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Full Length Article

Composition and Dynamic Variations of the Natural Volatiles of *Prunus armeniaca*

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Abstract

Plant-derived volatiles are used as attractants in pest control applications in the agricultural industry. *Scolytus seulensis* (a bark beetle native to Asia) is the major pest attacking *Prunus armeniaca* (apricot) in Xinjiang, China. To identify host volatiles that attract *S. seulensis*, dynamic headspace sampling was performed to collect volatiles from different parts of living *P. armeniaca* in a forest environment. The relative contents of the volatile components from the trunks and leaves of healthy and infected plants were compared in different seasons and at different times within a single day. The experiments revealed that the volatiles emitted from the trunks of the infected plants during the growth season (from May to September) were mainly alkanes, while those emitted from the leaves during this period were mainly esters. The composition of volatiles emitted by *P. armeniaca* varied with the month of sampling. The infected plants emitted some specific volatiles that were not emitted by healthy plants: hexanal, (E)-4-oxohex-2-enal, cyclohexane, β-ocimene, nonanoic acid and α-cubebene. The daily sampling showed that 1,3,5-cycloheptariene, α-pinene, acraldehyde, camphene, β-ocimene, and azulene were emitted only in small amounts by the infected plants. The composition and relative contents of the volatiles emitted from the trunks and leaves of the healthy plants were similar to those emitted from the infected plants between17:00 and 21:00 h. Our findings provide the basis for understanding the relationship between host selection by *S. seulensis* and the volatiles emitted by *P. armeniaca* during pest infection. Olfactometer bioassays were used to test response to host odors of Phloem of apricot versus and Phloem of apricot extract versus. This species also responded most quickly in the olfactometer, which is encouraging for successful biological control with this species. © 2018 Friends Science Publishers

Keywords: *Scolytus seulensis*; Herbivore-induced plant volatiles; Herbivory; Changing regularity

Introduction

Many studies have shown that the volatiles emitted by host plants provide an important guide that assists phytophagous insects in recognizing and locating hosts in complex environments (Barata *et al*., 2002; Halitschke *et al*., 2008). The compounds emitted from different parts of plants vary greatly in type and content (Loon *et al*., 2002; Wright and Smith, 2004). Even for the same part of a particular plant, the volatiles emitted at different growth periods may vary significantly (Zhang *et al*., 2000; Wu *et al*., 2010). Emitted volatiles are indicators of the physiological status of potential host plants; therefore, compositional differences in volatiles emitted by host plants influence insect behaviors (Fan *et al*., 2004). Tropism of insects to host volatiles is prevalent in a wide range of species (Poland *et al*., 2004; Pureswaran *et al*., 2004). For example, dying *Ulmus americana* (American elm) emit odors different from those emitted by healthy plants. By utilizing this difference, *Ambrostoma quadriimpressum* are able to find proper hosts within which to reproduce (Bin *et al*., 2010). The specific odor emitted by debilitated *Pinus yunnanensis* (Yunnan pine) regulates the host selection of *Monochamus alternatus* and *Scolytidae* species (Pureswaran *et al*., 2004; Wu *et al*., 2010). James (2003) caught *Deraeocoris brevis*, *Orius tristicolor*, *Geocoris pallens*, and hoverflies of the family Syrphidae using artificially synthesized methyl salicylate and cis-3-hexenyl acetate as decoy host volatiles in sticky traps. James and Price(2004) used methyl salicylate as a decoy host volatile and captured a range of predatory insects, including *Chrysopa nigricornis*, *Hemerobius* species, *Deraeocoris brevis*, *Stethorus punctum picipes* and *Orius tristicolor*. Birkett *et al.* (2000) placed (z)-jasmone traps in the field and demonstrated its repellant effect on *Phorodon humuli* feeding on *Pyrus communis.*

Scolytus seulensis (order Coleopter, family Scolytidae) mainly invades rosaceous fruit trees such as apricot, almond, peach, and plum. Infecting the area between the

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phloem and xylem of host plants, *S. seulensis* severely influences the growth of *Prunus armeniaca* (apricot) and eventually leads to the death of the trunk or branches. Because *S. seulensis* can produce devastating damage to *P. armeniaca*, this pest has gained widespread attention and has been a subject of considerable research. In previous studies, we found that the peak occurrence of *S. seulensis* occurs from mid-to-late May to early September. *P. armeniaca* branches at a height of 80–110 cm and those growing towards the east and south are most vulnerable to *S. seulensis* invasion. Volatiles emitted by host plants are the primary olfactory stimulants guiding *S. seulensis* invasion (Zhang and Schylter, 2004). Headspace analysis is of unique significance in odor analysis, because the combination of dynamic headspace sampling, thermal-desorption cold trap (TCT), and GC/MS techniques allows collection and analysis of plant volatiles under natural conditions, where the chemical composition of the gases emitted by the samples can be measured directly. Dynamic headspace sampling was adopted in this work to collect volatiles from living leaves and branches of *P. armeniaca* in order to explore variation in volatile composition and content under natural conditions, in different seasons and at different times of day, as well as to evaluate the impact produced by *S. seulensis*.

Materials and Methods

Sampling

The mainly growen Saimuiti apricot, which is local variety of Xinjiang, belonging to the common apricot cultivar (*Armeniaca vulgaris* Lam.), used as material in the experiment. Sampling was identified and carried out at Jiamu Experimental Station (Aksu, Xinjiang, China) in 2014 with permission of Ming Wang. Six healthy plants (without signs of infection) and 6 infected plants (with new and old holes, fresh and crusted resinosis, and feces) with a diameter at breast height of 20–30 cm (over 80% of the infected plants fell within this range) were selected. One day was selected during the middle of each month from May to September except July (the active period of adult *S. seulensis*) for sampling of the trunks (diameter 10–15 cm) and leaves of *P. armeniaca*, which was conducted from 09:00–12:00 h. July is the peak invasion period of *S. seulensis*. Sampling of trunks and leaves was performed once every 3 h from 09:00–21:00 h. Four continuous samplings were performed within a single day to measure daily variation in emitted volatiles. No protected species were sampled.

Polyethylene film (40 cm × 50 cm) was used to wrap the leaves and trunks of *P. armeniaca* separately to form a closed sampling chamber. An air sampler (Laoying, Qingdao) was used to remove the air from the sampling chamber, after which the air was filtered using activated carbon. When 3/4 of the sampling chamber was filled with air, the upper port was connected to a TANEX-TA GC column (PerkinElmer, Waltham, MA, USA). Cyclic gas sampling was performed in the closed system for 30 min at a gas flow rate of 100 mL/min. The absorption column was stored in a closed environment.

Analytical Instrumentation

The samples were subjected to automated thermal desorption (ATD)–gas chromatography (GC)–mass spectrometry (ATD–GC–MS). The chromatograph was a 7890A Network GC System interfaced with a 5975C Network MSD (Agilent Technologies, Santa Clara, CA, USA).The capillary column was an Agilent DB-5ms (60 m×0.25 mm; film thickness, 0.25 μm). The MS detector provided acquisition in the full-scan mode or selected ion monitoring (SIM) mode. Electron impact spectra were obtained at an electron energy of 70 eV. The temperatures of the GC–MS interface and source were both set to 250°C.

The GC oven was set at 45°C for 2 min, followed by an increase of 4°C per minute to 280°C and a final extension for 3 min at 280°C. Mass spectral data were acquired over a mass range of 29–500 amu for the full-scan mode. The qualitative identification of targeted compounds was based on retention times. Quantification of extracted ions was performed using the external standard method. To quantify BTEX in the SIM mode, the chosen precursor ions were 70 *m/z* (mass to charge ratio) for benzene and 91 *m/z* for the other evaluated compounds. Identification was based on the retention times of the quantified ions in addition to their ion ratios with qualifier ions (*m/z* 51, 65 and 106 for benzene, toluene and ethyl benzene, and xylenes, respectively). The dwell-time was 100 ms.

Sorbent Tube Analysis

Sorbent tube analyses were performed with 2 ATD-equipped systems (PerkinElmer, 350D, Waltham, MA, USA) and an auto-sampler. Thermal primary desorption of the sampling tubes was carried out at 250°C with a helium flow rate of 25 mL min^{-1} for 20 min in order to maintain conditions strictly similar to those used in the on-line sampling. The outlet split was also fixed to 5 mL min^{-1}.The cold trap was maintained at -30°C. During secondary desorption, the cold trap was rapidly heated from -30°C to 300°C and maintained at this temperature for 5 min. The analytes were injected onto the capillary column via a transfer line heated at 250°C. Chromatography conditions were identical to those used in the on-line analysis.

Component Identification

Identification of aromatic and volatile compounds was based on a comparison of their olfactory descriptions, mass spectra, and retention indices (RIs) with those of authentic standards and published data, as well as standard mass spectra in the NIST05. RI values were calculated using a

homologous series of *n*-alkane standards on HP-5 columns. By comparing the GC-peak area of each volatile compound with its relative content, the relative percentages of the detected peaks were obtained by peak-area normalization, with all relative response factors taken as one factor. Relative units were used to express the volatile contents (Kaseleht *et al*., 2011).

Olfactometer Methods

The response of individual beetles to foliage was measured as described in Arsenault *et al.* (2015) in a 30 by 30 by 3 cm 3 four-chambered olfactometer arena. The arena consisted of a base with air output, a walking chamber with four air inputs, and a 9 mm circular central opening to introduce insects and attach a vacuum source. Odor sources were placed in glass chambers attached to the arms of the arena. Four flow meters controlled airflow at a rate of 0.12 Mpa into the glass chambers that contained either a test material, or a blank control; these carried volatiles into the olfactometer. For experiments that required fewer than four arms, the airflow was turned off in the arms that were not in use. Volatiles were removed from the arena through the vacuum in the center, which maintained steady air flow.

Experiments were conducted generally between 08:00 and 20:00 the next day. For each experiment, an individual was placed into the center of the assay arena. Four fields of equal size in front of each odor source arm. Each source chamber contained a different prey host material and was positioned randomly prior to the bioassay for each individual. After every individual had been tested, the olfactometer was cleaned with ethanol and deionized water and treatments. The placement of the glass chambers was randomized on each run was switched to avoid position bias. For each test stimulus at least 20 beetles were tested.

Each beetle had the choice of leaving the central field to cross into one of the four delineated fields. The maximum time a beetle was allowed to walk in the arena without choosing a field was 10 min, after which the beetle was removed. When the beetle remained in one of the delineated fields for 60 s, the final position at the end of the behavioral assay was recorded, as well as the time required for the beetle to choose a field. When the beetle attempted to crawl into an odor source inlet arm, that treatment was considered its final choice, and the beetle was removed from the arena. Insects that did not make a decision within 5 min were considered as no response and discarded.

Results

Seasonal Variations of Volatiles Emitted by *P. armeniaca*

Compositional analysis of volatiles from emitted from the trunks of *P. armeniaca*: Plant volatiles are organic volatiles emitted from the leaf surface or other parts of the plant with molecular weight below 250 μg and a boiling point lower than 340°C. Plant volatiles include hydrocarbons, alcohols, aldehydes, ketones, esters, organic acids, cyanides, and organic sulfur compounds, all of which are secondary metabolic products Total ion chromatograms were obtained by ATD-GC-MS analysis with deduction of impurities from the background air. A total of 41 volatiles were emitted from the trunks of *P. armeniaca* from May to September (Table 1), including 10 alkanes, 8 alkenes, 5 aldehydes, 6 esters, 3 alcohols, 3 acids, 4 aromatic hydrocarbons, 2 ethers, and 1 ketone.

From May to September, 35 volatiles were emitted from the trunks of healthy plants. The ranking of the months by the number of types of identified volatiles was (in decreasing order): May>August>June>July. The differences in volatile emission among the tested months may have been related to weather conditions. During May (24 types) and August (20 types), high temperatures occur in the study area, along with little precipitation. In July (13 types), the amount of precipitation increases, which influences the emission of volatiles and reduces the number of types of volatiles. From May to September, 28 volatiles were emitted from the trunks of the plants infected by *S. seulensis*. The ranking of the months by the number of types of identified volatiles was (in decreasing order): June>May>July> August. The differences in the number of types of volatiles emitted each month by healthy and infected *P. armeniaca* may have been due to differences in the response to the stress caused by *S. seulensis.*

There were some differences in the contents of volatiles emitted from the trunks of *P. armeniaca* in different seasons. Methylene chloride, 1-butanol, n-butyl ether and β-myrcene were emitted from the trunks of healthy plants from May to August. With the exception of β-myrcene, the amount of each volatile emitted from the plants gradually decreased from May to August. In May, there were more types of volatiles emitted from the trunks of healthy plants than infected plants. The major volatiles emitted from healthy plants in May included ethylene chloride (16.062%), 1-butanol (10.984%), and n-butyl ether (18.294%). In June, the major volatiles emitted from healthy plants were ethylene chloride (16.596%), 1-butanol (17.725%), n-butyl ether (11.996%), and 1, 1′-binaphthalene (9.557%). In July and August, the major volatiles emitted from healthy plants included n-butyl ether (12.486 and 12.024%, respectively), isobutyl acrylate (18.259 and 15.74%, respectively), and β-myrcene (10.865 and 15.740%, respectively). The major volatiles emitted from the trunks of the infected plants were identical during from May to August. From May to August, the volatiles emitted by the infected plants, accounting for 67.677–81.288% of the total emitted volatile content, included methylene chloride, 1-butanol, 1,3,5-cycloheptatriene, n-butyl ether, isobutyl acrylate, propenoic acid, butyl ester, benzaldehyde, β-myrcene, nonanal, and naphthalene.1-Pentene, hexanal, ethylbenzene, and camphene were emitted by the infected plants, but not by the healthy plants.

Table 1: Seasonal variation of the relative contents of volatiles emitted from the trunks of healthy and infected *P. armeniaca*

No.	Retention time (min)	Compounds	Molecular formula	Relative molecular mass	Trunks of healthy plants				Trunks of infected plants			
					MAY	JUNE	JULY	AUGUST	MAY	JUNE	JULY	AUGUST
1	4.25	Ethanol	C2H6O	46.07	1.696± 0.031			4.140± 0.151	1.057± 0.015			
2	4.477	Ethene, methoxy-	C3H6O	58.08	3.1680 ±0.078			2.106± 0.054	2.245± 0.178	0.346± 0.038		
3	4.731	Methylene chloride	CH2Cl2	84.93	16.062 ±1.025	16.596 ±1.037	2.146± 0.108	5.662± 0.178	6.015± 0.957	2.661± 0.278	3.511± 0.375	1.981± 0.074
4	5.025	Pentane, 3-methyl-	C6H14	86.17				1.927± 0.037				
5	5.424	n-Hexane	C6 H14	86.17	3.485± 0.087	3.933± 0.195		9.032± 0.924	0.789± 0.028	0.327± 0.087	0.556± 0.055	
6	5.689	Ethyl acetate	C4H8O2	88.11	3.939± 0.095			1.839± 0.076	8.623± 1.025	0.641± 0.110		
7	6.242	1-Butanol	C4H10O	74.12	10.984 ±1.027	17.725 ±1.084	6.062± 0.168	8.284± 1.042	11.470 ±1.847	20.826 ±3.018	4.471± 0.785	8.189± 1.024
8	7.09	Methyl methacrylate	C4H6O2	86.09			8.158± 0.154	1.435± 0.084				
9	7.698	1-Pentene	C5H10	70.13					6.022± 0.758	1.597± 0.312	5.577± 1.398	
10	8.536	1,3,5-cycloheptatriene	C7H8	92.14	0.909± 0.052		7.660± 0.264	2.421± 0.149	3.939± 0.312	2.343± 0.276	3.241± 0.927	5.482± 0.897
11	9.359	Hexanal	C6H12O	100.16						0.591± 0.089		
12	9.603	Acetic acid, butyl ester	C6H12O2	116.16	6.560± 0.132		8.895± 0.325	6.927± 0.517	8.331± 1.020	12.768 ±2.014	7.105± 1.924	10.327± 1.198
13	11.172	Ethylbenzene	C8H10	106.16					1.041± 0.054			
14	11.383	n-Butyl ether	C8H18O	130.23	18.294 ±0.457	11.996 ±1.521	12.486 ±1.002	12.024± 1.042	28.410 ±2.748	34.912 ±4.218	14.224 ±2.879	16.077± 2.078
15	12.012	Isobutyl acrylate	C7H12O2	128.17	3.172± 0.015		18.259 ±0.985	15.74± 1.213	4.943± 0.125	5.982± 0.573	25.731 ±3.120	23.043± 4.213
16	12.218	Propenoic acid, butyl ester	C3H4O	56.06	4.080± 0.098		8.377± 0.356	7.023± 0.947	5.716± 1.076	8.303± 1.230	8.689± 1.495	10.040± 1.479
17	13.608	Camphene	C10H16	136.23							0.479± 0.087	0.837± 0.076
18	14.306	Benzaldehyde	C7H6O	106.12	1.941± 0.042		1.932± 0.084	2.507± 0.075	1.492± 0.079	1.487± 0.093	1.217± 0.201	1.811± 0.102
19	14.593	β-Myrcene	C10H16	136.23	1.356± 0.026	2.164± 0.124	10.865 ±1.097	9.988± 1.087	3.429± 0.142	2.170± 0.143	9.764± 1.843	11.255± 2.758
20	15.779	2-Propanol, 1,1′-oxybis-	C6H14O3	134.17	0.822± 0.035	5.835± 0.351						
21	16.157	2-Butenoic acid, butyl ester	C8H14O2	142.19			1.063± 0.121			1.092± 0.101		
22	16.374	Butylaldibutoxymethane	C9H20O2	160.25		2.838± 0.106	1.062± 0.095	1.018± 0.076		0.935± 0.095	0.403± 0.100	0.756± 0.134
23	17.208	Acetophenone	C8H8O	120.15	1.268± 0.095					0.424± 0.038		
24	17.862	Nonanal	C9H18O	142.24	1.400± 0.062		1.730± 0.091	2.591± 0.017	1.274± 0.095	1.422± 0.162	1.024± 0.059	1.015± 0.098
25	17.917	2-heptene	C7H14	98.2								1.169± 0.102
26	19.605	Benzoic acid	C7H6O2	122.12	2.090± 0.087							
27	20.083	Undecane	C11H24	156.31	0.829± 0.042							
28	20.493	Azulene	C10H8	128.17	2.631± 0.176		3.959± 0.096	1.085± 0.098	0.979± 0.028	0.513± 0.042	2.982± 0.356	2.395± 0.132
29	23.27	Benzocycloheptatriene	C11H10	142.19			5.150± 0.105	1.235± 0.21		0.383± 0.036	7.641± 1.292	3.436± 0.347
30	23.629	Naphthalene, 1-methyl-	C11H10	142.2			2.196± 0.067	3.016± 0.100			3.385± 0.375	1.573± 0.098

Table 1: Continued

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31	24.742	Tetradecane	C14H30	198.39	3.394± 0.124	2.901± 0.018	2.148± 0.187	
32	26.896	Pentadecane	C15H32	212.41	5.863± 0.541	4.498± 0.107	2.077± 0.258	0.614± 0.041
33	28.931	Hexadecane	C16H34	226.44	3.461± 0.106	3.902± 0.152		
34	29.759	2,6,10-Trimethyl-pentadecane	C18H38	254.49		2.638± 0.103		
35	30.853	Heptadecane	C18H38	254.49	0.897± 0.074	1.680± 0.036		
36	34.761	4-Hydroxybenzyl alcohol, bis(tert-butyldimethylsilyl) ether				4.939± 0.149		
37	36.947	Eicosenoic acid	C20H38O2	310.51		1.669± 0.062		
38	37.97	1,1′-Binaphthalene	C20H14	254.33		29.557 ±2.641	0.277± 0.048	
39	39.161	Docosane	C22H46	310.6		2.557± 0.127		
40	40.747	[1,1′:3′,1″-Terphenyl]-2′-ol	C18H14O	246.3	1.698± 0.035	2.783± 0.102		
41	41.478	2-Propenoic acid, 3-(4-Methoxyphenyl)-, 2-ethylhexyl ester	C18H26O3	290.4		1.789± 0.089		

The seasonal variation in the proportion of each volatile emitted from the trunks of *P. armeniaca* was analyzed comprehensively (Fig. 1); the proportion of hydrocarbons emitted from the trunks of healthy plants was lowest in July and highest in June, while the proportions of terpenoids and esters were highest in July and lowest in June. Differences in volatile emission were likely induced by changes in environmental factors such as temperature, illumination, water, and humidity, as well as by mechanical injury (Ping *et al*., 2001). The proportions of aldehydes (20.826%) and ethers (34.912%) emitted from the trunks of the infected plants were highest in June, while those of olefinic terpenes (29.684%) and esters (32.836%) were highest in July and August.

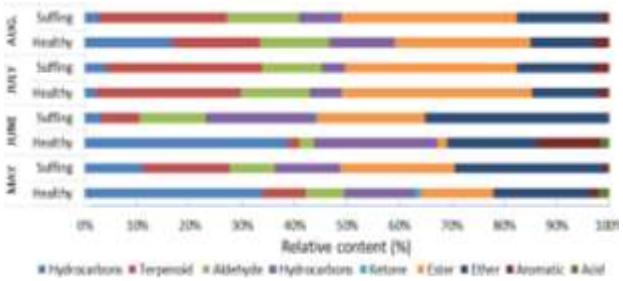

Fig. 1: Seasonal variation of volatiles emitted from the trunks of healthy & infected *P. armeniaca*

Compositional Analysis of Volatiles from the Leaves of *P. armeniaca*

A total of 45 volatiles were emitted from the leaves of *P. armeniaca* (Table 2), including 12 alkanes, 9 alkenes, 6 esters, 4 alcohols, 4 aldehydes, 4 aromatic hydrocarbons, 6 ethers, 3 ketones, 2 acids, and 2 other volatiles.

There were 38 volatiles emitted from the leaves of healthy plants. The number of types of volatiles varied with the season in the following order (from most to fewest): June>May>July>September>August. The pattern of variation observed in the number of types of volatiles emitted each month was consistent with that of the trunks. There were 26 volatiles emitted from the leaves of infected plants. Distinct from the healthy plants, the seasonal ranking by the number of types of emitted volatiles was (from most to fewest): June>August>May>September>July.

The composition of volatiles emitted by the leaves was different from that of the volatiles emitted by the trunks. The major volatiles emitted by the leaves were esters. The major volatiles emitted by the leaves of healthy plants included 3-hexen-1-ol, 3-methyl-4-penten-1-ol acetate, (E)-3-hexen-1-olacetate, 2-propenoic acid, and butyl ester. In addition to these compounds, the infected plants also emitted olefins such as azulene, benzocycloheptatriene, and styrene.

Emission of 3-methyl-4-penten-1-ol acetate mainly occurred from May to July, while emission of (E)-3-hexen-1-ol acetate mainly occurred from August to September. The patterns of variation in the emission of 3-methyl-4-penten-1-ol and (E)-3-hexen-1-ol acetate were consistent in healthy and infected *P. armeniaca*, from which both compounds were emitted in large amounts. For healthy plants, there were more volatiles emitted from the leaves in June, including (E)-3-hexen-1-ol acetate (32.592%), 3-hexen-1-ol (24.575%), and 1, 1′-binaphthalene (5.084%). For the infected plants, the emitted amounts of 3-hexen-1-ol, (E)-3-hexen-1-ol acetate, 3-methyl-4-penten-1-ol acetate were high, accounting for 64.74–94.775% of total emissions. Hexanal, (E)-4-oxohex-2-enal, cyclohexane, β-ocimene, nonanoic acid, α-cubebene were emitted by infected plants, but not by healthy plants.

Table 2: Seasonal variation of the relative contents of volatiles emitted from the leaves of healthy and infected *P. armeniaca*

NO	Retention time (min)	Compounds	Molecular formula	Relative molecular mass	Leaves of healthy plants					Leaves of infected plants				
					MAY	JUNE	JULY	AUG.	SEP.	MAY	JUNE	JULY	AUG.	SEP.
1	4.666	1-Propene,3-propoxy	C6H10O	98.00		0.589± 0.085								
2	4.737	Methylene chloride	CH2Cl2	84.93	0.515± 0.057	4.453± 1.102	1.717± 0.784	1.454± 0.985	2.545± 0.345	0.355± 0.076	0.497± 0.069	2.177± ±0.798	1.713± 0.105	1.525± 0.110
3	5.392	n-Hexane	C6H14	86.18		1.453± 0.120								
4	6.29	1-Butanol	C4H10O	74.12	0.255± 0.015	1.046± 0.953	1.775± 0.954							
5	6.869	3-Pentanone	C5H10O	86.13	1.226± 0.098	4.756± 1.023			1.736± 0.198	1.448± 0.618			0.784± 0.091	0.601± 0.049
6	8.542	Toluene	C7H8	92.14			0.966± 0.087		2.321± 0.281	0.546± 0.071		2.819± 0.814	5.064± 1.318	1.286± 0.512
7	9.078	Octane	C8H18	114.23				1.873± 0.752	1.641± 0.115		0.511± 0.081		1.425± 0.076	
8	9.338	Hexanal	C6H12O	100.16						0.250± 0.055				
9	9.603	Acetic acid, butyl ester	C6H12O2	116.16			7.760± 1.973							
10	10.978	3-hexen-1-ol	C6H12O	100.16	49.214 ±5.762	24.575 ±3.471	4.443± 1.025	5.576± 1.274	3.477± 1.031	38.294 ±4.151	14.785 ±3.975	11.643 ±3.027	1.818± 0.021	7.379± 1.754
11	11.383	n-Butyl ether	C8H18O	130.23			10.281 ±3.452						1.954± 0.318	
12	11.93	2-Propenoic acid, butyl ester	C7H12O2	128.17			11.559 ±3.785						0.874± 0.074	
13	12.212	2-Propenal	C3H4O	56.06			2.557± 0.216							
14	14.171	(E)-4-Oxohex-2-enal	C6H8O2	112.13						0.625± 0.071	0.700± 0.064			
15	14.355	2,4-hexadiene	C6H10	82.14	0.388± 0.046	1.472± 0.175								
16	14.615	β-Myrcene	C10H16	136.23	2.353± 0.125	3.864± 1.000	1.906± 0.108			0.994± 0.068				
17	14.902	4-Penten-1-ol, 3-methyl-, acetate	C8H14O2	142.20				85.032 ±6.425	66.954 ±5.147		4.958± 1.023		62.922 ±6.024	74.701 ±5.163
18	15.042	3-Hexen-1-ol, acetate, (E)-	C8H14O2	142.20	43.524 ±5.746	32.592 ±5.124	55.694 ±7.214			56.481 ±6.947	68.216 ±6.741	64.516 ±6.178		
19	15.135	Cyclohexane	C6H12	84.16							2.537± 0.948	0.975± 0.081		
20	15.795	1-Pentanol	C5H12O	88.15		0.568± 0.067					0.997± 0.079			
21	16.168	β-Ocimene	C10H16	136.23							1.872± 0.296			
22	17.235	Acetophenone	C8H8O	120.15		0.605± 0.072								
23	17.862	Nonanal	C9H18O	142.24	0.112± 0.075		0.645± 0.098	0.836± 0.045	3.538± 1.020		0.498± 0.038		1.257± 0.621	1.089± 0.137
24	17.944	2-Heptene	C7H14	98.20				1.255± 0.896			0.336± 0.015		1.841± 0.348	
25	20.563	Azulene	C10H8	128.17			0.697± 0.069	0.728± 0.087	7.647± 2.450		0.518± 0.062	7.118± 2.916	7.24±2. 954	4.297± 1.076
26	21.76	Nonanoic acid	C9H18O2	158.24							0.902± 0.084			
27	22.452	Tridecane	C13H28	184.36	0.226± 0.063									
28	23.27	Benzocycloheptatriene	C11H10	142.20				0.799± 0.095	6.875± 2.078			7.919± 2.795	8.912± 2.107	6.558± 2.174
29	23.833	Styrene	C8H8	104.15		0.536± 0.085			3.266± 1.025			2.833± 0.946	3.492± 0.651	2.564± 0.718
30	24.736	Tetradecane	C14H30	198.39	0.137± 0.034	1.121± 0.752				0.378± 0.071	0.454± 0.053			

Table 2: Continued

Table 2: Continued

31	25.424	Tocopherol	C29H50O2	430.71			2.447± 0.287	0.275± 0.019	0.562± 0.041	0.704± 0.095
32	26.896	Pentadecane	C15H32	212.41	1.644± 0.245	2.157± 0.956		0.354± 0.036	0.783± 0.096	
33	27.156	α-Cubebene	C15H24	204.35					0.412± 0.075	
34	28.644	Phenylmaleic anhydride	C10H6O3	174.15		1.359± 0.354				
35	28.937	Hexadecane	C16H34	226.44	0.121± 0.054	1.411± 0.196				
36	30.853	Heptadecane	C17H36	240.47		0.614± 0.084				
37	34.755	4-Quinolinol, 2-phenyl-	C15H11NO	221.25		0.633± 0.036				
38	37.37	Fenharmane	C18H18N2	262.35	0.285± 0.065	0.490± 0.062				
39	37.96	1,1′-Binaphthalene	C20H14	254.33		5.084± 1.020				
40	39.134	Docosane	C22H46	310.60		3.215± 0.321				
41	39.675	Benzoic acid, tetradecyl ester	C21H34O2	318.49		1.143± 0.098				
42	40.606	Hexacosane	C26H54	366.71		0.742± 0.087				
43	40.758	[1,1′:3′,1″-Terphenyl]-2′-ol	C18H14O	246.30		2.008± 0.100			0.462± 0.086	
44	41.175	Benzoic acid, tridecyl ester	C14H12O2	212.24		1.126± 0.135				
45	41.472	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	C18H26O3	290.40		2.388± 0.283				

The pattern of variation in volatile emission by the leaves from May to September was comprehensively analyzed, as shown in Fig. 2. For the healthy plants, emission of esters was highest from July to September. Eneynes were mainly emitted in September. Emission of alcohols gradually declined from May to September. For the infected plants, the amount of emitted alcohols also showed a decreasing trend, while eneynes emission gradually increased. There was little variation in ester emission from May to September.

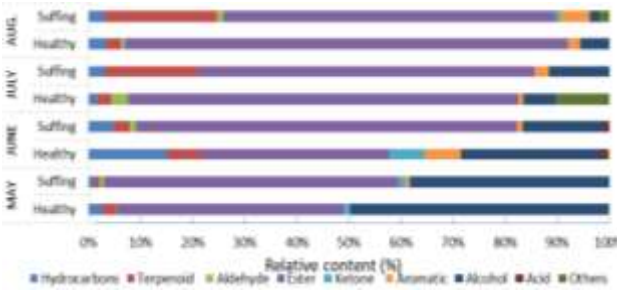

Fig. 2: Seasonal variation of volatiles emitted from the leaves of healthy & infected *P. armeniaca*

Daily Variation of Volatiles Emitted from the Trunks of *P. armeniaca*

According to the total ion chromatograms, 53 volatiles (see Table 3 for specific content and number of types) were identified, including 10 alkanes, 9 alkenes, 6 alcohols, 5 ketones, 6 aldehydes, 3 acids, 6 esters, 4 aromatic hydrocarbons, 2 ethers, and 1 other compound (Table 1). The major volatiles emitted from the trunks were alkanes.

The amounts of volatiles emitted from the healthy and infected plants varied over time. As shown in Table 3, methylene chloride, n-butyl ether, and pentadecane were emitted from the trunks of healthy plants at different times of day. It was found that more types of volatiles were emitted between 9:00 to 17:00 h than between 17:00 to 21:00 h.

The main volatiles emitted from the healthy plants were 1-butanol (27.107% between 9:00 and 17:00 h, 36.299% between 17:00 and 21:00 h), acetic acid butyl ester (12.671% between 9:00 and 17:00 h, 10.672% between 17:00 and 21:00 h), and n-butyl ether (24.126% between 9:00 and 17:00 h, 17.573% between 17:00 and 21:00 h). The major types of volatiles emitted from the infected plants were generally consistent throughout the day. Methylene chloride, 1-butanol, hexanal, acetic acid, butyl ester, n-butyl ether, 2-propenoic acid, butyl ester, 2-propenal, butanoic acid, butyl ester, 2-heptene, and decanal were continuously emitted from the infected plants and accounted for 89.8–94.5% of the total emissions.

Table 3: Dynamic daily variation of the relative contents of volatiles emitted from the trunks of healthy and infected *P. armeniaca*

NO	Retention time (min)	Compounds	Molecular formula	Relative molecular mass	Trunks of healthy plants				Trunks of infected plants			
					9:00	13:00	17:00	21:00	9:00	13:00	17:00	21:00
1	4.25	Ethanol	C_2H_6O	46.07		1.599± 0.098						
2	4.439	Methyl isobutyl ketone	C6H12O	100.16		1.766± 0.215			0.346± 0.061	0.286± 0.043		1.063± 0.085
3	4.731	Methylene chloride	CH2Cl2	84.93	16.596± 1.037	12.974 ±1.020	2.133± 0.291	3.918± 0.411	2.661± 0.246	5.726± 0.948	10.816 ±1.058	25.194 ±2.485
4	5.273	2-Butanone	C4H8O	72.11						0.206± 0.069		
5	5.424	n-Hexane	C6 H14	86.18	3.933± 0.195	4.876± 0.412			0.327± 0.096	0.337± 0.063		
6	5.689	Ethyl acetate	C4H8O2	88.11		1.627± 0.085			0.641± 0.068			
7	6.242	1-Butanol	C4H10O	74.12		2.498± 0.125	27.107 ±2.741	36.299 ±2.154	20.826 ±2.781	20.453 ±2.189	16.428 ±1.954	31.240 ±3.102
8	6.566	Acetic acid	C2H4O2	60.05	17.725± 1.084	16.648 ±1.278						
9	7.698	1-Pentene	C5H10	70.13					1.597± 0.062			
10	8.536	1,3,5-Cycloheptatriene	C7H8	92.14					2.343± 0.209			
11	9.18	Chalcone	C15H12O	208.26				0.968± 0.075				
12	9.359	Hexanal	C6H12O	100.16		1.811± 0.089	0.795± 0.064	1.172± 0.185	0.591± 0.091	0.714± 0.047	1.350± 0.103	1.700± 0.117
13	9.603	Acetic acid butyl ester	C6H12O2	116.16			12.671 ±1.856	10.672 ±1.476	12.768 ±1.020	14.81± 1.719	12.719 ±1.246	7.883± 0.987
14	10.886	C2H5CH=CHCH=CH2	C6H10	82.15			1.226± 0.369					
15	11.383	n-Butyl ether	C8H18O	130.23	11.996± 1.521	2.697± 0.158	24.126 ±2.183	17.573 ±1.956	34.914 ±3.046	34.936 ±3.147	28.769 ±2.875	13.542 ±1.795
16	11.93	2-Propenoic acid, butyl ester	C7H12O2	128.17			5.402± 0.459	5.142± 0.657	5.982± 1.025	6.404± ±1.327	5.64±0. 754	3.012± 0.130
17	12.222	2-Propenal	C3H4O	56.06			7.225± 0.981	7.445± 1.020	8.303± 1.237	8.52±1. 965	8.108± 0.847	4.959± 0.128
18	13.05	α-Pinene	$C_{10}H_{16}$	136.23						0.919± 0.091	1.709± 0.108	0.712± 0.051
19	13.608	Camphene	$C_{10}H_{16}$	136.23						0.319± 0.036	0.432± 0.071	
20	14.306	Benzaldehyde	C7H6O	106.12		2.011± 0.210	0.962± 0.038	1.657± 0.115	1.487± 0.200	0.569± 0.064	0.764± 0.049	
21	14.593	β-Myrcene	C10H16	136.23	2.164± 0.124		1.066± 0.095	1.714± 0.087	0.301± 0.076			
22	14.707	Butanoic acid, butyl ester	C8H16O2	144.21			1.529± 0.203	2.051± 0.108	1.869± 0.125	1.814± 0.103	1.210± 0.121	1.317± 0.143
23	15.07	2-Octen-1-ol	C8H16O	128.21			1.258± 0.107				0.835± 0.078	0.743± 0.076
24	15.779	1-Hexanol, 2-ethyl-	C8H18O	130.23	5.835± 0.351							
25	16.157	2-Butenoic acid, butyl ester	C8H14O2	142.2			0.646± 0.036	1.185± 0.102	1.092± 0.103	0.820± 0.089	0.770± 0.069	
26	16.374	Butylaldibutoxymethane	C9H20O2	160.25	2.838± 0.106		0.712± 0.065	1.123± 0.135	0.935± 0.069	0.989± 0.096	1.153± 0.102	
27	17.208	Acetophenone	C8H8O	120.15					0.424± 0.076			
28	17.862	Nonanal	C9H18O	142.24		2.399± 0.105	2.679± 0.201	3.231± 0.365	1.422± 0.100	0.758± 0.098	3.858± 0.375	2.458± 0.269
29	17.917	2-Heptene	C7H14	98.2							0.871± 0.068	0.739± 0.75
30	19.529	d-Camphora	C10H16O	152.23				1.573± 0.100			0.670± 0.071	

Table 3: Continued

Table 3: Continued

31	20.466	Decanal	C10H20O	156.26		2.81 ±0.116	1.524± 0.069	2.085± 0.128	0.513± 0.096	0.313± 0.046	0.996± 0.100	0.807± 0.079
32	23.27	Benzocycloheptatriene	C11H10	142.2					0.383± 0.073	0.401± 0.069		
33	23.914	Urea, 2-propenyl-	C3H7NS	100.12						0.262± 0.076	0.767± 0.076	1.201± 0.105
34	24.742	Tetradecane	C14H30	198.39	2.901± 0.018	3.120± 0.068	1.125± 0.123	0.939± 0.096				
35	25.408	Tocopherol	C29H50O2	430.71						0.444± 0.099	1.556± 0.106	2.618± 0.256
36	26.896	Pentadecane	C15H32	212.41	4.498± 0.107	6.945± 0.452	1.387± 0.098	1.253± 0.102			0.579± 0.079	0.811± 0.072
37	28.931	Hexadecane	C16H34	226.44	3.902± 0.152	4.879± 0.185						
38	29.759	2,6,10-Trimethyl-pentadecane	$C_{18}H_{38}$	254.49	2.638± 0.103							
39	30.717	1-Hexadecanol	C16H34O	242.44		2.322± 0.120						
40	30.853	Heptadecane	C17H36	240.47	1.680± 0.036	2.250± 0.132						
41	31.421	2H-1,4-Benzoxazine, 3,4-dihydro-	C8H9NO	135.16		1.269± 0.095						
42	34.761	4-Hydroxybenzyl alcohol, bis(tert-butyldimethylsilyl) ether	C6H14O3	46.07	4.939± 0.149	2.83±0. 152						
43	36.947	Eicosenoic acid	C20H38O2	100.16	1.669± 0.062	1.791± 0.075						
44	37.651	Heneicosane	C21H44	84.93		2.599± 0.105						
45	37.97	1,1′-Binaphthalene	C20H14	72.11	29.557± 2.641	4.464± 0.185			0.277± 0.021			
46	39.161	Docosane	$C_{22}H_{46}$	86.18		6.215± 0.468						
47	39.681	Benzoic acid, tetradecyl ester		88.11	2.557± 0.127							
48	40.119	Ethyl iso-allocholate	C12H26O	74.12			1.252± 0.093					
49	40.401	Squalene	C30H50	60.05			3.631± 0.378					
50	40.612	Hexacosane	C26H54	70.13		4.819± 0.207						
51	40.747	[1,1′:3′,1″-Terphenyl]-2′-ol	C18H14O	92.14	2.783± 0.102							
52	41.478	2-Propenoic acid, 3-(4-methoxyphenyl)-,2 -ethylhexyl ester	C18H26O3	208.26	1.789± 0.089							
53	41.727	9-Heptadecanol	C17H36O	100.16		2.782± 0.158	1.544± 0.087					

After comparing volatile emission from healthy and infected plants throughout the day, it was found that the composition and contents of volatiles emitted from the trunks were similar between 13:00 and 21:00 h. The volatiles specific to the infected plants included 2-butanone, 1-pentene, 1,3,5-cycloheptatriene, α-pinene, camphene, acetophenone, and 2-heptene. The volatiles specific to the infected plants were not emitted at high levels and were mostly alkenes. It has been reported extensively that alkenes can attract trunk-boring insects (Hare, 2010; Helms *et al*., 2014), demonstrating the importance of this result in the context of attraction of destructive insects to injured plants.

Daily variation in volatile emission from the trunks of *P. armeniaca* was analyzed, as shown in Fig. 3. For the healthy plants, alkanes and acids were emitted in large quantities at 13:00 h. The amounts of emitted aldehydes and alcohols gradually increased between 9:00 and 21:00 h, while the amounts of emitted ethers, esters and eneynes peaked at 17:00 h. For the infected plants, the major volatiles emitted from the trunks were detected throughout the day. The amounts of emitted alcohols and ketones showed an increasing trend between 9:00 and 21:00 h and reached maximum levels at 21:00 h. Emissions of ethers, esters, and aldehydes were greatest at 9:00 h.

Overall Compositional Analysis of the Volatiles Emitted by the Leaves of *P. armeniaca*

Daily variation in volatile emission from the leaves of *P. armeniaca* was analyzed using total ion chromatograms. A total of 47 volatiles were identified (see Table 4 for specific contents and types).

Table 4: Dynamic daily variation of the relative contents of volatiles emitted from the leaves of healthy and infected *P. armeniaca*

NO	Retention time (min)	Compounds	Molecular formula	Relative molecular mass	Leaves of healthy plants				Leaves of infected plants			
					9:00	13:00	17:00	21:00	9:00	13:00	17:00	21:00
1	4.666	1-Propene,3-propoxy	C6H10O	98.00	0.589± 0.085							
2	4.737	Methylene chloride	CH2Cl2	84.93	4.453± 1.102	2.543± 0.218	0.468± 0.072	1.356± 0.125	0.497± 0.042	0.394± 0.043	1.309± 0.245	2.205± 0.263
3	5.392	n-Hexane	C6 H14	86.18	1.453± 0.120							0.282± 0.023
4	6.29	1-Butanol	C4H10O	74.12	1.046± 0.953							
5	6.604	4-penten-2-ol, 3-methyl	C6H12O	100.16		0.502± 0.076	1.827± 0.168	3.238± 0.375		0.992± 0.079	1.191± 0.168	1.600± 0.173
6	6.869	3-Pentanone	C5H10O	86.13	4.756± 1.023		0.799± 0.092	1.982± 0.146		0.220± 0.009	0.352± 0.025	1.184± 0.096
7	9.078	Octane	C8H18	114.23			0.113± 0.046		0.511± 0.064	0.344± 0.75	0.699± 0.074	
8	9.191	Chalcone	C15H12O	208.26			0.288± 0.061	0.616± 0.081				0.350± 0.032
9	9.338	Hexanal	C6H12O	100.16						10.337 ±1.020	19.52± 1.527	3.916± 0.765
10	10.561	3-methyl-2-hexene	C7H14	98.19								0.823± 0.076
11	10.729	1-hexanol, 4-methyl	C7H16O	116.20		0.819± 0.094						0.229± 0.040
12	10.978	3-hexen-1-ol	C6H12O	100.16	24.575 ±3.471		20.789 ±2.965	35.598 ±3.152	14.785 ±1.213	36.221 ±3.985	21.599 ±2.174	37.898 ±5.120
13	12.212	2-Propenal	C3H4O	56.06							0.636± 0.049	0.282± 0.037
14	14.171	(E)-4-Oxohex-2-enal	C6H8O2	112.13				0.283± 0.087	0.700± 0.027	0.704± 0.042	0.385± 0.053	0.265± 0.026
15	14.355	2,4-hexadiene	C6H10	82.14	1.472± 0.175							
16	14.615	β-Myrcene	C10H16	136.23	3.864± 1.000	3.293± 1.025	0.230± 0.033					
17	14.902	4-Penten-1-ol, 3-methyl-, acetate	C8H14O2	142.20		23.634 ±2.956	18.036 ±1.213	30.592 ±2.915	4.938± 1.003	10.170 ±1.427	0.516± 0.061	10.008 ±2.015
18	15.042	3-Hexen-1-ol, acetate, (E)-	C8H14O2	142.20	32.592 ±5.124	67.21± 5.023	54.519 ±4.312	24.506 ±2.103	68.217 ±4.109	36.082 ±3.956	43.466 ±4.128	39.223 ±4.068
19	15.135	Cyclohexane	C6H12	84.16			0.337± 0.037		2.517± 0.196	0.350± 0.038	1.151± 0.130	0.685± 0.071
20	15.795	1-Pentanol	C5H12O	88.15	0.568± 0.067		0.332± 0.023	0.777± 0.072	0.997± 0.075			
21	16.168	β-Ocimene	C10H16	136.23					1.872± 0.108			
22	16.921	Methacrolein	C4H6O	70.09							0.367± 0.049	
23	17.235	Acetophenone	C8H8O	120.15	0.605± 0.072							
24	17.657	Chlorpyrifos	C9H11Cl3 NO3PS	350.59		1.026± 0.685	0.284± 0.042			0.261± 00.029		
25	17.862	Nonanal	C9H18O	142.24			0.199± 0.034	0.187± 0.036	0.498± 0.057	2.952± 0.132	6.08±1. 002	0.475± 0.042
26	17.944	2-Heptene	C7H14	98.20					0.336± 0.043		0.507± 0.057	0.234± 0.028
27	20.006	Dodecane	C12H26	170.34		0.972± 0.085	0.180± 0.015					
28	20.563	Azulene	C10H8	128.17					0.518± 0.072			
29	21.76	Nonanoic acid	C9H18O2	158.24					0.902± 0.062	0.224± 0.071		
30	22.452	Tridecane	C13H28	184.36			0.409± 0.064					

Table 4: Continued

Table 4: Continued

31	23.833	Styrene	C8H8	104.15	0.536± 0.085						
32	24.736	Tetradecane	C14H30	198.39	1.121± 0.752	0.487± 0.048	0.189± 0.024	0.454± 0.037			
33	25.424	Tocopherol	C29H50O2	430.71			0.232± 0.046	0.562± 0.046			
34	26.896	Pentadecane	C15H32	212.41	2.157± 0.956	0.556± 0.036	0.438± 0.075	0.783± 0.058	0.385± 0.048	1.125± 0.102	
35	27.156	α-Cubebene	C15H24	204.35				0.412± 0.031			
36	28.644	Phenylmaleic anhydride	C10H6O3	174.15	1.359± 0.354						
37	28.937	Hexadecane	C16H34	226.44	1.411± 0.196	0.147± 0.025				0.579± 0.061	
38	30.853	Heptadecane	C17H36	240.47	0.614± 0.084						
39	34.755	4-Quinolinol, 2-phenyl-	C15H11NO	221.25	0.633± 0.036						
40	37.37	Fenharmane	C18H18N2	262.35	0.490± 0.062						
41	37.96	1,1′-Binaphthalene	C20H14	254.33	5.084± 1.020						
42	39.134	Docosane	C22H46	310.60	3.215± 0.321						
43	39.675	Benzoic acid, tetradecyl ester	C21H34O2	318.49	1.143± 0.098						
44	40.606	Hexacosane	C26H54	366.71	0.742± 0.087						
45	40.758	[1,1′:3′,1″-Terphenyl]-2′-ol	C18H14O	246.30	2.008± 0.100			0.462± 0.041	0.364± 0.037	0.518± 0.049	0.341± 0.035
46	41.175	Benzoic acid, tridecyl ester	C14H12O2	212.24	1.126± 0.135						
47	41.472	2-Propenoic acid, 3-(4-Methoxyphenyl)-, 2-ethylhexyl ester	C18H26O3	290.40	2.388± 0.283						

Thirty-six volatiles were emitted from the healthy plants, while 29 volatiles were emitted from the infected plants.

In contrast to those emitted from the trunk, the major volatiles emitted from the leaves were esters. The major volatiles emitted from the leaves of healthy plants included dichloromethane, 3-hexen-1-ol, vinyl acetate, cis-3-hexenyl acetate, and 1,1′-binaphthyl. The major volatiles emitted from the leaves of infected plants included 3-hexen-1-ol, vinyl acetate, cis-3-hexenyl acetate and hexanal.

The amounts of volatiles emitted from the leaves of healthy and infected plants varied with time, while the major types of volatiles were relatively fixed. As shown in Table 2, dichloromethane and cis-3-hexenyl acetate were emitted from the leaves of the healthy plants throughout the day. The amount of dichloromethane emitted from the leaves gradually declined throughout the day. Cis-3-hexenyl acetate was emitted in a small amount in the morning and evening. The number of types of volatiles was greatest at 9:00, when the main volatiles were 3-hexen-1-ol (24.475%), cis-3-hexenyl acetate (32.610%), and 1-1′-binaphthyl (5.084%). The major volatiles emitted from the leaves of healthy plants at 13:00 were vinyl acetate (23.634%), cis-3-hexenyl acetate (67.210%), and 3-hexen-1-ol (20.789% at 17:00, 35.598% at 19:00), while during

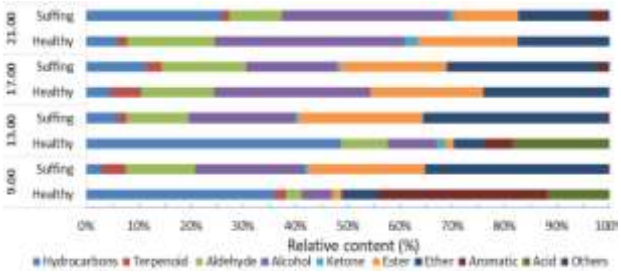

Fig. 3: Daily variation of volatiles emitted from the trunks of healthy & infected *P. armeniaca*

17:00 to 21:00 the major volatiles emitted from the leaves were vinyl acetate (18.036% at 17:00, 30.592% at 19:00) and cis-3-hexenyl acetate (54.519% at 17:00, 24.506% at 19:00).

For the infected plants, the volatiles include dichloromethane, 3-hexen-1-ol, vinyl acetate, cis-3-hexenyl acetate, hexanal, nonanal and 2,6-diphenylphenol were continuously emitted from the leaves over a 24-h period and accounted for 75.024%–92.614% of the total emitted volatiles. The amount of emitted dichloromethane gradually increased, consistent with the pattern of emission from the trunks, while cis-3-hexenyl acetate emission gradually declined throughout the day.

The composition and contents of volatiles emitted from the leaves of the infected and healthy plants were similar after 15:00, consistent with the variation in emission from the trunks. Volatiles emitted specifically by the infected plants included 3-methyl-2-hexene, acraldehyde, β-ocimene, azulene, and 2-crotonaldehyde. Volatiles specific to the infected plants were emitted at low levels and were mostly olefins. Volatile emission specific to the leaves of infected plants was similar to volatile emission specific to the trunks, suggesting that olefins can specifically attract trunk-boring insects (Zhang *et al*., 2007).

Daily variation in volatile emission from the leaves was analyzed (Fig. 4). For the healthy plants, eneynes and acids were mainly emitted from 9:00–13:00 h, while aromatic hydrocarbons and ketones were emitted mainly at 9:00 h and 21:00 h, respectively. For the infected plants, alkanes emission was highest at 9:00 h and then decreased sharply. Alcohol emission was higher at 21:00 h than at 9:00 h. Aldehyde emission was highest at 17:00 h and lowest at 9:00 h and 21:00 h.

Behavioral Response to Apricot Trunks Volatiles

In Olfactometer behavioral tests, the percentage of adult beetles responding to stimuli of phloem tested was generally high (Table 5). Dors from phloem of apricot elicited a significant attraction in both male and female Scolytus seulensis. When beetles were presented with phloem extract versus control hexane a significant preference for phloem extract was exhibited by both males and females.

Discussion

The odor emitted by plants is caused by the mixture of major components with trace quantities of secondary products. Insects are highly sensitive to particular plant volatiles. Odors can serve as complicated signals through diverse combinations of components with varying proportions. Due to the high sensitivity and selectivity of insect sense organs, chemicals can convey signals even in trace amounts. The number of types, composition, and relative proportions of volatiles emitted from plants are related to the physiological status of the emitting plants. External environmental factors such as illumination, temperature, water, CO_2 concentration and humidity, as well as the degree of invasion and stress from different insects, also influence volatile emission (Ping *et al*., 2001).

From May to August, a total of 41 volatiles were emitted from the trunks of *P. armeniaca*, while the number of types of emitted volatiles was reduced in July. The variation in volatile emission may have been related to weather conditions. In the sampling area, May and August are associated with high temperatures and little precipitation, while July has more precipitation. The major types of volatiles emitted from the trunks of the infected plants and healthy plants were similar from May to August. However, some volatiles were emitted at higher levels from the trunks of the infected plants than from the healthy plants, including methylene chloride, 1-butanol, 1,3,5-cycloheptatriene, n-butyl ether, isobutyl acrylate, propenoic acid, butyl ester, benzaldehyde, β-myrcene, nonanal, and naphthalene. Differences in the levels of emission of these volatiles may have been due to stress caused by *S. seulensis* on *P. armeniaca*. A total of 45 volatiles were emitted from the leaves of *P. armeniaca*. The major volatiles emitted from the leaves were esters. The volatiles specific to the infected plants were hexanal, (E)-4-oxohex-2-enal, cyclohexane, β-ocimene, nonanoic acid, and α-cubebene. The infected plants emitted greater amounts of azulene and benzocycloheptatriene from the leaves than the healthy plants. Changes in the proportions of volatiles emitted at trace levels may provide key chemical information to adult *S. seulensis* favoring *P. armeniaca* as hosts and sites of oviposition and mate seeking (Ruther and Kleier, 2005).

Semiochemicals have considerable diversity, complexity, and spatio-temporal variability; therefore, the sensory environment inhabited by insects is a complex dynamic system (Huber *et al*., 2001; Kigathi *et al*., 2009). Changes in emission of trace volatiles are particularly important, because such volatiles may serve as the basis for the development of efficient insect attractants. Through analysis of the specific volatiles emitted by the trunks and leaves of infected plants, it was found that some olefins were only emitted from the infected plants, including 1,3,5-cycloheptatriene, camphene, β-myrcene, β-ocimene, azulene, and benzocycloheptatriene. Although the olefins specific to the injured plants were not emitted at high levels, many reports have indicated that they are the major volatiles responsible for attracting trunk-boring insects to host plants.

There were changes in the relative proportions and composition of volatiles emitted from the trunks and leaves of healthy plants at different times of day. More types of volatiles were emitted from the leaves at 9:00 h and 17:00 h in comparison with the rest of the day, which may have been related to temperature and illumination intensity. Leaves are the primary site of respiration and photosynthesis in plants. High temperatures and strong illumination at noon can cause closure of the stomas on the leaves and suppress photosynthesis, leading to a decline in volatile emission. Moreover, temperature and illumination intensity are lower at night, which may suppress photosynthesis and volatile emission. However, there was little difference in the composition of volatiles emitted from the infected plants throughout the day. The composition and relative content of volatiles emitted from the trunks and leaves of healthy plants between 17:00 and 21:00 h were similar to those of the infected plants. In our field survey, we observed that adult *S. seulensis* generally emerge from their tunnels in the evening. Because the composition of volatiles emitted from the trunks of healthy plants is similar to that emitted by the infected plants in the evening, adult insects are induced to leave their tunnels and seek new hosts.

Table 5: Behavioral response of Scolytus seulensis adults in aolfactometer to different sources of Phloem of apricot odors

Odor sources	Male				Female			
	N[a]	Treated[b]	X^2	P Value[c]	N[a]	Treated[b]	X^2	P Value[c]
Phloem of apricot versus control air	20(20)	18	13.2	<0.001	20(20)	18	16.2	<0.001
Phloem of apricot extract versus control hexane	20(20)	18	11.6	<0.001	20(20)	18	12.6	<0.001

[a]Total sample size (*N*: number of individuals that made a choice in parentheses)
[b]Number of individuals (of those that made a choice) that chose the treated arm first
[c]d.f. = 1 for all χ^2 reported

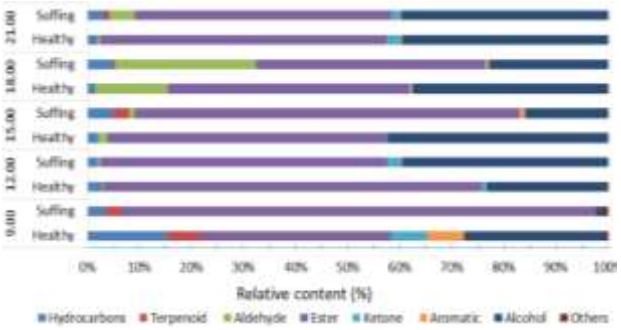

Fig. 4: Daily variation of volatiles emitted from the leaves of healthy & infected *P. armeniaca*

When seeking specific host plants, phytophagous insects mainly rely on their olfactory receptors to recognize the chemical fingerprints specific to the hosts (Barata *et al*., 2002; Zakir *et al*., 2013). The recognition of the chemical fingerprints of plant odors by insects is a complicated process, in which the concentrations of plant volatiles and the proportion of each volatile critically influence host selection, mating, and oviposition (Yan and Wang, 2006). *S. seulensis* are attracted to a complex mixture of exogenous odorous substances, rather than to a single substance. Strong attraction of phytophagous insects to host plants occurs due to the appropriate mixture of different volatiles emitted at different physiological phases.

In comparison with the standard GC, the ATD-GC/MS method has certain limitations. The absolute content of each volatile cannot be determined. Although many samples had consistent relative proportions as determined by GC analysis, the differences in the concentrations of volatiles emitted by the different samples were not evaluated. Therefore, the appropriate concentrations of volatiles in *S. seulensis* attractants must be determined by further biological tests on *S. seulensis*. The findings presented herein provide a foundation for the development and screening of botanical attractants for *S. seulensis* that could be used for ecological control.

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References

Barata, E.N., H. Mustaparta, J.A. Pickentt, L.J. Wadhams and J. Araujo, 2002. Encoding of host and non-host plant odours by receptor neurons in the eucalyptus woodborer *Phoracantha semipunctata* (Coleoptera: Cemmbycidae). *J. Comput. Physiol*., 188: 121–133

Bin, C., F. XiaoXia, H. Qi, Z. BaoMin, Z. DaMing, L. XingPeng, G. ChangQi and S. XiaoLing, 2010. Effects of Herbivore-Induced *Ulmus pumila* Volatiles on the Host Selection Process of *Ambrostoma quadriimpreeeum*. *Sci. Silvae Sin*., 46: 76–82

Birkett, M.A., C.A.M. Campbell, K. Chamberlain, E. Guerrieri, A.J. Hick, J.L. Martin, M. Matthes, J.A. Napier, J. Pettersson, J.A. Pickett, G.M. Poppy, E.M. Pow, B.J. Pye, L.E. Smart, G.H. Wadhams, L.J. Wadhams and C.M. Woodcock, 2000. New roles forcis-Jasmone as an insect semiochemical and in plant defense. *Plant Biol*., 97: 9329–9334

Halitschke, R., J.A. Stenberg, D. Kessler, A. Kessler and I.T. Baldwin, 2008. Shared signals 'alarm calls' from plants increase apparency to herbivores and their enemies in nature. *Ecol. Lett*., 11: 24–34

Hare, J.D., 2010. Ontogeny and season constrain the production of herbivore-inducible plant volatiles in the field. *J. Chem. Ecol*., 36: 1363–1367

Helms, A.M., C.M.D. Moraes, M.C. Mescher and J.F. Tooker, 2014. The volatile emission of *Eurosta solidaginis* primes herbivore-induced volatile production in *Solidago altissima* and does not directly deter insect feeding. *BMC Plant Biol*., 14: 1–9

Huber, D.P.W., J.H. Borden and M. Stastny, 2001. Response of the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae), to conophthorin and other angiosperm bark volatiles in the avoidance of non-hosts. *Agric. Entomol*., 3: 225–232

Fan, H., Y.J. Jin and J.Q. Li, 2004. Advances on plant volatile steriochemicals attracting herbivorous insects, *J. Beijing For. Uni*., 26: 76–81

James, D.G., 2003. Synthetic Herbivore-Induced Plant Volatiles as Field Attractants for beneficial Insects, *Environ. Entomol*., 32: 977–982

James, D.G. and T.S.Price, 2004. Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *J. Chem. Ecol.*, 30: 1613–1628

Kaseleht, K., T. Paalme, A. Mihhalevski and I. Sarand, 2011. Analysis of volatile compounds produced by different species of lactobacilli in rye sourdough using multiple headspace extraction. *Int. J. Food Sci. Technol.*, 46: 1940–1946

Kigathi, R.N., S.B. Unsicker, M. Reich, J. Kesselmeier, J. Gershenzon and W.W. Weisser, 2009. Emission of volatile organic compounds after herbivory from *Trifolium pratense* (L.) under laboratory and field conditions. *J. Chem. Ecol*., 35: 1335–1348

Ping, L.Y., Y.B. Shen, Y.J. Jin and J.H. Hao, 2001. Leaf volatiles induced by mechanical damage from diverse taxonomic tree species. *Acta Bot. Sin*., 43: 261–266

Poland, T.M., P.D. Groot, S. Burke, D. Wakarchuk, R.A. Haack and R. Nott, 2004. Semiochemical Disruption of the Pine Shoot Beetle, *Tomicus piniperda* (Coleoptera: Scolytidae). *Environ. Entomol.*, 33: 221–226

Pureswaran, D.S., R. Gries and J.H. Borden, 2004. Antennal responses of four species of tree-killing bark beetles (Coleoptera: Scolytidae) to volatiles collected from beetles and their host and non-host conifers. *Chemoecology*, 14: 59–66

Ruther, J. and S. Kleier, 2005. Plant-plant signaling: Ethylene synergizes volatile emission in *Zea mays* induced by exposure to (z)-3-hexen-1-o1. *J. Chem. Ecol*., 31: 2217–2222

Loon, J.J.A., Z.W. Chen, J.K. Nielsen, R. Gols and T.Q. Yu, 2002. Flavonoids from cabbage are feeding stimulants for diamondback moth larvae additional to glucosinolates: chemoreception and behavior. *Entomol. Exp. Appl.*, 104: 27–34

Wright, G.A. and B.H. Smith, 2004. Variation in complex olfactory stimuli and its influence on odour recognition. *Proc. Biol. Sci*., 271: 147–152

Wu, S.R., P.Y. Zhou, Z.Y. Li, R.J. Fu and S.Y. Yuan, 2010. Analysis of the volatile constituents of pine needles from health and debility *Pinus yunnanensis*. *Nat. Prod. Res. Dev*., 22: 1048–1052

Yan, Z.G. and C.Z. Wang, 2006. Identification of *Mythmna separata*-induced maize volatile synomones that attract the parasitoid *Campoletis chlorideae*. *J. Appl. Entomol*., 130: 213–219

Zakir, A., M. Bengtsson and M.M. Sadek, 2013. Specific response to herbivore-iduced de novo synthesized plant volatiles provides reliable information for host plant selection in a moth. *J. Exp. Biol*., 216: 3257–3263

Zhang, Q.H. and F. Schylter, 2004. Olfactory recognition and behavioral avoidance of angiosperm non-host volatiles by conifer-inhabiting bark beetles. *Agric. For. Entomol*., 6: 1–19

Zhang, Q.H., F. Schlyter and G. Birgersson, 2000. Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (Coleoptera: Scolytidae): Chemical and electrophysiological analysis. *Chemoecology*, 10: 69–80

Zhang, Q.H., F. Schlyter, G. Che and Y. Wang, 2007. Electrophysiological and Behavioral Responses of *Ips subelongatus* to Semiochemicals from Its Hosts, Non-hosts, and Conspecifics in China. *J. Chem. Ecol*., 33: 391–404

Arsenault, A.L., N.P. Havill, A.E. Mayfield and K.F. Wallin, 2015. Behavioral Responses of *Laricobius* spp. and Hybrids (Coleoptera: Derodontidae) to Hemlock Woolly Adelgid and Adelgid Host Tree Odors in an Olfactometer. *Environ. Entomol.*, 44: 1562–1570

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