Research Article

Olfactory and visual responses of the long-legged chafer *Hoplia spectabilis* Medvedev (Coleoptera: Scarabaeidae) in Qinghai Province, China

Qing-He Zhang,[a]* Jian-Hai Ma,[b] Qi-Qing Yang,[b] John A Byers,[c] Michael G Klein,[d] Feng-Yu Zhao[b] and You-Qing Luo[e]

Abstract

BACKGROUND: Monitoring traps and control methods are needed for the long-legged chafer, *Hoplia spectabilis* Medvedev, which has recently reached outbreak numbers in pastureland of Qinghai Province, China.

RESULTS: Field trapping experiments, using cross-pane funnel (barrier) traps, showed that *H. spectabilis* adults were not significantly attracted to branches of the host plant *Dasiphora fructicosa* (L.) Rydb. However, beetles were slightly attracted to similar host plant branches infested by conspecific beetles, possibly owing to weakly attractive volatiles, primarily (*Z*)-3-hexenyl acetate, released from beetle-damaged host leaves. This compound was weakly attractive when released from traps. However, *H. spectabilis* beetles showed strong visual responses to yellow- or white-painted trap panes, with weaker responses to blue, red or green panes, and least response to black panes. Black traps at 0.2–1.5 m above ground intercepted significantly more beetles than traps at 2.5 m. The mean flight height based on trap catches was 0.88 m (SD = 0.76), yielding an effective flight layer of 1.9 m. Flight response of beetles to colored barrier traps occurred between 10:00 and 18:00, and peaked between 12:00-14:00, when daily temperatures reached their maximum.

CONCLUSION: Unbaited yellow or white cross-pane funnel traps are recommended for both monitoring and mass-trapping programs against this economically and ecologically important scarab beetle.
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Keywords: scarab; barrier trap; visual; olfactory; plant volatile; (*Z*)-3-hexenyl acetate; monitoring; mass trapping

1 INTRODUCTION

In July 2005, a sudden and serious outbreak of a long-legged chafer was reported in the natural woody scrublands along the Habu River, Tianjun County, Qinghai Province, China.[1,2] It was identified as *Hoplia spectabilis* Medvedev (Coleoptera: Scarabaeidae), a newly recorded species for Qinghai Province.[1,2] *Hoplia spectabilis* was reportedly distributed in Sichuan[3] and Qinghai,[2] China, but its status as a pest had never been documented. Since 2005, severe outbreaks of this uncommon chafer have spread across most of the natural scrublands along the 200 km long Habu River. The scarab has caused significant damage, and even death, to several key species of woody shrubs, especially *Hippophae neurocarpa* Liu & Ho (Elaeagnaceae), *Dasiphora fruticosa* (L.) Rydb. (Syn. *Potentilla fruticosa* L.) (Rosaceae) and *Myricaria germanica* Desv. (Tamaricaceae). These shrubs are critically important for protection of the precious and fragile riverbanks in the high-altitude pasturelands (3000–4000 m asl).

Hoplia spectabilis has one generation per year in Qinghai Province and overwinters as second and third instars in the sandy pasture soil at about 60 cm below the surface. The grubs feed on the grass roots in the pastureland.[2] Adults emerge intensively during mid-June and early July and fly towards and aggregate on

the woody shrubs along the river for feeding (petals of flowers and leaves) and mating.[2] Since 2006, chemical control of adults has been implemented with traditional pesticides, via ground and aerial applications, in some heavily infested stands. However, owing to the relatively short flight/foraging period of adults and strong environmental concerns for pollution of the river, successful control with insecticides over the whole outbreak area cannot be sustained economically and ecologically. Therefore, efficient and

* Correspondence to: Qing-He Zhang, Sterling International, Inc., 3808 N. Sullivan Rd, Bldg 16, Spokane, WA 99216, USA. E-mail: qing-he@rescue.com

a Sterling International, Inc., Spokane, WA, USA

b Forest Pest Control and Quarantine Station of Qinghai Province, Xining, PR China

c USDA-ARS, US Arid-Land Agricultural Research Center, Maricopa, AZ, USA

d Department of Entomology, The Ohio State University, Wooster, OH, USA

e School of Forestry, Beijing Forestry University, Beijing, PR China

environmentally friendly alternative measures, such as trapping devices for both monitoring and control, are urgently needed.

Owing to its status as a new pest, knowledge of *H. spectabilis* is limited to its morphological description[1,3] and recently reported basic biology.[2] Nothing is known about its sensory ecology, such as visual and olfactory responses to ecologically relevant signals. However, female-produced sex pheromones and host plant volatiles such as kairomones have been reported in several phytophagous scarab beetles,[4–15] including two *Hoplia* spp., *H. communis* Waterhouse[16] and *H. equina* LeConte.[17] Practical applications for synthetic sex pheromones and plant volatile attractants have been developed for several economically important scarab species for detection, monitoring and even control of chafer populations in agricultural, turf and garden settings.[4,5,11,18–21]

The objectives of the present field study were: (1) to test the potential attraction of *H. spectabilis* to volatiles from host plants with or without conspecific beetle feeding; (2) to test-screen a series of known synthetic sex pheromone or plant volatile kairomone attractants for other scarab beetles on *H. spectabilis*, plus (Z)-3-hexenyl acetate, a major volatile component identified from freshly cut branches of its key host plants (Zhang *et al.*, unpublished data); (3) to determine visual responses of *H. spectabilis* to differently colored barrier traps; (4) to observe diurnal flight patterns using the barrier traps; (5) to test the effects of trap height on beetle catches and calculate mean height of flight ± SD.

2 MATERIAL AND METHODS

All field trapping experiments were carried out on the edge of pastureland next to a natural stand of mixed woody shrubs of *Hi. neurocarpa*, *D. fruticosa* and *M. germanica* on the northern riverbank of the Habu River in Tianjun County (36° 54′–39° 12′ N, 96° 49′–99° 42′ E), Qinghai Province, China, during mid-June to mid-July 2009. This shrub stand along the Habu River was surrounded by hundreds of km^2 of pastureland, and was heavily infested by *H. spectabilis* adults in the previous years. Cross-pane funnel traps (Pherobio Technology Co., Ltd, Beijing, China) (Fig. 1), hereafter simply referred to as traps, with a total of 6000 cm^2 active pane surface area (50 × 30 cm for each pane), were used in original black or painted different colors, depending on the experiments. Trapped beetles were counted individually if <100, or measured by volume using a scaled glass test tube. The sexes were separated by squeezing out the genitals of captured beetles, and sex ratios were determined from subsamples (≤100) of trap catches.

The following synthetic attractant candidates were obtained from various commercial and non-commercial sources: Japanese beetle sex pheromone [(R)-japonilure = (Z)-5-(1-decenyl) dihydro-2(3H)-furanone; 95%, Nitto Denko Inc., Japan]; Osaka beetle sex pheromone [(S)-japonilure; 95%, gift from Dr Aijun Zhang]; *H. equina* sex pheromone (2-tetradecanone; 98%, gift from AZ); oriental beetle sex pheromone [(Z)-7-tetradecen-2-one; 95%, Bedoukian Research Inc., USA]; *Phyllophaga* sex pheromone (L-valine methyl ester and L-isoleucine methyl ester; gift from Dr Paul S Robbins); plant volatiles [eugenol (99%), geraniol (98%) and phenethyl propionate (99%) from Vigon International, Inc., USA; (Z)-3-hexenol (98%), (Z)-3-hexenyl acetate (98%), octyl butyrate (98%), 2-phenylethanol (99%), valeric acid (99%), hexanoic acid (99.5%) and *trans*-anethole (99%) from Sigma-Aldrich, USA; cinnamyl acohol (>97%, TCI, Japan)].

Experiment 1 was carried out from 27 to 30 June 2009 to test the potential attraction of *H. spectabilis* adults to several known
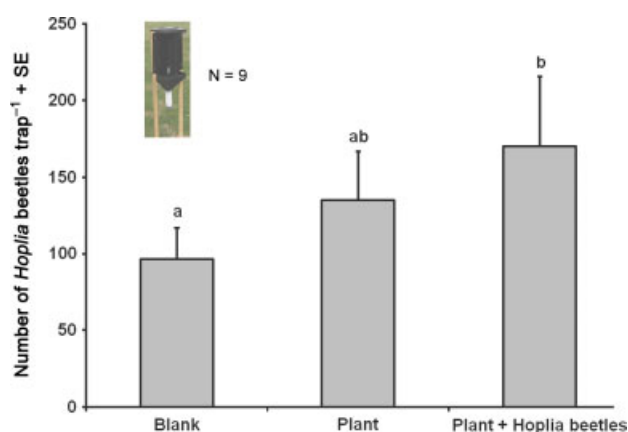

Figure 1. Mean captures of *Hoplia spectabilis* in black cross-pane funnel traps (photo insert) baited with fresh *Dasiphora fruticosa* branches with or without 200 live adult beetles feeding on them, or in an unbaited trap, 26–29 June 2009, Tianjun, Qinghai, China. Bars with the same letter are not significantly different ($P > 0.05$) by REGW multiple Q-test after ANOVA on log ($x + 1$) transformed trap catch data.

plant (food) volatiles, single components or mixtures for scarab beetles (Table 1). Four sets (blocks) of black traps were set up in lines on the pastureland (ca 0.5 m above ground) next to the mixed shrub forest stand, with ca 10 m between traps within each set, and 20 m between sets. Within each set, eleven traps were baited with the different known plant-based attractants, including a blank control. The dispenser type, loading and release rates are listed in Table 1. The positions of traps, together with dispensers within each set, were assigned randomly. To minimize any positional effects, the dispenser positions were re-randomized once[22] when >100 beetles had been captured in the best traps.

Experiment 2 was conducted from 27 to 30 June 2009 to test sex pheromones of several well-known scarab beetles, such as Japanese beetle, oriental beetle, Osaka beetle and a long-legged chafer, *H. equina*, in addition to a combination of Japanese beetle sex pheromone with a floral attractant mixture (see Table 1 for details on chemical candidates). Five sets of black traps were set up in lines, as in experiment 1, about 30 m from experiment 1. Within each set, seven traps were baited with different pheromones, a blend with food attractant mixture and a blank negative control. The dispenser types, loading and release rates of the semiochemicals are listed in Table 1. The positions of traps, together with dispensers within each set, were assigned randomly and re-randomized as in experiment 1.

Experiment 3 was carried out from 27 to 30 June 2009 to test two well-known sex pheromone components of *Phyllophaga* beetles and their combinations with different loading ratios (see Table 1 for detailed information on chemicals). Three sets of black traps were set up as in experiment 1, about 30 m from experiment 2. Within each set, six traps were baited with different sex pheromone treatments and a blank negative control. The dispenser types, loading and release rates of the tested semiochemicals are described in Table 1. The randomization of traps was as in experiment 1.

Experiment 4 was conducted from 26 to 29 June 2009 to test the potential attraction of *H. spectabilis* adults to fresh host plant leaves (*D. fruticosa*) with or without 200 beetles feeding on them. Three sets of black traps were set up in lines as in experiment 1, about 50 m from the other experiment trap lines, with ca 10 m between traps within each set, and 50–100 m between sets. Within each

Table 1. Field responses of *Hoplia spectabilis* adults to the known scarad beetle attractants, 27–30 June 2009, Tianjun, Qinghai, China

Chemicals	Dispenser type[a]	Loading (mg)	Release rate (mg day^{-1})[b]	Beetles trap^{-1} visit^{-1} [mean ($\pm$ SE)]	N
Experiment 1: food attractants					
Japanese beetle floral attractant mix (PEG)[c]	0.05 mm PE bag	1000	26.5	190 ($\pm$30.5)	8
(Z)-3-Hexenol	0.05 mm PE bag	1000	17.5	189 ($\pm$38.4)	8
2-Phenylethanol	0.05 mm PE bag	1000	8.5	245 ($\pm$55.4)	8
PEG + (Z)-3-hexenol	0.05 mm PE bag	1000 + 1000	26.5 + 17.5	257.5 ($\pm$39.7)	8
PEG + 2-phenylethanol	0.05 mm PE bag	1000 + 1000	26.5 + 8.5	173.5 ($\pm$34.6)	8
(Z)-3-Hexanol + 2-phenylethanol	0.05 mm PE bag	1000 + 1000	17.5 + 8.5	282 ($\pm$56.0)	8
PEG + (Z)-3-hexenol + 2-phenylethanol	0.05 mm PE bag	1000 + 1000 + 1000	26.5 + 17.5 + 8.5	268.5 ($\pm$49.2)	8
Cinnamyl alcohol	0.05 mm PE bag	1000	2	319 ($\pm$69.1)	8
trans-Anethole	0.30 mm PE bag	1000	20	210 ($\pm$41.7)	8
Rose chafer attractant mix[d]	0.15 mm PE bag	1000	32	175.5 ($\pm$32.8)	8
Blank	0.15 mm PE bag	0	0	327.5 ($\pm$63.1)	8
Experiment 2: sex pheromone and floral attractants					
Japanese beetle sex pheromone	Rubber septa (RS)	1	Unknown	120.0 ($\pm$22.9)	9
Oriental beetle sex pheromone	RS	1	Unknown	135.6 ($\pm$15.2)	9
Hoplia equina sex pheromone	RS	1	Unknown	128.9 ($\pm$21.6)	9
Osaka beetle sex pheromone	RS	1	Unknown	140.0 ($\pm$20.3)	9
Japanese beetle floral attractant mix (PEG)	0.05 mm PE bag g	1000	26.5	131.1 ($\pm$26.7)	9
Japanese beetle sex pheromone + PEG	RS + 0.05 mm PE bag	1 + 1000	Unknown + 26.5	131.1 ($\pm$26.9)	9
Blank	RS + 0.05 mm PE bag	0	0	135.6 ($\pm$17.6)	9
Experiment 3: sex pheromone attractants of *Phyllophaga* beetles					
L-Valine methyl ester	RS	4	Unknown	141.7 ($\pm$21.7)	6
L-Valine/L-isoleucine methyl esters	RS	3 + 1	Unknown	123.3 ($\pm$10.9)	6
L-Valine/L-isoleucine methyl esters	RS	2 + 2	Unknown	123.3 ($\pm$20.3)	6
L-Valine/L-isoleucine methyl esters	RS	1 + 3	Unknown	121.7 ($\pm$19.7)	6
L-Isoleucine methyl ester	RS	4	Unknown	146.7 ($\pm$16.1)	6
Blank	RS	0	0	153.3 ($\pm$38.5)	6

[a] PE bag: 3 × 5 cm with a substrate felt and film thickness ranging from 0.05 to 0.3 mm; RS: grey rubber septa.
[b] Release rates were measured at 22 °C in a laboratory fume hood.
[c] PEG: phenethyl propionate/eugenol/geraniol at 3/7/3.
[d] Valeric acid/hexanoic acid/octyl butyrate (1 : 1 : 1).

set, three traps were baited either with 200 g of fresh collected *D. fruticosa* branches alone or with 200 g of fresh branches with 200 live *H. spectabilis* adults (mixed sexes). They were caged in a blue mosquito screen bag, or a blank screen bag was used as the negative control. The positions of traps, together with the blue screen cages, within each set were assigned randomly, and, to minimize any positional effects, the dispenser positions were re-randomized twice when >100 beetles were caught in the highest catch traps.

Experiment 5 was conducted during the late flight season (19–24 July 2009) to test (Z)-3-hexenyl acetate against a blank control. (Z)-3-Hexenyl acetate had just been found to be a major volatile component from aeration samples of freshly cut branches of the key host plants *Hi. neurocarpa* and *D. fruticosa*, collected at the testing site in late June 2009 (Zhang *et al*., unpublished data). Ten pairs of yellow traps were set up (ca 0.5 m above ground) along the edge of the mixed shrub forest, with ca 10 m between two traps (one for blank, the other for treatment) within each pair, and 15 m between trap pairs. Polyethylene (PE) bag dispensers (film thickness 0.30 mm; bag size 3 × 5 cm) were loaded with 2 mL of (Z)-3-hexenyl acetate (99%) (ca 50 mg day^{-1} release). The positions of traps and dispensers (loaded or blank) within each pair were assigned randomly, and, to minimize any positional effects,

the dispenser positions were shifted once when >100 beetles had been caught in the highest catch traps.

Experiment 6 tested the effects of trap color on *H. spectabilis* catch from 23 to 29 June 2009, using traps at three sites: site 1, an open pastureland 50–100 m from the mixed species shrub forest stand; site 2, open spaces within a *Hi. neurocarpa* shrub stand; site 3, open spaces within an *M. germanica* shrub forest stand. The traps (black, as originally manufactured) were painted with black, white, red, yellow, blue and green oil paints (same brand from Baode Paint Co., Tianjin, China) 1 week before field testing to ensure no residual volatiles from the paints. Six sets of the six different colors of painted traps (36 total) were set up in lines at each site in a Latin-square design, i.e. each row and column had all six colors of traps. The distance between traps within each set was ca 10 m, with 20 m between the trap sets at each site.

Reflected light from 6 × 10 cm pieces of the colored traps used in the experiments were measured at 10:00 h under sunny conditions (~100 000 lux) on 17 July 2009. Reflectance spectra were measured by a USB2000 spectroradiometer using OOIBase32 v.2.0.2.2 software (Ocean Optics Inc., Dunedin, FL). A solarization-resistant, UV-transparent, optical fiber (400 µm) probe with an adjustable collimating lens was held perpendicular to the colored surface to capture the spectral reflection. Reflectance

intensity readings from near-UV through to visible wavelengths (300–850 nm) were automatically scanned using an integration time of 5 ms.

An inexpensive and readily available alternate method to spectroradiometers that can be used to describe colors of organisms objectively and quantitatively involves the use of a digital camera where pixels make up the images, and the pixels are colored based on an RGB system.[23] Each pixel combines the three components in ranges from 0 to 255 in intensity. To determine the RGB values, the colored pieces from traps were photographed, at the same time as the spectral measurements, using a Canon PowerShot A540 digital camera at the macro setting (2816 × 2112 pixel resolution). The resulting JPEG images were analyzed for RGB values using square areas of about 1600 pixels for each color with the software described by Byers.[23]

Experiment 7 evaluated the diurnal rhythm of *H. spectabilis* flight activity from 25 to 26 June 2009, using six differently colored traps. Three sets of six different colors of traps were set up in lines in pastureland >10 m from the shrub stand edge, with 10 m between traps within each set and 15 m between sets. The positions of traps within each set were assigned randomly. The traps were visited and emptied every 2 h from 6 : 00 to 22 : 00.

Experiment 8 tested the effects of trap height on *H. spectabilis* catches from 30 June to 14 July 2009, using the black traps. Three sets of traps were set up in lines in pastureland >20 m from the shrub stand edge. Each set consisted of four traps hung at 0.2, 0.5, 1.5 and 2.5 m above the ground on one pair of wooden poles, with 10 m between traps within each set and 30 m between sets. The positions of traps within each set were assigned randomly, and the traps were checked and emptied weekly with their positions re-randomized after the first visit, resulting in six trap–week replicates. Parameters of the vertical flight distribution were estimated from the four trap height catches.[24] A mean flight height based on trap heights where individual beetles were caught and the SDs were calculated, as well as the best-fitting normal curve of distribution with the same mean and SD.[25] Skewness and kurtosis of the distribution were also calculated and tested for significant differences from normality.[26] The effective flight layer (F_L) was calculated from the SD of the height catch data according to the formula[24]

$$F_L = SD \cdot \sqrt{2 \cdot \pi}$$

Trap catch data (for experiments with more than two treatments) were transformed by $\log(x + 1)$ to fit the assumption of homogeneity of variance for ANOVA. The means were compared by ANOVA, followed by the Ryan–Einot–Gabriel–Welsh (REGW) multiple Q-test (SPSS 16 for Windows) at $\alpha = 0.05$. Data from experiment 5 with only one treatment and one blank control were analyzed by paired t-tests at $\alpha = 0.05$ (two tails).

3　RESULTS

In experiments 1 to 3, 20 000, 8300 and 5000 *H. spectabilis* adults were captured, respectively, during the 3 day trapping period. All the unbaited control traps caught significant numbers of *H. spectabilis*, ranging from 135 to 320 beetles trap^{-1} visit^{-1} (95% confidence intervals did not include zero) and were not significantly different from any traps baited with the synthetic attractant candidates within each experiment ($P > 0.05$; Table 1). In other words, there is no evidence that any of the known scarab attractants (sex pheromones or kairomones) were attractive to *H. spectabilis*. The sex ratio (♀:♂) ranged from 1 : 1.15 to 1 : 2.2.

In experiment 4, 3615 *H. spectabilis* were captured during the 3 day trapping period. Blank control traps captured on average ca 100 beetles trap^{-1} visit^{-1}. Traps baited with host branches caught slightly more beetles, but were not statistically different. However, traps baited with plant materials and infested with 200 live *H. spectabilis* caught significantly more beetles than the blank traps (Fig. 1). The sex ratio of captured beetles was 1 : 2.1 (♀:♂), based on pooled subsamples, which was similar, and not statistically different, to that collected from several small shrubs in the open pastureland (♀:♂ = 1 : 1.7).

In experiment 5, 5086 *H. spectabilis* beetles were captured during the 5 day trapping period in late July. Blank traps captured ca 110 beetles trap^{-1} visit^{-1}, while traps baited with (Z)-3-hexenyl acetate caught significantly more beetles (Fig. 2). Interestingly, the sex ratio of captured beetles was about 1 : 0.11–0.14 (♀:♂) for both the treatment and blank control, a very strong female-biased ratio compared with that expected from experiment 4. The significant sex ratio change over the 3 week period from experiment 4 to experiment 5 indicates that males begin to perish after mating, while females live longer and continue to lay eggs.

In experiment 6, 65 107 *H. spectabilis* were captured during the 6 day trapping period. Most beetles (36 115) were caught in the open pastureland site (site 1), followed by the *M. germanica* site (site 3) (20 151), and the fewest at the *Hi. neurocarpa* site (site 2) (8841). The technical color parameters (reflectance intensity and RGB values) of the tested cross-pane funnel traps are summarized in Fig. 3 and Table 2. There were significant trap color effects on beetle catches ($P < 0.0001$; ANOVA). At all three sites, yellow and white traps caught the most beetles (both females and males), followed by blue traps, while the black, green and red caught the fewest (Fig. 4A/B/C). Yellow traps in the open pastureland site and in the *M. germanica* stand site caught significantly more beetles than blue, green, red or black traps (Fig. 4), whereas blue traps at the *Hi. neurocarpa* site showed no differences in catches from either yellow or white traps (Fig. 4C). Similarly to the yellow traps ($P > 0.05$), white traps also had significantly higher catches than the green, red or black traps at all three sites, and even the blue traps at the *M. germanica* site (Fig. 4). Blue traps seemed to catch more beetles than the green, red or black traps, but this was not statistically significant in most cases (Fig. 4). Black traps were the least attractive, but were not significantly different from green and red traps. The overall sex ratios of captured beetles
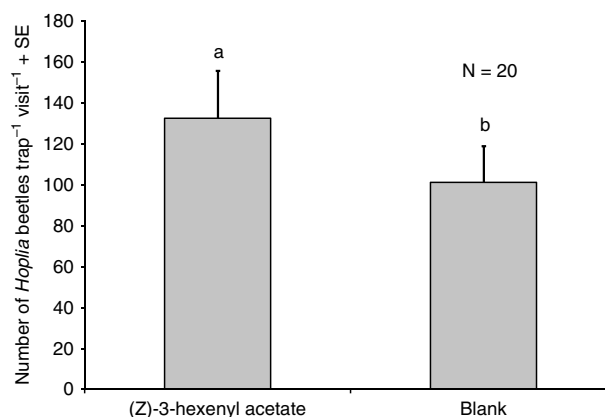
Figure 2. Mean captures of *Hoplia spectabilis* in yellow cross-pane funnel traps baited with either a (Z)-3-hexenyl acetate PE bag dispenser or a blank bag, 19–24 July 2009, Tianjun, Qinghai, China. Bars with different letters are significantly different ($P < 0.05$) by paired t-test (two tails).
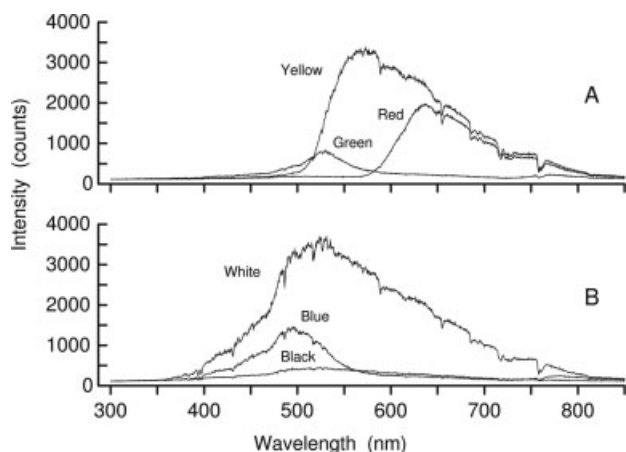
Figure 3. Spectrograms of sunlight reflected from various colors of painted plastic used in traps (from Tianjun, Qinghai, China): (A) yellow, red and green spectrograms; (B) white, blue and black spectrograms. Reflectance measurements were taken at 10:00 a.m. (17 July 2009) with a USB2000 spectroradiometer (Ocean Optics, Inc., Dunedin, FL) under clear skies in Mesa, Arizona.
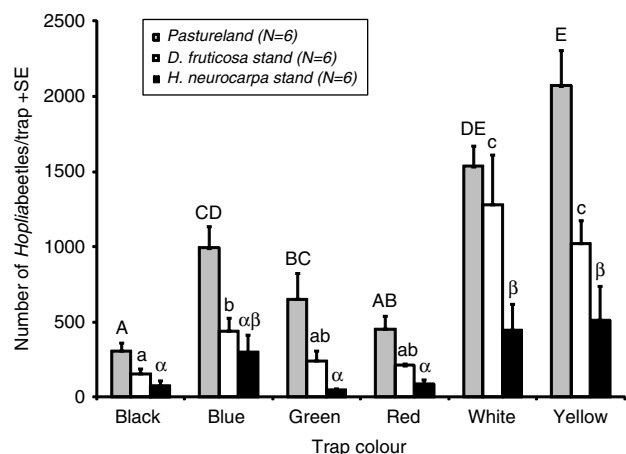

Figure 4. Mean captures of *Hoplia spectabilis* in six differently colored traps at three habitat sites, 23–29 June 2009, Tianjun, Qinghai, China. Bars with the same letter (capital, lower case or Greek) within each site are not significantly different ($P > 0.05$) by the REGW multiple Q-test after ANOVA on the $\log(x + 1)$ transformed trap catch data.

at the *M. germanica* site (♀:♂=1:0.71) and at the *Hi. neurocarpa* site (♀:♂=1:0.55) were more female biased than at the open pastureland site (♀:♂=1:0.91).

The higher catches on the white and yellow traps is correlated positively with intensity or brightness/lightness values (Table 2). Averaging of the RGB values shows an intensity order of white > yellow > blue = red > green = black, which corresponds well to the catches with these colors. Using HSV (the same as HSB) color systems gives a brightness order of white = yellow = red > blue > green > black, which models the catch except for red. The HSL color system gives a lightness order of white > red = yellow > blue > green = black, which again matches well except for red.

In experiment 7, a total of 11 868 *H. spectabilis* were captured. Flight response of *H. spectabilis* to the differently colored traps started just before 10:00 and peaked between 12:00 and 14:00 (Fig. 5). The flight activity declined dramatically after 16:00 and stopped after 18:00. No catches were recorded during the
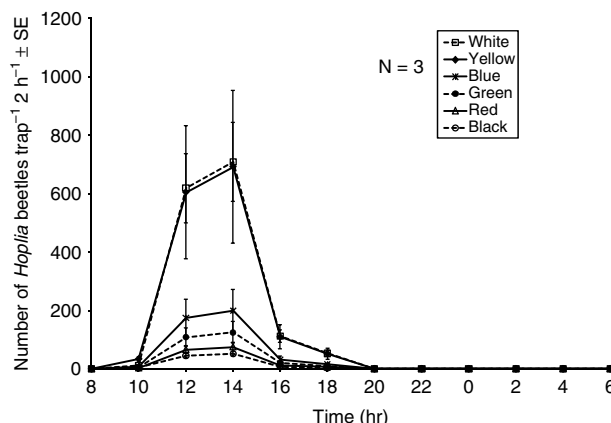

Figure 5. Diurnal flight activity of *Hoplia spectabilis* beetles to three sets of six differently colored traps, 25–26 June 2009, Tianjun, Qinghai, China.
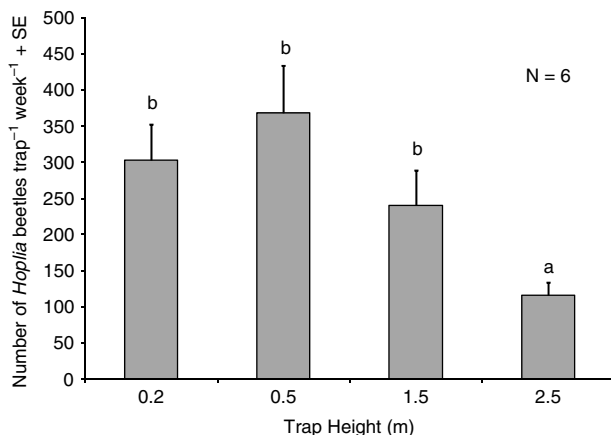

Figure 6. Mean captures of *Hoplia spectabilis* caught in black traps at four different heights in a pastureland next to a mixed shrub forest stand in Tianjun, Qinghai, China, 30 June–14 July 2009.

scotophase. Although there were significant differences in overall trap catches between variously colored traps, no color effect on the diurnal rhythm of *H. spectabilis* flight activity was observed (Fig. 5).

In experiment 8, a total of 6160 beetles were captured. The black traps at 0.2, 0.5 and 1.5 m above ground caught significantly more beetles than the traps at 2.5 m, with no significant differences in catches among the three lower trap heights (Fig. 6). The vertical flight distribution, as reflected in catches on traps (Y) at the four heights (X), approximated a normal distribution:

$$Y = 774 \cdot (\exp(-(X - 0.88)^2/(2 \cdot 0.76^2))/(0.76 \cdot \sqrt{2 \cdot \pi}))$$

with kurtosis = -0.293 and skewness = 1.0, tailing upwards in elevation ($P < 0.001$). The mean flight height of *H. spectabilis* ($N = 1026$) was calculated to be 0.88 m, based on the trap catches at the four heights, with an SD of 0.76 m. The SD was used to calculate an F_L (effective flight layer) of 1.9 m thickness.

4 DISCUSSION

This is the first report on the sensory ecology (olfactory and visual responses) of the long-legged chafer pest *H. spectabilis*. The chemical ecology of phytophagous scarab beetles has been intensively

Table 2. RGB values $\pm$ SD ($N = 1600$ each color) and intensity percentages of various colors of digital images of painted plastic used in cross-pane funnel traps near Tianjun (Qinghai Province, China). Percentages ($\pm$ SD) of red, green and blue components and CVs (coefficients of variation) are shown in parentheses

Color	R (percent; CV)	G (percent; CV)	B (percent; CV)	Intensity Percentage[a]	HSV/HSL[b]
White	198 ± 2 (35.5 $\pm$ 0.3; 0.8)	190 ± 2 (34.1 $\pm$ 0.3; 0.9)	170 ± 2 (30.5 $\pm$ 0.4; 1.3)	73	77/72
Yellow	200 ± 2 (56.5 $\pm$ 0.5; 0.9)	154 ± 1 (43.5 $\pm$ 0.4; 0.9)	0 ± 1 (0 $\pm$ 0.2; 0)	46	78/39
Green	38 ± 4 (24.2 $\pm$ 2.6; 10.7)	67 ± 4 (42.7 $\pm$ 2.4; 5.6)	52 ± 5 (33.1 $\pm$ 2.9; 8.8)	21	26/21
Red	189 ± 2 (78.4 $\pm$ 0.7; 0.9)	24 ± 2 (10.0 $\pm$ 0.7; 7.0)	28 ± 2 (11.6 $\pm$ 1.0; 8.6)	32	74/42
Blue	23 ± 5 (9.1 $\pm$ 2.0; 22)	88 ± 4 (34.9 $\pm$ 1.7; 4.9)	141 ± 4 (56.0 $\pm$ 1.7; 3.0)	33	55/32
Black	42 ± 3 (33.3 $\pm$ 2.3; 6.9)	42 ± 3 (33.3 $\pm$ 2.2; 6.6)	42 ± 3 (33.3 $\pm$ 2.6; 7.8)	17	16/16

[a] Intensity or luminosity as a percentage is defined as $100[(R + G + B)/3)/255]$ rounded to the nearest whole number.
[b] Intensity percentage for the HSV (and HSB) color system is defined as $100[(\text{maximum of } R, G, \text{ or } B)/255]$, while for HSL the percentage is $100[(\text{minimum of } R, G, \text{ or } B + \text{maximum of } R, G, \text{ or } B)/2/255]$.

studied since the late 1970s, with the focus on several economically important plant-feeding scarabs, such as the well-known Japanese beetle, *Popillia japonica* Newm.[4,15] Both sex pheromones and host plant volatile kairomone attractants have been identified for many phytophagous chafer species, and some of them have been commercialized for monitoring, mass trapping or mating disruption in agricultural, turf or home-garden settings.[4,5,11,18–21] The present field results from experiments 1 to 3 clearly showed that none of the known sex pheromone or host plant kairomone attractants for scarab beetles that were tested, including the attractants for two *Hoplia* species, *H. communis* (2-phenylethanol as food attractant)[16] and *H. equina* (2-tetradecanone as female-produced sex pheromone),[17] attracted *H. spectabilis*. The unbaited black traps caught significant numbers of *H. spectabilis* beetles (both sexes), which might indicate a strong visual response to the vertical object, or the effect of simple interception by a barrier under high population levels. Traps baited with the fresh host plant materials (*D. fruticosa* branches) caught slightly more beetles than did the controls, but were not statistically different. However, traps baited with plant materials plus 200 live beetles caught significantly more beetles (both males and females) than blank traps, but were not different from the plant materials alone. Such weak but statistically significant attraction by the plant materials infested by the live insects might be due to an increased release rate of host plant volatiles induced by feeding activity of the live beetles, or by conspecific volatile compounds released by the live beetles, or both. Present efforts to identify sex pheromone components of this beetle (by GC-MS analysis), based on aeration samples of live males or females, and solvent extracts of the male or female genitals, have not resulted in any positive evidence (Zhang *et al.*, unpublished data). Furthermore, recent GC-MS analysis of aeration samples of the freshly cut branches of the three major host species, *Hi. neurocarpa*, *D. fruticosa* and *M. germanica*, indicated that (*Z*)-3-hexenyl acetate is the major volatile component (Zhang *et al.*, unpublished data). The present field trapping experiment in late July 2009 showed that synthetic (*Z*)-3-hexenyl acetate was indeed weakly attractive to male and female *H. spectabilis* beetles. This compound was also reported as part of host kairomone attractants for the Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] (Chrysomelidae) and the Emerald ash borer (*Agrilus planipennis* Fairmaire) (Buprestidae).[27,28] It is a well-known green-leaf volatile[29] and is normally released from leaves damaged by insect feeding or other mechanical factors. It is the only synthetic compound showing any attraction to *H. spectabilis* beetles in the present study. Thus, the weak attraction to traps baited with freshly cut *D. fruticosa* branches + live *H. spectabilis* beetles might be due to the release of (*Z*)-3-hexenyl acetate from the plant in response to beetle feeding rather than to the release of a beetle-produced sex pheromone.

Hoplia spectabilis exhibited little or no olfactory responses to known scarab beetle semiochemicals. In contrast, the beetle showed a strong positive visual response to colored vertical objects. Trap color showed a significant effect on *H. spectabilis* catches, with the lighter colors (yellow and white) being significantly more effective than dark colors, especially black, red and green (Figs 3 and 4, Table 2). The intensity/brightness/lightness of colored traps according to the average RGB, HSV/HSB and HSL color systems (Table 2) correlated well with the chafer catches on the particular shades of colored traps. For example, white and yellow had the highest catches and intensities, blue was medium in intensity and catch, while green and black had low intensities and low catch. Red usually had a medium to high intensity calculated, but consistently was among the least attractive. The average RGB, HSV/HSB and HSL color systems are based on human perception of colors and would not be expected to function as well with beetles which should have UV (345–360 nm), blue (400–450 nm) and green (515–540 nm) receptors and none in the red region.[23] In scarabs, Gribakin[30] reported only a UV (355 nm) and a green (525 nm) receptor sensitivity in the genus *Lethrus*.

A Japanese long-legged chafer, *H. communis*, was also reportedly attracted to artificial objects with whitish surfaces such as clothes and cars.[16] Similar trap color effects on scarab beetle catches have been reported for the Japanese beetle. Yellow Japanese beetle traps were the most effective until the stronger dual pheromone/food lures were developed,[31] which were much better than unbaited color traps. White traps are best for the rose chafer, *Macrodactylus subspinosus* L.[32] Such strong visual responses by *Hoplia* to vertical objects with light colors might be due to newly emerged adults in the pastureland searching for white or yellow flowers for foraging and mating on the angiosperm bushes or tall vegetation around the grassland, before flying back to pastureland for egg laying. As shown in Fig. 4, the patterns of catch on the various trap colors were not significantly affected by the habitat. However, the overall beetle catches in the pastureland were significantly higher than those within the shrub stands, perhaps because beetles had landed on host plants nearby and

were not dispersing as much as over grasslands without such host plants. The low overall trap catches in the shrub stands might be due to competition between the shrub trees and traps, either owing to reduced silhouette contrast of traps by surrounding trees or to volatiles from injured leaf tissue, or to both. In contrast to scarab beetles, conifer bark beetles (Scolytidae)[33–35] and root weevils (Curculionidae)[36] are more attracted to dark colors (black or red) than to the lighter colors (white or yellow). These forest beetles are probably responsive to visual cues and traps of any color presenting dark silhouettes under certain light conditions that mimic conifer host tree trunks.

Flight response of *H. spectabilis* to differently colored traps occurred during the daytime (10:00 to 18:00) and peaked (12:00–14:00) when daily temperatures reached their maximum. Present results concur with earlier reports on the daily flight activities of some other phytophagous chafers.[4] No catches in funnel traps (this study) or in black-light traps[2] were observed during the scotophase.

The traps at 0.2–1.5 m caught significantly more beetles than traps at 2.5 m (Fig. 6). This response probably reflects dispersal flight height of this long-legged chafer in nature. In fact, most of the vegetation (grasses and shrubs) in this pastureland area was lower than 2 m. Similar results have been reported for the Japanese beetle.[37] It is possible that increased catches in the lower (0.2–1.5 m) traps were due to beetles flying lower against strong wind and turbulences in the open pastureland, or to the presence of low-level visual silhouettes (woody shrubs or tall grasses), or both. Fortunately, the optimal trap height (0.2–1.5 m) will be easily and conveniently implemented in practice for monitoring and mass trapping. Recent simulation models of mass trapping[38] have used the effective attraction radius (EAR) to represent attractive sources of pheromone (or colors). The EAR represents a spherical passive trap that would catch the same number of beetles as an attractant trap according to the equation

$$EAR = \sqrt{(A_c \cdot T_A)/(P_c \cdot \pi)}$$

where A_c is the catch on the attractant trap, T_A is the trap interception area, as seen from one direction, and P_c is the catch on the control (unbaited) trap.[39] In these models of mass trapping in two dimensions, the spherical EAR must be transformed into a circular dimension by a second equation:[24,40]

$$EAR_c = (A_c \cdot T_A/2)/(P_c \cdot F_L)$$

where F_L is defined by the SD of the vertical flight distribution, as reported above (experiment 8). An EAR can be calculated for the colored traps if the black trap is assumed to be unattractive. However, until it is determined that black traps are not visually attractive but simply intercept beetles, EAR calculations will not be considered appropriate. The value of F_L (1.9 m) is offered in anticipation of such experiments, so that mass trapping models using the EAR_c can be performed to aid control programs using colored traps.

In conclusion, *H. spectabilis* showed only weak olfactory responses to host plant materials infested by the conspecific beetles, possibly owing to some weakly attractive volatiles, such as (*Z*)-3-hexenyl acetate, released by host branches in response to the beetle damage. No evidence of a specific pheromone produced by either sex was found in the present experiments. Research is needed to identify more effective plant kairomones and to confirm the existence or absence of a sex pheromone. In contrast,

H. spectabilis showed strong visual responses by attraction to yellow or white trap surfaces. Thus, the unbaited yellow or white cross-pane funnel (barrier) traps are strongly recommended for both monitoring and mass-trapping operational programs against this new-outbreak scarab beetle pest. The barrier traps should be deployed in lines (1.5 m height or lower; at least 20 m between lines) in the pastureland, parallel to and 10–20 m away from shrub stand edges. Such an effort might efficiently intercept the dispersal flight of *H. spectabilis* beetles from the pastureland towards the shrub forests along the Habu River, thus reducing *H. spectabilis* damage to the vegetation on the riverbanks. Moreover, the yellow/white barrier trap with a modified collection device could also be used for autodissemination of entomophathogenic fungi into populations of *H. spectabilis* adults and larvae, as suggested by Klein and Lacey[18] for the Japanese beetle.

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