Mechanisms Underlying Host Plant Selection by *Holcocerus hippophaecolus* Adults

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We determined the mechanisms underlying host selection by adults of the seabuckthorn carpenterworm, *Holcocerus hippophaecolus* Hua, Chou, Fang et Chen. Four sea buckthorn (*Hippophae rhamnoides* L.) subspecies (varieties) with different degrees of resistance to *H. hippophaecolus* were chosen for artificial insect infection in cages. The results showed that olfactory and visual cues are very important for the selection of host plants by *H. hippophaecolus*, but that olfactory stimuli play a more vital role in this process. The relative abundance of branches and leaves had no effect on the likelihood that adults landed on plants from four subspecies (varieties), but did influence landing rates within the same subspecies (varieties). When considering only the most resistant sea buckthorn subspecies (varieties), the presence of luxuriant branches and leaves led to lower landing rates. These results provide a theoretical basis for the understanding of *H. hippophaecolus* damage to sea buckthorn and the means to implement effective measures of control.

Key words: Holcocerus hippophaecolus, *Hippophae rhamnoides*, Host Selection

Introduction

The larvae of *Holcocerus hippophaecolus* Hua, Chou, Fang et Chen (Lepidoptera, Cossidae), also known as seabuckthorn carpenterworm, attack mainly the roots and trunks of sea buckthorn (*Hippophae rhamnoides* L.), a commercially important deciduous shrub, thus making them hollow and eventually leading to the death of the plant (Lu *et al*., 2004; Zong *et al*., 2005). In recent years, *H. hippophaecolus* populations have sharply increased in Inner Mongolia and the provinces Liaoning, Shanxi, Ningxia, Shaanxi, and Gansu in China, which has aggravated the damage to sea buckthorn populations and severely impaired the local eco-environmental construction and economic activities based on this species.

The selection of host plants is characterized by six phases, which include search, orientation, location, landing, surface estimation, and acceptance or rejection of the host for oviposition. Host foraging encompasses the first four phases, whereas the last two phases are dedicated to host identification (Renwick and Chew, 1994). Visual, olfactory, and taste cues play a vital role in this process (Bell, 1990; Canale and Raspi, 2000; Campbell and Borden, 2006; Forbes and Feder, 2006; Duan *et al*., 2006; Goyret *et al*., 2007; An and Ren, 2007). Visual stimuli help insects to identify the coulor, shape, and size of host plants during foraging, whereas olfactory cues help insects to detect volatile components of host plants from long distances (Yang *et al*., 2008). Taste cues are also used for the identification of host plants, enabling insects to perceive nutritional differences among host plants before selection (Yin and Sun, 2000).

Currently, the function of olfaction and vision during host foraging is the subject of debate. Prokopy and Owens (1983) and Renwick and Chew (1994) have pointed out that vision is the most important sense employed by insects when foraging for hosts. For example, previous research with *Euhrychiopsis lecontei*, the weevil, has shown that vision is the most important sensorial ability used during foraging (Reeves *et al*., 2009). The results of field tests and Y-tube olfactometer tests conducted by Stenberg and Ericson (2007) have similarly shown that the smell of branches and leaves are unattractive to *Altica engstroemi*, which can locate its hosts using only visual

stimulus. Todd *et al.* (1990) have also suggested that visual stimuli are sometimes more important to aphids and leafhoppers (Homoptera), thrips (Thysanoptera), flies (Diptera), and butterflies (Lepidoptera). However, other studies pointed to a pivotal role of olfaction over vision in the selection of hosts. McPheron and Mills (2007) have shown, for instance, that olfaction is more important than vision during host foraging after conducting a series of host selection experiments with vespids. Goyret *et al.* (2007) have also indicated that olfactory stimuli can enhance the responsiveness of the tobacco hornworm more than visual stimuli, and Wu *et al.* (2005) have proposed that *Campylomma chinensis* Schuh is strongly attracted by the smell of *Lantana* but is not visually attracted by this plant. Lu *et al.* (2008) have reasoned that vision and olfaction both play leading functions in host location and landing.

To our knowledge, the mechanisms underlying the selection of host plants by *H. hippophaecolus* have not yet been studied. The present study is aimed at examining whether *H. hippophaecolus* adults exhibit selectivity towards different sea buckthorn subspecies (varieties), further at determining the role of visual and olfactory cues on host selection. The experiments were conducted with the use of artificial insect infestation in net houses under different treatments. The results presented here are also aimed at providing a theoretical basis for the understanding of *H. hippophaecolus* damage to four sea buckthorn subspecies (varieties), hence the means to implement effective measures to control *H. hippophaecolus* populations.

Material and Methods

Sea buckthorn

The sea buckthorn varieties examined were Chengse, Ayaganka, and Taiyang, which have been bred from the subspecies *Hippophae rhamnoides mongolica* and *H. rhamnoides sinensis*; for convenience, this paper will only refer to four varieties. Chengse and *H. rhamnoides sinensis* are both known to be susceptible to *H. hippophaecolus*, whereas Ayaganka and Taiyang show, respectively, a low and high level of resistance to *H. hippophaecolus*. Individuals of these four varieties used in the experiment were all healthy and not damaged.

Insects

During May and June 2009, we dug roots of sea buckthorn individuals infected by *H. hippophaecolus* from planted forests in the Yangwa countryside, Pengyang county, and Ningxia Hui Autonomous Region in China. The roots were then buried in soil and covered with a wire net to prevent the larvae and adults, from escaping. After eclosion, we chose those adults that were healthy and did not have damaged wings, for the artificial insect infestation.

Allocation of sea buckthorn

Three square net houses (2.5 m x 2.5 m x 2.5 m) were initially set up. Four individuals of each sea buckthorn variety were planted in every corner of each net house so that every variety was present in each net house. The diameter at breast height, plant height, and growth vigor of all plants were similar. *H. hippophaecolus* adults were released in the centre of the net house, which was 1 m from each sea buckthorn individual.

Influence of olfaction and vision on host selection

To determine the influence of visual and olfactory cues on host plant selection, the following treatments were employed: 1. *H. hippophaecolus* adults and the four varieties were untreated; 2. the four varieties were untreated, but the antennae of adults were cut off and their compound eyes marked with red colour; 3. adults were untreated, but all sea buckthorn trunks – except those from Taiyang – were covered with a green cloth; and 4. the compound eyes of adults were marked with red colour and the trunks of the four varieties were covered with green cloth. These procedures were repeated three times, with 30 to 93 adults being placed in each net house every time after ecloding. The landing rate on the four sea buckthorn varieties was recorded once every hour.

Influence of sea buckthorn branches on host selection

After the experiments described above were completed, the following treatments were applied to the four sea buckthorn varieties: 1. all branches of Chengse and Taiyang were cut and only trunks were left in net house 1; 2. all branches of every sea buckthorn variety were cut and only trunks were left in net house 2 except for *H. rhamnoides*

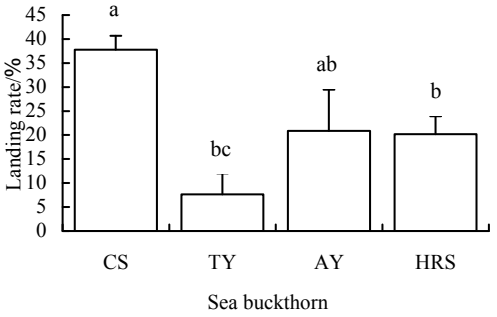

Fig. 1. Landing rate of untreated *H. hippophaecolus* adults on the four sea buckthorn varieties; the bars represent standard errors and different letters signify significant difference ($p < 0.05$). CS, TY, AY, and HRS signify, respectively, Chengse, Taiyang, Ayaganka, and *H. rhamnoides sinensis*.

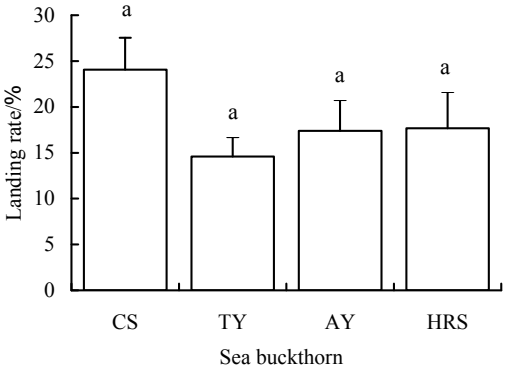

Fig. 2. Landing rate of *H. hippophaecolus* adults with their antennae cut off and compound eyes marked with red colour on the four sea buckthorn varieties; the bars represent standard errors and different letters signify significant difference ($p < 0.05$). CS, TY, AY, and HRS signify, respectively, Chengse, Taiyang, Ayaganka, and *H. rhamnoides sinensis*.

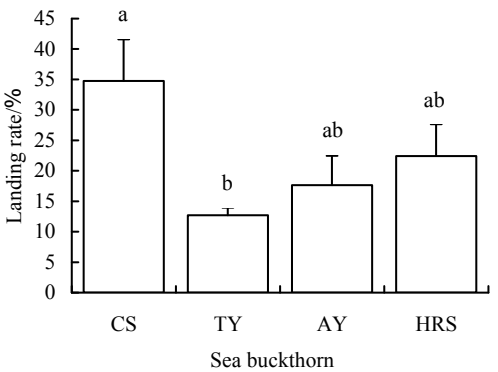

Fig. 3. Landing rate of *H. hippophaecolus* adults with their compound eyes marked with red colour on the four sea buckthorn varieties covered with green cloth; the bars represent standard errors and different letters signify significant difference ($p < 0.05$). CS, TY, AY, and HRS signify, respectively, Chengse, Taiyang, Ayaganka, and *H. rhamnoides sinensis*.

sinensis; and 3. all branches of Chengse were cut and only trunks were left in net house 3. These treatments were repeated 3 times, with 30 to 40 adults being released into each net house every time as they ecloded. Landing rates on the four sea buckthorn varieties were recorded once every hour.

Experimental data and significance of differences were statistically analysed by the software of Statistical Product and Service Solutions (SPSS) 16.

Results

Natural selectivity of H. hippophaecolus adults on host plants

The landing rate of newly emerged adults on the four sea buckthorn varieties is shown in Fig. 1. At the 5% significance level, there were significant changes between Chengse and Taiyang ($p = 0.005 < 0.05$), Chengse and *H. rhamnoides sinensis* ($p = 0.02 < 0.05$), and no significant changes among the other sea buckthorn varieties. Adults landed most frequently on Chengse and least frequently on Taiyang, hence showing a strong selectivity towards the former. Landing rates on *H. rhamnoides sinensis* and Ayaganka were intermediate.

Influence of olfaction and vision on host selection

The landing rate of *H. hippophaecolus* adults that had their antennae cut off and compound eyes marked with red colour on the four sea buckthorn varieties is shown in Fig. 2. There were no significant changes at the 5% significance level among the four varieties, however, compared with the results of Fig. 1, there were marked differences in the landing rates for untreated adults, which indicates that either olfaction or vision are important to host selection.

The four varieties were covered with a green cloth and the compound eyes of adults were marked with red colour. The landing rates of adults on each sea buckthorn variety are shown in Fig. 3. The highest and lowest rates were observed for Chengse and Taiyang, respectively. Af-

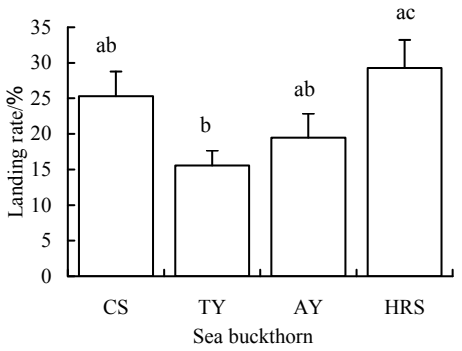

Fig. 4. Landing rate of untreated *H. hippophaecolus* adults on all sea buckthorn trunks which – except those from Taiyang – were covered with a green cloth; the bars represent standard errors and different letters signify significant difference ($p < 0.05$). CS, TY, AY, and HRS signify, respectively, Chengse, Taiyang, Ayaganka, and *H. rhamnoides sinensis*.

ter removing the green cloth from Taiyang, newly emerged adults were placed in the net house. The results are shown in Fig. 4. The landing rate on Chengse was still higher than on Taiyang. Taiyang was highly resistant to *H. hippophaecolus*, whereas Chengse was, conversely, highly susceptible. The results indicate that visual stimuli do not change the behaviour of adults towards host plants. In conclusion, vision seems to have a low influence on host selection, with the results being similar to those from the natural selection experiment.

The results shown in Figs. 1–3 indicate that the landing rates on Chengse decreased with the treatments when compared to those on untreated plants, whereas the opposite pattern was observed for Taiyang. These results indicate that covering trunks with a green cloth influenced the behaviour of adults to some extent, in this case by changing visual cues used for host selection. Landing rates on the four varieties which have different degrees of resistance to *H. hippophaecolus* were, however, clearly different. Specifically, adults landed much more frequently on Chengse than Taiyang plants. Moreover, even considering that *H. hippophaecolus* adults can visually detect Taiyang only, the landing rate on these plants is still the lowest (Fig. 3). In summary, olfaction seems to play a pivotal role in host selection, whereas vision seems to play a complementary role.

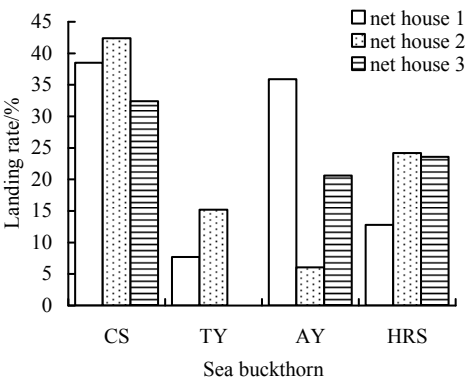

Fig. 5. Influence of the abundance of branches on host selection of *H. hippophaecolus* adults. CS, TY, AY, and HRS signify, respectively, Chengse, Taiyang, Ayaganka, and *H. rhamnoides sinensis*.

Influence of branches on host selection

Fig. 5 shows the results of host selection by *H. hippophaecolus* adults after the branches of the four sea buckthorn varieties had been cut. Landing rates on Chengse (now with its branches removed) were still the highest in all three net houses (38.5%, 42.4%, and 32.4%), which suggests that the presence of branches does not influence the host selection process. Landing rates on Taiyang and Ayaganka were increased and reduced, respectively, when their branches were cut. These results indicate that the differences in the abundance of branches and leaves have no effect on the probability of landing on the four varieties characterized by different levels of resistance to *H. hippophaecolus*. Within the same variety, however, a higher abundance of branches led to an increased landing rate.

Discussion

Volatile components of plants play a decisive role in the behavioural response of herbivorous insects toward host plants, especially, in the preference of the insect for the plant host and/or its habitat. Our results suggest that both olfaction and vision are important for *H. hippophaecolus* adults in the process of host selection, but olfactory cues play a more vital role during this process because the adults can respond to their host plants' volatiles and change from one host to another. Moreover, the results indicate that host selection by *H. hippophaecolus* adults is strongly determined by the detection of volatiles from different hosts, which seem to be especially distinct

between resistant and susceptible sea buckthorn varieties. At present, the volatile compounds of sea buckthorn leaves, berries, and oil have attracted considerable attention around the world concerning nutritional, medicinal, and taxonomic purposes (Tian *et al*., 2003; Tiitinea *et al*., 2006; Chen, 1990), however, the functions in host plant selection by *H. hippophaecolus* adults have still not been reported. So, future research should address the volatile compounds emitted by the four varieties and volatile attractants for *H. hippophaecolus*.

The antenna is one of the most pivotal olfactory organs of an insect and plays an important role in searching for and recognizing host plants (Canale and Raspi, 2000). After cutting off the antenna, *H. hippophaecolus* adults were no longer stimulated by volatiles released by the four sea buckthorn varieties for loss of olfaction, and the highest landing rate was less than 25%, with no significant changes at the 5% significance level among the four varieties. The results further show that olfaction plays a pivotal role in host selection of *H. hippophaecolus* adults.

Previous research has devoted little attention to the influence of the abundance of branches and leaves on host selection. A previous study by Stenberg and Ericson (2007) showed that the odour of the host plants' branches and leaves has no attractive effect on insects. The present study indicates that *H. hippophaecolus* is strongly attracted to the susceptible sea buckthorn varieties regardless of the abundance of branches. It also shows that the differences in the abundance of branches among plants have a stronger influence on landing rates within the same sea buckthorn variety. Future research should address the factors determining why plants with abundant branches are not chosen so frequently.

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