.58	1.65	1.03	0.21	0.91	0.72
30	3850	hexadecanal	C ₁₆ H ₃₂ O	0.45	0.20	0.42	0.36	0.47	0.29	0.28	0.35
31	4020	benzyl benzoate	C ₁₄ H ₁₂ O ₂	1.70	0.81	1.10	1.20	3.37	9.28	2.93	5.19
32	4085	octadecanal	C ₁₈ H ₃₆ O	0.21	0.19	0.29	0.23	0.25	0.33	0.12	0.23
33	4111	6,10,14-trimethyl-2-pentadecanone	C ₁₈ H ₃₆ O	0.65	0.68	0.84	0.72	0.79	0.71	0.62	0.71
34	4288	heneicosane	C ₂₁ H ₄₄	0.65	0.44	0.32	0.47	0.53	0.55	0.45	0.51

89 * The retention time was based on TIC of male flower bud of *P. × tomentosa* (M7).

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92 Table S2. Compound classes identified in the volatiles of MFB and FFB of *P. ×*
 93 *tomentosa*

Compound class	Relative mass fraction/%							
	MFB				FFB			
	M2	M7	M13	M̄	F9	F10	F13	F̄
alkane	4.79	3.45	4.00	4.08	2.11	2.01	2.23	2.11
alkene	17.47	11.02	15.5	14.66	12.51	10.13	12.15	11.61
aldehyde	21.52	18.39	20.97	20.3	25.82	21.63	23.8	23.75
alcohol	4.18	2.58	11.26	6.02	5.72	3.07	3.65	4.15
ketone	3.42	3.47	2.93	3.27	8.62	9.37	14.98	10.99
ester	28.14	35.27	28.11	30.5	31.27	39.72	30.02	33.67
aromatic	41.08	45.71	43.51	43.43	49.7	56.61	46.46	50.92

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MFB

FFB

Figure S1. The samples of *P. x tomentosa* (male buds in the left, female buds in the right)

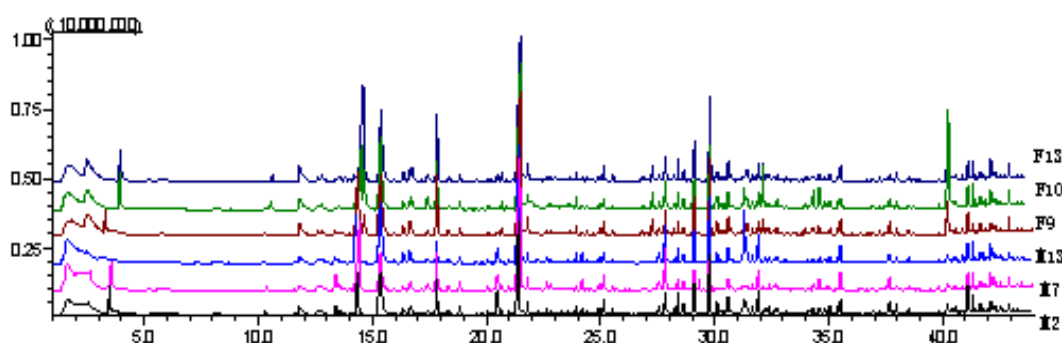


Figure S2. The total ion chromatogram of volatile components from the flower buds of *P. x tomentosa*

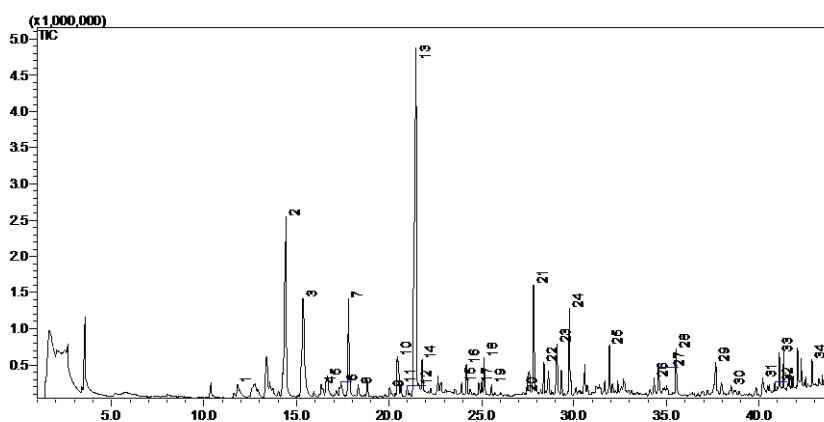
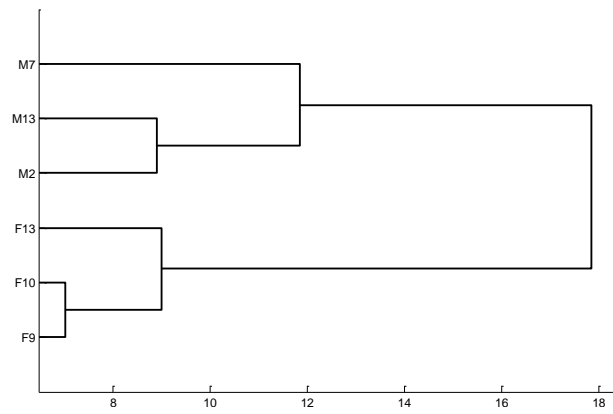


Figure S3. The total ion chromatogram of male flower bud of *P. x tomentosa* (M7)



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108 Figure S4. Hierarchical clustering dendrogram of the flower buds of *P. × tomentosa*

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(Ward's method)

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