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## RESEARCH ARTICLE

### Identification of *Caragana* plant volatiles, overlapping profiles, and olfactory attraction to *Chlorophorus caragana* in the laboratory

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*Chlorophorus caragana* (Coleoptera: Cerambycidae) is a trunk borer that feeds on *Caragana* shrubs in the desert. There are five species of *Caragana* plant in the distribution area of *Ch. caragana*. We investigated damaged *Caragana* plants in the field. Olfactory responses of female *Ch. caragana* to plants and identified volatile compounds from *Caragana* plants were further evaluated. *Caragana davazamcii* was severely damaged in the field, followed by *Caragana microphylla*. No damage was found to the other three species. Behavioral experiments showed that *C. davazamcii*, *C. microphylla*, and *Caragana korshinskii* were attractive to female insects. *Caragana ordosica* could repel and avoid female insects. *Caragana brachypoda* had no effect on the orientation behavior of female insects. Seventy volatile components were identified from the *Caragana* plants, and (*Z*)- $\beta$ -ocimene, 1,3-pentadiene, (*Z*)-3-hexenyl acetate, perillene, chrysanthenone, and limonene were the most abundant volatiles identified from the *Caragana* plants. The volatiles were categorized into three groups. Those most attractive to *Ch. caragana* consisted of chrysanthenone, 1,3-pentadiene, and (*Z*)- $\beta$ -ocimene. Those repelling *Ch. caragana* consisted of perillene, dibutyl phthalate, nonanal, and pentadecane, and those irrelevant to each other consisted of (*Z*)-3-hexenyl acetate, 1-octene, nonene, decanal, (*Z*)-3-hexenol, and  $\alpha$ -pinene.

**Keywords:** long-horned beetle; *Caragana*; volatiles; olfactory orientation; principal component analysis; hierarchical clustering analysis

#### Introduction

Volatile organic compounds (VOCs) are released by plants and consist of a mixture of multiple compounds. The chemical composition, proportion, and rate of release vary due to factors such as the plant type and its physiological status. Even if VOCs were from the same family or genera of plants, significant qualitative and quantitative differences were still present (Gao et al. 2005). Plant volatiles played a significant role in the orientation of phytophagous insects to host plant (Pettersson et al. 1998; Bruce et al. 2005). Phytophagous insects recognized their hosts by the specific VOC mixture which was used to decide whether the plants were suitable for feeding and laying eggs (Mustaparta 2002; Tasin et al. 2006; Anton et al. 2007; Cardé & Willis 2008; Piñero & Dorn 2009; Najar-Rodriguez et al. 2013).

Pests damaged several host plants based on certain common volatiles released by these plants (Bruce et al. 2005; Rajapakse et al. 2006; Leppik & Frérot 2012). On the other hand, VOCs released by nonhost plants were always perceived as irrelevant or repellent cues and led to nondirectional movement of phytophagous insects in feeding or laying eggs. Therefore, the similarity of volatiles released by different host plants could provide more information for volatiles essential for insects' attractiveness; however, the volatiles released by nonhost plants could be used for the development of insect repellents (Unsicker et al. 2009; Jactel et al. 2011).

*Caragana* plants are shrubs, which are crucial for water and soil conservation in deserts, and are widely distributed in the desert area of Ningxia, China. The larvae of *Chlorophorus caragana* feed on the wooden part of the plant, which leads to the death of the shrubs due to wind-breakage (Zong et al. 2012). Recently, the damage caused by *Ch. caragana* has been serious in *Caragana* plants. There are five species of *Caragana* plants, *Caragana davazamcii*, *Caragana korshinskii*, *Caragana microphylla*, *Caragana brachypoda*, and *Caragana ordosica*, distributed in the desert area of Ningxia, China. However, up to now, it is unclear which plant species are most attractive or repellent to *Ch. caragana*.

Here we (1) investigated natural insect damage on the five species of *Caragana* in the field, (2) collected and analyzed the VOCs from branches and leaves of the five species of *Caragana*, (3) evaluated the attractiveness of these VOCs to *Ch. caragana* females by olfaction selection experiments under laboratory conditions, and (4) applied principal component analysis (PCA) and clustering analysis of these VOCs to determine the common and noncommon components between VOCs derived from the five species of *Caragana*.

#### Materials and methods

##### *Plant materials and insects*

Five plant species were evenly distributed in the desert woods in Lingwu City. There *C. davazamcii*, *C.*

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Table 1. Distribution details of five *Caragana* species from Lingwu City.

Species	Abbreviation	Location	Northern latitude	East longitude	Elevation height (m)	Date of plant collection	Net plant weight (g)
<i>C. microphylla</i>	<i>C. mic</i>	Yanzhi Mountain	38°05'37.49"	106°23'04.04"	1186	20 July 2013	141.7
<i>C. davazamcii</i>	<i>C. dav</i>	Yanzhi Mountain	38°05'32.09"	106°23'16.72"	1194	22 July 2013	172.4
<i>C. korshinskii</i>	<i>C. kor</i>	Yongli	37°56'48.08"	106°39'14.38"	1423	24 July 2013	199.8
<i>C. brachypoda</i>	<i>C. bra</i>	Huimin Xiang	38°06'54.5"	106°39'48.30"	1323	26 July 2013	46.8
<i>C. ordosica</i>	<i>C. ord</i>	Yongli	37°54'48.5"	106°39'35.00"	1467	25 July 2013	41.6

*microphylla*, and *C. brachypoda* were distributed close to the urbanized city. *C. ordosica* and *C. korshinskii* were distributed in the suburbs. The distribution details are shown in Table 1.

*Ch. caragana* larvae were collected from the desert woods in Lingwu City and were maintained in laboratory artificial climate chests (Blue Pard MGC-2500, Shanghai, China) at L:D 14:10 and 60% relative humidity. The temperatures of day and night were 30°C and 25°C, respectively. The artificial diet consisted of yeast extract, agar, sugar, Wesson's salt mixture, casein, Vanderzant's vitamin mixture, sorbic acid, methylparaben, and distilled water. The artificial diet was replaced weekly and feces were removed at that time until larvae became pupae. Upon emergence, adults to be tested were sexed. Each adult was kept singly in a transparent plastic disposable cup (upper diameter × height × bottom diameter = 75 mm × 30 mm × 60 mm) and was provided with honey water. Robust unmated female adults were chosen for subsequent behavioral experiments.

#### Investigation of insect population density in the field

Five *Caragana* species were chosen in the corresponding desert woods in Lingwu City which is indicated in Table 1. For each plant species, six sample plots were investigated. Each plot (20 × 20 m<sup>2</sup>) included at least 30 plant individuals. Plants were individually investigated on each of four directions (N, S, E, and W) to elucidate the wood anatomy. The pest population density, various forms of insects, and the number of eclosion holes were recorded. The proportion of damaged plants and the pest population density per plant were calculated as an index of measurable damages by *Ch. caragana*.

#### Attraction of five species of *Caragana* to *Ch. caragana*

The attraction of *Caragana* plants to unmated female *Ch. caragana* was measured with a Y-tube olfactometer in June and July 2013. Healthy twigs from five *Caragana* species were obtained from the desert woodland in Lingwu (Table 1). Twigs and leaves from the five species of *Caragana* were cut off in the field at 6:00 am, and then placed in a clean zip lock bag with a wet cotton ball to prevent dehydration. We did not further cut the leaves from the excised twigs to avoid further adding new artificial wound, so the number of leaves from the twigs from the five species was not absolutely equal. The weight and length were also different (Table 1).

These twigs were then taken back to the Management Quarantine Station of Lingwu City to perform behavioral tests.

The olfactometer was a Y-shaped glass tube (diameter: 7.5 cm; selective arm length: 40 cm; common arm length: 50 cm). The selective arms were connected to two glass chambers (diameter: 7.5 cm; length: 10 cm), one with plant material and the other being a methylene chloride control. Tested insects were placed into the common arm. Air velocity was 1 L/min, controlled by an air sampler (type QC-1, the Beijing Municipal Institute of Labor Protection Science). Airflow was filtered through activated carbon and a gas-washing bottle, then pushed into the two glass chambers, finally reaching the test insects. The airflow was started 10 min before the test insects were placed into the chamber to ensure that the plant odor was sufficiently spread within the selective arm.

Experiments were performed under even light (incandescent simulation of sunlight) and temperatures of 24–25°C with 60–70% relative humidity. All experiments were carried out between 8:00 am and 6:00 pm. During the time, *Ch. caragana* was active in the field. An unmated female insect was randomly selected and placed at the entrance of the Y-tube. Each female was observed for a maximum of 10 min. When the insect had passed the 20 cm mark on the selective arm, it was considered to have made a choice; if the insect did not reach the mark, it was considered not to make a choice (Bertschy et al. 1997). For each plant sample, 30 female insects were tested, and the tests were repeated three times. After five insects were tested, the position of both glass arms and selective chambers were changed simultaneously to avoid deviation of odor. After a plant sample was tested, the entire device was cleaned in detergent solution, rinsed with ethanol, and then dried at 120°C.

#### Collection of VOCs from five species of *Caragana* plants

From 8:00 am to 1:00 pm on July 20–26, 2013, healthy plants of *C. davazamcii*, *C. microphylla*, *C. korshinskii*, *C. ordosica*, and *C. brachypoda* were selected for headspace collection of VOCs (Kappers et al. 2011). The plant twigs were placed into a sealed Teflon oven bag (Reynolds, 406 mm × 444 mm, Richmond, VA, USA). After being quickly evacuated, the bag was filled with air filtered by activated carbon. The closed loop system collected plant volatiles repeatedly, and finally,

the VOCs were adsorbed into an adsorption tube (CAMSCO, Houston, TX, USA; length: 8.89 cm; diameter: 0.635 cm) with 200 mg Tenax TA (60/80 mesh; Supelco, Bellefonte, DE, USA). Before the collection, the adsorption tube was activated for 120 min at 270°C using nitrogen blowing at 100 mL/min. The connecting tubes of the devices were made from Teflon (inner diameter: 0.6 cm; outer diameter: 0.8 cm). During collection, plants were checked to confirm that they were not damaged. Six samples from each plant were collected at the same time. The six samples were as adjacent as possible to ensure the similar habitat (Vallat et al. 2005). Meanwhile, collection using an empty bag was performed as a control. The flow rate was 500 mL/min, and each sample was collected consecutively over 4 h. To avoid the direct sunlight and excessive vapor from plants, the entire collection device was covered by a sunshade. After sampling, the adsorption tubes were maintained at -20°C.

#### **Automatic thermal desorption/gas chromatography/mass spectrometry (ATD/GC/MS)**

Automatic thermal desorption equipment (ATD 650 Turbo Matrix; PerkinElmer, Waltham, MA) was directly connected to the GC (Clarus 600; Perkin Elmer). The injection rate of VOCs dissociated from the adsorption tube to GC was 5.0%.

#### **Automatic thermal desorption (ATD)**

The first dissociation temperature of the sample was 260°C, which was maintained for 10 min. Before entering a cold trap (-25°C), the sample was heated to 300°C at a heating rate of 40°C/s, then maintained for 5 min.

#### **Gas chromatography (GC)**

A DB-5 chromatographic column was used (30 m long; internal diameter: 0.25 mm; film thickness: 0.15 µm; Agilent Technologies, Santa Clara, CA). The carrier gas was He (1.5 mL/min). The initial temperature of 40°C was maintained for 2 min. Next, the temperature was increased to 160°C at a rate of 4°C/min, before heating to 270°C in 20°C/min steps where it was maintained for 3 min. The split ratio was 2:1.

#### **Mass spectrometry (MS)**

The electrons were from an EI source; the electron was 70 eV; scanned mass-to-charge ratio range was 29–600 amu; interface temperature was 25°C; ion source temperature was 220°C; and quadrupole temperature was 150°C. Emission current was 150 µA. Using the full scan mode, each scan was performed for 0.2 s.

Chromatographic retention time and MS data in NIST of identified compounds and 34 standards (Table 3) were compared. Then, C5–C27 straight-chain alkanes were injected into the same equipment and analyzed by

the same temperature programming. Retention time was used to calculate the retention index (KI value). Through comparisons with published KI values, a qualitative diagnosis was performed (Ruther 2000; Adams 2007). No quantitative analysis was performed, and any statistical assessment was related to these 'peak areas.' Using total ion current peak area normalization, the relative amount of each identified component was calculated. The software Turbo Mass 5.4.2 (PerkinElmer, Waltham, MA) was used for data analysis.

#### **Chemicals**

Nonene (99.5%), heptaldehyde (95.0%), 1-heptanol (98.0%), 1-decene (99.5%), limonene (95.0%), nonanal (95.0%), (*E,E*)-alloocimene (90.0%), 1-dodecene (99.5%), decanal (95.0%), 1-tridecene (99.5%), and pentadecane (99.0%) were obtained from CNW technologies GmbH (Düsseldorf, Germany); methyl butyrate (99.0%), 1-octene (99.0%), octane (99.0%), (*Z*)-3-hexenol (99.5%), nonane (99.0%), benzaldehyde (95.0%), β-pinene (95.0%), decane (99.0%), octanal (97.0%), (*Z*)-3-hexenyl acetate (95.0%), 1-hexanol,2-ethyl- (99.0%), isophorone (99.0%), dodecane (99.0%), tridecane (98.0%), tetradecane (97.0%), hexadecane (98.0%), and dibutyl phthalate (99.0%) were obtained from Dr Ehrenstorfer (Augsburg, Germany); chrysanthenone (85.0%) was obtained from CHEMOS GmbH (Czech Republic, Germany); α-pinene (98.0%) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA); β-elemene (82.0%) was obtained from Skyrun Industrial Co. Limited (CSR Ind, China); 1,3-pentadiene (99.0%) was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan); (*Z*)-β-ocimene (95.0%) was obtained from BOC Sciences (New York, USA); and perillene (98.0%) was obtained from Shanghai BeiZhuo Biotechnology Co., Ltd (Shanghai, China). Compounds for which no standards were available were tentatively identified using the NIST database.

#### **Statistical analysis**

Chi-square test was applied to analyze results from the behavioral tests, including preference (percentage of adult insects choosing the odor source or the clean air), nonpreference (percentage of adult insects with no choice), and responsiveness (the ratio of adult insects making a choice). By means of one-way variance, the different response percentages of *Ch. caragana* to different odor sources from five *Caragana* species were compared (SPSS 16.0, SPSS Inc., Chicago, IL, USA). Multivariate analysis of variance (ANOVA) was performed to identify significant differences between volatiles (dependent variables) emitted by different *Caragana* species. If significant, one-way ANOVA and Tukey's HSD post hoc tests were further conducted to test for quantitative differences in concentrations of individual and total headspace volatiles emitted by different *Caragana* species (SPSS 16.0, SPSS Inc., Chicago, IL, USA).

Different plant VOCs were summarized with PCA and cluster analysis. PCA was applied to yield a 2D display of the multivariable set of data and to graphically determine whether clustering of five different *Caragana* species occurred based on their overall volatile profiles. Hierarchical clustering analysis of each sample were carried out using the between-group linkage method and Euclidean distance (SPSS 16.0, SPSS Inc., Chicago, IL, USA).

## Result and discussion

### Natural damage to five *Caragana* species by *Ch. caragana*

The result showed that *C. davazamcii* had the largest average larvae per plant rate ( $74.00\% \pm 13.00\%$ ) and population density of insects per plant ( $1.16 \pm 0.84$ ). It was followed by *C. microphylla*, with average larvae per plant rate ( $11.00\% \pm 2.00\%$ ) and population density of insects per plant ( $0.13 \pm 0.08$ ; Table 2). No damage was found on *C. ordosica*, *C. brachypoda*, and *C. korshinskii*.

### Olfactory response of *Ch. caragana* to five *Caragana* species

Female *Ch. caragana* was attracted by the odor of *C. korshinskii* ( $\chi^2 = 10.67$ ,  $N = 24$ ,  $P < 0.01$ ), *C. davazamcii*

( $\chi^2 = 7.35$ ,  $N = 23$ ,  $P < 0.01$ ), and *C. microphylla* ( $\chi^2 = 6.00$ ,  $N = 24$ ,  $P < 0.05$ ). The odor of *C. ordosica* ( $\chi^2 = 6.00$ ,  $N = 24$ ,  $P < 0.05$ ) repelled *Ch. caragana*. The effect of *C. brachypoda* odor was not significant ( $\chi^2 = 3.52$ ,  $N = 24$ ,  $P > 0.05$ ). Results from the one-way ANOVA showed that olfactory response rates of *Ch. caragana* to the five species VOCs were significant ( $F = 37.68$ ,  $P < 0.01$ ). The difference between the attraction of *C. korshinskii*, *C. davazamcii*, and *C. microphylla* was not significant. The difference between *C. ordosica* and *C. brachypoda* was also not significant. However, the difference between these two groups was found to be significant (Figure 1).

### VOC composition of five *Caragana* species

Through headspace collection and GC-MS analysis, 70 different compounds were identified from the twigs of five *Caragana* species. The identified components were divided into eight chemical classes, which comprised 5 alcohols, 7 ketones, 10 alkanes, 8 olefins, 11 esters, and 1 aromatic compound. No quantitative analysis were performed. The relative amount of each identified component was based on peak area normalization of total ion current (Table 3).

Thirty-five types of compounds were identified from *C. microphylla*. The most abundant compounds included

Table 2. Investigation of damage on five species of *Caragana* in the field.

Plant name	Investigated plants	Average larvae plant rate (%)	Average population density of insects/plant
<i>C. microphylla</i>	602	$11.00 \pm 2.00$	$0.13 \pm 0.08$
<i>C. davazamcii</i>	417	$74.00 \pm 13.00$	$1.16 \pm 0.84$
<i>C. ordosica</i>	180	0	0
<i>C. brachypoda</i>	180	0	0
<i>C. korshinskii</i>	180	0	0

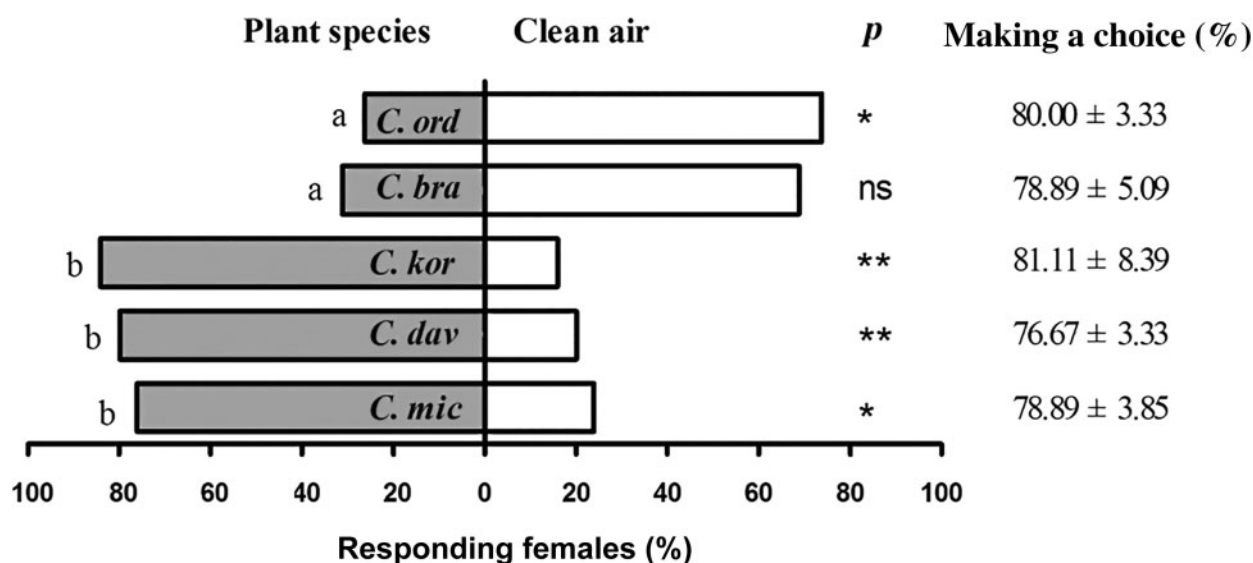


Figure 1. Olfaction selection preference of *Ch. caragana* to five *Caragana* species odors. P-values are based on chi-square test: \* $P < 0.05$ ; \*\* $P < 0.01$ ; ns,  $P \geq 0.05$ . Different lowercase letters on the left side of the bar indicate significant differences (one-way ANOVA followed by Turkey's multiple comparison test,  $P < 0.05$ ). *C. ord* = *C. ordosica*, *C. bra* = *C. brachypoda*, *C. kor* = *C. korshinskii*, *C. dav* = *C. davazamcii*, *C. mic* = *C. microphylla*.

Table 3. Relative TIC-peak areas of VOCs collected in the headspace of the twigs and leaves from species of *Caragana* plants.

Compound class and name	S <sup>a</sup>	CAS <sup>b</sup>	KI <sup>c</sup>	Composition (%)					<i>F</i>
				<i>C. mic</i> <sup>d</sup>	<i>C. dav</i> <sup>d</sup>	<i>C. kor</i> <sup>d</sup>	<i>C. bra</i> <sup>d</sup>	<i>C. ora</i> <sup>d</sup>	
Methyl butyrate <sup>s</sup>	DR	623-42-7	724	0a	0a	0a	0a	0.23 ± 0.06b	82.63*
1,3-pentadiene <sup>s</sup>	TCI	504-60-9	729	10.81 ± 3.24a	22.29 ± 3.46b	20.6 ± 3.81b	0c	0c	93.57*
1-octene <sup>s</sup>	DR	111-66-0	792	0.16 ± 0.04a	1.68 ± 0.14b	1.05 ± 0.14c	9.56 ± 0.77d	0.91 ± 0.07c	715.87*
Octane <sup>s</sup>	DR	111-65-9	800	0.22 ± 0.03a	2.08 ± 0.4c	2.33 ± 0.15c	4.92 ± 0.24d	1.17 ± 0.12b	372.59*
( <i>Z</i> )-3-hexenol <sup>s</sup>	DR	928-96-1	857	0.95 ± 0.2a	0.42 ± 0.05b	0c	3.05 ± 0.16d	2.02 ± 0.12e	585.25*
2-methyloctane	–	3221-61-2	861	0a	0a	0a	1.43 ± 0.48b	0a	53.32*
1-hexanol	–	111-27-3	867	0a	0a	0a	0a	0.2 ± 0.01b	2923.78*
Nonene <sup>s</sup>	CNW	124-11-8	891	0.5 ± 0.07ab	1.13 ± 0.33b	0a	10.35 ± 0.89c	0.73 ± 0.04b	639.99*
Nonane <sup>s</sup>	DR	111-84-2	899	0.34 ± 0.11a	0.82 ± 0.15ab	1.09 ± 0.4b	2.19 ± 0.65c	0.37 ± 0.03a	27.71*
Heptaldehyde <sup>s</sup>	CNW	111-71-7	900	0.3 ± 0.04ab	0.16 ± 0.04a	0.4 ± 0.07b	1.53 ± 0.23d	0.73 ± 0.04c	144.45*
Pentyl acetate	–	628-63-7	915	0.03 ± 0.01ab	0a	0a	0.81 ± 0.1c	0.08 ± 0.01b	332.46*
3-methyl-2-butenyl acetate	–	1191-16-8	918	0a	0a	0a	0a	0.07 ± 0b	2417.66*
4-penten-1-yl acetate	–	1576-85-8	919	0.04 ± 0.01a	0b	0b	0b	0b	131.37*
α-thujene	–	2867-5-2	923	0a	0a	0a	0a	0.45 ± 0.06b	300.61*
α-pinene <sup>s</sup>	SA	80-56-8	933	0a	0.37 ± 0.04a	0a	5.51 ± 1.28b	4.76 ± 0.59b	115.33*
Camphene	–	79-92-5	952	0a	0a	0a	0a	0.12 ± 0.04b	52.32*
Benzaldehyde <sup>s</sup>	DR	100-52-7	961	0.1 ± 0.04a	0.14 ± 0.01a	0.17 ± 0.02a	2.22 ± 0.49b	0a	110.98*
1-heptanol <sup>s</sup>	CNW	111-70-6	969	0a	0a	0a	0a	0.54 ± 0.09b	213.07*
β-pinene <sup>s</sup>	DR	127-91-3	980	0.42 ± 0.03a	5.61 ± 1.33b	0a	0a	0.9 ± 0.2a	94.32*
Sulcatone	–	110-93-0	985	0a	0a	0a	0a	0.48 ± 0.02b	3695.02*
1-decene <sup>s</sup>	CNW	872-05-9	991	0a	0.51 ± 0.1b	0.85 ± 0.17c	0a	0a	121.74*
Decane <sup>s</sup>	DR	124-18-5	999	0.14 ± 0.04a	0.23 ± 0.06a	0.68 ± 0.17b	0c	0c	68.14*
Octanal <sup>s</sup>	DR	124-13-0	1001	0a	0.51 ± 0.1ab	0.65 ± 0.06b	5.3 ± 0.74c	0a	271.14*
( <i>Z</i> )-3-hexenyl acetate <sup>s</sup>	DR	3681-71-8	1007	15.32 ± 0.96a	0.74 ± 0.1b	5.05 ± 0.55c	27.45 ± 1.39d	0b	1288.23*
Hexyl acetate	–	142-92-7	1008	0a	0a	0a	2.87 ± 0.27b	1.12 ± 0.34c	249.66*
1,3-dichlorobenzene	–	541-73-1	1021	0a	0a	0.67 ± 0.08b	0a	0a	420.53*
1-hexanol,2-ethyl- <sup>s</sup>	DR	104-76-7	1027	0a	0a	0a	0a	1.13 ± 0.04b	5745.83*
Limonene <sup>s</sup>	CNW	138-86-3	1031	0.37 ± 0.07a	20.66 ± 1.24b	0.61 ± 0.18a	0a	0a	1601.01*
( <i>Z</i> )-β-ocimene <sup>s</sup>	BOC	3338-55-4	1040	34.42 ± 2.78a	24.88 ± 4.44b	35.13 ± 3.32a	1.53 ± 0.19c	6.6 ± 1.02b	185.8*
( <i>E</i> )-β-ocimene	–	3779-61-1	1050	0a	0a	0a	0a	2.93 ± 0.38b	350.19*
Acetophenone	–	98-86-2	1065	0a	0a	0a	0.96 ± 0.09b	0a	726.55*
1-octanol	–	111-87-5	1072	0a	0a	0a	0a	0.31 ± 0.01b	10,770.33*
Nonanal <sup>s</sup>	CNW	124-19-6	1098	0.97 ± 0.2a	1.96 ± 0.06b	1.98 ± 0.11b	0c	7.24 ± 0.9d	271.83*
Perillene <sup>s</sup>	SBB	539-52-6	1099	0a	0a	0a	6.05 ± 0.35b	40.46 ± 2.58c	1376.99*
Undecane	–	1120-21-4	1100	0a	0.3 ± 0.07b	0.96 ± 0.16c	0a	0a	177.15*
Chrysanthenone <sup>s</sup>	CG	473-06-3	1123	17.27 ± 2.39a	2.47 ± 0.6b	4.49 ± 0.82c	0d	0d	230.04*
Isophorone <sup>s</sup>	DR	78-59-1	1135	0.21 ± 0.03a	0.11 ± 0.03a	0.51 ± 0.08a	2.36 ± 0.65b	0.15 ± 0.02a	64.37*
( <i>E,E</i> )-alloocimene <sup>s</sup>	CNW	673-84-7	1142	0.47 ± 0.08a	0.57 ± 0.07a	0.42 ± 0.06a	0b	2.55 ± 0.51c	109.49*
( <i>E</i> )-2-Nonenal	–	18829-56-6	1155	0a	0a	0a	0a	0.19 ± 0.06b	64.73*
( <i>Z</i> )-3-hexenyl butanoate	–	16491-36-4	1185	0.11 ± 0.01a	0b	0b	0b	0b	730.66*
Methyl salicylate	–	119-36-8	1190	0.16 ± 0.03a	0b	0b	0b	0b	227.71*
1-Dodecene <sup>s</sup>	CNW	112-41-4	1192	0.14 ± 0.02a	0.61 ± 0.2a	2.31 ± 0.68b	1.39 ± 0.29c	0.3 ± 0.05a	41*
Dodecane <sup>s</sup>	DR	112-40-3	1199	0.25 ± 0.05a	1.24 ± 0.17bc	1.54 ± 0.26c	1.09 ± 0.25b	0.12 ± 0.04a	71.61*
Decanal <sup>s</sup>	CNW	112-31-2	1204	0.34 ± 0.08a	1.24 ± 0.32b	1.18 ± 0.34b	5.75 ± 0.29d	2.83 ± 0.78c	150.99*

Table 3 (Continued)

Compound class and name	S <sup>a</sup>	CAS <sup>b</sup>	KI <sup>c</sup>	Composition (%)					F
				<i>C. mic</i> <sup>d</sup>	<i>C. dav</i> <sup>d</sup>	<i>C. kor</i> <sup>d</sup>	<i>C. bra</i> <sup>d</sup>	<i>C. ord</i> <sup>d</sup>	
1-Tridecene <sup>s</sup>	CNW	2437-56-1	1291	0a	1.23 ± 0.34b	2.15 ± 0.02c	3.65 ± 0.92d	0.97 ± 0.23b	56.29*
Tridecane <sup>s</sup>	DR	629-50-5	1299	0.87 ± 0.11a	1.74 ± 0.22b	2.42 ± 0.15c	0d	0.6 ± 0.17e	244.06*
8-methyl-1-Decene	–	61142-79-8	1347	0a	0.11 ± 0.05b	0a	0a	0a	31.78*
Octadecanal	–	638-66-4	1357	0a	0a	0a	0a	0.32 ± 0.08b	89.54*
2-Undecenal	–	2463-77-6	1365	0a	0a	0a	0a	0.4 ± 0.11b	81.04*
β-elemene <sup>s</sup>	SI	515-13-9	1375	7.27 ± 2.39a	0.62 ± 0.27b	0b	0b	0b	52.75*
α-Copaene	–	3856-25-5	1376	0a	0a	0a	0a	0.35 ± 0.12b	50.73*
β-bourbonene	–	5208-59-3	1380	0a	0.22 ± 0.09b	0a	0a	0.14 ± 0.07b	24.64*
1-Tetradecene	–	1120-36-1	1391	0a	0.65 ± 0.23b	0a	0a	0a	48.57*
Tetradecane <sup>s</sup>	DR	629-59-4	1399	1.01 ± 0.1a	1.81 ± 0.3b	3.44 ± 0.23c	0d	0.74 ± 0.19a	271.42*
Longifolene	–	475-20-7	1402	0a	0a	0a	0a	0.16 ± 0.06b	44.59*
Dodecanal	–	112-54-9	1407	0a	0a	0a	0a	0.22 ± 0.08b	40.02*
β-Caryophyllene	–	87-44-5	1418	0.84 ± 0.1a	0b	0b	0b	0.41 ± 0.07c	291.95*
Dihydro-β-ionone	–	17283-81-7	1433	0a	0a	0.14 ± 0.05b	0a	0a	47*
α-Bergamotene	–	17699-05-7	1434	0.83 ± 0.14a	0b	0b	0b	0b	211.2*
Geranyl acetone	–	3796-70-1	1448	0a	0a	0a	0a	0.21 ± 0.06b	62.99*
Alloaromadendrene	–	25246-27-9	1461	0a	0a	0a	0a	0.36 ± 0.13b	45.08*
β-Cadinene	–	523-47-7	1472	0a	0a	0a	0a	0.61 ± 0.16b	87.86*
Pentadecane <sup>s</sup>	CNW	629-62-9	1500	3.62 ± 0.49c	1.94 ± 0.42b	4.89 ± 0.41cd	0a	5.9 ± 1.54d	56.23*
1-Hexadecene	–	629-73-2	1593	0.21 ± 0.03a	0.18 ± 0.05a	0.62 ± 0.1b	0c	0.25 ± 0.06a	83.7*
Hexadecane <sup>s</sup>	DR	544-76-3	1600	0.47 ± 0.08a	0.55 ± 0.23a	1.73 ± 0.33b	0c	0.66 ± 0.11a	66.72*
Tetradecanal	–	124-25-4	1611	0.16 ± 0.03a	0.09 ± 0.02b	0.08 ± 0.03b	0c	0c	69.21*
Isopropyl myristate	–	110-27-0	1814	0a	0a	0a	0a	0.1 ± 0.02b	133.02*
Phytone	–	502-69-2	1845	0a	0a	0a	0a	0.29 ± 0.06b	158.88*
Disobutyl phthalate	–	84-69-5	1870	0.13 ± 0.02a	0.13 ± 0.04a	0.97 ± 0.4b	0a	0a	31.42*
Dibutyl phthalate <sup>s</sup>	DR	84-74-2	1922	0.18 ± 0.03a	0a	0a	0a	7.24 ± 1.2b	215.13*
Aldehyde compounds	–			1.87 ± 0.17a	4.09 ± 0.45b	4.45 ± 0.43b	14.81 ± 1.2d	11.93 ± 1.2c	180.4*
Terpene compounds	–			55.43 ± 1.78b	75.22 ± 1.89d	56.79 ± 0.82b	13.1 ± 1.66a	60.81 ± 1.66c	677.48*
Ketone compounds	–			17.49 ± 2.37d	2.58 ± 0.62ab	5.14 ± 0.78c	3.32 ± 0.57bc	1.13 ± 0.57a	189.02*
Ester compounds	–			15.97 ± 0.97a	0.87 ± 0.09b	6.01 ± 0.79c	31.13 ± 1.45d	8.84 ± 1.45e	730.31*
Alkane compounds	–			6.92 ± 0.4a	10.72 ± 1.15b	19.08 ± 0.82c	9.63 ± 0.9b	9.57 ± 0.9b	99.58*
Olefin compounds	–			1.01 ± 0.11a	6.1 ± 0.98c	6.97 ± 0.75c	24.95 ± 1.75d	3.15 ± 1.75b	579.81*
Alcohol compounds	–			0.95 ± 0.2c	0.42 ± 0.05b	0 ± 0a	3.05 ± 0.16d	4.19 ± 0.16e	947.37*
Aromatic compound	–			0 ± 0a	0 ± 0a	0.67 ± 0.08b	0 ± 0a	0 ± 0a	420.53*
Total identified	–			99.6	100	100	99.1	99.6	

<sup>a</sup>S: corresponding components were verified using a standard sample. The standards were obtained from Dr Ehrenstorfer, Augsburg, Germany (DR), Tokyo Chemical Industry Co., Tokyo, Japan (TCI), CNW technologies GmbH, Dusseldorf, Germany (CNW), Sigma-Aldrich Co., St. Louis, MO, USA (SA), BOC Sciences, New York, USA (BOC), Shanghai BeiZhuo Biotechnology Co., LTD, Shanghai, China (SBB), CHEMOS GmbH, Czech Republic, Germany (CG), Skyrun Industrial Co.Limited, CSR Ind, China (SI). '–' was noted as a standard not obtained.

<sup>b</sup>CAS: Registry Number in Chemical Abstracts Service.

<sup>c</sup>KI: Kovats index determined on the intermediately polar DB-5 column (<http://www.pherobase.com/>).

<sup>d</sup>*C. mic* = *C. microphylla*, *C. dav* = *C. davazancii*, *C. kor* = *C. korshinskii*, *C. bra* = *C. brachypoda*, *C. ord* = *C. ordosica*.

Different lowercase letters on the same line indicate significant differences (one-way ANOVA followed by Turkey's multiple comparison test,  $P < 0.05$ ). \*denotes a statistically significant difference below 0.05.

eight terpenes, two ketones, and seven esters, with relative amounts of 55.43%, 17.49%, and 15.97%, respectively. In addition, *C. microphylla* also included four olefins, eight alkanes, five aldehydes, and one alcohol. All of these four classes accounted for only 10.8% of the total amount. (*Z*)- $\beta$ -ocimene (34.42%  $\pm$  2.78%), chrysanthenone (17.27%  $\pm$  2.39%), (*Z*)-3-hexenyl acetate (15.32%  $\pm$  0.96%), 1,3-pentadiene (10.81%  $\pm$  3.24%), and  $\beta$ -elemene (7.27%  $\pm$  2.39%) were the principal components of *C. microphylla*, accounting for 85.09% of the total amount of VOCs identified (Table 3).

Thirty-six types of compounds were identified from *C. davazamcii*, including eight abundant terpenoid compounds, accounting for 75.2% of total amount. The relative amounts of terpenoids compounds in *C. davazamcii* were significantly higher than that in the other four plants ( $F = 677.48$ ,  $P < 0.05$ ). In addition, nine kinds of alkanes and eight kinds of olefins were identified, accounting for 10.7% and 6.1% of total amount, respectively. Then, six aldehydes, two ketones, one alcohol, and two esters were identified, with 8.0% of the total relative amounts. (*Z*)- $\beta$ -ocimene (24.88%  $\pm$  4.44%), 1,3-pentadiene (22.29%  $\pm$  3.46%), limonene (20.66%  $\pm$  1.24%), and  $\beta$ -pinene (5.61%  $\pm$  1.33%) were the principal components of *C. davazamcii*, accounting for 73.44% of the total amount (Table 3).

Thirty types of compounds were identified from *C. korshinskii*, including four abundant terpenoid compounds, accounting for 56.8% of total amount, followed by nine alkanes with 19.1% of total amount. In additions, five olefin (7.0%), two esters (6.0%), three ketones (5.1%), six aldehydes (4.5%), and one aromatic compound (0.7%) were also identified. (*Z*)- $\beta$ -ocimene (35.13%  $\pm$  3.32%), 1,3-pentadiene (20.6%  $\pm$  3.81%), (*Z*)-3-hexenyl acetate (5.05%  $\pm$  0.55%), pentadecane (4.89%  $\pm$  0.41%), and chrysanthenone (4.49%  $\pm$  0.82%) were the principal components of *C. korshinskii*, accounting for 70.16% of the total amount (Table 3).

Twenty-one types of compounds were identified from *C. brachypoda*, including three esters and four olefins, accounting for 31.1% and 25% of total amount, respectively. The two classes were significantly higher than those in the other four species (esters:  $F = 730.31$ ,  $P < 0.05$ ; olefins:  $F = 579.81$ ,  $P < 0.05$ ). Four aldehydes were identified, which were significantly higher than in the other four plants ( $F = 180.4$ ,  $P < 0.05$ ), accounting for 14.8% of total amount. In addition, three terpenes and four alkanes were identified, with 13.1% and 9.6% of total amount, respectively. Two ketones and one alcohol were identified, with 6.3% of total relative amount. (*Z*)-3-hexenyl acetate (27.45%  $\pm$  1.39%), nonene (10.35%  $\pm$  0.89%), 1-octene (9.56%  $\pm$  0.77%), perillene (6.05%  $\pm$  0.35%), decanal (5.75%  $\pm$  0.29%), and  $\alpha$ -pinene (5.51%  $\pm$  1.28%) were the principal components of *C. brachypoda*, accounting for 64.67% of the total amount (Table 3).

Forty-eight compounds were identified in *C. ordosica*, including 14 identified abundant terpenes, accounting for 60.8% of total amount. The other compounds present at high relative amounts were seven aldehydes

(11.9%), seven alkanes (9.6%), and six esters (8.8%). In addition, five alcohols, four olefins, and five ketones were identified, accounting for 8.5% of total amount. Perillene (40.46%  $\pm$  2.58%), dibutyl phthalate (7.24%  $\pm$  1.2%), nonanal (7.24%  $\pm$  0.9%), (*Z*)- $\beta$ -ocimene (6.6%  $\pm$  1.02%), pentadecane (5.9%  $\pm$  1.54%), and  $\alpha$ -pinene (4.76%  $\pm$  0.59%) were the principal components of *C. ordosica*, accounting for 72.2% of the total amount (Table 3).

### PCA and hierarchical cluster analysis

PCA clearly divided the VOCs from the five plant species into three groups. The PCA horizontal axis explained 28.71% of the total variance and the vertical axis a further 20.69% (Figure 2). From five *Caragana* VOCs, 16 compounds were selected based on three principles. First of all, these compounds should account for a relatively high percentage in one or several plant species, and their mean percentages in total VOCs were not less than 4.3%. Second, the amounts of selected VOCs were significantly different between the five plant species (ANOVA:  $P < 0.05$ ). Third, these compounds should cover most of the chemical classes. According to these principles, 16 compounds were marked in the biplot (1,3-pentadiene, 1-octene, (*Z*)-3-hexenol, nonene,  $\alpha$ -pinene,  $\beta$ -pinene, (*Z*)-3-hexenyl acetate, limonene, (*Z*)- $\beta$ -ocimene, nonanal, perillene, chrysanthenone, decanal,  $\beta$ -elemene, pentadecane, and dibutyl phthalate).

Hierarchical cluster analysis between-groups linkage (squared Euclidean distance) was used to analyze these

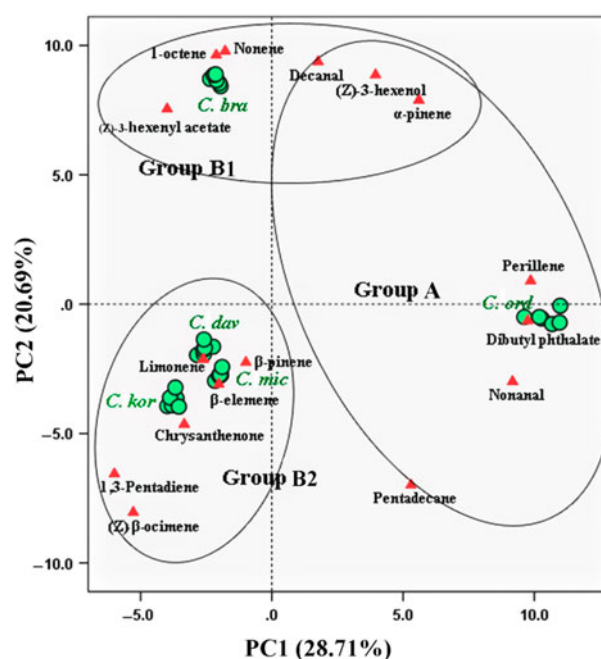


Figure 2. PCA score plot of five species of *Caragana* plant headspace VOCs. Green circles represented five species of *Caragana*. Black circles represented classification of five species of *Caragana*. Red triangles represented 16 different compounds, based on PCA of the component matrix. *C. ord* = *C. ordosica*, *C. bra* = *C. brachypoda*, *C. kor* = *C. korshinskii*, *C. dav* = *C. davazamcii*, *C. mic* = *C. microphylla*.



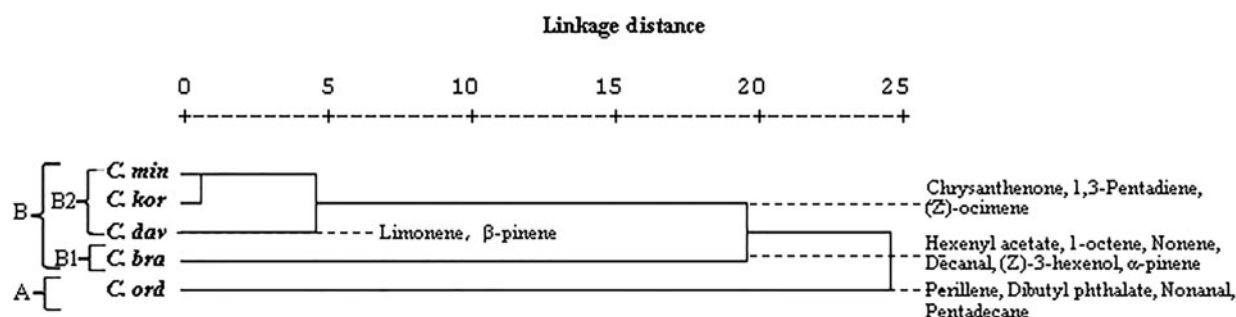


Figure 3. Phylogenetic tree of five species of *Caragana* plant VOCs based on Euclidean distance. Analyzed compounds were represented in the subgroups. The abbreviated species names are shown in Table 1. *C. mic* = *C. microphylla*, *C. kor* = *C. korshinskii*, *C. dav* = *C. davazamcii*, *C. bra* = *C. brachypoda*, *C. ord* = *C. ordosica*.

volatiles derived from five *Caragana* plant species, at a distance >5 and <20. They were divided into three clusters (B1, B2, and A; Figure 3).

PCA and system clustering results were consistent. The results showed VOC composition between the groups was significantly different. According to PCA, *C. davazamcii*, *C. korshinskii*, and *C. microphylla*, which attracted *Ch. caragana* in the behavioral test, belonged to Group B2. The common characteristics of the three species were the high percentage of (Z)-β-ocimene (35.13% ± 3.32% to 24.88% ± 4.44%), chrysanthenone (17.27% ± 2.39% to 2.47% ± 0.60%), and 1,3-pentadiene (22.29% ± 3.46% to 10.81% ± 3.24%). Furthermore, (Z)-3-hexenyl acetate (15.32% ± 0.96%) and β-elemene (7.27% ± 2.39%) of *C. microphylla* were also abundant. The VOCs of *C. davazamcii* differed slightly from *C. microphylla* and *C. korshinskii*, in terms of the proportions of limonene (20.66% ± 1.24%) and β-pinene (5.61% ± 1.33%; Figure 3). Overall, the main chemical characteristics of this Group B2 were the high proportions of terpenoids, ketone, and ester compounds.

Damage to *C. korshinskii* by *Ch. caragana* was not observable during the field investigation; however, this plant species was attractive to female insects during behavior experiments. The phylogenetic distance between *C. davazamcii*, *C. microphylla*, and *C. korshinskii* was close, as reported by Hou et al. (2006). In the present study, PCA and cluster analysis also indicated that plant VOCs of *C. korshinskii* are similar to *C. davazamcii* and *C. microphylla*. The behavior results from our study supported the powerful attraction ability of *C. davazamcii*, *C. microphylla*, and *C. korshinskii* to the beetle during the behavior experiment. However, why no damage was observed on *C. korshinskii* plants? In our investigation, no *Ch. caragana* population was found in the *C. korshinskii* suburbs (Table 2). Lower population densities in *C. korshinskii* suburbs of Lingwu City may explain the low damage rate by *Ch. caragana* on *C. korshinskii*.

Phytophagous insects select host plants on the basis of one or several substances released by the plant (Chin 1980; Zhang et al. 2001). Previous studies have shown that ocimene is an important defensive substance for plants. External stimuli could induce plants to release ocimene (Arimura et al. 2004). Using a Y-shaped tube,

it was found that (E/Z)-β-ocimene and (Z)-3-hexenyl acetate attracted *Mylocerinus aurolineatus* (Coleoptera: Curculionidae; Sun et al. 2010). Fewer studies have been performed on chemical communication by chrysanthenone and 1,3-pentadienes (Kostyk et al. 1993). (Z)-3-hexenyl acetate attracted *Pantomorus cervinus* (Coleoptera: Curculionidae; Wee et al. 2008); and β-elemene and limonene as kairomones affected the behavior of *Anoplophora glabripennis* (Coleoptera: Cerambycidae; Yasui et al. 2007; Yasui et al. 2011; Wei et al. 2013). Several studies have showed that some monoterpenes, such as α-pinene and β-pinene, can attract Cerambycidae, Curculionidae, and Scolytidae, which feed on coniferous trees. Furthermore, mixing ethanol with the chemical attractant strengthened its action (Mizell et al. 1984; Byers et al. 1985; Siegfried 1987; Sweeney et al. 2004; Wei et al. 2013). In total, all above-mentioned studies indicated that these compounds (e.g. ocimene, chrysanthenone, 1,3-pentadienes, β-elemene, limonene, and β-pinene) have biological activities on Coleoptera. So, we hypothesize they were also attractive to *Ch. caragana*. Furthermore, several other studies had shown that single compounds had no effect on phytophagous insects, but a mixture of multiple components formed a specific chemical signature to herbivores (Dicke 2000; Hammack 2001).

Group B1 (*C. brachypoda*) was the nonhost plant of *Ch. caragana*, and VOCs were characterized by high levels of (Z)-3-hexenyl acetate (27.45% ± 1.39%). Other species, except for *C. microphylla*, contained less of this substance. The contents of nonene (10.35% ± 0.89%) and 1-octene (9.56% ± 0.77%) were also more abundant than the other plant species. The contents of decanal (5.75% ± 0.29%), α-pinene (5.51% ± 1.28%), and (Z)-3-hexenols (3.05% ± 0.16%) were higher than in the other four plant species. It was clear that the chemical components of Group B1 mainly consisted of esters and olefin, and small amounts of aldehydes, alcohols, and terpenes were also present. In the bioassay, *Ch. caragana* did not perform significant orientation behavior to VOCs of *C. brachypoda*.

The nonhost plant *C. ordosica* that repelled *Ch. caragana* belonged to Group A. One of the characteristics of the VOCs in this group was the high level of perillene (40.46% ± 2.58%), which was less in other

species. Next, the contents of dibutyl phthalate ( $7.24\% \pm 1.20\%$ ), nonanal ( $7.24\% \pm 0.90\%$ ), and pentadecane ( $5.90\% \pm 1.54\%$ ) were all found to be higher in this species than in the others. In addition, the percentages of  $\alpha$ -pinene ( $4.76\% \pm 0.59\%$ ), (*Z*)-3-hexenol ( $2.02\% \pm 0.12\%$ ), and decanal ( $2.83\% \pm 0.78\%$ ) were slightly higher than that in *C. davazamcii*, *C. microphylla*, and *C. korshinskii*. The chemical components of Group A were mainly terpenes, with a slightly higher percentage of esters, aldehydes, and alkanes, as well as a small amount of alcohol.

The components of nonhost plant VOCs did not induce orientation behavior and even led to anti-directional movements. Poland and Haack (2000) indicated nonhost plant VOCs, including 1-hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, 3-octanol, and verbenone, interfered with host plant orientation by *Tomicus piniperda* (Coleoptera: Scolytidae). Erbilgin et al. (2007) showed that acetophenone had a strong repellent activity on western pine beetles. In our studies, perillene, dibutyl phthalate, nonanal, pentadecane,  $\alpha$ -pinene, (*Z*)-3-hexenol, and decanal, derived from *C. ordosica*, were repellent to *Ch. caragana*. However, we found Group B1 and Group A shared three common components, including  $\alpha$ -pinene, (*Z*)-3-hexenols, and decanal. Group B1 did not exhibit significantly repellent behavior by *Ch. caragana*, so these three compounds might not be the main repellent substances.

## Conclusion

Among the three *Caragana* species, *C. korshinskii*, *C. davazamcii*, and *C. microphylla* attracted *Ch. caragana* in laboratory. The common substance of the three species was a high proportion of terpene compounds ((*Z*)- $\beta$ -ocimene, 1,3-pentadiene,  $\beta$ -elemene, limonene, and  $\beta$ -pinene), as well as ketone compounds (chrysanthenone), and ester compounds ((*Z*)-3-hexenyl acetate). In the field, *C. davazamcii* and *C. microphylla* were damaged by *Ch. caragana*, but no damage to *C. korshinskii* was found in the field. The inconsistency for *C. korshinskii* might contribute to the inappropriate physical characteristics and nutrient status of *C. korshinskii*. *C. ordosica* repelled *Ch. caragana* and did not suffer from damage in the field. The chemical characteristics of this species mainly consisted of terpenes (perillene and  $\alpha$ -pinene), a slightly higher percentage of esters (dibutyl phthalate), aldehydes (nonanal and decanal), alkanes (pentadecane), and a small amount of alcohol ((*Z*)-3-hexenol). *C. brachypoda* did not suffer from damage in the field, and its chemical characteristics were mainly esters ((*Z*)-3-hexenyl acetate), olefinic (nonene and 1-octene), a small concentrations of aldehyde (decanal), alcohols ((*Z*)-3-hexenols), and terpenes ( $\alpha$ -pinene).

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