Front. For. China 2007, 2(4): 459–465
DOI 10.1007/s11461-007-0073-5

RESEARCH ARTICLE

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Rapid changes of induced volatile organic compounds in *Pinus massoniana*

Abstract Using the thermal-desorption cold trap gas chromatography/mass spectrometer (TCT-GC-MS) technique, the composition and relative contents of volatile compounds were analyzed in undamaged (control), insect-damaged (ID) and artificially-damaged (AD) leaves of *Pinus massoniana* in field at different times and levels of damage. Results showed that although volatile substances were highly released earlier in AD leaves plants, they were significantly less abundant in AD than in ID leaves treatments. Also, the damage level considerably influenced the changes of induced volatile products from leaves. Compared with the control, the emission rate of camphene, *β*-pinene, phellandrene, caryophyllene and (*E*)-farnesene was high after 1 h in 25%–40% ID-affected leaves, whereas that of tricyclene, myrcene, camphene, *β*-Pinene, phellandrene and caryophyllene reached its maximum after 24 h in 60%–75% ID-affected leaves. In the same manner, some volatile compounds in the AD leaves treatment displayed their peaks just after 1 h, but others after 24 h. The AD and ID leaves at the damage level of 25%–40% did not exhibit an obvious regularity with time; however, in 60%–75% AD leaves, peaks of volatile substances were attained after 1 or 2 h. Our results also showed that the relative content of *β*-pinene increased and was higher in damaged than control plants. *β*-pinene plays an important role in inducing the insect resistance of *P. massoniana* trees.

Keywords *Pinus massoniana*, induced volatile organic compounds (VOCs), rapid changes

Translated from *Scientia Silvae Sinicae*, 2006, 42(4): 65–70 [译自: 林业科学]

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1 Introduction

When plants are attacked by insects, the direct defense response is to release volatile organic compounds (Farmer, 2001). Paré and Tumlison (1999) reported that many of the volatiles, including (*E*,*E*)-*α*-farnesene, (*E*)-*β*-farnesene, (*E*)-*β*-ocimene, Linalool, are emitted following Lepidopteran larvae damage in cotton. According to Li's research (1998), among all the terpenes in the essential oil of feeding-damaged *Pinus tabulaeformis*, the amount of *α*-copaene increased obviously. When the ashleaf maple suffered from the feeding-damage of Asian longhorn beetles (ALB), the emission rate of most volatile compounds reached the first peak of 9 h after infestation, then decreased, and then increased again, reaching the maximum at 48 h (Li et al., 2002). Similarly, when the ashleaf maple were exposed to drought stress, the emission of nine volatile compounds increased, including butyl alcohol, pentyl alcohol, *trans*-2-hexen-1-al, *cis*-3-hexen-1-ol, pentanal, pentanoic acid, hexanal, during which butyl alcohol, pentyl alcohol and *cis*-3-hexen-1-ol, elicited antennal responses from ALB (Jin et al., 2004). *Pinus massoniana* is the main hardwood for afforestation and resin collection in South China; however, it has always been threatened by *Dendrolimus punctatus*, which break up cyclically in China. To examine the temporal dynamic changes of volatile organic compounds in *P. massoniana*, three treatments were performed, including undamaged, insect-damaged, and artificially-damaged. The ultimate purpose of this work is to confirm the quality and mechanism of the volatile compounds in plants, and try to put forward a new strategy for pest control.

2 Materials and methods

2.1 Materials

Pinus massoniana Lamb trees were employed in mountainous areas of Jiangxi Agricultural University. Larvae (3rd) of *Dendrolimus punctatus* Walker (at the fourth developmental stage) were provided by Agricultural College of Jiangxi Agricultural University (Jiangxi, China).

2.2 Collection of volatile compounds

The natural forests were selected and labeled in mountainous areas of Jiangxi Agricultural University in June, 2004. Three leaves treatments were used, which consisted of insect-damaged (IDL), artificially-damaged (ADL) and undamaged (UDL) leaves on six different and healthy pine trees. In the first treatment, leaves from the selected lateral branches of the trees were subjected to the attack of *D. punctatus* larvae for 72 h, resulting in 25%–40% and 60%–75% leaf damage, respectively. Leaves on the involved branches were manually cut with scissors in the second treatment, simulating the degree of the leaf damage caused by insects like above. Leaves in the third treatment were not damaged and were taken as controls. With dynamic headspace collection, volatile organic compounds of leaves were collected from the trees after 1, 2, 4, 8, 24, 48 and 72 h, directly preserved at a desiccator for further analysis.

2.3 Detection of volatile compounds

Volatile compound determination was performed according to a method described previously (Li et al., 2002). Volatile organic compounds were detected using a thermal-desorption cold trap (TCT) (CP-4010 PTI/TCT, CHROMPACK Company)- gas chromatography (GC) (TRACE TM GC 2000CE, INSTRUMENT Company)/ mass spectrometer (MS) (VOYAGER MASS, FINNIGAN Company) system with a DB-5 Low Bleed/MS column (60 m × 0.32 mm × 0.5 μm, J&W Scientific, Agilent Technologies, USA).

Volatile organic compounds were identified using Xcalibur 1.2 software, NIST 98 mass library and retention time. The relative contents of volatile compounds were quantified with the method of normalized area.

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3 Results and analysis

3.1 Rapid changes of volatile substances from *P. massoniana* induced by different means

The results of the volatiles with insect feeding and artificial damage from *P. massoniana* needles are indicated in Figs. 1 and 2. Compared with controls, under the slightly insect-damaged condition, the peak of α-pinene was registered 8 h after damage, camphene, β-pinene, phellandrene, caryophyllene, farnesene increased after 1 h, yet each reached its peak at different time: camphene at 72 h, β-pinene and phellandrene at 2 h, and caryophyllene and farnesene at 48 h after damage. Under the slightly artificially-damaged condition, α-pinene, camphene andcaryophyllene, β-pinene and phyllandrene reached their peaks at 8, 1 and 24 h, respectively. Despite of different damage modes, it was identical that the maximum of the relative content of α-pinene appears; on the contrary, there was no such a rule in other terpenes. Under the severely insect-damaged condition, all the volatile compounds increased in amount at 1 h except phellandrene and caryophyllene. α-pinene reached its maximum at 4 h, and tricyclene, myrcene, camphene, β-pinene, phellandrene and caryophyllene reached their peaks after 24 h, whereas farnesene obtained its maximum at 72 h. In 60%–75% AD leaves, α-pinene was continuously lower than controls in amount in the entire sampling sequence and the maxima of the other

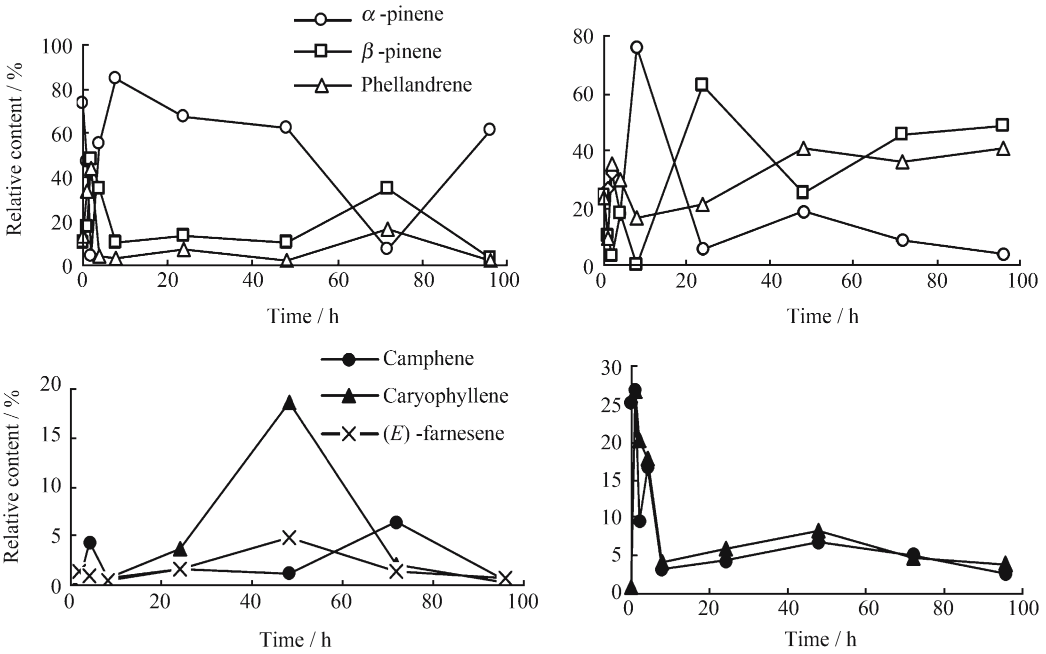

Fig. 1 Changes of volatile compounds of *P. massoniana* after slight ID (left) and AD (right)

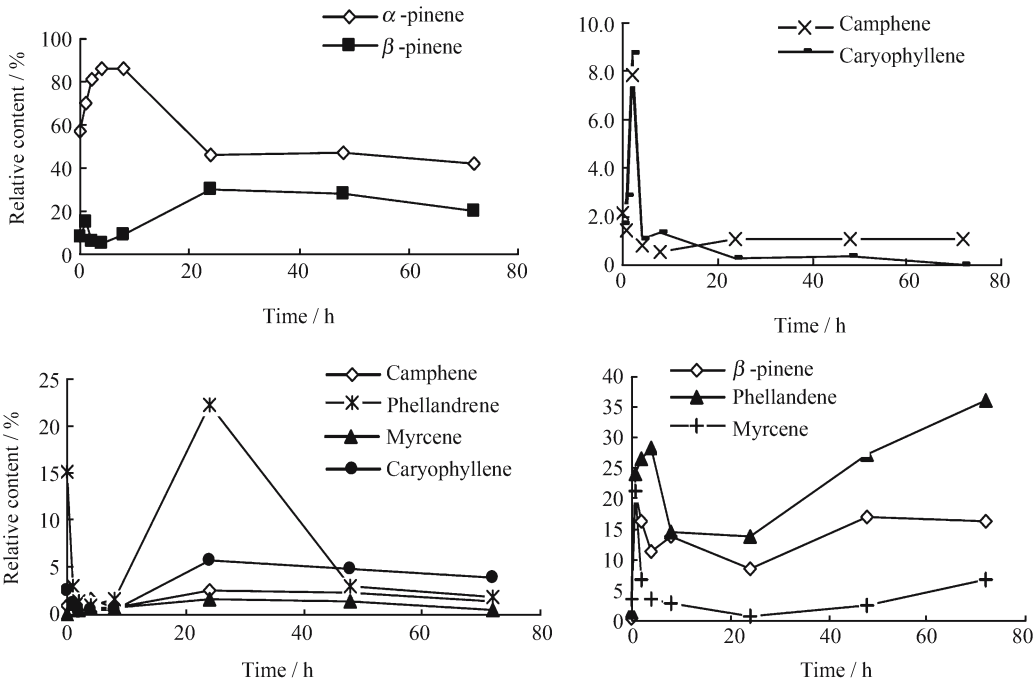

Fig. 2 Changes of volatile compounds of *P. massoniana* after serious ID (left) and AD (right)

volatile compounds mostly appears at 1 or 2 h. Thus, we can conclude that although volatile substances were highly released earlier in artificially-damaged leaves, they were significantly less than those in insect-damaged leaves, and that the peaks of α-pinene in different damage modes were not regular. The author suggested that α-pinene is just the main component in conifers. However, much less is known about the function of α-pinene in the plant resistance against insects. Furthermore, the reason why most data in 2 h were lower may possibly be attributed to the sampling time which is too close to the first spot, and the plant had not returned to its normal growing form.

Plants stand in a dynamic interaction between primary and secondary metabolizability. Volatile organic compounds constantly keep the lowest metabolic level in controlled *P. massoniana*, and their variations followed a much more pronounced diurnal rhythm, with low emission at night and high levels during the periods of maximal photosynthesis (Martin et al., 2003). When a plant is invaded, the resistance system, which is also in the dynamically varying condition, can emit large amounts of VOCs in a relative shorter period (Paré and Tumlison, 1999). This response is a kind of variation on the basis of time and produces rapid induced resistance (Liu et al., 2003).

Plants response to insect feeding damage by releasing a variety of volatile compounds from the damaged site, and the profile of the volatile compounds emitted is markedly different from those of undamaged or artificially-damaged plants. In cotton, the breakage of leaf glands cause stored terpenes to be released on much higher levels (Loughrin et al., 1994). Hence, there exist differences in release peaks and relative contents between the volatile compounds induced by insect damage and those induced by artificial damage.

3.2 Rapid changes of volatile substances on different insect-damaged levels

3.2.1 Induced changes of volatile compounds by slightly and severely insect-damage

Results from slightly and severely insect-damaged *P. massoniana* showed that there were more volatile compounds with high levels of herbivory. Besides the compounds present in slightly-ID materials, tricylene and mycrene emerged and changed significantly in severely-ID. The release peaks of the volatile compounds in severely-ID exhibited an obvious regularity with time: peaks of the volatile substances were attained after 24 h except α-pinene, (*E*)-farnesene; furthermore, the peaks of relative contents in severely-ID were lower than those in slightly-ID (Tables 1–3).

Terpenes are important insect-resistant substances in inducible defense of conifers, and it is the main way for conifer trees to secrete the high concentration in monoterpene-containing oleoresin. This has already been identified in our experiment. However, the concentration of the induced volatile compounds was greatly related with the damaging degrees and the time. The damaging degrees influenced the varieties of the volatile compounds from *P. massoniana*. When the loss of *P. massoniana* needles reached 25%–40%, the released volatile compounds increased significantly, and the ability to generate induced chemical defense reached its

Table 1 Changes of relative content of induced-volatile after slight feeding damage (unit: %)

Time /h	*α*-pinene	Camphene	*β*-pinene	Phellandrene	Caryophyllene	(*E*)-farnesene
0	73.89	0.24	9.37	13.4	0.43	ND
1	46.99	0.38	17.44	33.38	0.79	0.34
2	3.67	0.28	47.85	44.1	1.82	1.28
4	54.64	4.48	34.59	4.07	0.55	0.93
8	84.22	0.71	10.59	2.68	0.59	0.55
24	67.64	1.72	13.31	7.54	3.68	1.67
48	62.27	1.09	9.73	2.44	18.54	4.79
72	7.27	6.46	34.88	16.34	2.10	1.44
96	60.80	0.48	3.06	2.32	0.32	0.60

Table 2 Changes of relative content of induced-volatile after serious feeding damage (unit: %)

Time /h	Tricyclene	*α*-pinene	Camphene	*β*-pinene	Myrcene	Phellandrene	Caryophyllene	(*E*)-farnesene
0	ND	57.20	0.89	7.98	0.001	15.14	2.47	ND
1	0.07	70.15	1.35	15.28	1.23	2.91	1.22	5.87
2	0.04	81.06	0.63	5.60	0.55	1.38	0.25	3.57
4	ND	85.69	1.54	4.51	0.78	0.92	0.47	2.66
8	0.03	85.53	0.76	8.75	0.63	1.60	0.41	ND
24	0.16	46.32	2.62	29.88	1.63	22.18	5.77	1.34
48	0.13	46.95	2.21	27.64	1.40	2.97	4.73	6.68
72	0.10	42.44	1.40	20.30	0.46	1.84	3.94	22.76

Table 3 Changes of relative content of volatile compounds in undamaged *P. massoniana* (unit: %)

Time/ h	Tricyclene	*α*-pinene	Camphene	*β*-pinene	Myrcene	Phellandrene	Caryophyllene
1	0.09	68.25	1.02	16.10	1.40	2.21	3.14
2	0.06	75.71	0.29	16.10	1.21	3.83	0.90
4	0.08	63.02	0.60	25.70	2.32	2.79	2.78
8	0.10	56.03	1.13	34.21	1.16	5.01	0.96
24	0.15	77.73	1.61	15.58	0.90	1.30	0.90

maximum. When the loss reached 60%–75%, although the volatile compounds were higher than control, the quantity of most volatile compounds was lower than that in slightly-ID. It is suggested the reason was possibly that after severe insect damage, the great loss of leaves resulted in the discontinuity in water metabolism and photosynthesis, thus the primary metabolism was influenced, and the secondary metabolism was decreased and even terminated. The induced resistance of *P. massoniana* indicated the on-off effect caused by the damaging degrees rather than linear relationships (Lou and Chen, 1997). Moreover, the energy and substance used for the defense of plants was limited. To avoid excessive energy consumption and benefit the growth of trees, the damaged *P. massoniana* trees did not continuously generate terpenes with high concentration (Li et al., 1998), but rapidly, discontinuously and adjustably.

3.2.2 Induced changes of volatile compounds by slightly and severely mechanically-damage

Volatile organic compounds increased after slightly-AD and severely-AD. A lot of volatile compounds increased 1 or 2 h after damage. Artificially-damaged and insect-damaged leaves at the 25%–40% damage level did not exhibit an obvious regularity with time; however, in 60%–75% AD leaves, all kinds of volatile substances were lower than those in slightly-AD. The relative content of *α*-pinene was lower than control in severely-AD. Peaks of other volatile compounds except phellandrene reached its maximum after 1 or 2 h after damage (Tables 3–5).

The purpose of artificial damage is to examine whether there is coherence between the two modes in respect of the variety of VOCs, and whether the artificial damage can take the place of insect damage. The results showed that the relative contents of VOCs increased in both modes and the varieties were basically identical. Differences only existed in the time of peaks and release quantity. Ge et al. (1997) found that artificial damage, similar to insect damage, also had the regulative function to *D. punctatus*. In conclusion, artificial damage and imitating insect damage can achieve the purpose that the population of *D. punctatus* be regulated, the damage be lightened and the breaking cycle be delayed.

3.3 Induction of *β*-pinene from volatile chemicals of *P. massoniana*

β-pinene was concerned in the detected volatile chemicals. The change of *β*-pinene was significant after *P. massoniana* were treated using different means and levels (Fig. 3). Compared with the control, the relative content of *β*-pinene

Table 4 Changes of relative content of induced-volatile compounds after slight needle-cutting (unit: %)

Time/ h	*α*-pinene	Camphene	*β*-pinene	Phellandrene	Caryophyllene
0	25.18	25.38	24.59	23.81	1.04
1	26.58	26.79	10.53	9.30	26.79
2	32.08	9.54	2.81	35.23	20.33
4	17.74	16.51	17.88	30.12	17.74
8	75.91	3.36	0.11	16.49	4.13
24	5.41	4.32	62.99	21.21	6.08
48	19.14	6.96	24.71	40.72	8.46
72	8.75	5.18	45.47	35.96	4.64
96	4.31	2.66	48.50	40.67	3.87

Table 5 Changes of relative content of induced-volatile compounds after serious needle-cutting (unit: %)

Time/ h	Tricyclene	*α*-pinene	Camphene	*β*-pinene	Myrcene	Phellandrene	Caryophyllene
0	0.27	88.95	2.10	0.19	3.46	1.40	1.69
1	0.23	25.75	1.41	21.27	21.27	24.24	2.88
2	0.30	27.83	7.81	16.14	6.82	26.67	8.77
4	0.10	53.56	0.80	11.21	3.51	28.32	1.05
8	0.11	65.26	0.55	13.82	2.92	14.34	1.34
24	0.11	73.15	1.08	8.49	0.80	13.73	0.26
48	0.09	49.32	1.04	17.00	2.45	27.14	0.35
72	0.19	38.48	1.06	16.17	6.66	36.15	0.02

increased by 46.29% after 2 h and by 53.84% after 72 h in 25%–40% ID-affected leaves, whereas that of *β*-pinene increased by 46.12% after 24 h and by 20.7% after 72 h in 60%–75% ID-affected leaves. The relative content of *β*-pinene increased by 74.44% after 24 h and by 64.6% after 72 h in 25%–40% AD leaves treatment, whereas that of *β*-pinene increased by 24.33% after 1 h and 0.46% after 72 h in 60%–75% AD leaves treatment. However, the relative content of *β*-pinene decreased after 2 and 8 h. This may be a reason that the emission of volatile compounds from *P. massoniana* reduced after needles were cut. The role of *β*-pinene for *D. punctatus* will be studied further in the experiment. For example, *β*-pinene from *Pinus sylvestris* had a repellent effect on *Tomicus piniperda*. Cao (1984) pointed that the increase of *D. punctatus* population could be disadvantageous after *D. punctatus* fed on the needles of high *β*-pinene with insect-resistant. The Research Group of the Resistance of Masson pine to Masson pine moth (1990) indicated that the content of *β*-pinene from the No.11 Masson pine trees in a Masson pine forest increased 168.1% than the control. This pine tree showed significant resistance to *D. punctatus.* It exhibited a non-preference against the egg-laying of moths and an adverse effect on the growth of larvae. This was related to the content of *β*-pinene. Li et al. (1997) compared the resistance ability of *Pinus sylvestris* var. *mongolica* and *P. tabulaeformis* to that of *Dendrolimus spectabilis*. The results indicated that the content of *β*-pinene from *P. tabulaeformis* reached 16.9%, whereas that of *P. sylvestris* var. *mongolica* was only 8.7%. Their difference was nearly one-fold in the chemical analysis of needle oil. This may be a reason that *D. spectabilis* adults prefer to locate the needles of *P. tabulaeformis* as their oviposition sites and the mortality of the larva fed on *P. tabulaeformis* needles was higher than that of *P. sylvestris* var. *mongolica*. Zhao et al. (1995) found that the largest electroantennogram (EAG) response was elicited by *β*-pinene. This fact suggested that *β*-pinene could induce *D. punctatus.* Therefore, it is very important to further study the synthesis and release mechanism of *β*-pinene in plant defense response against insects.

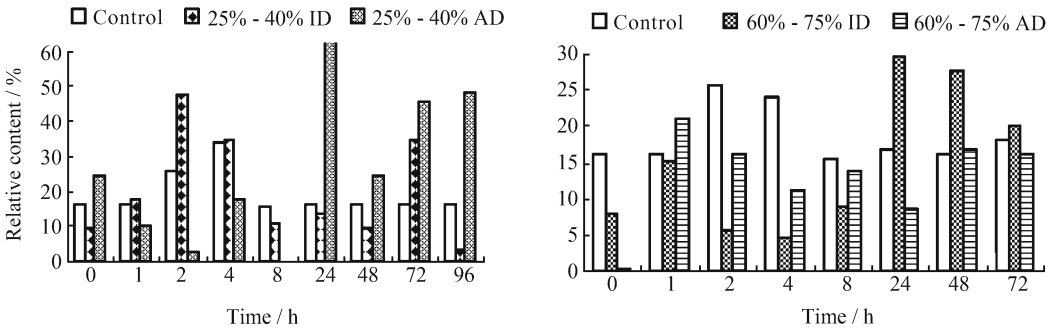

Fig. 3 Effects of contents of *β*-pinene after different treatments

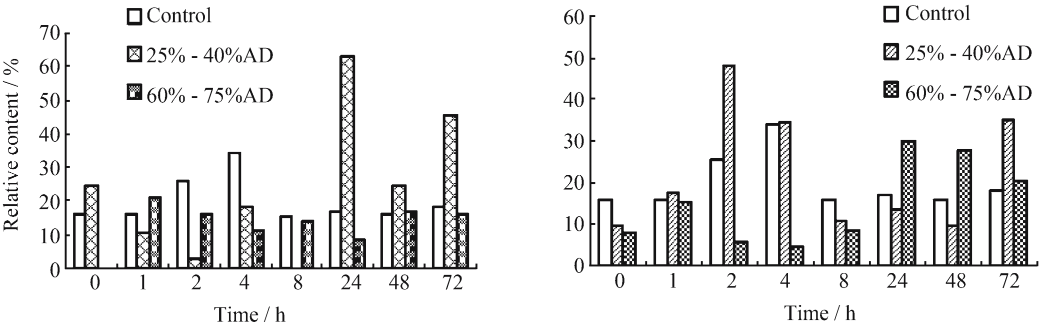

Fig. 4 Effects of content of β-pinene after different degrees

4 Conclusions and discussion

Plant leaves usually released a small amount of volatile chemicals, but when a plant is damaged by herbivorous insects, many more volatile compounds are released. The stressed response of plants is a change with time (Wang et al., 2001). Results showed that the relative contents of volatile compounds from undamaged (control), insect-damaged (ID) and artificially-damaged (AD) leaves of *Pinus massoniana* changed with different times and levels of damage. Although volatile substances were highly released earlier in plants of AD leaves, they were significantly less abundant in AD than in ID leaves treatment. Also, the damage level considerably influenced the changes of induced volatile products from leaves. Compared with the control, the emission rates of camphene, β-pinene, phellandrene, caryophyllene and (*E*)-farnesene were high after 1 h in 25%–40% ID-affected leaves, whereas the rates of tricyclene, myrcene, camphene, β-pinene, phellandrene and caryophyllene reached their maxima after 24 h in 60%–75% ID-affected leaves. In the same manner, some volatile compounds in AD leaves treatment displayed their peaks just after 1 h, but others after 24 h. Artificially-damaged and insect-damaged leaves at the 25%–40% damage level did not exhibit an obvious regularity with time; however, in 60%–75% AD leaves, peaks of volatile substances were attained after 1 or 2 h. Our results also showed that the relative content of β-pinene increased and was higher in damaged than control plants. β-pinene plays an important role in inducing insect resistance of *P. massoniana* trees.

The response based on plants' self-determination and their dynamic process of metabolism. When the stress was sustained, plants will make a corresponding response to the different kinds and intensity stresses (Wang, 1993). Terponoids are one of inducible defense chemicals of conifers. Volatiles emitted from a diverse group of plants and insects mediate key processes in the behavior of specific insects. They contribute to the natural enemies and parasitoids, distinguishing whether the plant was damaged, or to the host location or predation. It can also serve as airborne semiochemicals, promoting or deterring interactions between plants and insect herbivores and their natural enemies (Paré and Tumlison, 1999). The research not only recognizes the theory of relationships among plants and insect herbivores and their natural enemies, but also complements and perfects the management theory in practice (Lou and Chen, 1997). Also, it is important to study the biological control of pests. The contents and components of released volatile compounds were affected by the diurnal rhythm and environmental factors (Kessler and Baldwin, 2001). Although the study on the molecular mechanism of volatile compounds has acquired progress, their metabolic pathway and regulation will be studied deeply, which can explore the chemical mechanism about insect resistance.

The relationships between inducible volatile compounds and the behavior of *D. punctatus* will be identified by bioassay.

Acknowledgements This research was financially supported by the Key Project of the National Natural Science Foundation of China (Grant No. 30330490).

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