Development of Semiochemical Attractants for Monitoring and Controlling *Chlorophorus caragana*

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Chlorophorus caragana is an important wood-boring pest that infests *Caragana korshinskii*. The larvae bore into the stems to the point of hollowing them out, causing the whole tree to wither and even die. To control these infestations, volatile compounds were collected from *C. korshinskii* and used in electroantennography to ascertain which plant semiochemicals could be used to trap adult *C. caragana* in the field. Isophorone, *cis*-3-hexen-1-ol, 3-pentanone, dibutyl phthalate, and diisobutyl phthalate were the main volatile compounds produced by *C. korshinskii*. These compounds induced dose-dependent electrophysiological responses in the antennae of adult *C. caragana* to some degree. Accordingly, 58 different compound mixtures were tested in field trapping experiments over two consecutive years. Isophorone was most attractive to adult insects. In the field, the best traps were funnel-shaped ones hanging at a height of 1 m. The trapping efficiency was 63.8%. Adult beetles appear between mid June and late August, with an eclosion peak in mid July. The prototype trapping system developed could be used as a tool to monitor and control *C. caragana* adults.

Key words: Caragana korshinskii, *Chlorophorus caragana*, Volatile Compounds

Introduction

Caragana korshinskii Kom. (Leguminosae) is a deciduous shrub, which is currently one of the most important plant resources for vegetation restoration, ecological reconstruction, and regional economic development in arid and semi-arid areas of China (Niu, 2003). In recent years, the area of pure *C. korshinskii* forest has increased constantly. Global warming, frequent bad weather, and the special geographic environment of the northwest arid and semi-arid regions of China are all factors that have increased the infestation of *C. korshinskii* by some pests. Pests such as *Pterolophia multinotata*, *Cydia nigricana*, *Kytorhinus immixtus*, and *Bruchophagus neocaraganae* cause the whole tree to wither and even die, which has serious effects upon the environment, ecology, and local economic development in northwest China (Li and Qi, 1995; Yang and Gao, 2000; Li and Li, 2001; Luo *et al*., 2006).

Chlorophorus caragana (Cerambycidae, Coleoptera) was identified in 2009 as a wood-boring pest infesting *C. korshinskii* in Zhongwei City, Ningxia Hui Autonomous Region, China, and was subsequently described as a new species (Zong *et al.*, 2012). Its larvae bore into the stems of *C. korshinskii*. Most stems are bored to the point of being hollow, causing the whole tree to wither and die (Cao, 2010; Cao *et al.*, 2010). In recent years, infestation by *C. caragana* has occurred in large areas of Zhongwei, Lingwu, and Yanchi City of Ningxia in China, and constituted a huge threat to large areas of both planted and naturally growing *C. korshinskii* forests.

Throughout evolution, both insects and plants have formed various relationships that are beneficial to one or the other, or to both. The behavioural response of herbivorous insects toward host plants is an important core issue when studying such interspecies relationships. This response includes the particular preference of the insect for a host and/or its habitat, and the selection of particular feeding and/or spawning sites. The mechanisms used by the host plant to control the feeding or spawning activity of insects are also of importance. Volatile plant components play a key role in the latter process (Du and Yan, 1994;

Bolter *et al.*, 1997; Pare and Tumlinson, 1999; Dicke, 1999; Du, 2001). Consequently, the use of volatile plant components as efficient attractants is becoming a critical research area within the field of Integrated Pest Management (Andersen and Metcalf, 1986; Yoichi *et al.*, 1998; Miao *et al.*, 2003; Allison *et al.*, 2004; Tao, 2009; Zhao, 2011).

The use of volatile compounds derived from *C. korshinskii* to attract *C. caragana* has not been yet reported. Therefore, the aim of this study was to collect and identify volatile compounds from *C. korshinskii*, and check by electroantennography and field trapping which plant semiochemicals can be used to trap *C. caragana* in the field with a view to improve their monitoring and control.

Material and Methods

Experimental areas

The experiments were conducted in fields located in the cities of Zhongwei and Lingwu, Ningxia, China. Zhongwei City is located south of the Tengger Desert. The annual average temperature is 10.4 °C and the annual average precipitation is 186.6 mm. The experimental field was 13,300 ha of pure *C. korshinskii* shrubbery, although some other plants were present, notably *Artemisia scoparia*, *Salsola ruthenica*, *Agriophyllum squarrosum*, *Corispermum mongolicum*, *Pennisetum flaccidum*, and *Bassia dasyphylla*. The vegetation coverage by *C. korshinskii* was about 35%, the average plant height was 2.1 m, and the average basal stem diameter was 2.4 cm. The infestation rate was 81%, with an average population density of 3.1 larvae per plant.

Lingwu City is located at the junction of mid-Ningxia, the eastern Yellow River, the Yinchuan plain, and the Ordos platform. The annual average temperature is 8.8 °C and the annual average precipitation is 207.7 mm. The experimental field covered 15 ha of *C. korshinskii* plantation, but also included *Caragana microphylla*, *Artemisia ordosica*, *Artemisia sphaerocephala*, *Agriophyllum squarrosum*, *Glycyrrhiza uralensis*, *Peganum nigellastrum*, and *Artemisia halodendron*. The vegetation coverage was about 20–50%, the average plant height was 2.4 m, and the average basal stem diameter was 3.2 cm. The infestation rate was 36.2%, with an average population density of 4.3 larvae per plant.

Collection and identification of volatile compounds

Three healthy and three insect-damaged *C. korshinskii* plants were selected from a *C. korshinskii* forest in Zhongwei City for collection of volatile compounds by the dynamic headspace method as follows (Cheng *et al.*, 2009): Sets of stems to be tested were covered with microwaveable bags (48.26 cm x 23 cm x 1.27 cm; Reynolds, Richmond, CA, USA). The air was removed and the bag re-filled with air percolated through activated carbon and a GDX101 apparatus (60/80 mesh; Yansheng, Shanghai, China). Sampling was then started. Gas was cycled through the microwave bag, which was connected to a quartz glass tube (length, 16 cm; inner diameter, 321 μm) filled with the absorbent TenaxTA (60/80 mesh, 200 mg; Supelco, Bellefonte, DE, USA), a polymer packing material particularly suited to the trapping of volatile compounds. The volatile compounds produced by the stems and three controls without the stems were collected over an extraction time of 40 min with an exhaust flow rate of 100 ml/min.

Analysis of volatile compounds

The quality and quantity of volatile compounds produced by the *C. korshinskii* stems were analysed using thermal desorption cold-trap injector gas chromatography/mass spectroscopy (TCT-GC/MS) (CP 4020-Trace 2000/Voyager; Finnigan, ThermoQuest, San Jose, CA, USA).

The thermal desorption was performed under a system pressure of 20 kPa. The tube containing the adsorbates was heated to 250 °C for 10 min. The volatile compounds were thermally desorbed in turn and condensed within a short capillary column surrounded by liquid nitrogen. The inlet temperature was 260 °C. Upon injection, the column was rapidly heated from 50 °C to 260 °C, and the volatile compounds were allowed to flow into the GC column with the carrier gas.

The GC set-up comprised a DB-5 Low Bleed/MS column (60.0 m x 0.32 mm x 0.5 μm; J & W, Santa Clara, CA, USA) with helium as the carrier gas. The injector temperature was kept at 250 °C. The GC oven temperature was initially maintained at 40 °C for 3 min, but was then increased to 270 °C at a rate of 6 °C/min. The post-run was set at 280 °C for 5 min.

The MS conditions were: EI, 70 eV; I/F temperature, 250 °C; source temperature, 200 °C, emis-

sion current, 150 A; detector voltage, 300 V. A full scan was run within a mass range of *m/z* 19–350 and a scan rate of 0.4 s/scan.

Identification of volatile compounds

The NIST98 mass spectral library and retention time database was analysed using Xcalibur software (version 1.2) to identify the volatile compounds produced by *C. korshinskii*.

Normalization of the peak area was used to quantify the various types and relative contents of the volatile substances, and differences between the volatile compounds produced by the different plant subgroups were analysed using the least significant difference (LSD) multiple comparison method.

Electroantennograms

Instruments

An intelligent data acquisition controller (IDAC4; Syntech, Kirchzarten, Germany) with a programmable output control and a stimulus controller (CS55; Syntech) were used to record the electroantennograms of *C. caragana.* Data were recorded and saved using EAG Pro acquisition software (Syntech).

Insects

Large quantities of *C. korshinskii* branches damaged by *C. caragana* were collected from Zhongwei City (105° 18′ E, 37° 52′ N) in Ningxia, China, in April 2009. The branches were then placed into insect cages in an indoor facility until the eclosion of the adults. Highly active *C. caragana* individuals were used in the experiment.

Preparation

According to the identification of volatile compounds, isophorone (97%; Sigma-Aldrich, Shanghai, China), dibutyl phthalate (99%; Sigma-Aldrich), diisobutyl phthalate (99%; Sigma-Aldrich), *cis*-3-hexen-1-ol (98%; Sigma-Aldrich), and 3-pentanone (98%; Sigma-Aldrich) released by *C. korshinskii* were selected according to their relative concentrations in the samples. The compounds were diluted in paraffin oil to yield serial dilutions from 10^{-6} up to 10^{-1} ppm. A piece of filter paper (9 cm x 2 cm) was folded four times to produce 2 cm x 0.5 cm grids and then inserted into a Pasteur pipette. Each test solution (25 μl) was applied to the folded filter paper.

Ten antennae from both sexes were used in the experiments. *C. caragana* antennae were carefully removed from the scrobe and the tips of the flagella cut off using fine surgical scissors. The antennae were mounted between a Y-shaped metal electrode (by connecting the base of the antenna to the reference electrode and the cut tip to the recording electrode). The prepared antenna was then placed 0.5 cm from the stimulation flow, in a constant airflow of 20 ml/min. A puff of air (40 ml/min) was then blown through the Pasteur pipette, thus allowing the chemicals on the filter paper to flow over the antenna. Each chemical was tested in a series from low to high concentrations, reactions to the reference chemical were measured for each tested chemical. Each stimulation lasted 0.3 s with about 45–60 s between stimulations. 2-Hexen-1-ol (98%; Sigma-Aldrich) was used as the standard. Five replications were recorded for each antenna. Because the responses diminished as the experiment progressed, the electroantennogram responses were normalized to the relative response (in %) of the active standard control 2-hexen-1-ol at a concentration of 10^{-3} ppm (Visser, 1979; Dickens, 1984).

Field trapping

Lures

Depending on the electroantennogram results, the compounds emitted by *C. korshinskii* were used alone or mixed at a certain ratio. To begin with, 1 ml of a given volatile compound, dissolved in paraffin oil, was removed with a syringe and transferred into a 5-ml centrifuge tube with an air-tight cap. The tubes were shaken for 30 s in a vortex mixer (CS-H1; Boliyang, Beijing, China) to homogenize the compounds. Finally, a piece of absorbent cotton was placed in the centrifuge tube and saturated to ensure that the compounds were in a homogeneous state. The cap of the centrifuge tube was then opened. Fifty-eight different mixtures were used for the field trapping experiments.

Trapping

Three types of trap were used (Fig. 1). A triangular trap was made from a folded PVC board (1 mm thick) with a piece of thin wire used to form the triangle. This was tied to a branch. The lure was then fixed to the centre of a sticky board inside the triangular trap. A cross trap was made from a gauze bag, two black plastic boards in

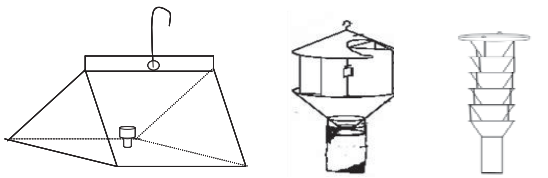

Fig. 1. The three traps used in the study.

the form of a cross (the angle between the two crossed boards was 90°) and black food-grade PVC covered the top and bottom, the bottom of the trap with the gauze bag forming a trapping room, making it difficult for the insects to walk on and easy to collect the trapped insects. The third trap was a funnel. A cover was placed over six connected black funnels with a white cylinder used for collecting insects at the bottom. The lure was fixed to the edge of the connected funnels and the captured insects slid down the funnels into the cylinder. A small amount of water was injected into the cylinders to prevent the insects from escaping.

Selection of the best attractant

During the emergence period of the adult *C. caragana* (June to September, both in 2009 and 2010), 29 mixtures were tested simultaneously in the field trapping experiments in Zhongwei and Lingwu of Ningxia, China. During these two years, lures were placed in the centre of the sticky board in the triangular traps, and the traps were randomly hung in *C. korshinskii* shrubberies. Each mixture was tested three times. Simultaneously, three blank controls comprising hung traps without attractant (a plastic tube filled with absorbent cotton instead of a lure) were set up in two separate shrubberies. The distance between traps was 50 m and the hanging height was 1 m. Depending on the weather conditions, numbers of captured adults were counted every 2 to 7 d, until no further adults were captured. During the experiments, the position of the traps was changed regularly to reduce any errors caused by the differing adult population densities in different places.

Optimal hanging height for the traps

In mid July 2010, during the peak emergence of adults, the most attractive compound was used as the lure and placed in the triangular sticky traps. The traps were then hung on the *C. korshinskii* branches at heights of 0.5 m, 1.0 m, and 1.5 m (threee traps at each height). The number of adults in each trap was then counted.

Optimal type of trap

When choosing the best-hanging height, the most successful attractant was placed in the three different types of trap, which were hung randomly in the *C. korshinskii* shrubberies. The distance between each trap was 50 m and the hanging height was 1 m. Three traps of each type were used. The number of adults in each of the different traps was then counted.

Trapping efficiency of the attractants

Because adult *C. caragana* leave circular emergence holes on plants' branches, the number of emergence holes is equivalent to the number of adults. From June to September 2009, all emergence holes in the Zhongwei experimental fields were marked in red before the trapping experiment. After trapping, the unmarked emergence holes (N) were counted along with the number of captured adults (S). The trapping efficiency was calculated as: $S/N \cdot 100\%$.

Statistical analysis

Differences between the relative antenna responses of male and female *C. korshinskii* to different concentrations of chemicals were analysed using one-way ANOVA (SPSS 16.0 for Windows) and two-way ANOVA (Graphpad Prism 5.0). Other experimental data were analysed using Microsoft Excel 2003.

Results

Volatile compounds isolated from the stems of healthy and insect-damaged C. korshinskii

GC/MS analysis revealed that the extract from the stems of healthy and insect-damaged *C. korshinskii* comprised 10 volatile compounds, including alcohols, aldehydes, esters, and ketones (Table I). Of these, 3-hexen-1-ol, 3-hexenyl acetate, and isophorone were the main volatile compounds in healthy *C. korshinskii*. The type and relative content of volatile compounds changed markedly in damaged plants. The relative levels of 3-hexen-1-ol and 3-hexenyl acetate were different and isophorone disappeared. Simultaneously, seven new chemicals appeared. 2-Hexen-1-ol, dibutyl

Table I. Volatile compounds produced by *Caragana korshinskii.*

No.	Retention time [min]	Compound	Relative level ± SE (%)	
			Healthy	Insect-damaged
1	9.43	3-Pentanone	–[a]	2.17
2	12.91	2-Methyl-4-pentenal	–	2.35 ± 0.01
3	14.96	2-Hexen-1-ol	–	11.09 ± 0.01
4	15.4	3-Hexen-1-ol	80.82 ± 0.02	63.75 ± 0.16
5	15.69	(*E*)-2-Hexen-1-ol	–	0.01 ± 0.01
6	16.68	Allyl methyl ketone	–	0.62
7	20.08	3-Hexenyl acetate	16.97 ± 0.01	17.38 ± 0.05
8	24.2	Isophorone	2.21 ± 0.11	–
9	40.98	Dibutyl phthalate	–	2.63 ± 0.02
10	41.13	Diisobutyl phthalate	–	0.01 ± 0.01

[a] Not detected.

phthalate, 2-methyl-4-pentenal, and 3-pentanone were confirmed as the main herbivore-induced volatile compounds produced by *C. korshinskii*.

Electroantennograms

Generally, the relative antenna responses of *C. caragana* became stronger as the concentrations of the test chemicals increased (Fig. 2). Stronger responses were noted for isophorone, *cis*-3-hexen-1-ol, and 3-pentanone, whereas weaker responses were observed for dibutyl phthalate and diisobutyl phthalate. With increasing concentrations, the responses of both sexes to isophorone increased moderately and almost linearly, whereas the responses to 3-hexen-1-ol and 3-pentanone increased suddenly at 10^{-3} ppm. There was no significant difference between the sexes in terms of the relative response to isophorone, 3-hexen-1-ol, 3-pentanone, or diisobutyl phthalate ($P > 0.05$; two-way ANOVA). However, the response of the male insects to dibutyl phthalate was significantly higher than that of the females at a concentration of 10^{-1} and 1 ppm ($P < 0.01$).

Optimal attractant for C. caragana

Twenty-nine compounds were mixed at different ratios with the five principle volatile components from *C. korshinskii* and tested in field trapping experiments in Zhongwei and Lingwu: A, isophorone; B, dibutyl phthalate; C, diisobutyl

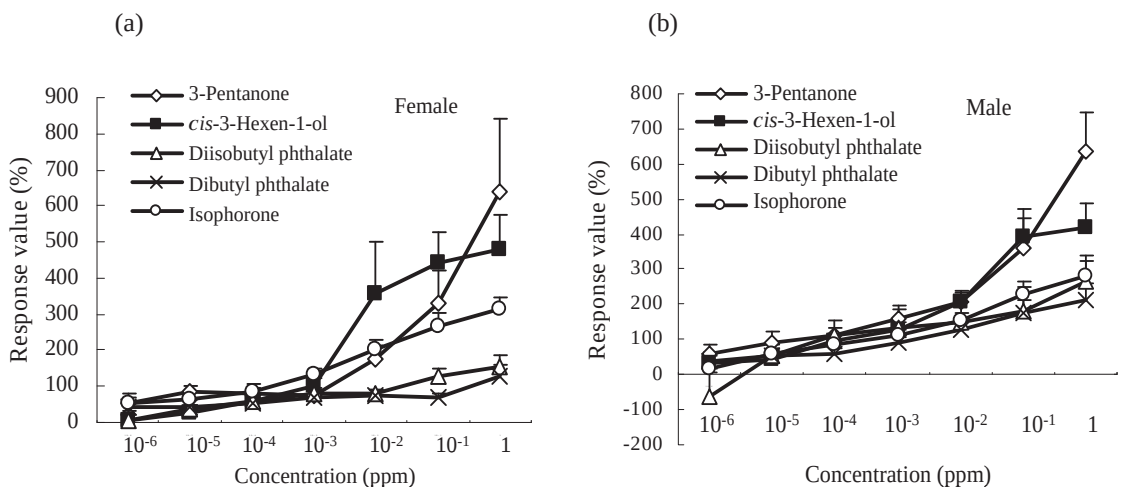

Fig. 2. Mean antenna responses of *C. caragana* to different dilutions of five test compounds relative to a standard compound.

phthalate; D, *cis*-3-hexen-1-ol; E, 3-pentanone; CK, contrast. Any compounds/mixtures that attracted no insects were removed. The remaining compounds/mixtures are shown in Fig. 3. In Zhongwei in 2009, 1/29 compounds/mixtures attracted different numbers of adults: mixture ABC was the best, trapping an average of 14.33 adults per trap. This was followed by A, AB, and CE. By contrast, only 10/29 compounds/mixtures attracted adults in Lingwu in 2010. Compound A was the best, followed by mixtures AB, AC, and ABC. The comprehensive 2-year-results showed that compound A, and mixtures AB and ABC had the best trapping effects, but there was no significant difference between them ($P > 0.05$).

Whether mixed with compound A alone, or all mixed together, neither compound B nor compound C enhanced the trapping effect. No adult beetles were trapped by compound D alone, but some were trapped when compound D was mixed with compound C or with compounds B and C (*i.e.* BCD). Compound E alone trapped some adult beetles, and its trapping efficiency improved somewhat when mixed with compounds C or B, but the difference was not significant ($P > 0.05$).

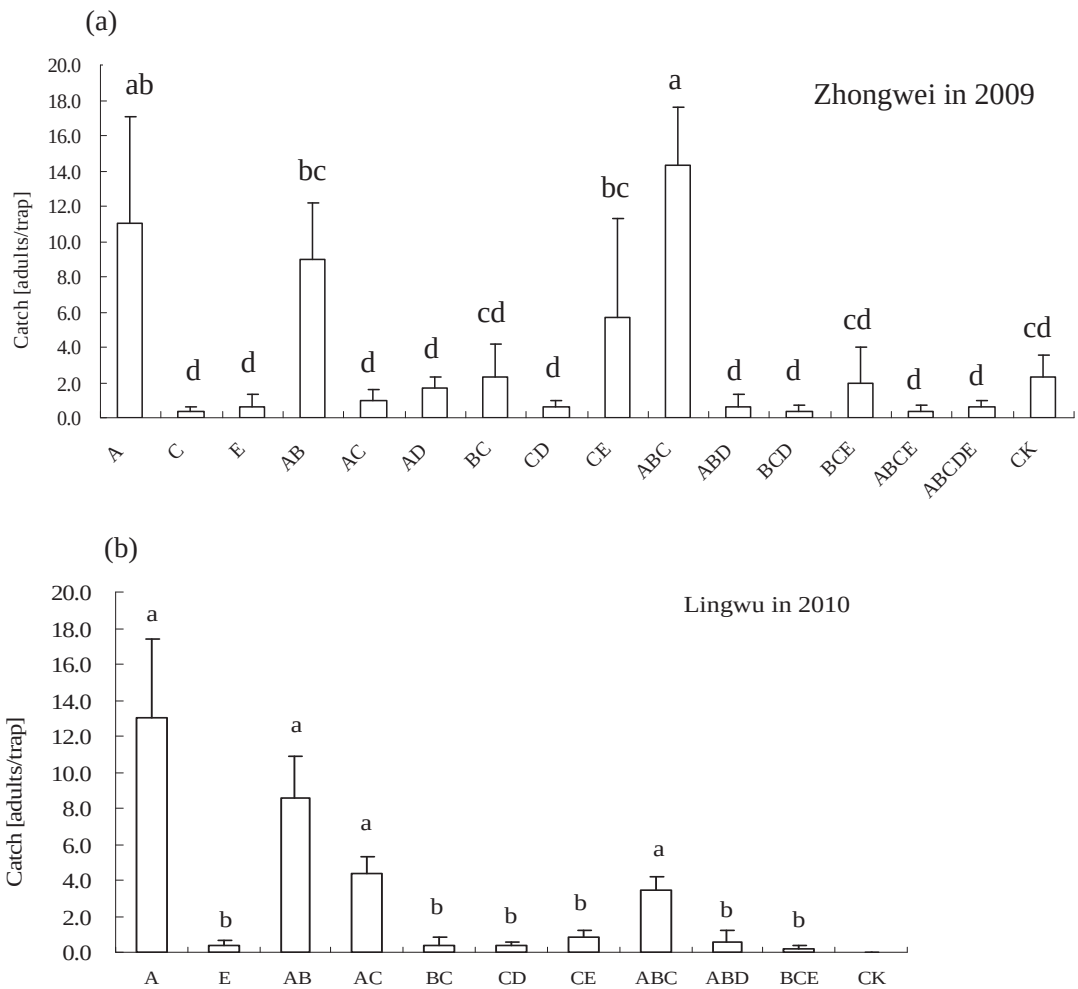

Fig. 3. Trapping effects of the different compounds/mixtures. A, isophorone; B, dibutyl phthalate; C, diisobutyl phthalate; D, *cis*-3-hexen-1-ol; E, 3-pentanone; CK, contrast. a, b, c signify the difference; the same letters signify no significant difference ($P > 0.05$).

Taking into consideration the trapping effects and associated costs, compound A (isophorone) was chosen as the best for attracting adult beetles. Therefore, isophorone was used in all subsequent fieldtrapping experiments.

Field application of the C. caragana attractants

Optimal trapping device

The nature of the lures is the most important factor for trapping adult beetles, but the trapping device itself cannot be ignored. The size, shape, and colour of the trapping device can all influence its effectiveness. A certain amount of isophorone in a lure was placed in the triangular, cross, and funnel-shaped traps, respectively, and they were hung in the *C. korshinskii* forests. The trapping efficiency of the three traps is shown in Fig. 4. There was no significant difference between the average number of adult beetles trapped in the triangular and funnel-shaped trapping devices; however, no adult beetles were trapped in the cross-shaped ones.

Practically speaking, although the triangular trapping devices are cheaper, they have limitations because they depend on a glue to capture the adult beetles. The adhesive area has a limited range, and when it is full of adult beetles it needs to be changed regularly. Also, when the glue board becomes wet, it will not work properly until it has dried again. The funnel-shaped trap costs more than the triangular trap, but it is not subject to changes in the weather, and it can be used for long periods. Therefore, the funnel-shaped trap was chosen as the most suitable for trapping adult beetles in practical situations.

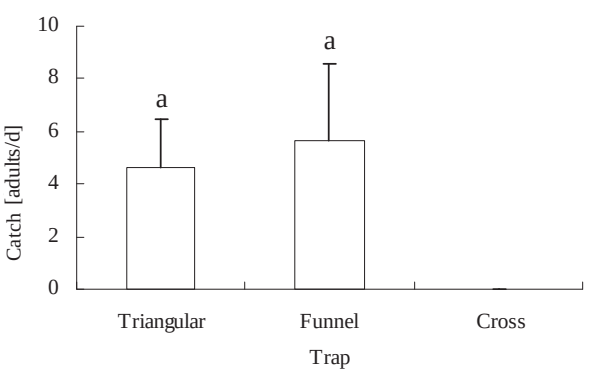

Fig. 4. Number of *C. caragana* adults caught in the different traps per day.

Optimal hanging height

C. caragana moved around the *C. korshinskii* forests at a certain height, and traps were therefore set at different heights. The results are shown in Fig. 5. Most beetles were trapped at a height of 0.5 m, followed by 1.0 m and 1.5 m. One-way ANOVA was used to analyse the trapping data at the three heights, and the results showed no significant difference between 0.5 m and 1.0 m; however, there was a significant difference at 1.5 m. As the average damaged *C. korshinskii* stands at over 2 m, the trapping devices were set at 1.0 m for convenience.

Trapping efficiency of the attractants

Mixture CE trapped an average of 5.76 adult beetles per trap, while mixture AB trapped an average of 8.6 (Fig. 3a). Although mixture AB trapped more adult beetles than mixture CE, it could not be proven that AB was better than CE because of differences in the average population density of *C. caragana* and the portion of affected trees in the two experimental forests. Also, the rates of adult eclosion were different. Therefore, the trapping efficiency was not based solely on the number of the trapped adult beetles but was rather determined according to the number of trapped adult beetles and the number of eclosion adults. The results showed that there were 320 current-year eclosion holes during the experimental period, and 204 adult beetles were trapped in the triangular trapping devices. Therefore, the trapping efficiency was 204/320 · 100% = 63.8%.

Monitoring of the adult eclosion period

The attractants were used to monitor the eclosion of *C. caragana* from 2009 to 2011. The adult beetles appeared from mid June to late August,

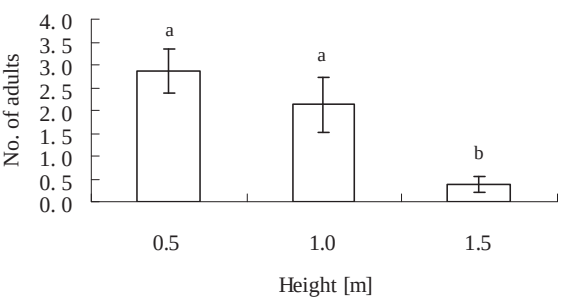

Fig. 5. Number of *C. caragana* adults trapped at different heights with a triangular trap.

with the largest number occurring from late June to early August, with a peak in mid July (Fig. 6).

Discussion

The main volatile substances produced by *C. korshinskii* are isophorone, *cis*-3-hexen-1-ol, and 3-pentanone. Isophorone naturally occurs in water, honey, mushrooms, and the flowers of many plants (Zarghami and Heinz, 1971; Pyysalo, 1976; Knudsen *et al.*, 1993; Jang and Shang, 1994; Soria *et al.*, 2003). It is an important solvent widely used in paints, pesticides, pharmaceuticals, plastics, and the spice industry (Ye *et al.*, 2002; Yao, 2010). Both *cis*-3-hexen-1-ol and 3-pentanone are important volatile substances produced by many higher plants and are found in both healthy and pest-infested plants. These compounds play a significant role in a pest's host choice and in the plant's indirect defences (Visser *et al.*, 1979; Baehrecke *et al.*, 1989; Kessler and Baldwin, 2001; Van Poecke *et al.*, 2001; James, 2005). Furthermore, volatiles such as dibutyl phthalate and diisobutyl phthalate, which were determined in our experiment, are also found in fruits, mushrooms, and insects, and they are important plasticizers used in the production of various plastics (Shaw and Wilson, 1982; Shiota, 1990; Jang and Shang, 1994; Fujii *et al.*, 2003). However, there are no reports regarding the use of isophorone, dibutyl phthalate, and diisobutyl phthalate as plant semiochemicals in pest monitoring and control.

The results of the field trapping experiments showed that, whether used alone or mixed with the four other monomers, isophorone had the best trapping effects. Therefore, for practical control applications, we suggest that isophorone can be used as the attractant for *C. caragana*. The material for synthesizing isophorone is freely available in many countries and the method of its synthesis is straightforward (Ye *et al.*, 2002; Yao, 2010); therefore, isophorone should have a broad application for monitoring and controlling *C. caragana*.

The *C. caragana* attractants trapped both female and male adult beetles. During the eclosion period, the attractants were placed into the traps and then hung in the *Caragana* shrubs. The distance between two traps was 50 m, and the hanging height was 1 m. The traps monitored the occurrence, growth, and decline of the adult beetles both timely and accurately. If the adult beetles are trapped and killed during the period of their emergence, they cannot breed successfully, and damage to plants will consequently be reduced.

Currently, however, there are practical problems. The validity of the lure is the most important. In the field, lures need to be changed three or four times during the whole adult stage of the beetles to achieve better monitoring and control, thus greatly increasing the cost of preven-

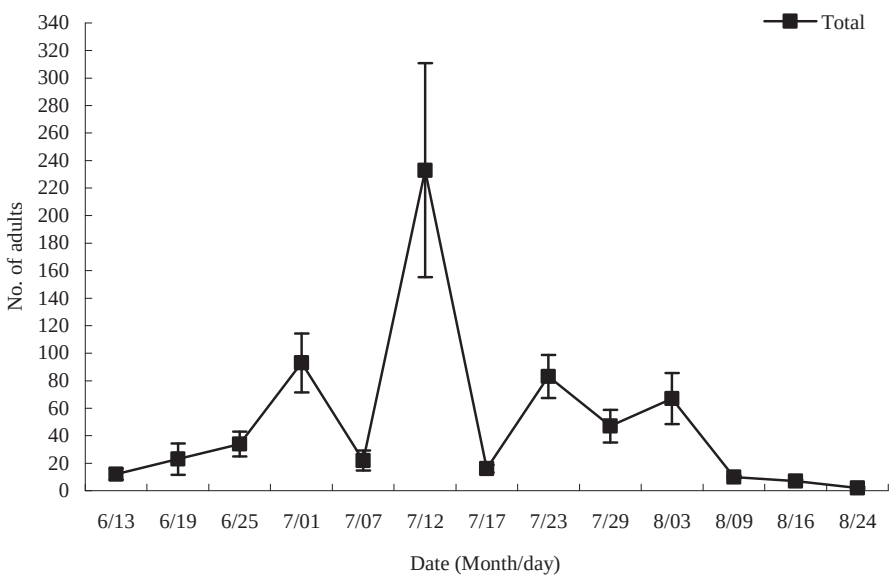

Fig. 6. Monitoring of the appearance of *C. caragana* adults between June and August (2009 – 2011) in Lingwu.

tion. Therefore, a suitable carrier must be chosen. Also, further studies regarding sustained release technology and the persistence of the lure are required. At the same time, the most suitable distance between two traps, the minimum number of hanging traps required, and the hanging position need further investigation.

C. korshinskii grows in thickets and has a small ground diameter. The larvae of *C. caragana* mainly damage the trunk and live within this concealed environment. Diachoresis also occurs in the interior of the trees. Thus, it is hard to judge whether the host plant is harmed by inspection of the outer foliage. Many control methods are available, but their efficiencies are not ideal, and they are very difficult to use in practice. Therefore, the use of plant semiochemicals to trap both female and male adult beetles will play an important role in the control of *C. caragana*.

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