Pak. J. Bot., 52(2): 375-382, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-2(31)

DYNAMICS OF VOLATILE ORGANIC COMPOUNDS IN MASSON PINE (*PİNUS MASSONİANA* LAMB.) SEEDLING NEEDLES UNDER DROUGHT STRESS

WENXUAN QUAN[1, 2], CHAO ZHAO[1,2] AND GUIJIE DING[1*]

[1]*Guizhou Provincial Forest Resources and Environment Research Centre / Key Laboratory of Plateau-mountain Forest Cultivation, Guizhou University, Guiyang 550025, China*
[2]*Key Laboratory of Mountainous Environmental Protection, Guizhou Normal University, Guiyang 550001, China*
[*]*Corresponding author's email: gjdinggzu@126.com*

Abstract

The aim of this study is to evaluate whether volatile organic compounds (VOCs) of Masson pine adapted their physiological responses under drought stress or not. The needle length, root length, root/shoot ratio and the VOCs of Masson pine seedlings under drought stress were investigated by comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS). The results showed that the growth index was enhanced with the increase of stress strength, and the seedling growth, biomass, needle length, and root length were inhibited compared with the well-watered treatment. The amounts of total VOCs under drought stress were increased, the concentration of terpenoids, acids and esters, under drought stress were higher than the well-watered treatment, but the concentration of alcohols and carbonyl compounds was slightly decreased under the moderate drought treatment. Light drought, moderate drought, and severe drought compared with the well-watered treatment, increased the release of VOCs by 49, 50, and 49%, respectively. The results show a key role of the VOCs released in the needles and therefore play a possible key role in drought resistance. This study provides evidence that the needles of Masson pine release more VOCs to adapt to drought conditions.

Key words: Masson pine; Seedlings; Drought; VOCs; GC×GC-TOFMS.

Abbreviations: VOCs: volatile organic compounds; 2D-GC×GC/TOFMS: comprehensive two-dimensional gas chromatography time of flight mass spectrometry; SWC: soil water content; WW: well-watered; LD: light drought; MD: moderate drought; SD: severe drought; SPME: solid-phase microextraction; The mean ± SD: the mean and standard deviation; RSD: relative standard deviation; TIC: total ion chromatogram

Introduction

The Masson pine (*Pinus massoniana* Lamb.) has been considered one of the pioneer tree species adapted to the Red Earth Area of subtropical China (Zhang *et al*., 2013). The species is a common tree in plantation forestry that replaces or compensates for the loss of the natural forest, it is a fast-growing tree widely grown in central and southern China (Chau & Lo, 1980; Chen *et al*., 2015a). Chinese rosin is obtained mainly from the turpentine of this pine, the wood is widely used to make pulp for the paper industry, and the pine needles were used as medicine for a long time (Feng *et al*., 2010; Yang *et al*., 2010; Zhang *et al*., 2011). Because of this, it has become an important tree for afforestation in South China.

Masson pine is the major pioneer species for vegetation reforestation due to its strong adaptability, preferable tolerance to drought and infertility in southern China. However, most of those forests show poor stand structure and low productivity due to human or natural factors, so that the composition of forest ecosystems is reduced, covered with exiguous vegetation and subjected to severe soil erosion, resulting in the poor eco-efficiency of such forests in the conservation of water and soils. Accordingly, the water absorption capacity of the Masson pine root system, as well as mycorrhizal fungi, have been suggested as likely explanations of this adaptation, and Masson pine has become one of the major pioneer species of vegetation reforestation. It has strong resistance to the drought, low phosphorous, acid rain, and the other extreme conditions (Wang *et al*., 2013; Hu *et al*., 2014; Li *et al*., 2017; Zhang *et al*., 2017).

The biosynthesis and storage' of volatile organic compounds (VOCs), is much more important issue when working with the VOC storing pine needles (Loreto & Schnitzler, 2010). VOCs play an important role in the stress resistance of the coniferous trees (Su *et al*., 2009; Ioannou *et al*., 2014; Quan & Ding, 2017). Several recent studies have focused on the chemical nutrient elements and biomedical prospects of Masson pine seedlings (Mohamed *et al*., 2014; Guan *et al*., 2015; Wang *et al*., 2015). Some scientific papers dedicated the comparative proteomic analyses on Masson pine under the acid rain stress (Hu *et al*., 2014). Therefore, it's necessary to analyze the composition and changes of needle VOCs to provide scientific guidance for the artificial afforestation of Masson pine.

Drought is an environmental constraint that seriously affects the plant growth and productivity, hindering the development of plant production, and therefore monitoring the VOCs changes in Masson pine seedling under drought conditions is necessary. Therefore, the objective of this research aimed to evaluate the growth and needle VOCs responses of Masson pine seedlings to different level of drought stress conditions.

Materials and Methods

Study site: The seeds of Masson pine used in this experiment were from the same mother tree in Majiang County, Guizhou Province. Sowing time was in December 2015, and the one-year-old Masson pine seedlings were used for the drought stress in April 2016, which were potted in the greenhouse (26°35′ N, 106°43′ E).

Treatments: The soil moisture content in the pot experiment was determined by the method of Zhou *et al*., (2015). Soil water content (SWC) in this study was expressed as follows:

$$SWC\ (g\ g^{-1}) = (W1-W2)/W2$$

where W1 is the fresh weight of soil, and W2 is the dry weight of soil (Watt *et al*., 1994).

The soil moisture content was maintained by weighing method (Quan & Ding, 2017). The experimental seedlings were divided into 4 groups for processing: well-watered (WW, 70-80% field moisture capacity), light drought (LD, 60-70% field moisture capacity), moderate drought (MD, 50-60% field moisture capacity), and severe drought (SD, 35-50% field moisture capacity) (Xu & Zhou, 2011). The measurements were carried out 60 d after the seedlings were subjected to relatively long-term drought stress treatment, and each group had 12 pots. After treatment, the saplings were used for this study. The needles were harvested and analyzed at the same time. The shoots and roots of the seedlings of each treatment were harvested separately to determine the root/shoot ratio (Chen *et al*., 2015b).

Pre-treatment method: Seedling needles were cut into pieces, and SPME procedures followed the method with some minor changes (Xiang *et al*., 2015). The samples (20 mg) were added to the headspace bottle and phenylethyl acetate solution (1 μL) was also added. The headspace bottle was placed in a dry heat block adjusted to 80.0°C for 10 min, and then the SPME fiber (Supelco, PDMS/DVB/CAR) was manually inserted into the sample bottle headspace for 15 min (Xiang *et al*., 2014). The SPME-coated fiber with trapped VOCs was introduced manually into the GC×GC two-dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS, ZOEX, ZX-1) injection port to allow desorption. The bottle with cap as the headspace bottle (Agilent, 22mL), and phenylethyl acetate as the internal standard (Sigma-Aldrich, purity >99.0%). All measurements were made 3 repetitions per treatment.

Instrument condition: The GC×GC-TOFMS were as follows: DB-1MS phase (30 m × 0.25 mm, 1.00 μm), second capillary column was DB-wax phase (1.2 m × 0.1 mm, 0.10 μm); temperature program: 60°C (held for 2 min) to 240°C (held for 1 min), increased at 3°C min^{-1}; inlet pressure: 30 psi (hold 2 min) to 48 psi (hold 1 min), 0.3 psi min^{-1}, and the carrier gas was helium; running time: 63 min, inlet temperature: 250°C, injection mode: splitless, purge time: 3.5 min, modulation period: 9 s, continuous period: 300 ms, the hot jet: 375°C and the pressures is 40 psi, the cold jet nitrogen flow rate:18 L min^{-1}. The characteristics of the TOFMS system were as follows: ion source: 70 eV EI; ion source temperature: 280°C; transfer line temperature: 300°C; mass range: 45-450 amu; FWHM resolution: 4000; and acquisition rates: 100 Hz.

Statistical analysis: The mean and standard deviation (SD) values of three replicates were calculated. Clustering analysis using R to draw a heatmap from VOCs data (Eisen *et al*., 1998). The data were processed using the Zoex GC-Image system. The qualitative identification of the VOCs of the Masson pine was retrieved by the standard spectral library (NIST2.0 mass spectral library and Wiley08 mass spectral library). The quantitative analysis use the internal standard method (Peng *et al*., 2004; Xiang *et al*., 2015), in which phenylethyl acetate served as the internal standard without considering calibration factors.

Results

Growth status for seedlings of Masson pine under drought stress: The experimental drought stress significantly reduced the needle length, root length and biomass. In general, the seedling biomass for each treatment was affected by the drought stress, except that no significant difference in the seedling height for Masson pine seedlings occurred between the well-watered and other treatments (Table 1).

Drought stress significantly inhibits the increase of seedling biomass, the aboveground, belowground and total biomass of seedlings under drought stress is lower than those of the well-watered treatment. The root length was the highest under light drought stress (22.10 cm), and the root/shoot ratio was the highest under moderate drought stress. moderate drought stress had been found to promote root/shoot ratio and hence improve the water-use efficiency (Franco *et al*., 2011; Wurzburger & Miniat, 2014), which may be one of the major reasons that the moderate drought promote the root/shoot ratio.

The VOCs of Masson pine seedling needle samples: To confirm the feasibility of the VOCs determination method used in this experiment, the qualitative and quantitative verification of VOCs was also carried out. The chromatogram is shown in Fig. 1.

For the chemometric analysis, the concentrations of 68 VOCs were analyzed (Table 2). The comprehensive analyses of the needle VOCs showed significant quantitative and qualitative differences between the samples. The terpenoids dominated the needle VOCs, with the presence of α-pinene and caryophyllene characterizing most of the needle VOCs.

Table 1. The Masson pine seedlings status under different drought stress treatments.

Treatments	Needle length (cm)	Root length (cm)	Seedling height (cm)	Shoot dry weight (g)	Root dry weight (g)	Root/shoot ratio
WW	9.55 ± 0.88a	16.45 ± 0.66b	19.27 ± 2.58a	1.82 ± 0.02a	0.59 ± 0.09a	0.32 ± 0.05b
LD	7.11 ± 0.76b	22.10 ± 1.42a	18.98 ± 1.93a	1.07 ± 0.09b	0.44 ± 0.04b	0.41 ± 0.02a
MD	5.87 ± 0.72c	12.51 ± 1.67c	18.33 ± 1.60a	1.06 ± 0.11b	0.49 ± 0.06ab	0.46 ± 0.07a
SD	5.47 ± 0.45c	9.79 ± 0.89d	16.87 ± 1.92a	0.99 ± 0.04b	0.39 ± 0.05b	0.39 ± 0.04ab

Mean ± standard deviation (n=12). Values sharing same letters differ non-significantly (p>0.05)

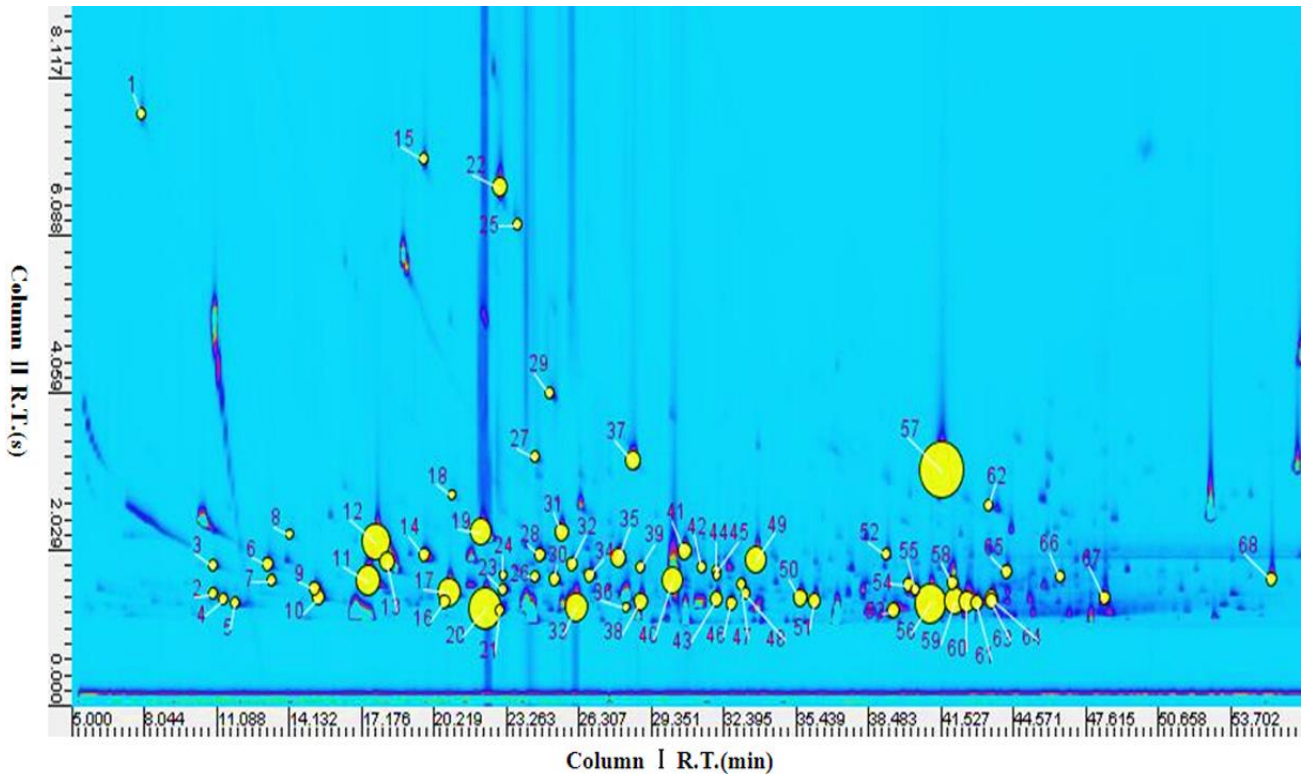

Fig. 1. Total ion chromatogram (TIC) of VOCs from the Masson pine needles.

This is an earlier investigation by 2D-GC×GC-TOFMS on the chemical composition of Masson pine needle VOCs under drought stress (Quan & Ding, 2017). In the chemical composition, we classified these VOCs following the metabolic pathways:

(1) The groups of terpenoids. Terpenoids are dimers, or combinations of a 5 carbon precursor called isoprene, are a large and diverse class. The terpenoids group is a basic VOC substance in pine needles, and it is the main flavor component of pine needles. The terpenoids concentration of LD, MD, and SD are 1.67, 2.03, and 2.58 times of the WW treatment. Terpene hydrocarbons include *α*-pinene, camphene, *β*-thujene, terpinolen, *β*-cubebene, caryophyllene, *β*-caryophyllene, isoledene, *β*-cadinene, and calamenene. The oxygenated terpenes include borneol, *α*-terpieol, isoborneol, *β*-cyclocitral, bornyl acetate, cedran-8-ol, and manoyl oxide. The norsoprenoids include α-ionone, *β*-ionone, dihydroactinidiolide, and geranyl acetone.

(2) The groups of acids and esters. The acids and esters group is an important group of VOCs in pine needles, and the content of each component has a great influence on Masson pine; the acids and esters group is represented mainly by undecanoic acid, 10-methyl-, methyl ester and benzeneacetic acid, methyl ester. The acids and esters concentration of LD, MD, and SD were 1.51, 1.52, and 3.65 times of the WW treatment, respectively. This group included acetic acid, 2-methyl-2-butenoic acid, hexanoic acid, methyl ester, (E)-3-hexenoic acid, methyl ester, (E)-3-hexenoic acid, (E)-2-hexenoic acid, 2,4-hexadienoic acid, methyl ester, methyl (3E)-3-nonenoate, butanedioic acid, methyl-, dimethyl ester, benzoic acid, methyl ester, octanoic acid, methylester, benzoic acid, benzeneacetic acid, methyl ester, nonanoic acid, methyl ester, acetic acid, 2-phenylethyl ester, decanoic acid, methyl ester, nonanoic acid 9-oxo-methyl ester, undecanoic acid 10-methyl-methyl ester, (Z)-hex-3-enyl benzoate, methyl tetradecanoate, hexadecanoic acid, methyl ester, and thymol methyl ether.

(3) The groups of alcohols. The alcohols group is an important VOC substance in pine needles, and represented mainly by 1-octanol. The alcohols concentration of LD, MD, and SD were 1.25, 0.70, and 2.67 times of the WW treatment. The alcohols included 1-penten-3-ol, 2-(Z)-pentenol, 3-(Z)-hexen-1-ol, 1-hexanol, 1-nonen-3-ol, benzenemethanol, 2-ethyl-1-hexanol, 1-octanol, benzeneethanol, and 6-methyl-5-hepten-2-ol.

(4) The groups of carbonyl compounds. The carbonyl compounds group is an important VOCs substance in pine needles, they are emitted from a variety of plants, and they affect plant growth. Most parts of carbonyl compounds can be metabolized into organic acids, amino acid, etc., by the endoenzymes in needles. The carbonyl compounds concentration of LD, MD, and SD were 1.17, 0.76, and 1.45 times of the WW treatment. The compounds included 1-penten-3-one, 2-methyl butanal, 2-(E)-pentenal, 2-methyl-4-pentenal, hexanal, 2-hexenal, (E,E)-2,4-hexadienal, benzaldehyde, 5-hepten-2-one, 6-methyl-, octanal, benzeneacetaldehyde, and decanal.

(5) The others. The other components were detected in lower percentages in needles, not exceeding 1%. It includes 2-ethylfuran, pyridine, and 5-ethyl-2(5H)-furanone.

Table 2. Qualitative and quantitative analysis of volatile components in needles of Masson pine seedlings.

ID	Compound name	Formula	Relative conc.± SD (ng g^{-1})			
			WW	LD	MD	SD
1.	Acetic acid	$C_2H_4O_2$	66.82 ± 4.73	191.56 ± 13.65	209.11 ± 11.37	368.06 ± 25.47
2.	1-Penten-3-one	C_5H_8O	41.89 ± 2.56	216.16 ± 15.38	41.12 ± 4.15	48.22 ± 4.11
3.	1-Penten-3-ol	$C_5H_{10}O$	61.18 ± 3.36	158.41 ± 9.98	66.86 ± 4.55	126.06 ± 10.72
4.	2-Methyl butanal	$C_5H_{10}O$	59.54 ± 3.28	42.77 ± 3.69	67.81 ± 5.61	21.13 ± 1.91
5.	2-Ethylfuran	C_6H_8O	18.45 ± 1.15	14.31 ± 0.91	11.58 ± 1.79	12.88 ± 1.38
6.	Pyridine	C_5H_5N	153.23 ± 8.43	34.07 ± 2.15	118.20 ± 9.04	116.83 ± 10.93
7.	2-(E)-Pentenal	C_5H_8O	76.21 ± 4.29	184.58 ± 13.63	83.96 ± 6.71	177.06 ± 8.05
8.	2-(Z)-Pentenol	$C_5H_{10}O$	35.40 ± 1.97	340.88 ± 31.48	29.69 ± 3.02	33.32 ± 3.83
9.	2-Methyl-4-pentenal	$C_6H_{10}O$	213.44 ± 15.74	235.10 ± 24.81	159.57 ± 16.85	222.83 ± 15.94
10.	Hexanal	$C_6H_{12}O$	317.90 ± 27.48	240.51 ± 25.15	166.58 ± 13.13	252.66 ± 18.48
11.	2-Hexenal	$C_6H_{10}O$	3834.42 ± 326.23	5630.97 ± 454.75	3513.49 ± 338.92	3760.46 ± 319.64
12.	3- (Z)-Hexen-1-ol	$C_6H_{12}O$	5764.04 ± 441.58	8855.35 ± 657.89	2664.99 ± 282.22	7154.81 ± 608.16
13.	1-Hexanol	$C_6H_{14}O$	743.29 ± 43.85	715.36 ± 65.07	308.97 ± 31.01	1366.82 ± 116.18
14.	(E,E)-2,4-Hexadienal	C_6H_8O	239.13 ± 14.11	399.47 ± 26.17	169.60 ± 12.53	189.47 ± 17.10
15.	2-Methyl-2-Butenoic acid	$C_5H_8O_2$	117.01 ± 6.92	51.73 ± 4.26	30.87 ± 3.15	457.92 ± 48.71
16.	Hexanoic acid, methyl ester	$C_7H_{14}O_2$	135.44 ± 7.99	120.72 ± 7.61	172.08 ± 12.70	279.69 ± 23.77
17.	(E)-3-Hexenoic acid, methyl ester	$C_7H_{12}O_2$	3446.98 ± 313.37	2790.74 ± 275.82	4012.58 ± 372.86	2494.92 ± 184.60
18.	5-ethyl-2(5H)-Furanone	$C_6H_8O_2$	31.35 ± 1.87	54.71 ± 4.45	30.85 ± 3.10	28.17 ± 3.30
19.	Benzaldehyde	C_7H_6O	2084.37 ± 122.98	1246.48 ± 88.53	824.23 ± 66.05	5218.16 ± 407.02
20.	*α*-Pinene	$C_{10}H_{16}$	8289.26 ± 589.07	10323.38 ± 850.37	13924.24 ± 846.85	18502.22 ± 1143.17
21.	Camphene	$C_{10}H_{16}$	51.83 ± 3.16	44.69 ± 3.82	105.94 ± 7.21	140.08 ± 10.93
22.	(E)-3-Hexenoic acid	$C_6H_{10}O_2$	984.46 ± 59.18	221.11 ± 23.93	228.89 ± 15.56	252.02 ± 18.66
23.	5-Hepten-2-one, 6-methyl-	$C_8H_{14}O$	106.20 ± 7.27	168.93 ± 11.64	265.93 ± 19.08	199.35 ± 16.15
24.	1-Nonen-3-ol	$C_9H_{18}O$	39.49 ± 3.43	66.08 ± 5.16	94.30 ± 9.41	170.09 ± 14.78
25.	(E)-2-Hexenoic acid	$C_6H_{10}O_2$	44.95 ± 2.67	15.99 ± 1.21	16.97 ± 1.45	33.27 ± 2.59
26.	2,4-Hexadienoic acid, methyl ester	$C_7H_{10}O_2$	305.53 ± 18.13	271.98 ± 18.13	348.28 ± 31.68	477.59 ± 37.68
27.	Octanal	$C_6H_8O_2$	44.11 ± 2.72	34.07 ± 2.16	53.57 ± 3.64	61.93 ±5.02
28.	Methyl (3E)-3-nonenoate	$C_{10}H_{18}O_2$	50.56 ± 2.98	57.77 ± 3.64	28.52 ± 1.34	32.24 ±2.71
29.	Benzenemethanol	C_7H_8O	212.52 ± 12.54	180.33 ± 11.46	95.85 ± 4.52	214.15 ± 18.35
30.	2-Ethyl-1-hexanol	$C_8H_{18}O$	74.96 ± 4.12	46.56 ± 3.93	20.82 ± 1.42	140.50 ± 12.38
31.	Benzeneacetaldehyde	C_8H_8O	134.45 ± 8.87	64.78 ± 4.18	76.05 ± 5.30	129.68 ± 11.50
32.	Butanedioic acid, methyl-, dimethyl ester	$C_7H_{12}O_4$	493.29 ± 31.08	230.97 ± 14.55	260.39 ± 15.49	745.14 ± 60.36
33.	*β*-Thujene	$C_{10}H_{16}$	186.71 ± 15.76	199.65 ± 13.58	668.75 ± 47.48	782.28 ± 58.35
34.	1-Octanol	$C_8H_{18}O$	2746.70 ± 193.04	1731.25 ± 119.07	3092.07 ± 289.54	15238.86 ± 1676.27
35.	Benzoic acid, methyl ester	$C_8H_8O_2$	137.18 ± 9.64	175.73 ± 12.07	169.11 ± 15.01	738.54 ± 81.24
36.	Terpinolen	$C_{10}H_{16}$	883.65 ± 75.67	5184.10 ± 426.61	8229.14 ± 587.27	11018.93 ± 910.81
37.	Benzeneethanol	$C_8H_{10}O$	21.24 ± 2.17	23.82 ± 1.51	39.81 ± 3.83	528.66 ± 58.15
38.	Octanoic acid, methylester	$C_9H_{18}O_2$	855.51 ± 67.05	1047.14 ± 65.97	1306.10 ± 72.73	1352.33 ± 128.76
39.	6-Methyl-5-hepten-2-ol	$C_8H_{16}O$	619.46 ± 54.07	803.42 ± 51.62	840.91 ± 68.70	2608.63 ± 276.94
40.	Benzoic acid	$C_7H_6O_2$	89.61 ± 9.93	53.33 ± 3.46	64.80 ± 5.60	109.97 ± 12.10
41.	Benzeneacetic acid, methyl ester	$C_9H_{10}O_2$	2540.45 ± 239.72	4616.18 ± 391.82	4971.10 ± 352.96	13079.36 ± 1238.73
42.	Borneol	$C_{10}H_{18}O$	571.82 ± 31.45	2120.96 ± 143.62	1182.08 ± 79.93	4279.69 ± 410.77
43.	Decanal	$C_{10}H_{20}O$	87.74 ± 4.83	40.19 ± 3.53	48.67 ± 4.46	223.46 ± 20.56
44.	*α*-Terpieol	$C_{10}H_{18}O$	299.84 ± 16.69	315.50 ± 29.88	505.75 ± 45.91	398.68 ± 36.68
45.	Isoborneol	$C_{10}H_{18}O$	10.05 ± 0.78	79.13 ± 5.89	50.96 ± 4.62	160.63 ± 14.58
46.	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	18.48 ± 1.54	33.05 ± 2.18	20.15 ±2.43	77.96 ± 7.35
47.	*β*-Cyclocitral	$C_{10}H_{16}O$	108.85 ± 9.49	65.63 ± 5.13	71.57± 6.08	358.13 ± 32.96
48.	Thymol methyl ether	$C_{11}H_{16}O$	111.78 ± 8.42	84.23 ± 6.31	63.78 ± 5.53	77.74 ± 7.15
49.	Acetic acid, 2-phenylethyl ester	$C_{10}H_{12}O_2$	78.48 ± 6.12	19.06 ± 2.21	66.25 ± 5.70	56.43 ± 5.19
50.	Bornyl acetate	$C_{12}H_{20}O_2$	613.44 ± 47.95	322.11 ± 21.29	445.48 ± 41.63	1129.20 ± 113.89
51.	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	348.92 ± 27.62	360.07 ± 24.68	455.56 ± 32.34	1702.02 ± 156.59
52.	Nonanoic acid, 9-oxo-methyl ester	$C_{10}H_{18}O_3$	84.02 ± 7.55	255.25 ± 26.08	126.94 ± 9.01	124.42 ± 11.45
53.	*β*-Cubebene	$C_{15}H_{24}$	322.61 ± 35.16	960.32 ± 60.50	1950.54 ± 88.49	1663.75 ± 143.07
54.	*α*-Ionone	$C_{13}H_{20}O$	198.82 ± 15.51	116.49 ± 8.34	111.47 ± 5.91	103.89 ± 6.03
55.	Geranyl acetone	$C_{13}H_{22}O$	99.88 ± 4.79	90.51 ± 6.70	109.72 ± 7.79	84.02 ± 4.87
56.	Caryophyllene	$C_{15}H_{24}$	7174.61 ± 659.62	12246.83 ± 971.55	13791.52 ± 979.20	18405.85 ± 1067.54
57.	Manoyl oxide	$C_{20}H_{34}O$	3187.49 ± 348.62	3504.26 ± 421.77	31.24 ± 3.22	11.54 ± 0.77
58.	*β*-Ionone	$C_{13}H_{20}O$	230.76 ± 18.45	207.43 ± 23.07	148.40 ± 11.54	267.86 ± 15.64
59.	*β*-Caryophyllene	$C_{15}H_{24}$	2770.68 ± 162.39	5700.11 ± 459.11	6894.36 ± 489.50	6162.68 ± 357.44
60.	Undecanoic acid, 10-methyl-methyl ester	$C_{13}H_{26}O_2$	1305.25 ± 72.79	6151.26 ± 387.53	4783.23 ± 339.61	15504.62 ± 898.27
61.	Isoledene	$C_{15}H_{24}$	323.85 ± 17.91	689.27 ± 44.42	1041.68 ± 83.96	1358.72 ± 78.81
62.	Dihydroactinidiolide	$C_{11}H_{16}O_2$	44.06 ± 2.42	29.27 ± 1.84	24.53 ± 2.74	67.81 ± 5.93
63.	*β*-Cadinene	$C_{15}H_{24}$	383.38 ± 21.79	867.20 ± 54.63	2134.13 ± 131.52	838.28 ± 60.03
64.	Calamenene	$C_{15}H_{24}$	448.37 ± 34.66	735.83 ± 48.36	1701.03 ± 120.77	1974.05 ± 146.08
65.	(Z)-Hex-3-enyl benzoate	$C_{13}H_{16}O_2$	135.69 ± 7.46	550.20 ± 24.66	155.38 ± 10.13	1097.38 ± 91.21
66.	Cedran-8-ol	$C_{15}H_{26}O$	71.19 ± 5.92	83.53 ± 6.37	84.84 ± 9.02	92.31 ± 8.83
67.	Methyl tetradecanoate	$C_{15}H_{30}O_2$	192.56 ± 17.69	371.56 ± 23.41	206.18 ± 9.64	2808.51 ± 207.83
68.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	202.58 ± 11.24	130.28 ± 8.21	135.44 ± 7.62	1026.70 ± 95.98

Mean ± standard deviation (n=3)

These VOCs include terpenoids, acids and esters, alcohols, carbonyl compounds, etc. Almost every sample of a different treatment was characterized by the abundance of the group of terpenoids, acids and esters, alcohols. *α*-pinene, as well as caryophyllene and terpinolen, were the dominant members of the terpenoids group. Drought stress had a profound impact on the concentration of the terpenoids, acids and esters, which were significantly higher under LD, MD and SD than in WW. However, the concentration of alcohols and carbonyl compounds were slightly decreased under the MD treatment, compared with the WW treatment. Using Ri386 to draw a heatmap from VOCs data (Fig. 2). In the heatmap, we could see that the change of terpenoids was the most obvious under four drought treatments, followed by the groups of acids and esters.

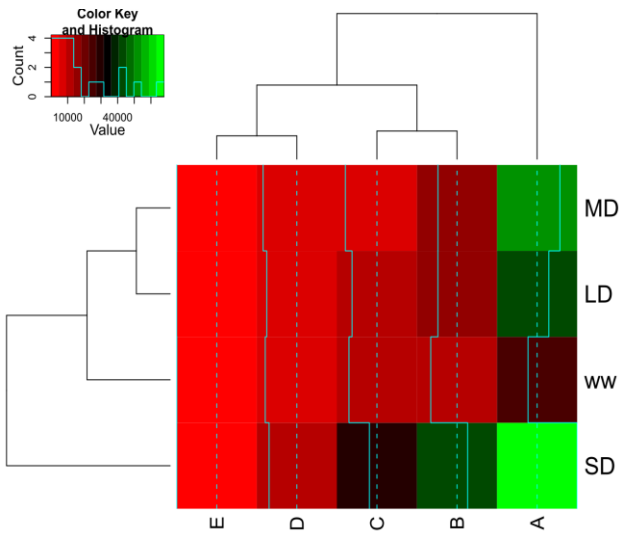

Fig. 2. Clustered image maps of the VOCs of needles in the different treatments. The pine needle VOCs are shown in rows and drought treatments are shown in columns. (A. terpenes, B. acids and esters, C. alcohols, D. carbonyl compounds, E. others).

For LD, MD, and SD compared with WW treatment, the amount of VOCs was increased by 49%, 50%, and 49%, respectively. Some main VOCs, such as 3-(Z)-hexen-1-ol, benzaldehyde, *α*-pinene, 1-octanol, 6-methyl-5-hepten-2-ol, benzeneacetic acid, methyl ester, borneol, decanoic acid, methyl ester, *β*-cubebene, caryophyllene, *β*-caryophyllene, undecanoic acid 10-methyl-methyl ester, isoledene, calamenene, and methyl tetradecanoate were increased with the deterioration of drought stress. Moreover, only a small number of VOCs were reduced by the drought is deepened, such as (E)-3-hexenoic acid, methyl ester, *α*-ionone, and manoyl oxide. The results showed a key role of the VOCs released in the needles and therefore played a possible key role in drought resistance.

Discussion

Effects of drought stress on plant growth and biomass: Drought disaster is an important factor that restricts the sustainable development of forestry in south China, and drought has devastating effects on many needle trees (Kolb & Robberecht, 1996; Gonzalez-Moro *et al*., 1999; Gruber *et al*., 2010; Swidrak *et al*., 2011; Vacchiano *et al*., 2012), and impacts ecosystems and species richness (Klos *et al*., 2009; Allen *et al*., 2010; Holmstrup *et al*., 2012; Weed *et al*., 2013; Vose *et al*., 2016). However, a recent study implied that moderate drought enhanced the competitive ability of forest trees (Wurzburger & Miniat, 2014).

The drought also induces changes in the cellular biochemistry of seedlings, such as reduced photosynthesis, a reduced growth rate, altered water balance (Pichler & Oberhuber, 2007; Thabeet *et al*., 2009). *Pinus* is a staple genus of tree of high economic value in middle and south China. However, drought stress is a severe factor limiting its growth and reducing its biomass. Some previous studies showed that Scots pine was more affected by summer droughts (Merlin *et al*., 2015). Our results showed that the severe drought condition inhibited the growth of needle and roots.

Effects of drought stress on plant VOCs: The accretion in the concentration of VOCs in Masson pine needles was due to drought stress, as indicated by the unaltered needle length. However, the functioning of the needle tissues did not appear to be altered, as the number of VOCs was not reduced due to the effect of drought stress. When the coniferous trees suffer from the stress, they release VOCs to enhance their defenses, such as *Pinus ponderosa* (Schade & Goldstein, 2001), *Pinus halepensis* (Filella *et al*., 2009), *Pinus pinaster* (Sampedro *et al*., 2011; Blanch *et al*., 2012), and *Pinus sylvestris* (Achotegui-Castells *et al*., 2013; Lundborg *et al*., 2016). The VOCs play a key role in plant resistance (Ruther & Kleier, 2005; Kännaste *et al*., 2009; Su *et al*., 2009; Dicke & Loreto, 2010; Loreto & Schnitzler, 2010; Bracho-Nunez *et al*., 2011; Azeem *et al*., 2013), and moreover, also a possible key role in pollination (Peñuelas *et al*., 2014). The efficacy of terpenoids as the research genetic markers, geographic variation and evolution has been resolved (Hanover, 1992). However, some researchers have implied that terpenoids are frequently used as chemosystematic markers due to the genetic control of their biosynthesis (Baradat & Yazdani, 1988; Incerti *et al*., 2013).

In these VOCs, terpenoids in the Masson pine play a major defensive role (Achotegui-Castells *et al*., 2013), and *α*-pinene is the most abundant metabolites, which is commonly found in the needles (Yatagai & Hong, 1997; Ioannou *et al*., 2014). In this study, the main VOCs were *α*-pinene (12.42%), caryophyllene (12.36%) and terpinolen (7.40%), followed by *β*-caryophyllene (4.14%) and borneol (2.87%).

The advantage of 2D-GC×GC-TOFMS in investigating the plant VOCs: Generally, GC×GC can identify the large range of target analytes; in addition, GC×GC has the higher resolution and sensitivity than one-dimensional gas chromatography and yields structured chromatograms that facilitate the identification of unknown compounds (Hoggard *et al*., 2009). The method resolves the overlapping peaks and interference peak and provides useful identification information (Fig. 3).

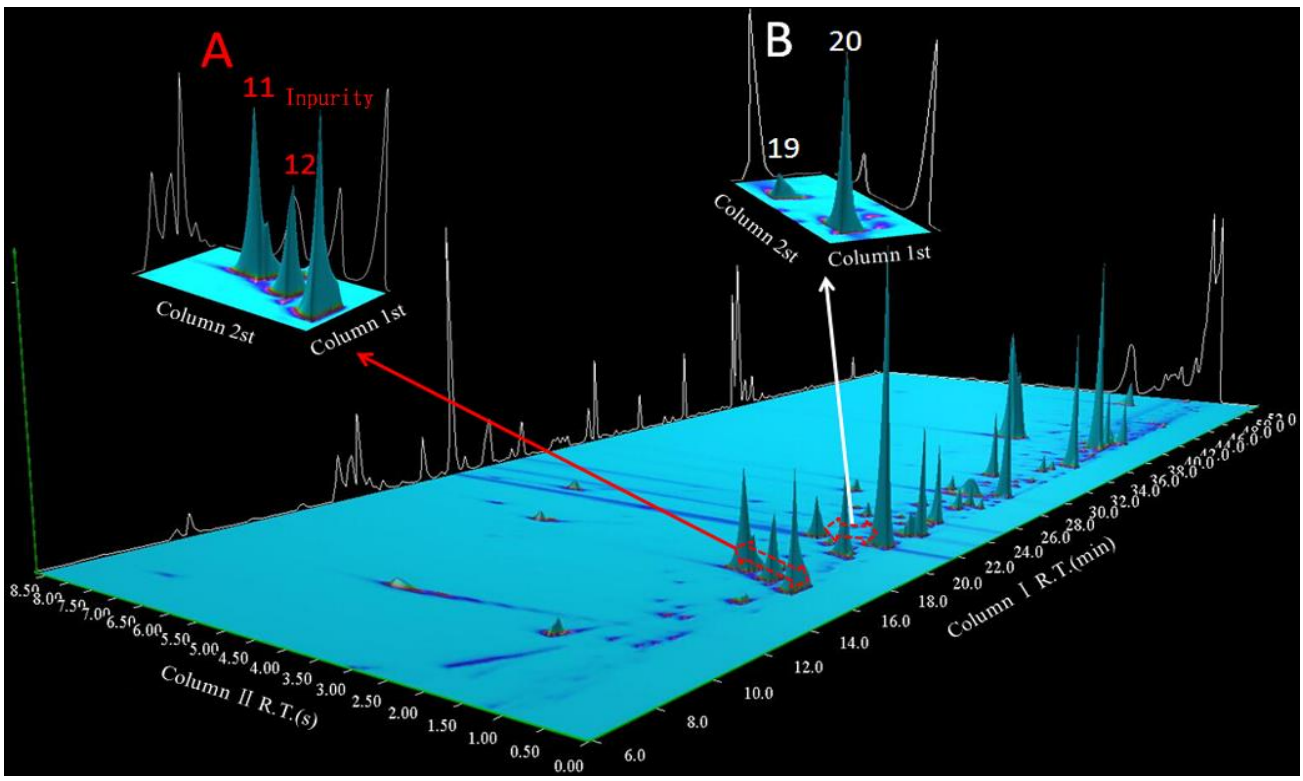

Fig. 3. Surface views showing the use of 2D in the separation of four VOCs, 2-hexenal, 3- (Z)-hexen-1-ol, benzaldehyde and *α*-pinene (A. The peaks (11 and 12) were inseparable and effectively eliminate the interference peak; B. the peaks (19 and 20) were inseparable under the chromatographic conditions used).

In prior studies, solvent extraction and distillation were generally used for extraction of VOCs in Masson pine, and identification by conventional methods based on one-dimensional gas chromatography (Su *et al*., 2009; Liu *et al*., 2015). At present, only a handful number of studies reported using HS-SPME combined with GC×GC-TOFMS technology, these included tobacco (Zhu *et al*., 2005; Ding *et al*., 2013; Xiang *et al*., 2015), wine (Danielle *et al*., 2005; Zhu *et al*., 2007), medicine (Qiu *et al*., 2007), milk (Yue *et al*., 2015), honey (Čajka *et al*., 2007), coffee beans (Humston *et al*., 2009), toxic waste (Vos *et al*., 2011), and essential oils (Jiang *et al*., 2015). The 2D-GC×GC-TOFMS method could be an effective technical means for the identification of VOCs in Masson pine needles, this study provided an insight into changes in the VOCs of coniferous trees as they adapted to drought stress.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (No. 31660200; 31960301), the National Science and Technology support Program during the 12th Five-year Plan Period (No. 2015BAD09B01), the National Key Research and Development Program of China (No. 2017YFD0600302), and Guizhou Provincial Joint Foundation (No. QKH LH [2017]7360; QLKH [2018]11). We thank Drs. Z.M. Xiang and K. Cai (Guizhou Academy of Tobacco Science, Guiyang, P. R. China) for advice on statistics. Thanks also due to Ph. D. Z.N. Yang, Ph. D. J.W. Hu (Guizhou Normal University, Guiyang, P. R. China) and the AJE (American Journal Experts) team, for improving the language.

References

Achotegui-Castells, A., J. Llusia, J.A. Hodar and J. Penuelas. 2013. Needle terpene concentrations and emissions of two coexisting subspecies of Scots pine attacked by the pine processionary moth (*Thaumetopoea pityocampa*). *Acta Physiol. Plant.*, 35: 3047-3058.

Allen, C.D., A.K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D.D. Breshears, E.H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J.H. Lim, G. Allard, S.W. Running, A. Semerci and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecol. Manag.*, 259: 660-684.

Azeem, M., G.K. Rajarao, H. Nordenhem, G. Nordlander and A.K. Borg-Karlson. 2013. *Penicillium expansum* volatiles reduce pine weevil attraction to host plants. *J. Chem. Ecol.*, 39: 120-128.

Baradat, P. and R. Yazdani. 1988. Genetic expression for monoterpenes in clones of *Pinus sylvestris* grown on different sites. *Scand. J. Forest Res.*, 3: 25-36.

Blanch, J.S., L. Sampedro, J. Llusia, X. Moreira, R. Zas and J. Penuelas. 2012. Effects of phosphorus availability and genetic variation of leaf terpene content and emission rate in *Pinus pinaster* seedlings susceptible and resistant to the pine weevil, *Hylobius abietis*. *Plant Biol.*, 14: 66-72.

Bracho-Nunez, A., N.M. Knothe and W.R. Costa. 2011. Root anoxia effects on physiology and emissions of volatile organic compounds (VOC) under short- and long-term inundation of trees from Amazonian floodplains. *Springerplus*, 9: 1-16.

Čajka, T., J. Hajšlová, J. Cochran, K. Holadová and E. Klimánková. 2007. Solid phase microextraction–comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the analysis of honey volatiles. *J. Sep. Sci.*, 30: 534-546.

Chau, K.C. and W.K. Lo. 1980. The *Pinus* scrub community as an indicator of soils in Hong Kong. *Plant Soil*, 56: 243-254.

Chen, Z., H. Shang, J. Cao and H. Yu. 2015a. Effects of ambient ozone concentrations on contents of nonstructural carbohydrates in *Phoebe bournei* and *Pinus massoniana* seedlings in subtropical China. *Water Air Soil Pollut.*, 226: 1-8.

Chen, D.Q., S.W. Wang, B.B. Cao, D. Cao, G.H. Leng, H.B. Li, L.N. Yin, L. Shan and X.P. Deng. 2015b. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Front. Plant Sc*i., 6: 1241. doi:10.3389/fpls.2015.01241.

Danielle, R., W. Peter, S. Jason, A. Malcolm and M. Philip. 2005. Analysis of methoxypyrazines in wine using headspace solid phase microextraction with isotope dilution and comprehensive two-dimensional gas chromatography. *J. Sep, Sci*, 28: 1075-1082.

Dicke, M. and F. Loreto. 2010. Special Issue: Induced biogenic volatile organic compounds from plants. *Trends Plant Sci.*, 15: 154-166.

Ding, Y., L.J. Zhu, S.M. Liu, H.Q. Yu and Y. Dai. 2013. Analytical method of free and conjugated neutral aroma components in tobacco by solvent extraction coupled with comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. *J. Chromatogr. A*., 1280: 122-127.

Eisen, M.B., P.T. Spellman, P.O. Brown and D. Botstein. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci*., 95: 14863-14868.

Feng, S., W.C. Zeng, F. Luo, J. Zhao, Z.R. Yang and Q. Sun. 2010. Antibacterial activity of organic acids in Aqueous extracts from pine needles (*Pinus massoniana* Lamb.). *Food Sci. Biotechnol.*, 19: 35-41.

Filella, I., J. Penuelas and R. Seco. 2009. Short-chained oxygenated VOC emissions in *Pinus halepensis*, in response to changes in water availability. *Acta Physiol. Plant.*, 31: 311-318.

Franco, J.A., S. Bañón, M.M.J. Vicente, J. Miralles and J.J. Martínez-Sánchez. 2011. Root development in horticultural plants grown under abiotic stress conditions - a review. *J. Hort. Sci. Biotech.*, 86: 543-556.

Gonzalez-Moro, B., A. Munoz-Rueda and M. Lacuesta. 1999. Sequential effects of acidic precipitation and drought on water relations of *Pinus radiata* seedlings. *J. Plant Physiol.*, 155: 93-100.

Gruber, A., S. Strobl, B. Veit and W. Oberhuber. 2010. Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. *Tree Physiol.*, 30: 490-501.

Guan, H., H.Y. Xiao and C.Q. Liu. 2015. Sulfur isotopic signatures of water-soluble sulfate in needles of *Pinus massoniana* Lamb in two chinese areas. *Environ. Earth Sci.*, 73: 1805-1811.

Hanover, J.W. 1992. Applications of terpene analysis in forest genetics. *New Forest.*, 6: 159-178.

Hoggard, J.C., W.C. Siegler and R.E. Synovec. 2009. Toward automated peak resolution in complete GC × GC–TOFMS chromatograms by PARAFAC. *J. Chemometr.*, 23: 421-431.

Holmstrup, M., J.G. Sørensen, K. Maraldo and B. Ehlers. 2012. Increased frequency of drought reduces species richness of enchytraeid communities in both wet and dry heathland soils. *Soil Biol. Biochem.*, 53: 43-49.

Hu, W.J., J. Chen, T.W. Liu, Q. Wu, W.H. Wang, X. Liu and H.L. Zheng. 2014. Proteome and calcium-related gene expression in *Pinus massoniana* needles in response to acid rain under different calcium levels. *Plant Soil*, 380: 285-303.

Humston, E.M., Y. Zhang, G.F. Brabeck, A. Mcshea and R.E. Synovec. 2009. Development of a GC×GC–TOFMS method using SPME to determine volatile compounds in cacao beans. *J. Sep. Sci.*, 32: 2289-2295.

Incerti, G., A. Romano, P. Termolino and V. Lanzotti. 2013. Metabolomic fingerprinting using nuclear magnetic resonance and multivariate data analysis as a tool for biodiversity informatics: a case study on the classification of *Rosa damascena*. *Plant Biosyst.*, 147: 947-954

Ioannou, E., A. Koutsaviti, O. Tzakou and V. Roussis. 2014. The genus *Pinus*: a comparative study on the needle essential oil composition of 46 pine species. *Phytochem. Rev.*, 13: 741-768.

Jiang, H., J. Wang, L. Song, X. Cao, X. Yao, F. Tang and Y. Yue. 2015. GC×GC-TOFMS analysis of essential oils composition from leaves, twigs and seeds of *Cinnamomum camphora* L. Presl and their insecticidal and repellent activities. *Molecules*, 21: 423.

Kännaste, A., H. Nordenhem, G. Nordlander and A.K. Borg-Karlson. 2009. Volatiles from a mite-infested spruce clone and their effects on pine weevil behavior. *J. Chem. Ecol.*, 35: 1262-1271.

Klos, R.J., G.G. Wang, W.L. Bauerle and J.R. Rieck. 2009. Drought impact on forest growth and mortality in the southeast USA: an analysis using forest health and monitoring data. *Ecol. Appl.*, 19: 699-708.

Kolb, P.F. and R. Robberecht. 1996. High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. *Tree Physiol.*, 16: 665-672

Li, D.Y., Y.C. Zhou and J.F. Gong. 2017. Manganese accumulation and anatomical changes in masson pine (*Pinus massoniana*) growing under high concentrations of manganese. *Pak. J. Bot.*, 49(4): 1317-1322.

Liu, Q.H., Z.C. Zhou, Y.C. Wei, D.Y. Shen, Z.P. Feng and S.P. Hong. 2015. Genome-wide identification of differentially expressed genes associated with the high yielding of oleoresin in secondary xylem of masson pine (*Pinus massoniana* Lamb) by transcriptomic analysis. *PLoS ONE* 10(7): e0132624.

Loreto, F. and J.P. Schnitzler. 2010. Abiotic stresses and induced BVOCs. *Trends Plant Sci*., 15: 154-166.

Lundborg, L., G. Nordlander, N. Björklund, H. Nordenhem and A.K. Borg-Karlson. 2016. Methyl jasmonate-induced monoterpenes in Scots pine and Norway spruce tissues affect pine weevil orientation. *J. Chem. Ecol.*, 42: 1237-1246.

Merlin, M., T. Perot, S. Perret, N. Korboulewsky and P. Vallet. 2015. Effects of stand composition and tree size on resistance and resilience to drought in sessile oak and Scots pine. *Forest Ecol. Manag.*, 339: 22-33.

Mohamed, H.A., C.L. Hsieh, C. Hsu, C.C. Kuo, H.S. Chang, C.K. Lee, T.H Lee., J.B. Wu, C.I. Chang, Y.H. Kuo and C.I. Chang. 2014. Two new acidic diterpenoids from the heartwood of *Pinus massoniana* Lamb. *Helv. Chim. Acta*, 97: 1146-1151.

Peng, F.M., L.Q. Sheng, B.Z. Liu, H.W. Tong and S.M. Liu. 2004. Comparison of different extraction methods: steam distillation, simultaneous distillation and extraction and headspace co-distillation, used for the analysis of the volatile components in aged flue-cured tobacco leaves. *J. Chromatogr. A*, 1040: 1-17.

Peñuelas, J., G. Farréarmengol, J. Llusia, A. Gargallogarriga, L. Rico, J. Sardans, J. Terradas and I. Filella. 2014. Removal of floral microbiota reduces floral terpene emissions. *Sci. Rep.*, 4: 6727.

Pichler, P. and W. Oberhuber. 2007. Radial growth response of coniferous forest trees in an inner Alpine environment to heat-wave in 2003. *Forest Ecol. Manag.*, 242: 688-699.

Qiu Y.Q., X. Lu, T. Pang, S.K. Zhu, H.W. Kong and G.W. Xu. 2007. Study of traditional Chinese medicine volatile oils from different geographical origins by comprehensive two-dimensional gas chromatography–time of flight mass spectrometry (GC×GC–TOFMS) in combination with multivariate analysis. *J. Pharm. Biomed.*, 43: 1721-1727.

Quan W.X. and G.J. Ding. 2017. Root tip structure and volatile organic compound responses to drought stress in Masson pine (*Pinus massoniana* Lamb.). *Acta Physiol. Plant.* 39(12):258.

Ruther, J. and S. Kleier. 2005. Plant-plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (z)-3-hexen-1-ol. *J. Chem. Ecol.*, 31: 2217-2222.

Sampedro, L., X. Moreira and R. Zas. 2011. Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *J. Ecol.*, 99: 818-827.

Schade G.W. and A.H. Goldstein. 2001. Fluxes of oxygenated volatile organic compounds from a ponderosa pine plantation. *J. Geophys. Res-Atmos*., 106: 3111-3123.

Su, J.W., J.P. Zeng, X.W. Qin and F. Ge. 2009. Effect of needle damage on the release rate of Masson pine (*Pinus massoniana* Lamb.) volatiles. *J. Plant Res.*, 122: 193-200.

Swidrak, I., A. Gruber, W. Kofler and W. Oberhuber. 2011. Effects of environmental conditions on onset of xylem growth in *Pinus sylvestris* under drought. *Tree Physiol.*, 31: 483-493.

Thabeet, A., M.Vennetier, C. Gadbin-Henry, N. Denelle, M. Roux, Y. Caraglio and B. Vila. 2009. Response of *Pinus sylvestris* L. To recent climatic events in the French Mediterranean region. *Trees*, 23: 843-853.

Vacchiano, G., M. Garbarino, E. Borgogno Mondino and M. Motta Renzo. 2012. Evidences of drought stress as a predisposing factor to Scots pine decline in Valle d'Aosta (Italy). *Eur. J. Forest Res.*, 131: 989-1000.

Vos J.D., R. Dixon, G. Vermeulen, P. Gorst-Allman, J. Cochran and E. Rohwer. 2011. Comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS) for environmental forensic investigations in developing countries. *Chemosphere*, 82: 1230-1239.

Vose, J.M., C.F. Miniat, C.H. Luce, H. Asbjornsen, P.V. Caldwell, J.L. Campbell, G.E. Grant, D.J. Isaak, S.P. Loheide and G. Sun. 2016. Ecohydrological implications of drought for forests in the United States. *Forest Ecol. Manag.*, 380: 335-345.

Wang, S.L., P. Wang and C.Q. Fan. 2015. Distribution of aluminum fractionation in the acidic rhizosphere soils of masson pine (*Pinus massoniana* Lamb). *Commun. Soil Sci. Plant Anal.*, 46: 2033-2050.

Wang, X.Q., Z. Liu, L. Niu and B. Fu. 2013. Long-term effects of simulated acid rain stress on a staple forest plant *Pinus massoniana* Lamb: a proteomic analysis. *Trees*, 27(1): 297-309.

Watt, M., M.E. McCully and M.J. Canny. 1994. Formation and stabilization of rhizosheaths of *Zea mays* L.(Effect of soil water content). *Plant Physiol*., 106: 179-186.

Weed, A.S., M.P. Ayres and J.A. Hicke. 2013. Consequences of climate change for biotic disturbances in North American forests. *Ecol. Monogr.*, 83: 441-470.

Wurzburger, N. and C.F. Miniat. 2014. Drought enhances symbiotic dinitrogen fixation and competitive ability of a temperate forest tree. *Oecologia*, 174: 1117-1126.

Xiang, Z.M., K. Cai, G.L. Liang, S.P. Zhou, Y.H. Ge, J. Zhang and Z.L. Geng. 2014. Analysis of volatile flavour components in flue-cured tobacco by headspace solid-phase microextraction combined with GC×GC-TOFMS. *Anal. Methods*, 6: 3300-3308.

Xiang, Z.M., K. Cai, S.P. Zhou, Z.L. Geng and W.J. Pan. 2015. Analysis of nitrogenous organic compounds from mainstream cigarette smoke using low-temperature solvent extraction followed by comprehensive two-dimensional gas chromatography with high-resolution time-of-flight mass spectrometry. *J. Sep. Sci.*, 38: 2655-2663.

Xu, Z.Z., and G.S. Zhou. 2011. Responses of photosynthetic capacity to soil moisture gradient in perennial rhizome grass and perennial bunchgrass. *BMC Plant Biol*., 11: 21-32.

Yang, N.Y., L. Liu, W.W. Tao, J.A. Duan and L.J. Tian. 2010. Diterpenoids from *Pinus massoniana* resin and their cytotoxicity against a431 and a549 cells. *Phytochemistry*, 71: 1528-1533.

Yatagai, M.A. and Y.B. Hong. 1997. Chemical composition of the essential oil of *Pinus massoniana* Lamb. *J. Essent. Oil Res.*, 9: 485-487.

Yue, J., Y.R. Zheng, Z.M. Liu, Y. Deng, Y.F. Jing, Y.L. Luo, W.J. Yu and Y.Y. Zhao. 2015. Characterization of volatile compounds in microfiltered pasteurized milk using solid-phase microextraction and GC×GC-TOFMS. *Int. J. Food Prop*., 18: 2193-2212.

Zhang T., X.P. Wen and G.J. Ding. 2017. Ectomycorrhizal symbiosis enhances tolerance to low phosphorous through expression of phosphate transporter genes in masson pine (*Pinus massoniana*). *Acta Physiol. Plant*., 39(4):101.

Zhang, Y.M., Z. Zhou and Q. Yang. 2013. Genetic variations in root morphology and phosphorus efficiency of pinus massoniana under heterogeneous and homogeneous low phosphorus conditions. *Plant Soil*, 364: 93-104.

Zhang, Z.F., M.L. Zhang, F.J. Wu and W.X. Peng. 2011. Analysis on function components and biohealth function of *Pinus massoniana* Lamb biomass. *Appl. Mech. Mater*., 55: 157-160.

Zhou, L., S.Q. Wang, Y.G. Chi, Q.K. Li, K. Huang and Q.Z. Yu. 2015. Responses of photosynthetic parameters to drought in subtropical forest ecosystem of china. *Sci. Rep*., 5(5):18254.

Zhu, S.K., X. Lu, L. Dong, J. Xing, X.L. Su, H.W. Kong, G.W. Xu and C.Y. Wu. 2005. Quantitative determination of compounds in tobacco essential oils by comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry. *J. Chromatogr. A*, 1086: 107-114.

Zhu, S.K., X. Lu, K.L. Ji, K.L. Guo, Y.L. Li, C.Y. Wu and G.W. Xu. 2007. Characterization of flavor compounds in Chinese liquor Moutai by comprehensive two-dimensional gas chromatography/time of flight mass spectrometry. *Anal. Chim. Acta*, 597: 340-348.

(Received for publication 11 August 2018)