

Oviposition of diamondback moth *Plutella xylostella* females is affected by herbivore-induced plant volatiles that attract the larval parasitoid *Cotesia vestalis*

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Abstract A mixture of four volatile compounds, (*Z*)-3-hexenyl acetate, α -pinene, sabinene and *n*-heptanal, emitted from cabbage plants infested by diamondback moth [DBM; *Plutella xylostella* (L.)] larvae attracts *Cotesia vestalis* (Haliday), a major parasitoid of DBM larvae. The volatiles may affect other organisms, such as DBM conspecifics, other herbivores and carnivores. Here, we studied whether the volatiles affect the oviposition behavior of DBM females. In a climate-controlled room, five pots of komatsuna plants (*Brassica rapa* var. *perviridis* L. cv. Rakuten; leaf vegetable) were placed in an acrylic box. For the treatment, we placed a bottle-type dispenser of the volatiles (0.01% in a triethyl citrate solution) next to the center pot. For the control experiment, we used a container with plants and triethyl citrate only. The presence of the volatiles did not affect the number of eggs per plant. Interestingly, DBM females laid more eggs on the adaxial leaf surfaces in the treatment compared with the control. This is the first study showing that plant volatiles affect the oviposition site preference of herbivores on leaves. The

results are discussed in relation to the application of attractants for DBM control in commercial greenhouses.

Keywords *Plutella xylostella* · *Cotesia vestalis* · Herbivore-induced plant volatiles · Attractants · Oviposition preference

Introduction

Plants emit a specific mixture of volatiles in response to the damage caused by herbivorous arthropods, and a well-known ecological function of such volatiles is to attract carnivorous natural enemies of the currently infesting herbivorous arthropods (Dicke et al. 1990; Arimura et al. 2009; Takabayashi 2014). *Cotesia vestalis* (Haliday), a parasitic wasp of the diamondback moth [DBM; *Plutella xylostella* (L.)] larvae, and an effective biological control agent of DBM populations (Talekar and Shelton 1993), is attracted to volatiles from DBM larvae-infested cabbage plants (Shiojiri et al. 2000). The volatiles involved in attracting *C. vestalis* to DBM-infested cabbage plants are a mixture of (*Z*)-3-hexenyl acetate, α -pinene, sabinene and *n*-heptanal (Shiojiri et al. 2010). Interestingly, only this mixture (hereafter called attractants) attracts *C. vestalis* (Shiojiri et al. 2010).

We are studying the effective use of native *C. vestalis* to control DBM in greenhouses in the satoyama environment (the zone between the mountain foothills and arable flat land) using artificial attractants (Abe et al. 2007; Urano et al. 2011; Uefune et al. 2012; Shimoda et al. 2014). Under field conditions, *C. vestalis* females are more attracted to uninfested komatsuna plants placed near artificial attractants than to uninfested plants without the attractants (Uefune et al. 2012). Furthermore, the incidence of

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parasitism of DBM larvae by the wasps on komatsuna plants near artificial attractants is higher than that on komatsuna plants without the attractants under field conditions (Uefune et al. 2012).

Volatiles emitted by infested plants are exploited by natural enemies and by conspecific, and/or heterospecific, herbivores. Adult female herbivores are either attracted to the volatiles emitted by infested plants (Bolter et al. 1997; Horiuchi et al. 2003) or avoid them (Dicke 1986; De Moraes et al. 2001; Horiuchi et al. 2003; Oluwafemi et al. 2011). For example, Horiuchi et al. (2003) reported that *Tetranychus urticae* Koch are attracted to lima bean plants that are slightly infested by conspecifics but are repelled by those heavily infested by conspecifics. Herbivores in their larval stages also react to volatiles emitted by infested plants. Sixth stadium *Spodoptera frugiperda* (Smith) larvae are attracted to corn plants infested by conspecifics (Carroll et al. 2006).

We have reported that DBM females prefer to lay eggs on DBM-infested cabbage plants compared with uninfested ones (Shiojiri and Takabayashi 2003). If attractants stimulate the oviposition of female DBM, then the use of attractants for DBM control would be a double-edged sword: not only attracting *C. vestalis* from the surroundings but also stimulating the oviposition of female DBM that have invaded the attractant-treated greenhouses from the surroundings. For the application of artificial attractants to control DBM, understanding the oviposition preference of DBM females is important. The objective of this study was to determine whether the attractants mentioned before are involved in the oviposition preference of DBM females to plants. Based on the results, the possible use of artificial attractants for DBM control was discussed.

Materials and methods

Plants and insects

Komatsuna plants (*Brassica rapa* var. *perviridis* L. cv. Rakuten) were cultivated in a greenhouse (25 ± 3 °C, $60 \pm 10\%$ RH, 16 L:8 D). Five plants were reared from seeds per plastic pot (upper diameter: 9 cm, lower diameter: 7 cm, depth: 7 cm) for 4–5 weeks. Then, 12 pots were placed in a plastic tray (37 cm × 27 cm × 6 cm depth) containing water for the plants. These pots were used for the experiments.

DBM larvae were collected in a field near Kyoto, Japan, and mass-reared on potted komatsuna plants in a climate-controlled room (25 ± 3 °C, $60 \pm 10\%$ RH, 16 L:8 D) to obtain adults. Eggs were collected every day, and hatched larvae were reared on cut plants in small cages (25 cm × 15 cm × 10 cm high). Newly emerged adults of

DBM were maintained separately in acrylic cages (35 cm × 25 cm × 30 cm high) and provided a 50% (v/v) honey solution as food in a climate-controlled room (25 ± 3 °C, $60 \pm 10\%$ RH, 16 L:8 D) to ensure mating. After 4 days, DBM females were used for experiments.

Attractants

Pure (Z)-3-hexenyl acetate, *n*-heptanal, α -pinene and sabinene (RC Treatt, Suffolk, UK; Wako Chemicals Co. Ltd., Osaka, Japan; Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) compounds were mixed in a 1.8:1.3:2.0:3.0 ratio, respectively (Shiojiri et al. 2010). The mixture was dissolved in triethyl citrate (TEC) to achieve slow volatilization. The above ratio was adjusted to that released by an infested cabbage plant as herbivore-induced volatiles using a gas chromatograph equipped with a flame ionization detector.

To expose the blend to DBM females, we used a bottle-type dispenser described in Uefune et al. (2012). The glass bottle (diameter: 35 mm, height: 55 mm) of the dispenser was filled with 20 g of the attractant blend, which was the mixture dissolved to 0.01% in TEC. Bottles of the same type containing 20 g of pure TEC were used as controls. The lids of the bottles have a hole (diameter: 12 mm) through which a piece of polypropylene thread (diameter: 12 mm, length: 70 mm) was strung. The purpose of the thread was to facilitate the evaporation of the chemicals. We used the 0.01% dose for the experiments because *C. vestalis* females are attracted to uninfested plants placed near dispensers containing 0.01% of the blend compared with uninfested plants only (Uefune et al. 2012).

Oviposition experiments

Each komatsuna pot, containing five komatsuna plants, was placed in a plastic cup (diameter: 9 cm, depth: 4.5 cm). Then, five komatsuna pots were placed in an acrylic box (60 cm × 60 cm × 60 cm) in the “dice five” formation in a climate-controlled room (25 ± 2 °C; 50–70% RH; 16 L:8 D). For treatment experiments, we placed a dispenser containing 0.01% of the artificial attractants next to the center pot. The other plants are referred to as plants without the attractants. The distance between the center plant and the surrounding four plants was 20 cm. We conducted control experiments where we again placed five plants in the “dice five” formation, but the dispenser near the center pot contained TEC only.

There were two windows (40 cm × 40 cm, covered with nylon gauze), so that the volatiles would not saturate the interior of the box. We released three male and female pairs of DBM in the box at 16:00. We counted the number of eggs laid on the plants 24 h later. We also recorded the distribution of the eggs on the leaves. The experiments

were repeated six times. In each experiment, we used a newly cleaned box.

Statistical analyses

To determine whether there were differences in the oviposition preferences of DBM females between plants with and without artificial attractants, we used randomized-block two-way ANOVAs. We added 0.5 to the number of eggs on leaves because we had zero data, and then used a Box–Cox transformation before the analyses. The adaxial proportion of eggs on the leaf surfaces was angular transformed before the analyses. Statistical analyses were conducted with the JMP software package (version 11.2.1; SAS Institute, Cary, NC, USA).

Results

The number of eggs

The treatments (the treated experiments vs the control experiments) did not affect the number of eggs laid on leaves of komatsuna ($df = 1, 10, F = 0.0398, P = 0.8459$, randomized-blocks two-way ANOVA) (Fig. 1). Further, the locations of the komatsuna pots did not affect the number of the eggs ($df = 4, 40, F = 0.6922, P = 0.5018$, randomized-blocks two-way ANOVA). The interaction between the treatments and locations was also not significant for the number of eggs ($df = 4, 40, F = 0.9086, P = 0.4683$, randomized-blocks two-way ANOVA) (Fig. 1).

Egg distribution on a leaf

The treatments affected the adaxial proportion (%) of eggs laid on the leaf surface ($df = 1, 7.456, F = 11.7686, P = 0.01$, randomized-blocks two-way ANOVA). The proportion was significantly higher on plants in the treated boxes than on those in the control boxes (Fig. 1). By contrast, the locations of the komatsuna pots did not affect the adaxial proportion of the eggs ($df = 4, 28.28, F = 0.7357, P = 0.5754$, randomized-blocks two-way ANOVA). The interaction between the treatments and locations was also not significant for the adaxial proportion ($df = 4, 28.28, F = 0.5052, P = 0.7322$, randomized-blocks two-way ANOVA) (Fig. 1).

Discussion

Shiojiri and Takabayashi (2003) reported that, in an acrylic box (30 cm × 25 cm × 30 cm), which was smaller than that used in this study, DBM females laid more eggs on

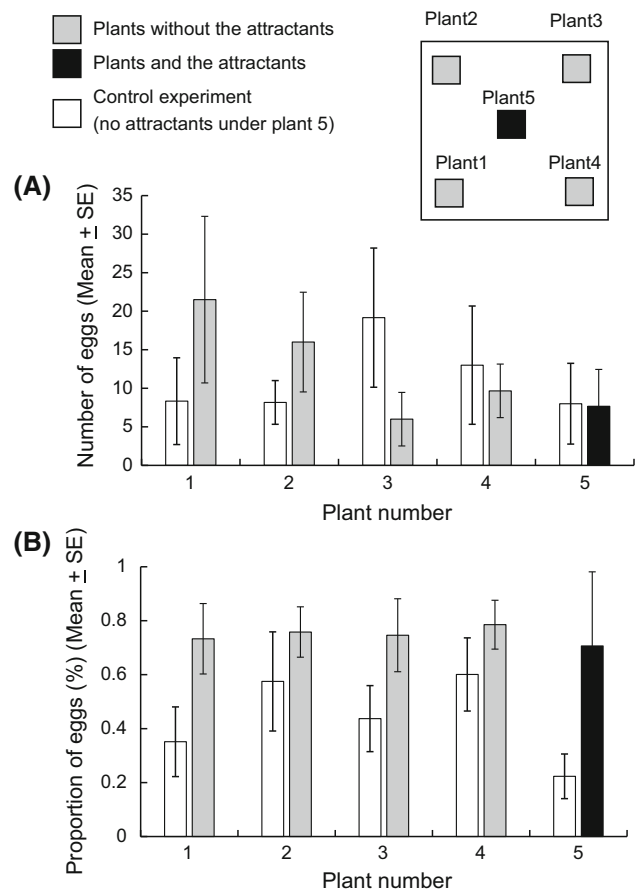


Fig. 1 The number of eggs laid by diamondback moth (DBM; *Plutella xylostella*) females on leaves (a) and the proportion (%) of eggs laid by DBM females on adaxial leaf surfaces (b)

DBM-infested cabbage plants than on uninfested ones. However, in this study, the mixture of (*Z*)-3-hexenyl acetate, *n*-heptanal, α -pinene and sabinene, which attract *C. vestalis* at a 0.01% dose (Uefune et al. 2012), did not affect the number of eggs that were oviposited by DBM females. Thus, the compounds that attract *C. vestalis* would not be involved as active principals from DBM-infested plants that stimulate DBM oviposition on infested plants. Zakir et al. (2013) reported the effects of volatiles from infested plants on the oviposition of *Spodoptera littoralis* females. A significant reduction in oviposition by females was found on undamaged cotton and alfalfa plants adjacent to *S. littoralis* larvae-damaged cotton plants that attract parasitoids and predators.

Badenes-Perez et al. (2011) studied the amounts of an oviposition stimulant of DBM females, glucosinolates, on the surface of three Crucifer species, *Barbarea rupicola*, *B. verna* and *B. vulgaris*. They found higher concentrations (twofold–tenfold) of glucosinolates on the abaxial surface than on the adaxial surface of *B. rupicola* and *B. verna* leaves. However, there were no differences in oviposition

by DBM between adaxial and abaxial surfaces on the *Barbarea* species or on *B. vulgaris*. Thus, glucosinolates are not involved in the site preferences by DBM females. In this study, $44 \pm 0.06\%$ (mean \pm SE) of eggs were laid on the adaxial leaf surfaces of komatsuna plants in control boxes. However, when DBM females were exposed to the artificial attractants in treated boxes, the proportion, $75 \pm 0.05\%$ (mean \pm SE), of eggs on the adaxial leaf surfaces was greater than that in the control boxes. To our knowledge, this is the first study showing that volatiles emitted by infested plants affect oviposition sites on leaves.

A possible adaptive function of the change would be to avoid damage caused by conspecific and heterospecific larvae. Philips et al. (2014) reported that DBM larvae move to the undersides of leaves after hatching. The first-instar larvae mine the spongy mesophyll tissue, whereas older larvae feed from the lower leaf surface and usually consume all of the tissue except the wax layer on the upper surface (Talekar and Shelton 1993). Such larvae would eventually have negative effects (injury and predation) on newly deposited DBM eggs on the abaxial surface of a leaf. Because the presence of the attractants indicates that there are DBM larvae on a plant, a change in the leaves' preferred oviposition sites in response to the attractants could, in part, be explained by the avoidance of negative effects by currently inhabiting DBM larvae. Larvae of *Mamestra brassicae* and *Spodoptera litura*, which infest a wide range of plant species including crucifer plants, have been reported to induce volatiles in plants (Menzel et al. 2014; Sugimoto et al. 2014). Early stages of larvae of these two species are known to stay on the abaxial leaf surface. Whether volatiles induced by these herbivores also affect the distribution of DBM eggs will be studied in the future.

Rainfall and overhead irrigation are important mortality factors for DBM (Kobori and Amano 2003; Talekar and Shelton 1993 and citations therein). Thus, for plant fitness, the increase in the number of eggs on the adaxial surface of a leaf caused by the attractants would be favorable for the plants, because eggs on the adaxial surface are more exposed to risk of water. In open agricultural fields and greenhouses, this shift would facilitate DBM control. The use of attractants for the parasitic wasp *C. vestalis* would, therefore, not be a double-edged sword as discussed in the introduction, but could produce twice the beneficial effect when used to control DBMs in greenhouses.

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