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RESEARCH PAPERS

Volatile Organic Compound Analysis of Host and Non-Host Poplars for *Trypophloeus klimeschi* (Coleoptera: Curculionidae: Ipinae)[1, 2]

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Abstract—*Trypophloeus klimeschi* Eggers was first discovered in Xinjiang Province and had strong selection specificity for *Populus alba* var. *pyramidalis* Bunge. There was an outbreak of this beetle in the northwest shelter forest of China, resulting in significant economic losses and loss of ecological benefits. Based on a prior long-term field investigation, *T. klimeschi* had a different extent of injuries for different ages of *P. alba* var. *pyramidalis* and other *Populus* in the same area were not selected by *T. klimeschi*. To further explore the specificity volatile compounds, this study involved selecting host and non-host trees to analyse the volatile chemical profile of host and non-host poplars of *T. klimeschi*. The main volatile compounds of the host poplar *P. alba* var. *pyramidalis* for different physiological statuses and those of three other non-host poplars (*P. alba* L., *P. tomentosa* Carr., and *P. dakuanensis* Hsu) were analysed through solid-phase micro extraction (SPME) coupled with thermal desorption and gas chromatography-mass spectrometry (GC-MS). The major compound groups were aldehydes, esters, alcohols, ketones, phenols, terpenes and alkanes. Comparative analysis of the changes in the different physiological stages of *P. alba* var. *pyramidalis* and other non-host *Populus* volatile substances was conducted, and the results showed that 2-hydroxy-benzaldehyde, nonanal, decanal, 2-methyl-butanal, (Z)-3-hexen-1-ol benzoate, methyl benzoate, methyl salicylate, geraniol and salicyl alcohol might act as attractants for *T. klimeschi*, and 2-hexenal, hexanal, 2-cyclohexen-1-one, caryophyllene, eugenol, benzyl alcohol, and eucalyptol could be deterrents for *T. klimeschi*. These experiments may lead to the optimisation of a synthetic lure that may be used to detect and monitor *T. klimeschi*.

Keywords: Populus spp., *Trypophloeus klimeschi*, volatile compounds, attractants, deterrents

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INTRODUCTION

Trypophloeus klimeschi Eggers, pertain to Coleoptera, Curculionidae, Ipinae, was first discovered in Xinjiang province in China and had strong species-specificity to *Populus alba* var. *pyramidalis* Bunge [1]. The beetle had been occurred in China's northwest shelter forest outbreak hazards, resulting in significant losses in economic, ecological and social benefits. After spending the entire immature portion of their life cycle under the bark of a host tree, newly emerged adult *T. klimeschi* leave the protection of the brood host and search for a new host in which to reproduce. After an outbreak in 2003 in Xinjiang Province, *T. klimeschi* spread rapidly to the adjacent areas. The insect is now found in Dunhuang and identified as *T. klimeschi* by morphology. Current management strategies primarily rely on synthetic pesticides because no simple and reliable sampling schemes are available. *T. klimeschi* had strong host-selection specificity, which can cause serious damage to *P. alba* var. *pyramidalis* Bunge by feeding on the phloem of branches and stems. However, other local poplar species, such as *P. alba* L., *P. tomentosa* Carr, and *P. dakuanensis* Hsu, are not damaged by this beetle. Some specific chemical volatile compounds might play an important role in host selection.

Populus is the preferred afforestation tree species for greening and shelterbelts in the North China Plain, especially in the "Three-North Area". Moreover, *P. alba* var. *pyramidalis*, the poplar in China, is extremely important to reforestation efforts in China. Many new *P. alba* var. *pyramidalis* in "Three-North shelterbelt" provide a continuous corridor for *T. klimeschi* spread from northwestern China to eastern region. Among woody plants, the poplar is a model organism because of its ecological and economic importance. A publication has identified over 75 volatile compounds in the headspace of young black poplar (*P. nigra*) trees by

[1] The article is published in the original.
Abbreviations: SPME—solid phase micro extraction; GC-MS—gas chromatography-mass spectrometry; DBH—diameter at breast height; VOCs—volatile compounds.

[2] Supplementary materials are available for this article at doi 10.1134/S1021443718060067 and are accessible for authorized users.

GC-MS after invasion by gypsy moth (*Lymantria dispar*) caterpillars for almost 2 d [2]. The volatiles that were emitted included homoterpenoids, monoterpenoids, sesquiterpenoids, green leaf volatiles, nitrogen-containing volatiles and aromatic compounds [2]. Forty eight components from the fresh and air-dried leaf buds of *P. nigra* were isolated using a Likens-Nickerson apparatus and analysed using gas chromatography-mass spectrometry (GC-MS) [3]. Another study also reported compounds of volatiles from withered black poplar leaves at different physiological stages, nineteen components in the odour were separately identified, and sixteen of them were identified as aldehyde, alcohol, phenol, ketone, acid and heterocyclic compounds [4].

Each plant has its own volatile secondary material with a different chemical composition and a certain proportion of the chemical composition of the plant fingerprints (chemical fingerprint) [5]. Different parts of the same plant contain a volatile oil composition that is also different. For example, the essential oil of the bark of the Lauraceae plant contains cinnamic aldehydes, the leaves mainly contain eugenol while the roots and the xylem in the stem mainly contain camphor [5]. Plant volatiles play a key role in host detection and recognition for herbivorous insects [5]. Almost all species of insects use chemical odours emitted by hosts to determine whether they are suitable for their own use. The recognition of host plants by these olfactory signals could occur via either species-specific compounds or specific ratios of ubiquitous compounds. For example, some Chrysomelidae beetles are attracted to host plants that have been infested or wounded by conspecifics, which causes an increase in pheromone production after feeding on the host plant [6]. Based on this view, researchers have studied all types of plants with pests that might be attracted to a specific active ingredient, and they believe these active ingredients will play a role in the comprehensive management of pests. Once identified, semiochemicals can have useful pest management applications, such as improved monitoring and sampling of natural enemies.

In this study, we focused on the volatile compound composition of four poplars to better understand the relationship between poplar volatile compounds and *T. klimeschi* selection. The four *Populus* varieties, *P. alba* var. *pyramidalis* (in different physiological stages), *P. alba*, *P. tomentosa* and *P. dakuanensis*, were analysed for their volatile compound compositions. The identified compounds could be used as the basis for the ecological control of *T. klimeschi* and provide a reference for the prevention and control of other poplar pests. Researchers hope that such investigations will lead to the optimisation of a synthetic lure that is capable of attracting *T. klimeschi*, which could be used in forestry practice for advanced pest control.

MATERIALS AND METHODS

Plant material. The plant materials were collected from the shelter belt of Dunhuang City (40°06′50.61″ N, 94°36′10.24″ E), Gansu Province, China. Dunhuang is located in northwestern Gansu Province, which has a temperate continental climate with the Gobi Desert as the main landform.

Collection of the *P. alba* var. *pyramidalis* host trees. Based on a prior long-term field investigation, *T. klimeschi* had different extents of injuries for different ages of *P. alba* var. *pyramidalis* Bunge. Due to the difficulty of determining age, the diameter measured at breast height (DBH) was used instead of age. According to the infestation percentage, *P. alba* var. *pyramidalis* at 10–20 cm DBH suffered the worst with 74.6%; *P. alba* var. *pyramidalis* at <10 cm DBH was 53.8%; and *P. alba* var. *pyramidalis* with more than 30 cm DBH had strong resistance with 23.8%. To analyse the changes in volatile compounds for different ages of *P. alba* var. *pyramidalis* as well as the changes after damage from *T. klimeschi*, we used the DBH in lieu of age, including three physiological states (<10 cm DBH, 10–20 cm DBH, and >30 cm DBH). Additionally, the infected tree was selected as a representative of *P. alba* var. *pyramidalis* at 10–20 cm DBH. The trees thick with wormholes were selected as the infected tree. Each sample (four samples: <10 cm DBH, 10–20 cm DBH, >30 cm DBH and infected *P. alba* var. *pyramidalis*) had three repetitions, twenty trees mixed in each repetition.

Collection of non-host trees *P. alba, P. tomentosa*, and *P. dakuanensis*. In surveys, we found that other poplars in the same area were not selected by *T. klimeschi*. Such specificity might be mediated by different chemicals of poplar varieties. To further explore the volatile compounds that have attractive or avoidance effect on *T. klimeschi*, the volatile compounds of *P. alba*, *P. tomentosa*, *P. dakuanensis* were collected and analysed. Each sample (three samples: *P. alba*, *P. tomentosa*, *P. dakuanensis*) had three repetitions, twenty trees mixed in each repetition.

The collected site was located in one man-made windbreak, which was planted in 2001. Additionally, their volatile compounds were compared to *P. alba* var. *pyramidalis* in 10–20 cm DBH.

Collection of volatiles. The plant leaves were separated from the bark. Either 5 g of leaves or bark was weighed in the Organization Grinding Apparatus (DHS TL-2020). The plant material was vibration grinded at 4°C for 2 min at a frequency of 75 Hz. Then, all samples were loaded into a Headspace Sample Vial (20 mL) for further volatiles analysis by gas chromatography-mass spectrometry (GC-MS).

Gas chromatography-mass spectrometry. Both fractions of the essential oil as well as the overall materials were analysed by gas chromatography/mass spectrometry (Thermo TRACE 1310ISQLT). At the beginning, the solid phase microextraction (SPME) head was

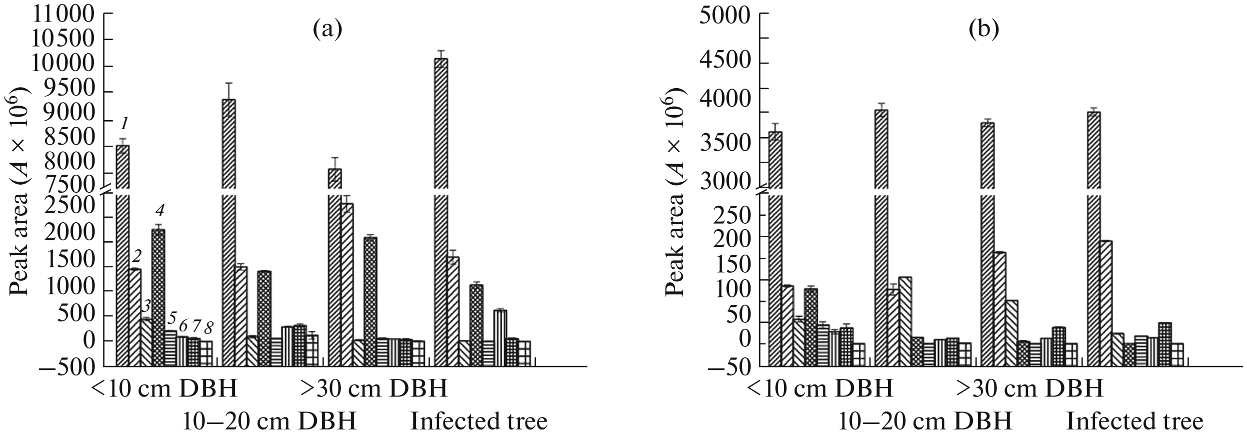

Fig. 1. Chemical composition and content of volatile substances of the leaves and barks in different physiological status of *P. alba* var. *pyramidalis*. (a) Leaf; (b) bark. *1*—Aldehydes; *2*—esters; *3*—alcohols; *4*—ketones; *5*—phenols; *6*—terpenes; *7*—alkanes; *8*—acids.

placed at the inlet of the gas chromatograph at 250°C for 5 min. The fibre was coated with 65 μm polydimethylsiloxane (PDMS)/divinylbenzene (DVB). Then, the volatiles were collected by the SPME fibre assemblies for 30 min at 50°C. Following the sampling, the SPME fibre was immediately inserted into the GC injector, and the volatiles bound to the fibre were thermally desorbed for 5 min at 250°C. A Varian VF-5MS (5% dipheny-95% polymethylsiloxane) capillary column (30 mm × 0.25 mm × 0.25 μm) was used for volatile separation. Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature programme consisted of 40°C (hold 1 min), 3°C/min to 130°C, and 25°C/min to 280°C (hold 5 min). The quadrupole mass detector operated at 150°C in the electron impact ionisation (EI) at 70 eV. The ion source temperature was set at 280°C, and the transfer line was set at 280°C. The mass acquisition range was 40–400 m/z.

Volatile compound identification and data processing. After SPME-GC-MS analysis of the four types of *Populus* leaves and barks, individual peaks were identified based on their fragmentation patterns, and the compound identities were confirmed by comparing the mass spectra with data system libraries. The data of the samples were computed from the GC peak areas. The retention times and mass spectra of compounds detected in the samples were compared to authentic compound samples. The test data were analyzed by SPSS software. The graph processing was conducted in Excel and OriginPro 8.

RESULTS

Volatile Compounds in Populus alba var. pyramidalis

Among the compounds identified, the main ones were aldehydes, terpenes, esters, alcohols, ketones, phenols, and alkanes. There were 33, 37, and 36 compounds identified from the *P. alba* var. *pyramidalis* leaves in <10 cm the DBH, 10–20 cm DBH, and >30 cm DBH, respectively. The identified components and their percentages are shown in Table S1 in supplementary for the original data used to perform this analysis, where the components are grouped by chemical class. In the bark, 33, 30, and 32 volatiles were also identified, respectively (Table S2).

Volatile Compounds of the Leaves in Populus alba var. pyramidalis

Through the fingerprints of leaves of *P. alba* var. *pyramidalis* in different physiological states, we found that the aldehyde content was the highest, with the contents of esters and ketones being the second-highest. There were substantial changes in the content of aldehydes, esters, ketones and terpenes (Fig. 1a).

The aldehydes mainly were represented by 2-hydroxy-benzaldehyde, 2-hexenal, nonanal, hexanal and decanal; the compounds in esters mainly included methyl benzoate and (Z)-pentanoic acid 3-hexenyl ester; the ketones were mainly 2-cyclohexen-1-one; and both 3-ethyl-1,5-octadiene and geraniol were the major compounds for the terpenes (Fig. 2).

The trend of detecting 2-hydroxy-benzaldehyde, nonanal and decanal among the aldehydes was consistent. The aldehyde content was the highest in 10–20 cm DBH, and the aldehyde content in 30 cm DBH was the lowest. In contrast, the highest contents of 2-hexenal and hexanal were detected in 30 cm DBH *P. alba* var. *pyramidalis*, and the lowest amounts were found in 10–20 cm DBH. And there were significant changes of 2-hydroxy-benzaldehyde, nonanal, decanal, 2-hexenal and hexanal in *P. alba* var. *pyramidalis* ($F_{2\text{-hydroxy-benzaldehyde}} = 853.039$, $df_{2\text{-hydroxy-benzaldehyde}} = 2$, $P_{2\text{-hydroxy-benzaldehyde}} = 0.000$; $F_{nonanal} = 2136.900$, $df_{nonanal} = 2$, $P_{nonanal} = 0.000$; $F_{decanal} = 94.222$, $df_{decanal} = 2$, $P_{decanal} = 0.000$;

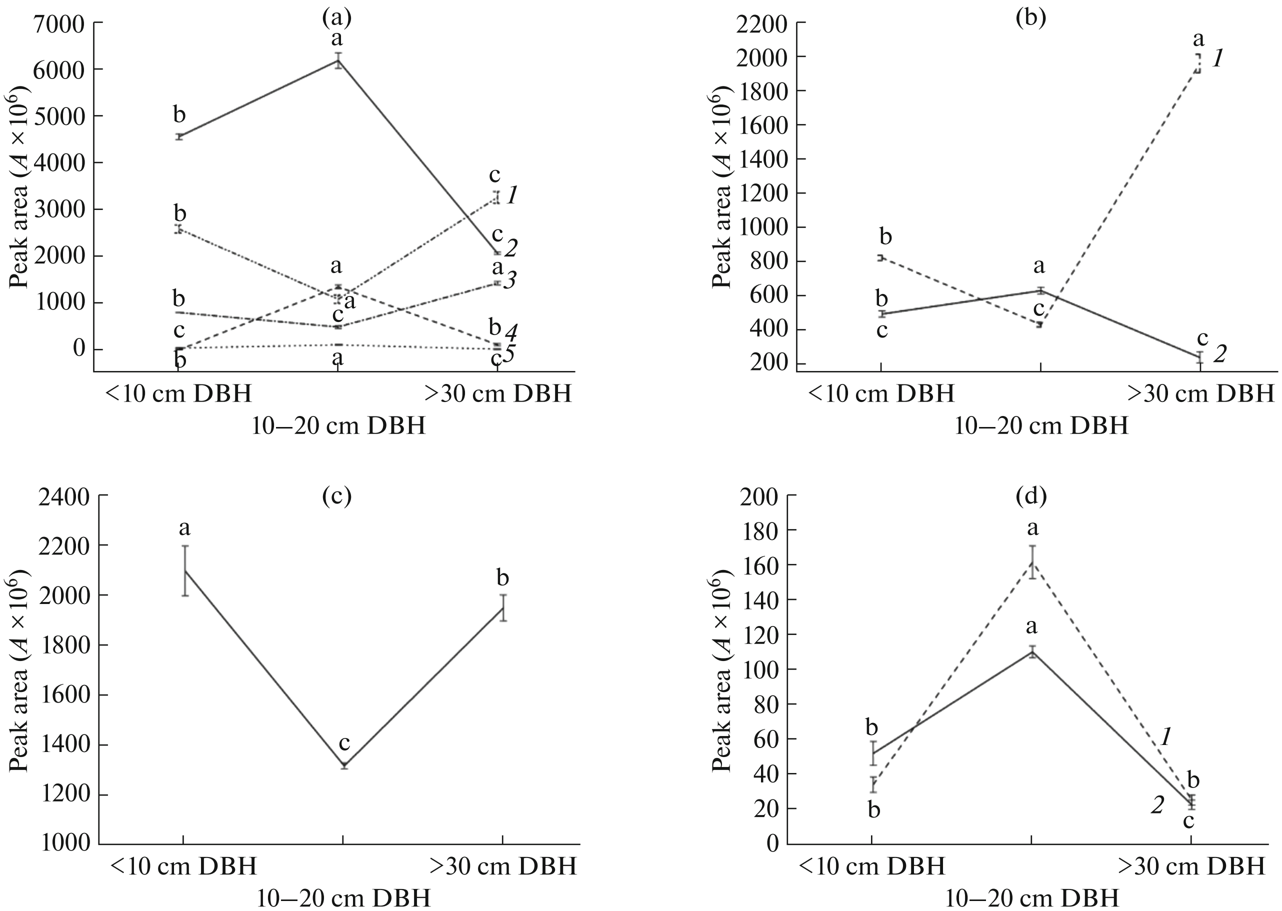

Fig. 2. Main compounds trend in leaves of *P. alba* var. *pyramidalis*. (a) Trend of aldehydes: *1*—2-hexenal; *2*—2-hydroxy-benzaldehyde; *3*—hexanal; *4*—nonanal; *5*—decanal; (b) trend of esters; *1*—(Z)-pentanoic acid 3-hexenyl ester; *2*—methyl benzoate; (c) trend of ketones: 2-cyclohexen-1-one; (d) trend of terpenes: *1*—3-ethyl-1,5-octadiene; *2*—geraniol.

$F_{2\text{-hexenal}} = 327.003$, $df_{2\text{-hexenal}} = 2$, $P_{2\text{-hexenal}} = 0.000$; $F_{\text{hexenal}} = 935.590$, $df_{\text{hexenal}} = 2$, $P_{\text{hexenal}} = 0.000$) (Fig. 2a).

The contents of methyl benzoate and (Z)-pentanoic acid 3-hexenyl ester were high, and the change was significant for esters ($F_{\text{methyl benzoate}} = 196.950$, $df_{\text{methyl benzoate}} = 2$, $P_{\text{methyl benzoate}} = 0.000$; $F_{\text{(Z)-pentanoic acid 3-hexenyl ester}} = 1702.031$, $df_{\text{(Z)-pentanoic acid 3-hexenyl ester}} = 2$, $P_{\text{(Z)-pentanoic acid 3-hexenyl ester}} = 0.000$). The two substances had changes that occurred in the opposite direction, and the content of (Z)-pentanoic acid 3-hexenyl ester was much higher in 30 cm DBH *P. alba* var. *pyramidalis* (Fig. 2b).

For ketones, only the content of 2-cyclohexen-1-one was higher, and the change was significant ($F_{\text{2-cyclohexen-1-one}} = 121.551$, $df_{\text{2-cyclohexen-1-one}} = 2$, $P_{\text{2-cyclohexen-1-one}} = 0.000$) (Fig. 2c).

In terpenes, geraniol and 3-ethyl-1, 5-octadiene had obvious changes, while the content was high ($F_{\text{geraniol}} = 273.211$, $df_{\text{geraniol}} = 2$, $P_{\text{geraniol}} = 0.000$; $F_{\text{3-ethyl-1, 5-octadiene}} = 452.588$, $df_{\text{3-ethyl-1, 5-octadiene}} = 2$, $P_{\text{3-ethyl-1, 5-octadiene}} = 0.000$) (Fig. 2d).

Volatile Compounds of the Barks in Populus alba var. pyramidalis

Compound species of the barks in *P. alba* var. *pyramidalis* were different from leaves. Through the fingerprints of the barks of *P. alba* var. *pyramidalis* in different physiological states, we found fewer species of compounds in barks than in leaves, the aldehyde content was the highest, and the relative content as high as 90%. The content of esters and alcohols were the second highest content. There were great changes in the relative content of aldehydes, esters and alcohols (Fig. 1b).

In the bark, aldehydes mainly were represented by 2-hydroxy-benzaldehyde, hexanal and 2-hexenal. The compounds in esters mainly included (Z)-3-hexen-1-ol benzoate. Salicyl alcohol was the major compound found for the alcohols (Fig. 3).

The trend of change in the content of 2-hydroxy-benzaldehyde was consistent with the leaves, and the content was highest in 10–20 cm DBH *P. alba* var. *pyramidalis* ($F_{\text{2-hydroxy-benzaldehyde}} = 56.828$,

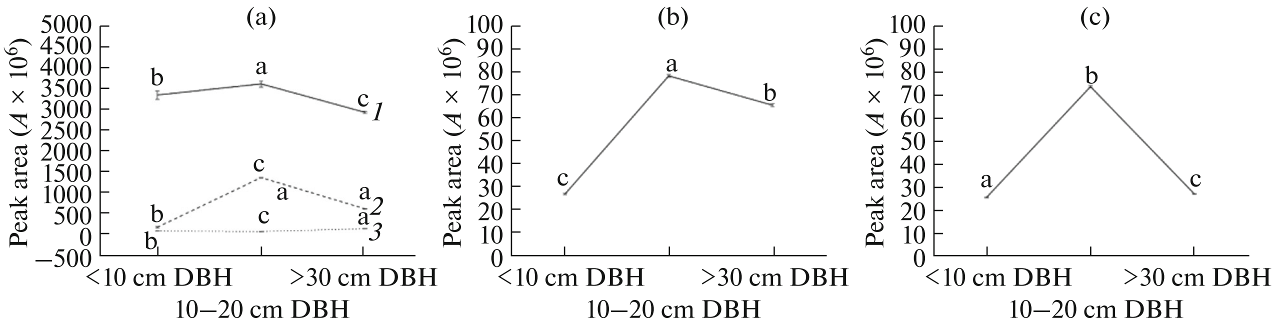

Fig. 3. Main compounds trend in barks of *P. alba* var. *pyramidalis*. (a) Trend of aldehydes: *1*—2-hydroxy-benzaldehyde; *2*—2-hexenal; *3*—hexanal; (b) trend of esters: (Z)-3-hexen-1-ol benzoate; (c) trend of alcohols: salicyl alcohol.

$df_{2\text{-hydroxy-benzaldehyde}} = 2$, $P_{2\text{-hydroxy-benzaldehyde}} = 0.000$). The trend for hexanal and 2-hexenal was also consistent with leaves, and the content was highest in 30 cm DBH *P. alba* var. *pyramidalis* ($F_{hexenal} = 1279.144$, $df_{hexenal} = 2$, $P_{hexenal} = 0.000$; $F_{2\text{-hexenal}} = 2550.440$, $df_{2\text{-hexenal}} = 2$, $P_{2\text{-hexenal}} = 0.000$) (Fig. 3a). For esters, only the content of (Z)-3-hexen-1-ol benzoate was higher, and the change was significant ($F_{(Z)\text{-3-hexen-1-ol benzoate}} = 7126.027$, $df_{(Z)\text{-3-hexen-1-ol benzoate}} = 2$, $P_{(Z)\text{-3-hexen-1-ol benzoate}} = 0.000$) (Fig. 3b). In alcohols, salicyl alcohol was the special compound, and the trend was consistent with that of (Z)-3-hexen-1-ol benzoate. The contents of (Z)-3-hexen-1-ol benzoate and salicyl alcohol were apparently higher in 10–20 cm DBH *P. alba* var. *pyramidalis* than they were in <10 cm DBH and 30 cm DBH ($F_{salicyl\ alcohol} = 25821.530$, $df_{salicyl\ alcohol} = 2$, $P_{salicyl\ alcohol} = 0.000$) (Fig. 3c).

Changes of Volatile Compounds of Infected and Healthy Leaves of Populus alba var. pyramidalis

The production of volatiles became different after suffering from *T. klimeschi*. In leaves, with the damage from *T. klimeschi*, the content of three compounds decreased significantly, specifically that of 2-hexenal, hexanal, and 2-cyclohexen-1-one, but seven other compounds increased significantly, including 2-hydroxy-benzaldehyde, nonanal, decanal, methyl benzoate, (Z)-pentanoic acid 3-hexenyl ester, 3-ethyl-1,5-octadiene and geraniol (Table 1).

There was variability among the types of compounds in the leaves and bark. In bark, the variety of volatile compounds was less than that in the leaves. The most common compounds were 2-hydroxy-benzaldehyde and 2-hexenal and the trends were consisting with the leaves. The amount of 2-hydroxy-benzaldehyde increased and 2-hexenal decreased. With the damage of *T. klimeschi*, the relative content of (Z)-3-hexen-1-ol benzoate, and salicyl alcohol decreased significantly (Table 2).

Volatile Compounds in Four Poplars Varieties

The total ion chromatograms (TICs) showed that the four varieties had remarkable qualitative and quantitative differences in their volatile profiles. The compounds were identified by GC-MS retention times along with synthetic standards, including aldehydes,

Table 1. The change of VOCs constitute in leaves of infected *P. alba* var. *pyramidalis*

Volatile compounds	Healthy leaves	Infected leaves	C	T	df	P
Hexanal	502.68 ± 30.33	353.35 ± 11.71	↓	12.492	2	0.006
2-Hexenal	1096.16 ± 89.26	918.52 ± 130.42	↓	7.039	2	0.020
2-Hydroxy-benzaldehyde	6189.80 ± 164.56	6744.82 ± 30.85	↑	4.200	2	0.050
Nonanal	1360.25 ± 41.64	1920.99 ± 51.01	↑	14.479	2	0.003
Decanal	123.20 ± 8.67	171.94 ± 5.78	↑	5.358	2	0.033
Methyl benzoate	634.61 ± 19.94	715.67 ± 12.11	↑	4.851	2	0.040
(Z)-pentanoic acid 3-Hexenyl ester	436.09 ± 12.82	615.24 ± 5.66	↑	52.509	2	0.000
2-Cyclohexen-1-one	1315.46 ± 11.86	1056.49 ± 47.73	↓	8.851	2	0.013
3-Ethyl-1,5-octadiene	160.30 ± 9.31	443.87 ± 22.57	↑	17.633	2	0.003
Geraniol	109.18 ± 3.43	139.53 ± 2.35	↑	8.430	2	0.014

↑ Compared with health leaves, the relative content of volatile compounds in infected leaves increased. ↓ Compared with health leaves, the relative content of volatile compounds in infected leaves decreased. Values—the peak area of each compound. T, df, P—data analyzed by *t*-test. Method of identification: materials were analyzed by gas chromatography/mass spectrometry (Thermo TRACE 1310ISQLT).

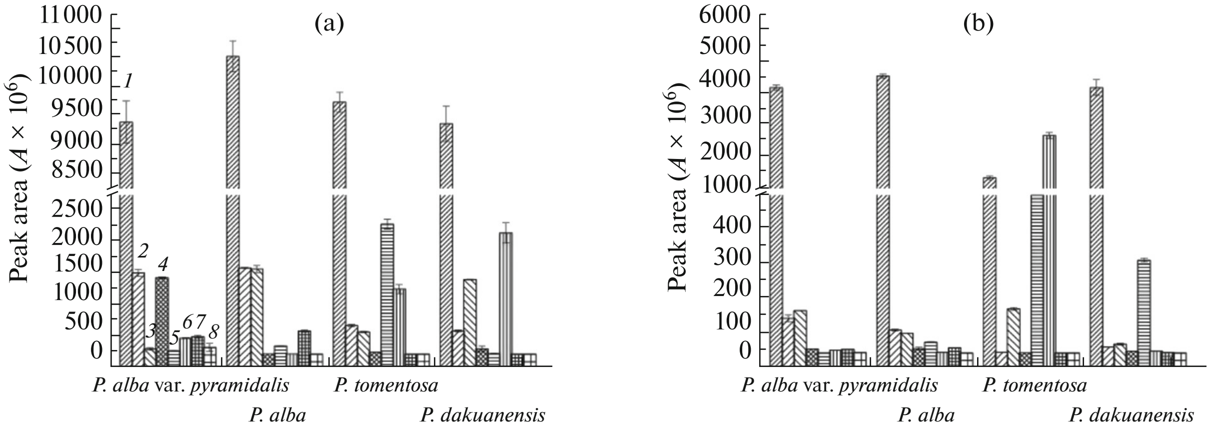

Fig. 4. Chemical composition and content of volatile substances of the leaves and barks in four *Populus* species. (a) Leaf; (b) bark. *1*—aldehydes; *2*—esters; *3*—alcohols; *4*—ketones; *5*—phenols; *6*—terpenes; *7*—alkanes; *8*—acids.

terpenes, esters, alcohols, ketones, phenols, and alkanes. There were 37, 27, 20, and 23 compounds that were identified from *P. alba* var. *pyramidalis*, *P. alba*, *P. tomentosa* and *P. dakuanensis* leaves, respectively (Table S3). In the bark, 30, 32, 39, and 32 compounds were identified in the species, respectively (Table S4).

Volatile Compounds of Leaves in Four Species of Poplar

Through the fingerprints of leaves from four species of poplars (*P. alba* var. *pyramidalis*, *P. alba*, *P. tomentosa* and *P. dakuanensis*), the content of esters and ketones in *P. alba* var. *pyramidalis* were higher than those in the other three non-host poplars (F_{esters} = 516.105, df_{esters} = 3, P_{esters} = 0.000; $F_{ketones}$ = 1850.242, $df_{ketones}$ = 2, $P_{ketones}$ = 0.000) (Fig. 4a).

In the leaves of *P. alba* var. *pyramidalis*, 2-hydroxy-benzaldehyde (48.14 ± 0.02%), nonanal (10.58 ± 0.01%), and 2-cyclohexen-1-one (10.24 ± 0.01%) were the major compounds. In the leaves of *P. alba*, the major compounds were 2-hydroxy-benzaldehyde (38.92 ± 0.02%), 2-hexenal (20.07 ± 0.01%) and phenylethyl alcohol (10.61 ± 0.01%). In the leaves of *P. tomentosa*, 2-hexena (34.14 ± 0.01%), 2-hydroxy-benzaldehyde (30.72 ± 0.01%), eugenol (15.45 ± 0.01%) and 3-ethyl-1,5-octadiene (6.79 ± 0.01%) were the major compounds. In the leaves of *P. dakuanensis*, as with the other three species of poplars, the major compounds were 2-hexenal (36.56 ± 0.02%), 2-hydroxy-benzaldehyde (28.12 ± 0.01%) and 3-ethyl-1,5-octadiene (15.23 ± 0.01%).

In the leaves of *P. alba* var. *pyramidalis*, the proportion of 2-hydroxy-benzaldehyde and nonanal were significantly higher than those in the other three poplars ($F_{2\text{-hydroxy-benzaldehyde}}$ = 57.645, $df_{2\text{-hydroxy-benzaldehyde}}$ = 3, $P_{2\text{-hydroxy-benzaldehyde}}$ = 0.000). Additionally, 2-cyclohexen-1-one, benzoic acid, methyl benzoate, 2,4-dimethyl-hexane and geraniol were unique compounds in the leaves of *P. alba* var. *pyramidalis* as well as represented a high proportion. In the leaves of *P. alba*, 3-ethyl-1,5-octadiene was a unique compound. In the leaves of *P. tomentosa*, eugenol was significantly higher than that in the other three poplars. In the leaves of *P. dakuanensis*, the proportion of 2-hydroxy-benzaldehyde was the lowest among the four *Populus* species, but 3-ethyl-1,5-octadiene was the highest ($F_{3\text{-ethyl-1, 5-octadiene}}$ = 213.622, $df_{3\text{-ethyl-1, 5-octadiene}}$ = 3, $P_{3\text{-ethyl-1, 5-octadiene}}$ = 0.000), and benzyl alcohol was a unique compound.

Volatile Compounds of Barks in Four Species of Poplar

Through the fingerprints of the bark of four species of poplar (*P. alba* var. *pyramidalis*, *P. alba*, *P. tomentosa*

Table 2. The change of VOCs constitute in barks of infected *P. alba* var. *pyramidalis*

Volatile compounds	Healthy barks	Infected barks	C	T	df	P
2-Hexenal	105.92 ± 2.27	36.26 ± 0.15	↓	57.593	2	0.000
2-Hydroxy-benzaldehyde	3595.73 ± 74.07	3774.33 ± 51.23	↑	7.818	2	0.016
(Z)-3-Hexen-1-ol benzoate	77.69 ± 0.57	12.46 ± 0.23	↓	140.711	2	0.000
Salicyl alcohol	73.13 ± 0.45	20.38 ± 0.46	↓	740.220	2	0.000

↑ Compared with health barks, the content of volatile compounds in infected barks increased. ↓ Compared with health barks, the content of volatile compounds in infected barks decreased. Values—the peak area of each compound. T, df, P—data analyzed by *t*-test. Method of identification: materials were analyzed by gas chromatography/mass spectrometry (Thermo TRACE 1310ISQLT).

and *P. dakuanensis*), the content of esters in *P. alba* var. *pyramidalis* was higher than those in the other three non-host poplars, and the results was similar for the leaves ($F_{esters} = 230.411$, $df_{esters} = 3$, $P_{esters} = 0.000$) (Fig. 4b).

In the bark of *P. alba* var. *pyramidalis*, the major compounds were 2-hydroxy-benzaldehyde (85.86 ± 0.01%), 2-hexenal (2.54 ± 0.01%), (Z)-3-hexen-1-ol benzoate (1.86 ± 0.00%), salicyl alcohol (1.75 ± 0.00%) and hexanal (1.75 ± 0.01%). In the bark of *P. alba*, 2-hydroxy-benzaldehyde (93.66 ± 0.01%) were the major compounds. In the bark of *P. tomentosa*, the major compounds were 2-hydroxy-benzaldehyde (25.01 ± 0.01%), eucalyptol (11.08 ± 0.01%), α-caryophyllene (9.07 ± 0.01%) and α-copaene (8.91 ± 0.00%). In the bark of *P. dakuanensis*, 2-hydroxy-benzaldehyde (88.64 ± 0.01%) and eugenol (10.00 ± 0.01%) were the major compounds.

The contents of 2-methyl-butanal and (Z)-3-hexen-1-ol benzoate in the bark of *P. alba* var. *pyramidalis* were significantly higher compared to those in the other three poplars ($F_{2\text{-methyl-butanal}} = 1424.099$, $df_{2\text{-methyl-butanal}} = 3$, $P_{2\text{-methyl-butanal}} = 0.000$; $F_{(Z)\text{-3-hexen-1-ol-benzoate}} = 13257.518$, $df_{(Z)\text{-3-hexen-1-ol-benzoate}} = 2$, $P_{(Z)\text{-3-hexen-1-ol-benzoate}} = 0.000$). In the bark of *P. alba* and *P. dakuanensis*, except for 2-hydroxy-benzaldehyde, the content of the other substances were lower. The proportion of eugenol in the bark of *P. dakuanensis* was the highest among the four *Populus* species. However, eugenol was not detected in *P. alba* var. *pyramidalis* or *P. alba*. The bark of *P. tomentosa* was rich in terpenoids, but the other three poplars did not contain or contained only a small amount of terpenes. Eucalyptol and caryophyllene were unique compounds to *P. tomentosa*.

DISCUSSION

Overall, in four varieties of *Populus*, the major compound groups were aldehydes, esters, terpenes, alcohols, ketones, and phenols. The results are consistent with previous studies [4]. However, there were many differences in chemical composition and relative content among the four varieties. And insect-induced quantitative changes in the blends were found released from *P. alba* var. *pyramidalis* in our research. Since *T. klimeschi* adults were significantly attracted by *P. alba* var. *pyramidalis* especially the *P. alba* var. *pyramidalis* in 10–20 cm DBH, it is speculated that *T. klimeschi* were attracted or deterrent by volatiles that different between *P. alba* var. *pyramidalis* in different physiological states and other three non-host trees.

In our experiment, the content of 2-hydroxy-benzaldehyde was higher in *P. alba* var. *pyramidalis*. Additionally, the 2-hydroxy-benzaldehyde level was higher in susceptible trees (10–20 cm DBH *P. alba* var. *pyramidalis*), and the content increased significantly after damage by *T. klimeschi*. The principal chemicals included benzaldehyde, benzyl acetate, and phenylacetaldehyde, which were found in *Cestrum nocturnum*, and they may provide optimal attraction of the cabbage looper moth *Trichoplusis ni* Hubner to its flowers [7]. The compounds 2-hydroxy-benzaldehyde, leaf alcohol and eugenol were shown to mix with each other to enhance the activity of EAG (electroantennogram) of *Clostera anastomosis* [8].

The trends for nonanal and decanal were the same as that for 2-hydroxy-benzaldehyde, and the content of nonanal and decanal were higher in *P. alba* var. *pyramidalis*. Moreover, the nonanal and decanal levels were higher in susceptible trees, and the content increased after damage from *T. klimeschi*. After odorant binding protein binding assays and gas chromatography electroantennographic detection of nonanal, trimethylamine and skatole, nonanal was shown to have equivalent attraction to the infusion-based lure for *Culex quinquefasciatus* [9]. One study observed that 4,8-dimethyl decanal was a common pheromone of four flour beetle species, including *T. castaneum*, *T. confusum*, *T. freemani*, and *T. madens* [10]. With Y-tube olfactometer bioassay testing, the decanal emitted by the cotton-fed host *Heliothis virescens* was identified as a key compound that contributed to the attractiveness of the parasitoid *Microplitis croceipes* [11].

The compounds 2-hexenal and hexenal are known to be components of the "green leaf odour" of plants. Moreover, we found that the relative content of 2-hexenal and hexanal were higher in the resistant tree (<10 cm DBH, >30 cm DBH *P. alba* var. *pyramidalis*), and they decreased after damage by *T. klimeschi*. Furthermore, the contents were much higher in the other three non-host trees compared to *P. alba* var. *pyramidalis*. Many researchers have reported that 2-hexenal has high antibacterial [12] and antifungal properties [13]. Furthermore, 2-hexenal caused accumulation of plant toxins in *Gossypium hirsutum* [14]. Additionally, 2-hexenal was shown to be a biocidal molecule that was produced in response to bacterial pathogenesis [12].

Additionally, 2-methyl-butanal was a unique compound in *P. alba* var. *pyramidalis*. Previous tests in a wind tunnel indicated that the compounds 3-methyl-1-butanal, decanal, 3-methyl-1-butanol, 2-pentenol, 2-hexenol, and 2-heptanone compounds were more attractive than clean air to *Ceratitis capitata* females [15].

The compound 3-ethyl-1,5-octadiene was a special substance in *P. alba*. Additionally, 3-ethyl-1,5-octadiene was recently detected in our experiment, and its specific biological activities need to be further validated.

Methyl benzoate was a unique compound in *P. alba* var. *pyramidalis*. Additionally, its relative content was higher in the susceptible tree, which increased after damage from *T. klimeschi*. The level of methyl salicylate was higher in *P. alba* var. *pyramidalis*. A previous study reported that methyl benzoate (MeBA) was structurally similar to methyl salicylate (MeSA). MeSA released from cabbage plants infested by cabbage moths was shown to inhibit the oviposition of conspecifically mated female moths [16]. Rice plants that are damaged by fall armyworm (*Spodoptera frugiperda*) larvae (FAW) emit approximately 30 volatiles, including MeSA and MeBA. FAW-induced volatiles are highly attractive to female parasitic wasps (*Cotesia marginiventris*), which are carnivorous enemies of FAW [16].

The compound (Z)-3-hexen-1-ol benzoate was mainly detected in the bark of *P. alba* var. *pyramidalis*. Additionally, the content was higher in the susceptible tree, and it decreased obviously after damage from *T. klimeschi*. This report focused less on (Z)-3-hexen-1-ol benzoate. However, some research studies on (Z)-3-hexen-1-ol acetate have been reported. *Holotrichia oblita* showed marked electroantennographic and behavioral responses to (Z)-3-hexen-1-ol acetate [17].

In our study, the content of (Z)-pentanoic acid 3-hexenyl ester was much higher in the resistant tree, and it increased after damage by *T. klimeschi*. This compound was detected for the first time in our experiment.

Geraniol was a special substance in *P. alba* var. *pyramidalis*. Geraniol showed an obvious change in different physiological states of *P. alba* var. *pyramidalis*, and its relative content was higher after damage by *T. klimeschi*. Geraniol was shown to be an active pheromone for the honey bee (*Apis mellifera*) [18]. An oxygenated monoterpene, geraniol, elicited a significantly larger response from the tarnished plant bug, *Lygus lineolaris*; females rather than males also elicited significantly greater EAGs in dose-response studies [19].

The trend of 3-ethyl-1,5-octadiene in different physiological states of *P. alba* var. *pyramidalis* showed a significant increase after damage. Additionally, the relative content was higher in the non-host trees *P. tomentosa* and *P. dakuanensis*. The compound 3-ethyl-1,5-octadiene was a new compound found in the press. Therefore, there are existing contradictions between the results. Thus, 3-ethyl-1,5-octadiene needs to be further confirmed through biological analysis.

Eucalyptol and caryophyllene were unique substances to *P. tomentosa*. Eucalyptol was a major component of eucalyptol oil and other plants as well as a monoterpene substance. The toxicity of eucalyptol against *Rhyzopertha dominica* and *Tribolium castaneum* has been recorded [20]. It has been reported that β-caryophyllene is a volatile compound emitted by plants into the atmosphere in response to herbivore attack and due to changes in abiotic factors [21]. The compound (E)-β-caryophyllene thus appears to serve as a defence against pathogens that invade floral tissues and similar to other floral volatiles, may play multiple roles in defence and pollinator attraction [22].

Salicyl alcohol was mainly detected in the bark. The content was higher in *P. alba* var. *pyramidalis*, especially in the vulnerable trees. Additionally, the level decreased after damage by *T. klimeschi*. Salicyl alcohol was detected in the branches of the Chinese wing-nut tree, and *Pterocarya stenoptera* attracted the cotton bollworm, *Helicoverpa armigera* [23].

Benzyl alcohol was a unique substance in *P. dakuanensis*. A previous study suggested that the presence of the cyano group at the α-position of the benzyl alcohol moiety of synthetic pyrethroids enhanced both the sodium current and insecticidal activities [24].

The compound 2-cyclohexen-1-one was the unique substance in the leaves of *P. alba var. pyramidalis* and was found in high proportions. Furthermore, the content was higher in the resistant tree, and it was increased after damage from *T. klimeschi*. The compound 2-cyclohexen-1-one was less often reported in the forest, but 3-methyl-2-cyclohexen-l-one has been the focus of some research. The natural anti-aggregation pheromone 3-methyl-2-cyclohexen-l-one was identified from *Dendroctonus pseudotsugae* and can presumably be used in forest protection without ecological disruption [25]. It was also reported that 3-methyl-2-cyclohexen-1-one can serve as an anti-aggregation pheromone. In *Dendroctonus pseudotsuga*, *D. rufipennis* and *D. ponderosae*, the pheromone decreased the response of both sexes [26].

Eugenol was the highest substance in *P. tomentosa* and *P. dakuanensis* and was found only in those species. The anti-bacterial activity of eugenol against *Salmonella typhi* is due to the interaction of eugenol on the bacterial cell membrane [27]. In vitro results indicated that both carvacrol and eugenol exerted an anticandidal effect by a mechanism implicating an important envelop damage [28].

The substance 2,4-dimethyl-hexane was a unique compound in the leaves of *P. alba* var. *pyramidalis*. This compound was newly detected in our experiment. By further analysing 2,4-dimethyl-hexane, we hope to understand its biological activity.

Benzoic acid derivatives from *Piper* species showed significant trypanocidal activity in vitro against promastigote forms of *Leishmania*, *Trypanosoma cruzi*, and *Plasmodium falciparum* [29]. Isopropyl salicylate had obvious anti-bacterial activity [30]. Benzoic acid and isopropyl salicylate were tree-resistant materials, although they were substances specific to *P. alba* var. *pyramidalis*, but they did not correlate with the attracting action and might be resistant to other diseases or insects.

In summary, in our study, the volatile constituents of the dedicated host tree *P. alba* var. *pyramidalis* and other non-host trees, including *P. alba*, *P. tomentosa* and *P. dakuanensis*, were analysed, and some key substances were analysed and investigated further. A comparative analysis of volatile compounds in different physiological conditions showed that 2-hydroxy-benzaldehyde, nonanal, decanal, methyl benzoate, geraniol, (Z)-3-hexen-1-ol benzoate and salicyl alcohol may be attractive to *T. klimeschi*. Additionally, 2-hexenal, hexanal, and 2-cyclohexen-1-one may have a resistance or avoidance effect on *T. klimeschi*. A comparative analysis of volatile substances in the host *P. alba* var. *pyramidalis* and the other three non-host poplars revealed that 2-hydroxy-benzaldehyde, nonanal, 2-methyl-butanal, (Z)-3-hexen-1-ol-benzoate, methyl benzoate, methyl salicylate and geraniol may have a certain attraction to *T. klimeschi*. Caryophyllene, eugenol, benzyl alcohol and eucalyptol may have a resistance or avoidance effect on *T. klimeschi*.

According to the results of the present study, it is possible that 2-hydroxy-benzaldehyde, nonanal, decanal, 2-methyl-butanal, (Z)-3-hexen-1-ol benzoate, methyl benzoate, methyl salicylate, geraniol and salicyl alcohol may have certain attractive effects on *T. klimeschi*. Additionally, 2-hexenal, hexanal, 2-cyclohexen-1-one, caryophyllene, eugenol, catechol, benzyl alcohol and eucalyptol may have resistance or avoidance effects on *T. klimeschi*. However, further behaviour tests are needed to clarify this hypothesis. This study screened a number of substances that were either enticing or repelling to *T. klimeschi*. The result enriched the current data on *Populus* volatile substances and provided a reference for the effective control of other poplar pests. Furthermore, information on close-range visual and chemosensory cues may help improve the utility of volatile chemical-based lures for pest management.

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