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Research

Biological Control - Parasitoids and Predators

Synchronous Occurrences of the Diamondback Moth (Lepidoptera: Plutellidae) and its Parasitoid Wasp *Cotesia vestalis* (Hymenoptera: Braconidae) in Greenhouses in a Satoyama Area

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Abstract

We characterized the correlation between the occurrences of diamondback moth, *Plutella xylostella* (L.), larvae and their dominant native parasitoid wasp, *Cotesia vestalis* (Haliday), in commercial greenhouses in a satoyama area, called Miyama, in Kyoto, Japan. In the three greenhouses used in this study, cruciferous 'mizuna' (*Brassica rapa* var. *laciniifolia* [Brassicales: Brassicaceae]) crops were grown. Pesticides against diamondback moth were not routinely applied in the greenhouses. We confirmed that populations of diamondback moth and *C. vestalis* were maintained on the wild crucifer plant *Rorippa indica* in the surrounding area from March to December. In the greenhouses, we observed several occurrences of diamondback moth larvae that were, in most cases, followed by occurrences of *C. vestalis*. We found that *C. vestalis* females were attracted by volatiles emitted from mizuna plants that were lightly infested with second-stadium diamondback moth larvae under laboratory conditions. The synchronous appearance of diamondback moth larvae and *C. vestalis* could be explained by the latter being attracted by the volatiles emitted from mizuna plants infested by diamondback moth larvae in the greenhouses.

Key words: attractants, choice chamber, plant volatiles, mizuna plants, diamondback moth

The rural agricultural landscape that consists of forest, village, small-scale wet rice paddy fields, crop fields and greenhouses in Japan is called 'satoyama' (Kobori et al. 2003). Satoyama landscapes are also found in other countries (Takeuchi et al. 2003). An ecological characteristic of satoyama is that several pest insects and their carnivorous natural enemies are present in the surrounding natural environment (Kagawa and Maeto 2009, Katoh et al. 2009). Invasions of agricultural fields by pest insects from the surroundings are common events in satoyama areas.

Miyama is a satoyama located in Kyoto Prefecture, Japan, where the crucifer crop 'mizuna' (*Brassica rapa* var. *nipposinica*) is a major greenhouse crop. A diamondback moth, *Plutella xylostella*, population exists in the surrounding area, and the diamondback moth is an important pest of mizuna plants in greenhouses in Miyama. Farmers have reduced the use of pesticides, and therefore, pesticides against diamondback moth are not routinely applied in the greenhouses. *Cotesia vestalis* is an effective parasitoid of diamondback moth larvae (Talekar and Shelton 1993, Furlong et al. 2013), and a *C. vestalis* population also exists in Miyama as the dominant natural enemy of diamondback moth larvae (J. Abe, personal observation).

The foraging *C. vestalis* females are attracted to volatiles that are emitted from cruciferous plants infested by diamondback moth larvae. This attraction was determined under laboratory conditions using cabbage plants (*Brassica oleracea* var. *capitata*), komatsuna plants (*B. rapa* var. *perviridis*), Japanese radish plants (*Raphanus sativus* var. *longipinnatus*) and *Arabidopsis thaliana* (Shiojiri et al. 2000, 2010; Ohara et al. 2017; Yoneya et al. 2018). The attraction was also confirmed under field conditions using a synthetic volatile blend, which was based on a natural blend that originated in the headspace of cabbage plants infested with diamondback moth larvae (Shiojiri et al. 2010, Uefune et al. 2012).

Here, we showed a positive correlation between the occurrence of diamondback moth larvae and that of *C. vestalis* in greenhouses in Miyama. We also revealed that *C. vestalis* females were attracted to volatiles emitted from mizuna plants that were infested with one second-stadium diamondback moth larvae for 1 d. Based on the laboratory and field data, we discussed the possible reasons for the synchronous occurrence of the two species.

Materials and Methods

Insects and Plants

Diamondback moth larvae were collected from fields in Ayabe, Kyoto, Japan (35°N, 135°E) in 2001 and were reared on potted komatsuna plants, *B. rapa* var. *perviridis* (Brassicales: Brassicaceae), in a climate-controlled room (25 ± 3°C, 60 ± 10% relative humidity [RH], 16:8 (L:D) h photocycle). Newly emerged diamondback moth adults were maintained in acrylic cages (35 × 25 × 30 cm) in the climate-controlled room. They were provided with a 50% (v/v) honey solution as food and potted komatsuna plants. Komatsuna plants with eggs were collected daily.

C. vestalis were obtained from parasitized diamondback moth larvae collected in Ayabe, Kyoto, Japan. They were kept in a plastic box with detached komatsuna leaves in the climate-controlled room. Cocoons on leaves were collected and kept in closed-ended glass tubes until emergence. Thereafter, they were maintained in glass tubes (25-mm inner diameter, 120-mm length) in a climate-controlled room (18 ± 3°C, 60 ± 10% RH, 16:8 (L:D) h photocycle). They were a maximum of 10 d old (after emergence from the host). They were acclimatized for 1–2 h in a climate-controlled room (25 ± 3°C, 60 ± 10% RH) before the experiments started.

Mizuna (*B. rapa* var. *nipposinica* 'Jounan-Sensuji') and komatsuna (*B. rapa* var. *perviridis* L. 'Rakuten') plants were cultivated in a climate-controlled glass house (25 ± 3°C, 60 ± 10% RH) for 4–5 wk. Four plants were grown per plastic pot (diameter: 72 mm, depth: 65 mm). These potted plants were used as the volatile sources in the laboratory experiments.

Response of *C. vestalis* to Diamondback Moth-Infested Plants

The potted mizuna or komatsuna plants that had received damage from one second-stadium diamondback moth larva per plant for 24 h were used as infested plants. Prior to the tests, the larvae, their silk and their feces were removed from the infested plants with the aid of a fine brush.

The flight responses of *C. vestalis* females towards a pot of diamondback moth-infested mizuna plants versus a pot of uninfested mizuna plants were tested in an acrylic cage (25 × 30 × 35 cm; three nylon gauze-covered windows and one door) under fluorescent lighting (20 W, 3,000 lux) in a climate-controlled room (25 ± 3°C, 60 ± 10% RH). We also compared the flight responses of the parasitoid wasps toward a pot of diamondback moth-infested mizuna plants versus a pot of diamondback moth-infested komatsuna plants. There was no wind in the cage.

Females were released individually from a glass tube (25-mm inner diameter, 120-mm length) positioned halfway between the two plant-containing pots. Upon their first visit to one of the test plants (defined as landing), they were removed with an insect aspirator. Ten wasps were tested using the same set of potted plants. Each wasp was tested only once, and the experiments were repeated on 3 or 4 d. Two-choice data under laboratory conditions were analyzed using a replicated *G*-test (Sokal and Rohlf 1995). Wasps that did not choose either plant were discarded from this analysis.

Field Observation

Four greenhouses owned by a farmer which were set in a 'dice four' arrangement with 2–3 m distance between each. We used three mizuna greenhouses (Greenhouse 1–3) (Supp Fig. 1 [online only]). The area of each greenhouses was ca. 270 m^2. The greenhouses were surrounded by open agricultural fields, a thicket and a river (Supp Fig. 1 [online only]). In this study, Greenhouse 4 was not used because the farmer sometimes abandoned its mizuna production after the occurrence of diamondback moth. The greenhouses were covered with 1-mm nylon mesh to try to prevent the invasion of pest insects. However, there were some slits along the entrance door and seams of the mesh that allowed diamondback moth larvae to invade the greenhouses. It is unlikely that the diamondback moth larvae in the surrounding area invaded the greenhouses by crawling (J. Abe, unpublished data). *Cotesia vestalis* adults can pass through 1-mm nylon mesh (Uefune et al. 2012).

We observed the numbers of diamondback moth larvae (third- and the fourth-stadiums), pupae and *C. vestalis* cocoons on mizuna plants in the greenhouses in 2004. The number of mizuna plants observed varied from 30 to 250 depending on the observation day. Thus, the densities were calculated. Observations were made every 7 d during the observed period. After November, since the densities off diamondback moth larvae and *C. vestalis* were low, the observation were made every 14 d. We also observed the numbers of diamondback moth larvae and *C. vestalis* cocoons on a wild cruciferous species, *Rorippa indica*, which was growing in the area surrounding the greenhouses. The areas in which *R. indica* plants were observed are shown in Supp Fig. 1 [online only]. The observed area was ca. 1,500 m^2. We checked all of *R. indica* plants at each observation time.

Diamondback moth larvae found on mizuna and *R. indica* plants were reared in a climate-controlled room in the laboratory (25 ± 2°C, 50–60% RH, 16:8 (L:D) h photocycle) to determine the incidence of parasitism. The number of incidences was added to the number of *C. vestalis* cocoons.

Results

Field and Greenhouse Observations

In the surrounding areas, *R. indica* plants were found throughout the observation period with some fluctuations (Fig. 1A). The diamondback moth larvae were observed from April to December. *Cotesia vestalis* cocoons and the parasitized larvae were also observed from May to November. In most cases, diamondback moth larvae and *C. vestalis* occurred at the same time (Fig. 1A).

In the three greenhouses (Fig. 1B–D), changes in the numbers of mizuna plants represented the planting and harvesting of the mizuna plants. In Greenhouse 1 (Fig. 1B), diamondback moth larvae occurred from May 30 to June 6 (the first), from June 21 to June 26 (the second) and from July 12 to August 4 (the third). These three occurrences were followed by occurrences of *C. vestalis*. After August 23, there were constant low-density occurrences of diamondback moth larvae (eight occurrences). Among them, four diamondback moth occurrences were followed by occurrences of *C. vestalis* (August 23, September 20, October 4, and November 15). Pesticides against diamondback moth larvae were used two times: from July 19 to 26 and from August 4 to 9.

In Greenhouse 2 (Fig. 1C), diamondback moth occurred from April 26 to May 9 (the first), from June 13 to June 26 (the second), from July 19 to August 23 (the third) and from September 14 to

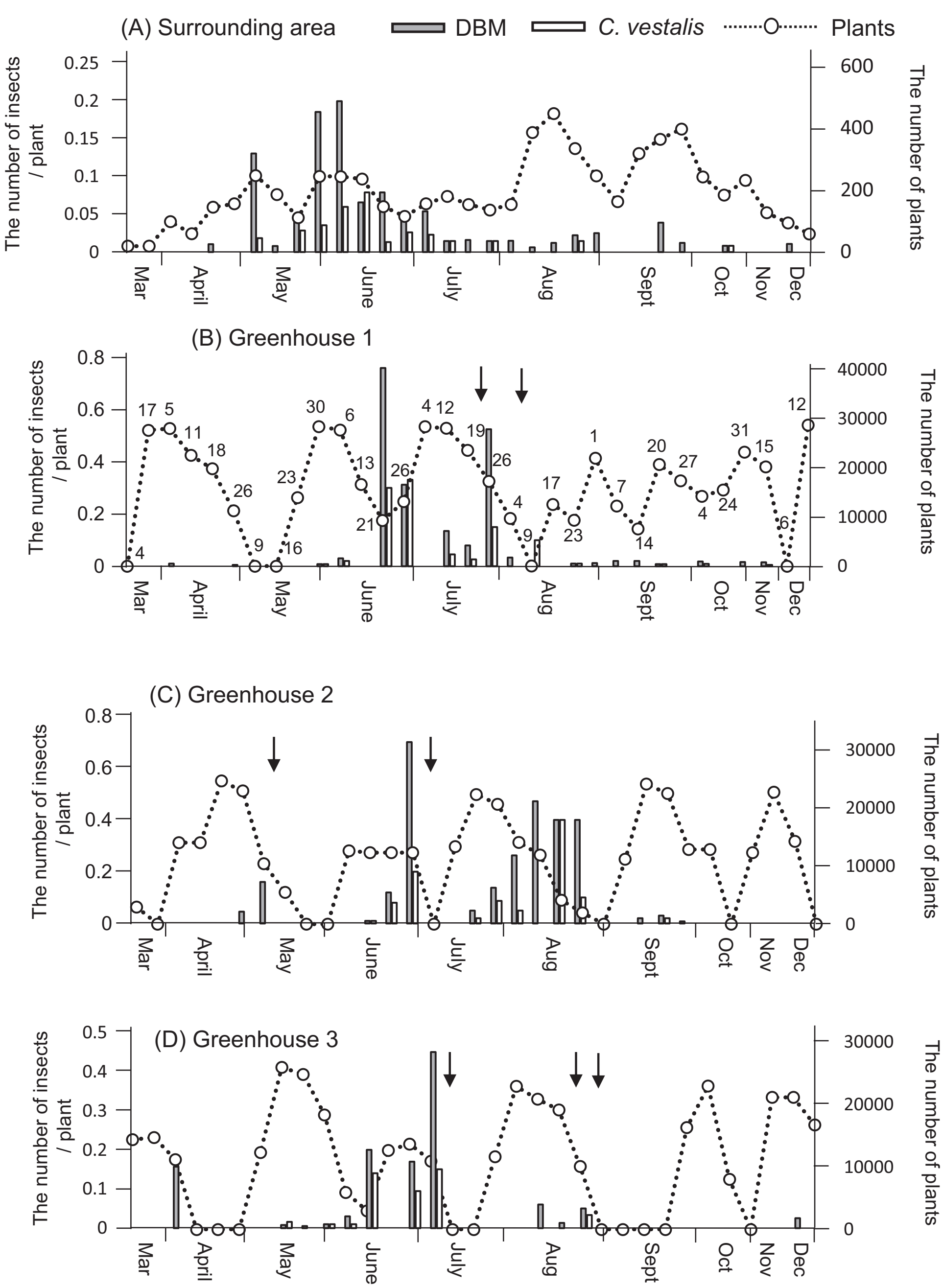

Fig. 1. Seasonal changes in the occurrences of diamondback moth larvae (gray bars) and its parasitoid wasp *Cotesia vestalis* (cocoons and parasitized larvae) (white bars) in greenhouses and their surrounding areas. The numbers of host plants (mizuna and *R. indica*) (dashed lines with white circles) are also shown. (A): Surrounding area, (B): Greenhouse 1, (C): Greenhouse 2, and (D): Greenhouse 3. Arrays indicated the use of pesticides

September 27 (the fourth). All the occurrences, except the first, were followed by occurrences of *C. vestalis*. Pesticides against diamondback moth larvae were used two times: from May 9 to 16 and from June 24 to July 4.

In Greenhouse 3 (Fig. 1D), diamondback moth occurred on April 5 (the first), from May 16 to June 13 (the second), from May 26 to July 4 (the third) and from August 9 to August 23 (the fourth). These occurrences, except the first, were followed by occurrences of *C. vestalis*. Diamondback moth larvae were not observed on the other observation days, except for December 5. Pesticides against diamondback moth larvae were used three times: from July 4 to 12, from August 23 to September 1 and from September 1 to 7.

Olfactory Responses of *C. vestalis* to Diamondback Moth Larvae-Infested Plants

We offered diamondback moth-infested mizuna plants and uninfested mizuna plants to *C. vestalis* females in a choice chamber. *C. vestalis* females preferred infested mizuna plants over uninfested mizuna plants (G_P = 7.1034, df = 1, P = 0.0077; G_H = 0.5977, df = 2, P = 0.7417; G_T = 7.7011, df = 3, P = 0.0526, replicated *G*-test) (Fig. 2: upper bar). We then offered *C. vestalis* females diamondback moth-infested mizuna plants versus diamondback moth-infested komatsuna plants in a choice chamber. *C. vestalis* females showed an equal distribution between the two odor sources (G_P = 1.6108, df = 1, P = 0.2044; G_H = 0.8402, df = 2, P = 0.8398; G_T = 2.4511, df = 3, P = 0.6534, replicated *G*-test) (Fig. 2: lower bar).

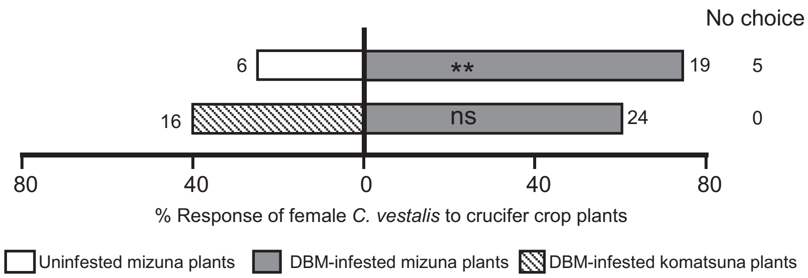

Fig. 2. Olfactory responses of *C. vestalis* females to cruciferous plants receiving different treatments. The experiments were repeated on three experimental days (upper bar) and four experimental days (lower bar). The data were pooled and subjected to a G-test. Numbers next to bars indicate the numbers of *C. vestalis* that responded to the volatiles. When the wasps did not land on either of the plants, they were called 'no choice' individuals. ** $0.01 > P > 0.001$; ns: not significantly different.

Discussion

Our results revealed that the area surrounding the greenhouses, which contained *R. indica* as the major cruciferous plant distributed in the observational area (Supp Fig. 1 [online only]), harbored source populations of diamondback moth and *C. vestalis*. The occurrences of diamondback moths in greenhouses could not always be explained by their seasonal density in the surrounding area. There were some differences in the occurrences of diamondback moth and *C. vestalis* among the three greenhouses. This may result from differences in their positions relative to the surrounding environment (Supp Fig. 1 [online only]). Furthermore, the fluctuations of diamondback moths in the field did not always correlate with the fluctuations of diamondback moths in the greenhouses (especially in July and August in Greenhouses 1 and 2).

The following two common observation occurred in the three greenhouses throughout the observation period: 1) peak occurrences of diamondback moth larvae were followed by peak occurrences of *C. vestalis* cocoons and/or parasitized larvae on mizuna plants and 2) occurrences of *C. vestalis* were recorded only when occurrences of diamondback moth larvae were recorded. An exception was Greenhouse 1 on August 9, when almost all the mizuna plants were harvested the day before and some cocoons were observed on the remaining plants. A similar synchronized occurrence was reported by Shimomoto (2002), in which an invasion of five native parasitoid species was observed in eggplant greenhouses in which leafminers (*Liriomyza trifolii*) existed.

Under laboratory conditions, we found that the mizuna plants that had received minor damage, such as one small infested edge, from one second-stadium diamondback moth larva attracted *C. vestalis*. Furthermore, the attractiveness of such infested mizuna plants was equivalent to that of komatsuna plants each infested by one second-stadium diamondback moth larva. Komatsuna plants infested by diamondback moth larvae can attract *C. vestalis* females under greenhouse (7 × 10 × 5 m) conditions (Ohara et al. 2017, Ozawa et al. 2018). These results, in combination with the field data, led us to hypothesize that the volatiles emitted from mizuna plants infested by diamondback moth larvae in greenhouses attracted native *C. vestalis* from the surrounding area, resulting in their synchronous occurrences in the greenhouses. To test this behavior, further olfactory or behavioral studies are needed to demonstrate their long-distant orientation from the field to greenhouses.

Abe et al. (2007) reported that single releases of five *C. vestalis* into experimental greenhouses (120 m^2, 4,000 komatsuna plants), in which the density of diamondback moth larvae was set to be 0.05 per plant (economic injury level in Miyama), resulted in the successful suppression of subsequent diamondback moth populations to under 0.05 larvae per plants for ~40 d. They observed diamondback moth outbreaks in the control greenhouses (initial conditions: 0.05 diamondback moth larvae per plant without the release of *C. vestalis*) (Abe et al. 2007). Thus, in the early stage of a diamondback moth occurrence in a greenhouse (~0.05 diamondback moth larvae per plant), the presence of approximately five *C. vestalis* females can maintain the diamondback moth larvae at the same density or lower. Consequently, the use of pesticides was not required. In this study, however, although synchronized occurrences of diamondback moth larvae and *C. vestalis* were detected, the use of pesticides was required in each greenhouse. Thus, we concluded that the synchronized occurrence of *C. vestalis* did not always meet the required conditions shown by Abe et al. (2007) to suppress diamondback moth larvae in greenhouses.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Supplemental Fig. 1 Map of the four greenhouses. The pale green area indicates where *R. indica* plants were observed. This map was revised from an original one produced by Google Maps (Google, Menlo Park, California, USA)

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References Cited

Abe, J., S. Urano, K. Nagasaka, and J. Takabayashi. 2007. Release ratio of *Cotesia vestalis* to diamondback moth larvae (*Plutella xylostella*) that suppresses the population growth of diamondback moth feeding *Brassica* leaf vegetables in a greenhouse. Bullet. Nat. Agr. Res. Centr. West. Region. 6: 125–132. (In Japanese.)

Furlong, M. J., D. J. Wright, and L. M. Dosdall. 2013. Diamondback moth ecology and management: problems, progress, and prospects. Annu. Rev. Entomol. 58: 517–541.

Kagawa, Y., and K. Maeto. 2009. Spatial population structure of the predatory ground beetle *Carabus yaconinus* (Coleoptera: Carabidae) in the mixed farmland-woodland satoyama landscape of Japan. Eur. J. Entomol. 106: 385–391.

Katoh, K., S. Sakai, and T. Takahashi. 2009. Factors maintaining species diversity in satoyama, a traditional agricultural landscape of Japan. Biol. Conserv. 142: 1930–1936.

Kobori, H., and R. B. Primack. 2003. Participatory conservation approaches for satoyama, the traditional forest and agricultural landscape of Japan. Ambio 32: 307–311.

Ohara, Y., T. Uchida, K. Kakibuchi, M. Uefune, and J. Takabayashi. 2017. Effects of an artificial blend of host-infested plant volatiles on plant attractiveness to specialist parasitic wasps. J. Appl. Entomol. 141: 231–234.

Ozawa, R., Y. Ohara, K. Shiojiri, T. Uchida, K. Kakibuchi, S. Kugimiya, M. Uefune, and J. Takabayashi. 2018. Uninfested plants and honey enhance the attractiveness of a volatile blend to a parasitoid *Cotesia vestalis*. J. Appl. Entomol. 142: 978–984.

Shimomoto, M. 2002. Control of *Liriomyza trifolli* (Burgess) by indigenous and imported parasitoids on eggplant in forcing culture. Bull. Kochi Agric. Res. Cent. 11: 37–44.

Shiojiri, K., J. Takabayashi, S. Yano, and A. Takafuji. 2000. Flight response of parasitoid toward plant-herbivore complexes: a comparative study of two parasitoid-herbivore systems on cabbage plants. Appl. Entomol. Zool. 35: 87–92.

Shiojiri, K., R. Ozawa, S. Kugimiya, M. Uefune, M. van Wijk, M. W. Sabelis, and J. Takabayashi. 2010. Herbivore-specific, density-dependent induction of plant volatiles: honest or "cry wolf" signals? PLoS One 5: e12161.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research, 3rd ed. W.H. Freeman and Company, New York, NY.

Takeuchi, K., R. D. Brown, I. Washitani, A. Tsunekawa, and M. Yokohari. 2003. Satoyama: the traditional rural landscape of Japan. Springer, Japan.

Talekar, N. S., and A. M., Shelton. 1993. Biology, ecology, and management of the diamondback moth. Ann. Rev. Entomol. 38: 275–301.

Uefune, M., Y. Choh, J. Abe, K. Shiojiri, K. Sano, and J. Takabayashi. 2012. Application of synthetic herbivore-induced plant volatiles causes increased parasitism of herbivores in the field. J. Appl. Entomol. 136: 561–567.

Yoneya, K., M. Uefune, and J. Takabayashi. 2018. Parasitoid wasps' exposure to host-infested plant volatiles affects their olfactory cognition of host-infested plants. Anim. Cogn. 21: 79–86.