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Learning is involved in the response of parasitic wasps *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) to volatiles from a broad bean plant, *Vicia faba* (Fabaceae), infested by aphids *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae)

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Abstract

It has been reported that volatiles from broad bean plants, *Vicia faba* (cv. '*the Sutton*') infested by the pea aphid, *Acyrthosiphon pisum*, attract a specialist parasitoid *Aphidius ervi*, collected from populations in England, Italy and Bulgaria, which had no previous experience of the host-infested plant volatiles. *Aphidius ervi* collected in Hokkaido, Japan were also attracted to volatiles from host-infested broad bean plants (cv. '*Nintoku Issun*') in preference to those from intact plants in a Y-tube olfactometer when the wasps were allowed to emerge on the infested plants, but wasps that had emerged in a clean Petri dish showed no significant choice between the two odor sources. When artificially exposed to the infested plant volatiles during emergence from the mummy, the wasps showed a significant preference for infested plant volatiles over those from intact plants. In further studies in a wind tunnel, significantly more wasps landed on infested plants than on intact plants when wasps were exposed to infested plant volatiles. Naïve wasps, however, did not show a higher landing response to infested plants. These data suggest that learning is required by *A. ervi* of the Hokkaido strain for their response to infested plant volatiles in their host searching behavior.

Key words: *Aphidius ervi*; host-searching behavior; plant volatiles; learning

INTRODUCTION

In many tritrophic interactions involving plants, herbivorous arthropods, and carnivorous natural enemies of herbivores (e.g. predators and parasitoids), infested plants attract herbivores' natural enemies (e.g. Takabayashi and Dicke, 1996 for review; Dicke, 1999; Sabelis et al., 2007). Responses by natural enemies to volatiles from prey-infested plants are often specific in terms of plant species, plant cultivars, plant developmental stages, herbivore species, herbivore strains and developmental stages of the herbivore (Takabayashi and Dicke, 1996 for review; Dicke, 1999; Sabelis et al., 2007). For example, when broad bean plants (*Vicia faba*) were infested by aphids (*Acyrthosiphon pisum*), they emitted herbivore-induced plant volatiles that attracted the parasitic wasp *Aphidius ervi* (Du et al., 1996, 1998). Volatiles from broad bean plants infested with non-host aphids (*Aphis fabae*) did not attract wasps (Du et al., 1996).

Parasitic wasps respond to host-infested plant volatiles even when the wasps are naïve in many tritrophic systems (i.e. no experience of hosts or host plants since emergence from the mummy). Further, the response of naïve wasps may be modified by familiarity with the volatiles associated with some reward (e.g. parasitization success, finding a cue for host searching such as feces etc.) (Turlings et al., 1993). For example, the flight response of the parasitic wasp *Cotesia kariyai* to volatiles emitted from corn plants infested by larvae of the host, *Mythmna separata*, is enhanced after associative learning (Fukushima et al., 2002).

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By contrast, several wasp species do not have an innate response to host-infested plant volatiles, and start responding only after associative learning experiences (Storeck et al., 2000; Girling et al., 2006; Blande et al., 2007).

Aphidius ervi is a solitary endoparasitoid occurring across Eurasia (Marsh, 1977). This wasp attacks several aphid species, such as *Acyrthosiphon pisum* on legume plants, *Macrosiphum euphorbiae* on tomato, *Aulacorthum solanii* on potato and *Sitobion avenae* on cereals (Takada and Tada, 2000). In Japan, the distribution of *A. ervi* is limited to northern areas and the recorded host species are *A. pisum* and *Acyrthosiphon kondoi* only (González et al., 1979). This wasp has been used for the biological control of *A. pisum* and related species in many regions of the world. *Acyrthosiphon pisum* is an economically important pest species that attacks many plant species belonging to several genera, such as *Pisum*, *Vicia*, and *Medicago* (Maiteki and Lamb, 1985; Lane and Walters, 1991). This aphid is also known to be a vector of several plant viruses (Sylvester, 1989; Bosqueperez and Buddenhagen, 1990).

Previous studies have shown, using wind tunnel and Y-tube olfactometer bioassays, that volatiles from broad bean plants infested by the aphid *A. pisum* attracted *A. ervi* females collected from England, Italy, and Bulgaria without experience of the host infested plant volatiles prior to bioassays. We report here that *A. ervi* collected in Hokkaido, Japan were attracted to volatiles from broad bean plants (cv. '*Nintoku Issun*') infested by *A. pisum* in preference to those from intact plants when the wasps were allowed to emerge on the infested plants, but wasps that had emerged in a clean Petri dish showed no significant choice between the two odor sources. The possible involvement of learning is discussed.

MATERIALS AND METHODS

Plants and insects. Three broad bean plants (*Vicia faba* var. Nintoku Issun) were grown in a vinyl cup (ca. 300 ml) under laboratory conditions (20±2°C, 16L8D). Seedlings of ca. 10 cm height with 4 trifoliate leaves (ca. 2 weeks after germination) were used for experiments and rearing of aphids.

Aphids, *Acyrthosiphon pisum*, were obtained from the culture maintained by Dr. Yoshitaka Nakashima (Obihiro University of Agriculture and Veterinary Medicine) in April 2006. They were reared on broad bean plants in the laboratory (20±2°C, 16L8D).

Infested plants were prepared by placing 100 randomly selected aphids on one broad bean plant, which was then kept for 3 days in a climate controlled room (20±2°C, 16L8D, 50–70% relative humidity (rh)). Uninfested plants were kept under the same conditions.

Aphidius ervi were obtained from the culture maintained by Dr. Yoshitaka Nakashima in April 2006. They were kept under laboratory conditions (20±2°C, 16L8D, 50–70% rh) on broad bean plants with *A. pisum* as hosts. Groups of wasps were subjected to one of three different treatments as follows:

1. Wasps emerged on a host-infested plant. The mummies were kept on the infested plants until adult wasp emergence. Mating generally occurred immediately after emergence. One day after emergence, we collected the wasps for use in bioassays. They were kept in a small glass vial (2 cm diam., 12 cm long) with 50% aqueous solution of honey as food for one day in a climate room (20±2°C, 16L8D, 50–70% rh). These wasps are hereafter referred to as plant-host complex-experienced wasps.

2. Naïve wasps. Mummies were removed from infested plants and kept in a plastic cage (35×30×25 cm) without aphids and plants (20±2°C, 16L8D, 50–70% rh). One day after emergence, female wasps were collected and kept individually in small vials (2 cm diam., 12 cm long) with a 50% aqueous solution of honey as food for one day (20±2°C, 16L8D, 50–70% rh) until they were used in bioassays.

3. Host-infested plant volatiles-experienced wasps. Mummies were removed from infested plants and kept in a PET tube (6 cm diam., 15 cm long, with the openings covered with gauze). Before emergence of naïve wasps, mummies were exposed to host-infested plant volatiles by placing the mummies in a cage next to host plants infested by *A. pisum* for 3–7 days. One day after emergence, wasps were collected individually in small vials (2 cm diam., 12 cm long) with a 50% aqueous solution of honey as food and kept for one day (20±2°C, 16L8D) until they were used in bioassays.

Bioassay. We examined the olfactory preference of *A. ervi* females when exposed to infested plant volatiles vs. intact plant volatiles using a Y-tube olfactometer and a wind tunnel.

1. Y-tube olfactometer bioassay. A Y-tube olfactometer (3.5 cm inner diam., 13 cm long for each branch) was used to test the walking response of *A. ervi* in a climate controlled room (20±2°C, 40–60% rh). Air was passed through activated charcoal and odor source bottles, in which either infested or intact plants were placed, to a branch of the olfactometer at the rate of 800 ml/min. A small vial with one *A. ervi* was placed at the base of the Y-tube with its opening facing upwind to release the wasp. When the wasp walked across a line marked in each branch, 7 cm from the Y junction, and stayed there for at least 1 min, we judged that the wasp had made a choice. A wasp that did not make a choice within 5 min was recorded as a non-responder. The control and experimental ends of the olfactometer were alternated every 6 bioassays. Odor sources were replaced every 12 bioassays. For each comparison, 60 wasps were used. A binomial test was used to test the statistical significance of the difference between the distribution of wasps that made choices of either of the two odor sources tested. Uninfested and infested plants were used as the two odor sources compared in all bioassays.

2. Wind tunnel bioassay. Flight responses to odor from infested and uninfested plants were tested in a wind tunnel. The flight chamber was a rectangular acrylic resin chamber (150×50×50 cm). Air flow was produced by a fan and also vented by an exhaust fan from the downwind end of the tunnel to outside the room. The air was purified by passing through an activated charcoal filter and the flow made laminar by honeycomb-shaped slats embedded at the upwind end of the flight chamber. The wind speed was adjusted to 20–25 cm/sec. The light intensity in the flight chamber was ca. 3,600 lx at the release point and was provided by a fluorescent light and diffused by a patterned, indented, plastic plate placed over the top. A yellow and green striped pattern was placed beneath the flight chamber floor for visual orientation of the wasps. All experiments were carried out at 24–28°C and 50–60% rh. Individual parasitoids were used only once and released from a glass vial placed on a platform (10×10 cm, 11 cm high) and the distance between the release point and target was 45 cm. The wasps were observed for 5 min from the time of release. The number of wasps that landed on the target within 5 min was counted and the percentage of landed wasps was calculated from the 40 individuals released. Naïve female wasps and host-infested plant volatiles-experienced wasps (40 individuals each) were used for bioassays.

RESULTS

Y-tube olfactometer bioassays

Naïve wasps did not show a preference between volatiles from intact plants and those from infested plants (34 wasps to infested plant volatiles and 26 wasps to intact plant volatiles: $p=0.4426$, binomial test) (Fig. 1). Wasps that had emerged on infested plants showed a significant preference for infested plant volatiles over intact plant volatiles (42 wasps to infested plant volatiles and 18 wasps to intact plant volatiles: $p=0.0027$, binomial test) (Fig. 1). When host-infested plant volatiles-experienced wasps were used, they preferred volatiles from host-infested plants over intact plants (45 wasps to infested plant volatiles and 15 wasps to intact plant volatiles: $p<0.001$, binomial test) (Fig. 1).

Wind tunnel bioassays

The number of host-infested plant volatiles-experienced wasps landing on host-infested plants was significantly larger than the number landing on intact plants (20 wasps on plant-host complex and 8 wasps on intact plants, $p<0.05$, Fisher's exact probability test) (Fig. 2). In contrast, naïve wasps

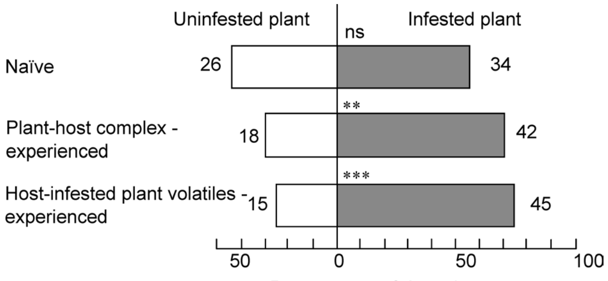

Fig. 1. Choice tests by *A. ervi* females in a Y-tube olfactometer. ** $p<0.01$, *** $p<0.001$ by binomial test. Numbers of individuals selecting each odor are indicated next to each bar. Treatments of wasps; naïve, plant-host complex-experienced, or host-infested plant volatile-experienced are indicated on each bar.

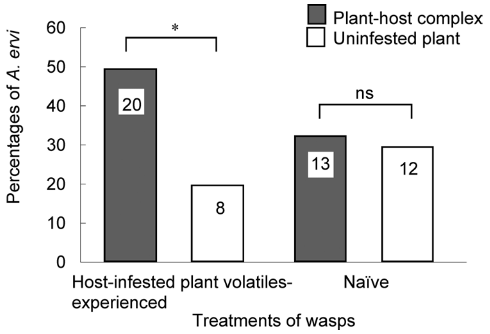

Fig. 2. Landing response by naïve or host-infested plant volatile-experienced *A. ervi* females to plant-host complex or intact plant in a wind tunnel. The number of *A. ervi* landing on the target is indicated on each bar. $*p<0.05$ by Fisher's exact probability test.

equally landed on either host-infested plants or intact plants (13 wasps on plant-host complex and 12 wasps on intact plants) (Fig. 2).

DISCUSSION

Powell et al. (1998) showed that although naïve *A. ervi* females were attracted to plants infested by their hosts, their responses to host-infested plants were enhanced further by coming into contacting with those plants after mummy emergence. In the present study, however, naïve *A. ervi* females did not respond to host-infested plants, while experienced females showed a significant preference for host infested plants. We found here that only experienced wasps showed a significant preference for host-infested plant volatiles. It has been demonstrated that the odor preference of *A. ervi* females develops during mummification: adult parasitoids exposed to vanilla odor at the larval ventral opening of the mummies showed a significant preference for vanilla odors in olfactometer bioassays, regardless of excision from the mummies (Gutierrez-Ibanez et al., 2007). In our study, developmental conditioning during mummification had no or little effect on the olfactory response of *A. ervi* for the following reason. Parasitized aphids were mummified on host-infested plants, and then the mummies were treated in three different ways; i.e. naïve wasps, plant-host complex-experienced wasps and host-infested plant volatiles-experienced wasps. Naïve wasps showed different olfactory responses to infested plant volatiles compared with the responses of both groups of experienced wasps. If developmental conditioning during mummification was involved in the response to infested plant volatiles, naïve wasps would have responded to the volatiles. Our data show that learning about host-infested plant volatiles after adult emergence is important for wasps to develop their preference for volatiles.

Host-plant preference exhibited during host selection in *Aphidius colemani* is induced by chemical cues on the mummy case at the time of adult emergence (Storeck et al., 2000). In this study, it was not possible to determine whether the odor response of *A. ervi* was induced by contacting volatiles from infested plants that were impregnated into the mummy case, or by exposure to volatiles in the air during/after their emergence from the mummy. Here we showed that the olfactory response of *A. ervi* could be conditioned not only during mummification (Gutierrez-Ibanez et al., 2007), but also during/after emergence. Learning during the host-finding process also affects the odor preference of *A. ervi* (Du et al., 1997; Guerrieri et al., 1997).

The reason why previously reported responses of naïve *A. ervi* to host-infested plant volatiles were not detected in this study remains unknown. The timing at which the olfactory response of *A. ervi* is determined could be genetically different between Japanese and European *A. ervi* populations, which are geographically isolated from each other. The percentage of parasitism recorded for Hokkaido (SPR) and Dutch (EVP) strains of *A. ervi* was significantly different when attacking several aphid species (Takada and Tada, 2000). Further, SPR and EVP strains differed morphologically and in their esterase banding patterns. Cross-mating experiments showed that SPR and EVP strains are partially reproductively isolated from each other (Takada and Tada, 2000). Alternatively, different combinations of bean varieties and host lines might affect conditioning. The cereal aphid specialist *Aphidius rhopalosiphi* was able to distinguish between different cultivated varieties of winter wheat in a Y-tube olfactometer (Wickremasinghe and van Emden, 1992). Different responses of parasitic wasps to different cultivated varieties infested by the same host species have also been reported in larval parasitoids and egg parasitoids (Elzen et al., 1986; Hoballah et al., 2002; Lou et al., 2006). Since we were unable to import into Japan broad bean plants cv. '*the Sutton*' used in previous studies (Du et al., 1996, 1997, 1998; Guerrieri et al., 1999), we could not test this possibility during this study. Responses to odors from the same plant species and variety should be compared in experiments with *A. ervi* whose physiological state is uniformly controlled.

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