Response to host-infested plants in females of *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae)

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Abstract

Responses of *Diadegma semiclausum*, a specialist solitary parasitoid of diamondback moth (*Plutella xylostella*) larvae, to host-infested cabbage plants were studied. Females were more attracted to host-infested plants than uninfested plants in a wind tunnel. The origin of the attraction was neither the hosts nor their associated products, such as feces, silk and exuviae, but the infested plant itself. Once on the infested plant, the females initiated specific antennal contact with a host-damaged site on a leaf to search for hosts. Based on these data, the effect of chemical cues from infested plants on host searching by females is discussed.

Key words: Diamondback moth; *Diadegma semiclausum*; host searching

INTRODUCTION

For parasitic wasps, contact chemical cues of host origin are reliable but have low detectability. Conversely, constitutively-produced host-food plant volatiles are detectable from some distance by parasitic wasps but have low reliability for the detection of their host (Vet and Dicke, 1992). In addition, several parasitic wasps are known to use “herbivore-induced plant volatiles” (HIPVs) for host searching (for review, Takabayashi and Dicke, 1996; Dicke, 1999). As the emission of HIPVs is the result of a specific induced response caused by the herbivore damage, HIPVs are reliable cues with high detectability (Vet and Dicke, 1992). The use of HIPVs by parasitic wasps has been reported for many tritrophic systems (for review, see Takabayashi and Dicke, 1996; Dicke, 1999).

After the location of host-infested plants, parasitic wasps use several contact chemicals of host origin such as feces, exuviae and silk on the host food plant as reliable cues. Furthermore, several studies show that chemical cues from a plant-herbivore complex play an important role in host finding. For example, *Cotesia glomerata*, a parasitoid of cabbage-butterfly (*Pieris rapae*) larvae, showed specific antennal contact with a host-damaged site on a leaf (further referred to in this paper as ‘host-

damaged edge’) of crucifer plants (Sato, 1979; Horikoshi et al., 1997; Sato et al., 1999). Interestingly, the cue originates from the plant-herbivore complex. The combination of the host regurgitant and plant juice is essential for the formation of this cue (Sato, 1979; Horikoshi et al., 1997). *Cotesia plutellae*, a parasitoid of diamondback moth larvae (*Plutella xylostella*) also shows a specific response to the host-damaged edge but not to a nonhost (*P. rapae*) damaged edge or an artificially damaged edge (Shiojiri et al., 2000b).

Diadegma semiclausum, a solitary parasitoid of *P. xylostella* larvae, is thought to have originated in Eurasia (Fitton and Walker, 1992) and be an effective biological control agent of *P. xylostella*. In the Papua New Guinea highlands, the release and successful establishment of *D. semiclausum* since 1995 has substantially reduced crop losses by *P. xylostella* (Saucke et al., 2000). The wasp has also been introduced into the Philippines (Amend and Basedow, 1997) and Taiwan (Talekar, 1996).

In 1993, this wasp was introduced into Japan from Taiwan to test its effectiveness in Japan, and released in Iwate Prefecture, northern Japan, from 1996 to 1999 (Noda et al., 2000). However, detailed studies on the host finding behavior of this wasp have not yet been carried out. In this study, we examined the response of females of *D. semi-*

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clausum to the volatile chemicals of host-infested plants and the response of females to the contact chemicals along the host-damaged edge. The role of infochemicals from infested plants on host finding is discussed.

MATERIALS AND METHODS

Plants and insects. Cabbage plants (*Brassica oleracea* cv. Shikidori) about 1 month old (i.e. with about five leaves) were grown in a growth chamber ($25 \pm 2^\circ\text{C}$; 60% RH; 16L:8D) and utilized for Experiment 1 as described below. Leaves from plants cultivated in the experimental field at Kyoto University campus were used in Experiment 2.

For Experiment 1, the progeny of the strain of *P. xylostella* which had been reared in Tohoku National Agricultural Experiment Station were used. For Experiment 2, adult females of *P. xylostella* were collected from crucifer crops in a field in Kyoto City. Both strains were kept in a controlled room ($25 \pm 2^\circ\text{C}$; 50–70% RH; 16L:8D) with crucifer leaves (predominantly cabbage, sometimes Chinese radish) as food.

D. semiclausum were obtained from the stock culture of Tohoku National Agricultural Experiment Station. Adults of the parasitoid species were fed honey and maintained in a plastic cup (12 cm in diam., 5 cm in height) in a controlled room ($20 \pm 2^\circ\text{C}$; 50–70% RH; 16L:8D) for 2 days after eclosion to ensure mating. Females were then kept in a

controlled room at $15 \pm 2^\circ\text{C}$, 50–70% RH and 16L:8D. At least 1 h before the start of each experiment, oviposition-inexperienced female wasps were transferred to another controlled room ($25 \pm 2^\circ\text{C}$, 50–70% RH) under light conditions. Each wasp was used only once for Experiment 1. After the experiment, each wasp was released into the cage with host-infested plants for further rearing. Some of these wasps were used for the bioassays in Experiment 2. However, we kept these wasps for at least 24 h in the room ($15 \pm 2^\circ\text{C}$, 50–70% RH) prior to the experiment to normalize the responses.

Experiment 1. Response of *D. semiclausum* to volatiles emitted from cabbage plants infested with *P. xylostella* larvae. To investigate whether the female wasps are attracted to the host-infested plants or just to the host and associated products, we used a wind tunnel (50 cm in diam., 150 cm in length, Fig. 1). The wind tunnel with a fan was placed in a room ($25 \pm 2^\circ\text{C}$, 50–70% RH), and air was first cleaned with charcoal at the end of the tunnel. Light intensity inside the wind tunnel was 1,000 lx. The following odour sources were used for the experiments.

1. Host-infested plant: a plant infested with 40 third instars for 1 day.
2. Host-infested plant without hosts: a host-infested plant from which all hosts were removed with a fine brush and the infested leaves were gently washed with water to remove the associated products of the larvae.

Fig. 1. A wind tunnel. A wasp was released from the 'release point'. The wasp was observed for 5 min and the number of wasps landing on one of the odour sources within 5 min was counted. Light intensity inside the wind tunnel was 1,000 lx. The wind tunnel with a fan was placed in an experimental room (25°C) and air was first cleaned with charcoal at the end of the tunnel.

3. Artificially damaged plant: leaves were partly damaged with a cork borer (10 mm in diam.) at one time to imitate the damage made by larvae. About ten holes were made per plant.

4. Hosts and their associated products on a dummy plant: To compare the attractiveness of hosts and their associated products vs. an intact plant, we made a dummy of the same shape as an intact plant. Hosts and the associated products were placed on the dummy. As visual factors of a dummy and a plant were similar, we could compare the attractiveness of an intact plant vs. that of hosts and their associated products without the effect of visual factors. A dummy was made with a round green paper (for leaves) and a wooden stick (for a stem). We collected feces from 40 third instars for one day and placed the feces on the dummy as hosts associated products. Then 40 third instar larvae were confined in nylon gauze and placed on the dummy.

5. Intact plant: intact cabbage plant.

Two odour sources for the above 5 treatments were set in the wind tunnel. The combinations of the two odour sources were as follows. (1) Host-infested plant vs. intact plant. (2) Host-infested plant without hosts vs. intact plant. (3) Host-infested plant without hosts vs. artificially damaged plant. (4) Artificially damaged plant vs. intact plant. (5) Intact plant vs. hosts and their associated products on a dummy plant. The height of the odour source plants was about 25 cm. The distance between the odour sources (from pot edge to pot edge) was 20 cm. In each observation, a wasp in a glass tube (3 cm in diam., 9 cm in length) was released at the downwind side of the wind tunnel (Fig. 1). The number of wasps which landed on either of the two odour sources were counted. Observations were stopped when a wasp landed on the odour source or 5 min elapsed. The non-landing wasps were considered as no choices. Each experiment combination was replicated with more than 30 wasps. The experiments were carried out over three to seven experimental days. A newly prepared odour source was prepared each experimental day.

Experiment 2. Response of *D. semiclausum* to a cabbage leaf damaged by *P. xylostella* larvae. Pieces of leaves that had been either infested by *P. xylostella* or were artificially damaged were tested. To set up the former, cabbage leaves were offered to *P. xylostella* larvae. After 24 h, the larvae were

removed and the leaves gently washed with water to remove the associated products such as feces, exuviae and silk from the leaves. Then a piece (10×20 mm) of each leaf was cut out with a herbivore-infested edge. Due to the feeding habits of the herbivores, the damaged edges of the leaves were not straight, but rather roughly concave in shape. To set up the latter, pieces (10×20 mm) were cut out of intact cabbage leaves.

Wasp searching behavior on a sample was observed in a glass tube (3 cm in diam., 9 cm in length) for 5 min. The total time that a wasp showed antennal contact with a sample (antennal contact time) was recorded. The sample was renewed every 30 min (ca. every 3–4 bioassays).

To elucidate the factors responsible for host-searching behavior of *D. semiclausum* at host-infested sites on a leaf, the responses of wasps to (1) regurgitant and (2) cut leaves to which the regurgitant was applied artificially were tested. The samples were renewed every 30 min (ca. every 3–4 bioassays).

(1) Regurgitant. To collect regurgitant, the bodies of fourth instar larvae were gently pinched with fingers. The regurgitant was impregnated on a piece of filter paper (10×20 mm). Regurgitant from four host larvae was applied to one piece of filter paper. The larvae were starved for 24 h before collecting the regurgitant to exclude the possible effect of chemicals originating from host-food plants.

(2) The regurgitant vomited from four larvae (4th instar) was directly applied by pinching larvae with fingers along one side of the cut edge of each leaf section (10×20 mm).

The response of wasps to feces of the host was also tested. Feces (ca. 200 granules) from five host-infested plants with host larvae of all stages was collected. Feces was transferred to a piece of moist filter paper (10×20 mm). The response of the wasps to the feces was tested with this paper.

To determine whether the antennal contact time was significantly different among treatments, we used a one way ANOVA and Scheffe's post hoc multiple mean comparison test.

Fig. 2. The flight responses of *D. semiclausum* females to cabbage plants with different treatments. Numbers next to the bars indicate the number of wasps that landed on the plant. N. C. (no choice) mean number of wasps that did not land within 5 min.

RESULTS

Experiment 1. Response of *D. semiclausum* to volatiles emitted from cabbage plants infested with *P. xylostella* larvae

Female wasps showed a significant preference for plants infested by the host larvae over uninfested plants (Fig. 2) (binomial test: $p < 0.001$). The wasps showed a significant preference for the infested plants from which all the host larvae and their associated products were removed over the uninfested plants (binomial test: $p < 0.001$), and over artificially damaged plants (binomial test: $p = 0.0012$). Wasps were significantly more attracted to the artificially damaged plants than the uninfested plants (binomial test: $p < 0.001$). The wasps also preferred uninfested plants over hosts and their associated products placed on the dummy plant (binomial test: $p < 0.001$).

Experiment 2. Response of *D. semiclausum* to the edge of a cabbage leaf made by *P. xylostella* larvae

The antennal contact time on the edge with five different treatments by females was significantly different (Fig. 3) ($F_{4,127} = 28.742$, $p < 0.0001$, one way ANOVA). The antennal contact times of female wasps on the host-damaged edge (mean $\pm$ SE: 108.4 ± 7.9 s, $n = 29$), the artificially damaged edge to which the regurgitant from the starved host larvae was applied (87.5 ± 5.5 s, $n = 28$) and the host feces (83.0 ± 6.9 s, $n = 29$) were significantly longer than those on the artificially damaged edge (25.0 ± 4.1 s, $n = 28$) and the filter paper impregnated with regurgitant (38.4 ± 7.8 s, $n = 18$) (Scheffe's test, $p < 0.05$).

Fig. 3. Antennal contact time of *D. semiclausum* females to various treatments: (1) a piece of leaf infested by *P. xylostella*, (2) a piece of leaf with artificial damage, (3) a piece of leaf with artificial damage plus host regurgitant, (4) host regurgitant on a filter paper, (5) host feces on a filter paper. Bars represent the means $\pm$ standard errors (SE) of the means. Bars with different letters were significantly different ($p < 0.05$) using Scheffe's post hoc multiple mean comparisons. Numbers on bars indicate number of wasps tested.

DISCUSSION

Our results show that *D. semiclausum* females were attracted to the volatiles emitted from cabbage plants infested by host larvae. Several parasitic wasps are known to be attracted to volatiles from host-infested plants (Dicke, 1999; Dicke and Vet, 1999). For *D. semiclausum*, the order of the olfactory response intensity to the plants (and hosts plus their associated products) was: host-infested plants > artificially damaged plants > uninfested plants > hosts and their associated products. In their host-infested plant findings, the volatiles from the host-infested plants appear to play an important

role for *D. semiclausum* females (Vet and Dicke, 1992). Shiojiri et al. (2001) chemically analysed the volatiles emitted from cabbage plants with different treatments (uninfested plants, artificially damaged plants, plants infested by *P. rapae*, plants infested by *P. xylostella* and plants infested by both herbivore species). Their results showed that host-infested plant volatiles were qualitatively and quantitatively different from uninfested and artificially damaged plants. The above preference of *D. semiclausum* to the host-infested plant volatiles can thus be explained in part by recognition of the qualitative and quantitative differences in the blends of the volatiles.

C. plutellae, a dominant parasitic wasp of *P. xylostella*, also is more attracted to the host-infested cabbage plants than either uninfested, artificially damaged or nonhost (*P. rapae* larvae) infested plants (Shiojiri et al., 2000a) and seems to recognize the qualitative and quantitative differences in the blends of the volatiles. Further, *C. plutellae* does not respond to the host larvae or their associated products (Shiojiri et al., 2000a). Although Shiojiri et al. (2000a) reported a specific response of *C. plutellae* to the host-infested plants, they did not show the order of preferences we showed above. Turlings et al. (1990, 1991) reported that *Cotesia marginiventris*, a generalist parasitoid that attacks many different host species (at least 20) on a wide range of plant species, was attracted to host-induced maize plant volatiles. *C. marginiventris* is more attracted to host-damaged maize plants than either the artificially damaged plants or the host larvae (*Spodoptera exigua*) as was the case with *D. semiclausum* (Fig. 2).

Females displayed long antennal contact with a host-damaged edge but not to the artificially damaged edge (Fig. 3). As the possibility of the existence of hosts would be high around the host-damaged edge, a specific response to the host-damaged edge would enhance the possibility to find hosts. The plant juices and host regurgitant were important for eliciting host searching by *D. semiclausum* females (Fig. 3). In another two tritrophic systems, one consisting of cabbage plants, *P. rapae* larvae and *C. glomerata* (Sato, 1979; Horikoshi et al., 1997), and the other consisting of cabbage plants, *P. xylostella* larvae and *C. plutellae* (Shiojiri et al., 2000b), a mixture of regurgitant and plant juice was also found to be important in eliciting host

searching by the respective wasps. In addition, female wasps in this study displayed antennal contact with the host feces (Fig. 3). Thus, both the host-damaged edge and the host feces are important for host searching in *D. semiclausum*.

In this paper, we demonstrated that *D. semiclausum* showed specific responses to host-damaged plants. Understanding the mechanisms involved in the production of the chemical cues used by this wasp and the elucidation of the chemical structures involved, is our plans for future study by which we may be able to enhance the effectiveness of *D. semiclausum* under field conditions.

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