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Color and Height Influence the Effectiveness of an Artificial Feeding Site for a Larval Endoparasitoid, *Cotesia Vestalis* (Haliday) (Hymenoptera: Braconidae)

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Abstract

An effective means of supplying food to *Cotesia vestalis*, the larval endoparasitoid of the diamondback moth, *Plutella xylostella*, was examined in a laboratory and an experimental small greenhouse. Compared to the red and blue boards, yellow boards significantly attracted parasitoids in the laboratory and in the small greenhouse, the yellow feeding site was more effectively utilized by parasitoids than the white one. The parasitoid tended to utilize a feeding site hung 50 cm above ground more than one hung 200 cm above ground, hence we concluded that color and height were important factors for the recognition of the feeding sites by *C. vestalis.*

Discipline: Insect pest
Additional key words: *Plutella xylostella*, artificial food supply, integrated pest management (IPM)

Introduction

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), is one of the most serious pests attacking crucifer crops worldwide[24, 28, 35]. This pest is also known to develop resistance to various kinds of insecticides[4, 21, 34]. Failures to control it due its widespread insecticide resistance have stimulated interest in integrated pest management (IPM) of *P. xylostella*[28]. To achieve a successful IPM program, the utilization of effective native natural enemies is crucial[38]. In many countries, *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae) is the dominant native solitary endoparasitoid that attacks the larval stages of *P. xylostella*[1, 16, 25, 26, 36], and is reported to be an effective biocontrol agent for suppressing the population density of *the latter*[5, 17, 19, 35, 36]. It is thus important to consider how to conserve and utilize *C. vestalis* in agricultural systems for successful IPM programs.

For the effective utilization of parasitoid wasps, the diet of adult female parasitoids is important[8, 11, 15]. Adult females of many parasitoid species obtain the required materials for maintenance and survival by feeding on a number of sugar sources[15]. The lack of a food source strongly decreases activity in terms of the wasps' host-searching behavior[33]. However, the availability of sugar sources varies in both natural and agricultural settings. For most parasitoids, there are only limited reports about food sources in the wild[13, 14], but we can readily assume that sugar sources in the field include floral nectar and extrafloral varieties as well as honeydew excreted by homopteran insects[7, 13, 30]. Conversely, many authors have noted that a lack of sugar availability may temporarily or permanently limit the reproductive success of parasitoids in agricultural systems[3, 6, 11, 29]. Most greenhouses cultivate only one or a few commercial crops and the food sources available for parasitoids are not always present[11]. Under most circumstances in Japan, natural enemies can-

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not utilize honeydew because naturally occurring homopteran insects in greenhouses behave as pests for crops and are prevented by farmers. Release experiments in experimental and commercial greenhouses showed that nearly all-adult *C. vestalis* were dead within 3 days of release (Abe et al., personal communication). Under these conditions, an artificial food supply is important to ensure the effectiveness of biocontrol agents[20, 29].

The techniques of artificial food supply for biocontrol agents currently consist of two methods. One is direct field spraying of artificial food[9, 32, 37]. Here, although advantageous in terms of ease of use, the actual usability of this method is significantly restricted because the commercial value of crops may be strongly decreased by staining their surface with sticky artificial food. Another method is the retention or encouragement of flowering plants in and around crops[18, 29] which has the merit of avoiding any impact on the commercial value of crops. The application of this method in greenhouses, however, is difficult for several reasons. First, the usable space for flowering plants in greenhouses may be scarce because the cropping area mainly dictates commercial greenhouse productivity. Second, the mean age of farmers, especially in Japan, is high and they dislike additional labors.

The alternate method of artificial food supply we considered is the installation of a vessel containing honey solution in greenhouses. This is easy and does not impair the commercial value of crops. The problem is, however, that we do not know what conditions (for example, in terms of the height and color of feeding site) optimally encourage the utilization of the food vessel for natural enemies. It is known that some parasitoid wasps are attracted to the color of flowers[2]. We thus assume that the color of the artificial feeding site is a key factor for attracting parasitoid wasps.

Mitsunaga et al.[22] showed that an artificial food supply strongly increased the longevity and daily parasitization ability of *C. vestalis* in the laboratory. However, additional information is needed to develop an effective method of artificial food supply that guarantees increased longevity and reproductive ability of *C. vestalis.* In this paper, we demonstrate the importance of the color and height of an artificial feeding vessel via change in the time-dependent parasitization rate of *C. vestalis* in a small greenhouse.

Materials and methods

1. Insect rearing

All insects were maintained in an environmental chamber controlled at 25±1˚C, 60±10% r.h., and a photoperiod of L16: D8. The laboratory cultures of *P. xylostella* and *C. vestalis* were established from larvae of *P. xylostella* collected in Ayabe, Kyoto Prefecture, Japan, in 2001 and 2002, respectively. The culture of *P. xylostella* had been reared on Komatsuna Mustard Spinach, *Brassica rapa* var. *perviridis* L. for more than 40 generations before we used these moths for experiments. *C. vestalis* wasps from the laboratory culture for more than 30 generations after establishment were used for experiments.

Fifty to 100 *P. xylostella* adult moths were released to allow copulation and oviposition on Komatsuna about one month old (about 20 cm high) within a netted plastic cage (25 × 35 cm, 30 cm in height). Larvae were fed on the same plants for 3 days after hatching, transferred to a paper padded plastic box (23 × 20 cm, 8 cm in height) with fresh Komatsuna leaves and reared until pupation. The Komatsuna leaves were protected against desiccation by enclosing the stems in wet cotton and covering them with plastic wrap. Some newly emerged adults were introduced into the netted plastic cage to maintain the culture.

To maintain the parasitoid stock culture, about 200 second-instar host larvae were presented to about 50 mated female wasps for 2 days in a netted plastic cage. The parasitized host larvae were reared on fresh Komatsuna leaves until the emergence of parasitoids in a paper padded plastic box. Just after emergence, wasps were released to mate in a netted plastic cage with a small cotton ball soaked in honey solution.

For the experiments, wasp cocoons were removed from the stock culture and kept separately in glass tubes (5.5 cm long × 1.5 cm in diameter) until adult emergence. Newly emerged (< 4 h old) wasps were subsequently released to mate in a netted plastic cage with a small cotton ball soaked in honey for 1 day and females were used for experiments.

2. Experiment 1: The preference for color boards of *C. vestalis* in the laboratory

Experiments were conducted in an environmental chamber controlled at 24±1˚C, 70±10% r.h. A red (Acrylite #115, wavelength = 644 ~ 648 nm), yellow (Acrylite #235, 588 ~ 591 nm), or blue (Acrylite #315, 468 ~ 474 nm) color board (20 × 20 cm, 1 cm in thickness, Acrylite, Mitsubishi Rayon Co., Ltd, Tokyo, Japan) was placed in a netted plastic cage (25 × 35 cm, 30 cm in height), into which 10 female wasps were also introduced. Adhesive agent (Kinryu, Miyatane Corporation, Miyazaki, Japan) was sprayed on each board. After 6 hours, the trapped females on each board were counted.

Ten replications were performed of each treatment. Data were analyzed using univariate ANOVA to evaluate

the effect of color.

3. Experiment 2: The influence of colored feeding site on the parasitization activity of *C. vestalis* in a small greenhouse

Experiments were conducted in a small greenhouse (7 × 7 m, 3 m in height), with temperature ranging from 24 to 30˚C and humidity of about 70±10% r.h. Two second-instar larvae of *P. xylostella* were allowed to feed on a Komatsuna plant about one month old for 24 h before exposure to the wasps. Ten *P. xylostella*–infested Komatsuna plants and 15 non-infested Komatsuna plants were arranged 5 × 5 at 30 cm intervals at the center of the greenhouse. The potted plants were arranged randomly. The experimental host distribution and density were based on the distribution and tolerance pest density of *P. xylostella* in commercial greenhouses cultivating Mizuna Mustard Spinach, *Brassica rapa* var. *laciniifolia* L. at Miyama, Kyoto Prefecture, Japan (Urano, personal communication).

A feeding site was installed by hanging from the center of the greenhouse ceiling and consisted of a 50%-honey-soaked sponge (5 cm in diameter and 2 cm in height) placed on a paper tray (9 cm in diameter). The height of the feeding site was 50 cm above ground. Two colors of feeding site were compared: white and yellow (measured wavelength = 588 ~ 595 nm). The white and yellow feeding sites were colored using a color spray (Alesco Hit Spray, Kanpe Hapio Corporation, Osaka, Japan). In the preliminary experiment, *C. vestalis* females showed neither preference nor evasion in response to the smell of the color spray.

Five female wasps were introduced into the greenhouse and allowed to oviposit into the *P. xylostella* larvae for 24 h. A day later, all plants and host larvae were removed. Three days after the introduction of the wasps, 10 newly *P. xylostella*–infested Komatsuna plants and 15 non-infested Komatsuna plants were rearranged 5 × 5 at 30 cm intervals at the center of the greenhouse, whereupon female wasps were allowed to oviposit into the *P. xylostella* larvae for 24 h again. If wasps were unable to utilize the feeding site effectively, we did not expect them to survive until the second trial because the mean longevity of non-fed *C. vestalis* females is 2.1[22] hence a low parasitization rate was expected. One day after the second trial, all plants and host larvae were removed.

The host larvae were reared, the number of emerged wasps was recorded, and the parasitism rate (the number of emerged wasps/the number of recaptured host larvae) was calculated. Five replications were performed in each color treatment. The arcsine-square-root-transformed data of the parasitism rate were compared using two-factor ANOVA to evaluate the effect of color and the number of days after the introduction of the wasps[31].

4. Experiment 3: The influence of the height of the feeding site on the parasitization activity of *C. vestalis* in a small greenhouse

Experiments were conducted in the same small greenhouse under the same conditions as in Experiment 2. The color of the feeding site was yellow. Two heights of feeding site were compared; respectively 50 and 200 cm above ground. The former height was decided based on the average plant height and the latter was due to convenience for farmers during harvest work and when stretching for rest. The parasitism rate was determined and statistics were recorded as in experiment 2.

Results

1. The preference for color boards shown by *C. vestalis* in the laboratory

The numbers of trapped females differed significantly according to the colors (Fig. 1). The mean number of trapped females on the yellow board was 5.9, significantly exceeding that of the females on blue (average number 2.4) and red (average number 1.9)(Tukey-Kramer's test, $p < 0.05$; Result of univariate ANOVA; df = 2, MS = 47.5, F-value = 11.43, $p = 0.0003$). The mean number of trapped females on the blue board did not differ significantly from that of females on the red board (Tukey-Kramer's test, $p > 0.05$).

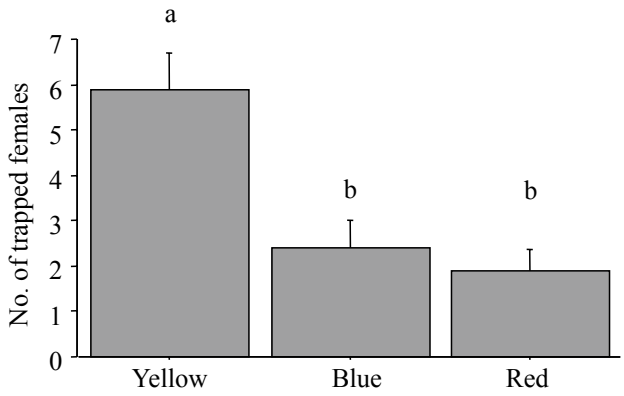

Fig. 1. Effect of colors on the number of *Cotesia vestalis* females trapped on sticky boards

Error bars show the standard error. Values with the same letter are not significantly different (univariate ANOVA followed by Tukey-Kramer's test, $p > 0.05$).

2. The influence of the colored feeding sites on the parasitization activity of *C. vestalis* in a small greenhouse

The mortality of the *P. xylostella* larvae before pupation of moths or wasps was low (4 %), hence the effect of this mortality was neglected. As regards the parasitism rate of *C. vestalis*, interaction between the color of the feeding site and the days after the introduction of the wasps was observed (Fig. 2, Table 1). This interaction was explained by the variation in the parasitism rate between white and yellow feeding sites only on day 3 (Fig. 2).

3. The influence of the height of the feeding site on the parasitization activity of *C. vestalis* in a small greenhouse

On the parasitism rate of *C. vestalis*, interaction between the height of the feeding site and days after the introduction of wasps was observed (Fig. 3, Table 2). This interaction was explained by the difference in parasitism rate between heights of 50 and 200 cm only when on day 3 (Fig. 3).

Discussion

Many authors have claimed that the diet of adult parasitoids significantly affects longevity and parasitization ability[8, 10, 11, 15, 27, 33]. However, practical methods for artificial food supply in commercial greenhouses have not been discussed.

C. vestalis in a plastic cage preferred the yellow board to those of other colors (Fig. 1). This result suggests that the optical response of *C. vestalis* is activated in the range of the middle wavelength region. Based on this result, we confirmed the usability of a yellow feeding site by comparison with a white one in a small greenhouse, and observed a significant difference in terms of the extended utilization by *C. vestalis* as estimated by the change of parasitism rate between yellow and white feeding sites (Fig. 2, Table 1). This difference was explained by the difference of the *C. vestalis*' survival rate until three days after the introduction in the greenhouse. Apparently, the parasitism rate under a white feeding site was low because *C. vestalis* adults were unable to use the feeding site effectively. Our results suggest that the opti-

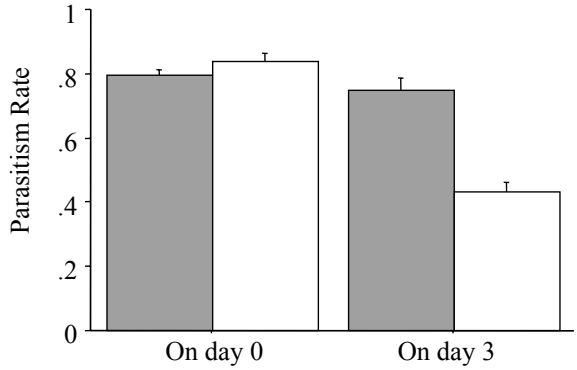

Fig. 2. Effect of the feeding site color (yellow, white) on the parasitism rate by *Cotesia vestalis* on different days after introduction
Error bars show the standard error.
■ : Yellow, □ : White.

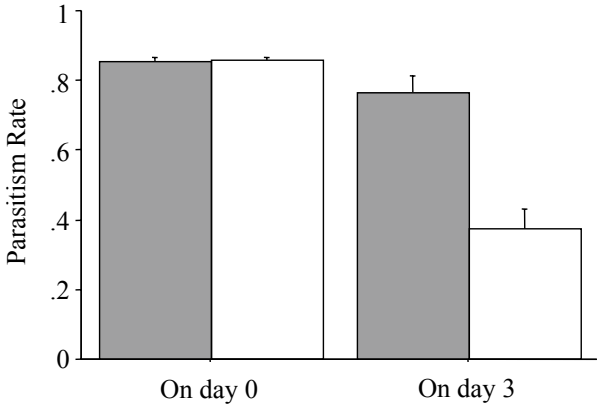

Fig. 3. Effect of the feeding site height (50 and 200 cm) on the parasitism rate by *Cotesia vestalis* on different days after introduction
Error bars show the standard error.
■ : 50 cm, □ : 200cm.

Table 1. Results of the two-factor ANOVA on the effects of the color of feeding site and days after the wasp introduction on the parasitism rate of *Cotesia vestalis*

Factors	df	Mean Square	*F*-value	*P*-value
Color	1	0.130	13.963	0.0018
Days	1	0.482	51.950	< 0.0001
Color * Days	1	0.292	31.428	< 0.0001
Residuals	16	0.009		

Table 2. Results of the two-factor ANOVA on the effects of the height of feeding site and days after the wasp introduction on the parasitism rate of *Cotesia vestalis*

Factors	df	Mean Square	*F*-value	*P*-value
Height	1	0.300	24.617	0.0001
Days	1	0.772	63.278	< 0.0001
Height * Days	1	0.317	25.950	0.0001
Residuals	16	0.012		

cal information is crucial for searching the feeding site for *C. vestalis* and showed that yellow is the preferred color. To further optimize the artificial feeding site, we have to survey a more precise wavelength region as well as the potential effect of learning on color preference.

When comparing the heights of the feeding sites, that of 50 cm was more effectively utilized by *C. vestalis* than 200 cm (Fig. 3, Table 2). This difference was shown by the difference of *C. vestalis*' survival rate until three days after its introduction into the greenhouse[22]. Our results suggest that the height at which the feeding site is installed strongly affects the efficiency of the utilization of wasps, and that a feeding site at a height of 50 cm is a preferable altitude for *C. vestalis.* To avoid potential disruption to farm work, the artificial feeding site should be set up at the edges of greenhouses.

As shown above, *C. vestalis* could only locate their artificial feeding site successfully under specific conditions (Figs.2 and 3). This means that, for the practical use of artificial feeding sites, more detailed information concering the optical responses for wavelength, flight ability, and the process of feeding-site searching behavior of *C. vestalis* must be surveyed.

Very few braconid parasitoids show host-feeding behavior[12]. This makes it particularly crucial to consider the addition of adult feeding sites for supplying sugar source to ensure the more effective utilization of braconid wasps as biological control agents, especially in commercial greenhouses[29]. In laboratory experiments, the artificial food supply can elongate to seven times the longevity of *C. vestalis*[22]. Conversely, artificial food supply may involve risks to facilitate the reproduction of pests. For example, an artificial food supply may double the expected lifetime fecundity of the pea leafminer *Chromatomyia horticola* at maximum[23]. Thus, an effective food-supply system may improve the effectiveness of biological control agents in IPM, and further optimization remains a future task in the sustainable utilization of natural enemies.

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