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Effects of conspecific herbivory and mating status on host searching and oviposition behavior of *Plutella xylostella* (Lepidoptera: Plutellidae) in relation to its host, *Brassica oleracea* (Brassicales: Brassicaceae)

Suk Ling Wee[1,2,*]

Abstract

Knowledge of host chemical volatiles has tremendously informed the fundamental understanding of plant-insect interactions as well as revealed opportunities for the creative use of plant-derived chemicals in the food and flavor industries and in insect pest management. This study was undertaken to assess the host searching behaviors of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) females in relation to their mating status and conspecific larval herbivory. This involved measurement of odor-modulated upwind flight of female moths in a wind tunnel. Subsequently, the host location, recognition and acceptance behaviors of gravid females in relation to either intact uninfested or larvae-infested cabbage, *Brassica oleracea* L. (capitata group; Brassicales: Brassicaceae) hosts were compared in no-choice and dual-choice tests. Mating status had little effect on the female responses to host odor and flight duration. However, female moths were significantly more attracted to conspecific larvae-infested cabbage plants and had significantly shorter flights than in relation to intact uninfested cabbage hosts. Behavioral responses of gravid females were consistent when offered intact and larvae-infested cabbage hosts in both no-choice and choice tests in observation cages. Antennal rotation and ovipositor probing were found to be important in host searching and recognition, respectively, before a host was accepted for egg laying. Female moths oviposited significantly more eggs on larvae-infested cabbage than on intact uninfested cabbage and in particular more on leaves than on other parts of the cabbage plant. These results indicate the potential of developing a brassica host-derived kairomone attractant as a useful monitoring tool for female diamondback moths in area-wide integrated pest management programs that have a sterile insect technique (SIT) component.

Key Words: diamondback moth; virgin; mated female; larvae-infested cabbage; odor-modulated flight; oviposition activity

Resumen

El conocimiento de los volátiles químicos de los hospederos ha ayudado enormemente en la comprensión fundamental de las interacciones planta-insecto, así como las oportunidades reveladas para un uso creativo de los productos químicos derivados de plantas en la industria alimentaria y de sabor y en el control de plagas de insectos. Se realizó este estudio para evaluar el comportamiento de las hembras de la polilla de la col (*Plutella xylostella* L.; Lepidoptera: Plutellidae) de buscar el hospedero en relación con su estado de apareamiento y la herbivoría larval conespecífica. Este se trato de medir el vuelo contra el viento de las polillas hembras modulado por el olor de las hembras en un túnel de viento. Posteriormente, el comportamiento de las hembras grávidas de ubicar al hospedero, reconocer y aceptar en relación con el hospedero de repollo (*Brassica oleracea* L., grupo *capitata*; Brassicales: Brassicaceae) no infestado o infestado con larvas fueron comparados en pruebas de no elección y de doble elección. El estado de apareamiento tuvo poco efecto sobre la respuesta de las hembras hacia el olor y la duración del vuelo. Sin embargo, las polillas hembras fueron significativamente más atraídas por las plantas de repollo infestadas con larvas de la misma especie y tenía vuelos significativamente más cortos que en relación con las plantas hospederas de repollo intactas no infestadas. La respuesta de comportamiento de las hembras grávidas fueron consistentes cuando se les ofrece hospederos de repollo intactos e infestados con larvas tanto en las pruebas de no-elección y de elección en las jaulas de observación. La rotación antenal y la acción de probar con el ovipositor resultaron ser importante en la búsqueda de hospedero y el reconocimiento, respectivamente, antes de que se aceptó un hospedero para la puesta de huevos. Las polillas hembras ovipositaron significativamente más huevos en el repollo infestados con larvas que en el repollo no infestado intacto y, en particular, más en las hojas que en otras partes de la planta del repollo. Estos resultados indican el potencial de desarrollar un atrayente de kairomona derivado de una hospedera brassica como una herramienta útil para el monitoreo de las hembras de polilla de dorso de diamante en los programas de manejo integrado de plagas de área amplia que tienen un componente de la técnica del insecto estéril (TIE).

Palabras Clave: polilla de dorso de diamante; virgen; hembra apareada; repollo infestado por larvas; vuelo modulado por el olor; actividad de oviposición

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Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a specialist pest of brassicas. Its mobility and rapidly evolved resistance against many insecticides makes the management of this major pest species extremely difficult (Talekar & Shelton 1993; Chapman et al. 2002; Zhao et al. 2002). Due to the high commercial value of brassica crops (FAO 1992), much research has been conducted on the biology and ecology of *P. xylostella* to develop better control and management strategies (Hartcourt 1957; Gupta & Thorsteinson 1960; Chow et al. 1977; Renwick & Radke 1990; Talekar & Shelton 1993; Reddy & Guerrero 2000; Furlong et al. 2013). The diamondback moth became the first insect pest to develop resistance to the environmentally friendly microbial biopesticide *Bacillus thuringiensis* (Tabashnik et al. 1990), and since then, the demand for more sustainable management of this moth pest has intensified, leading to a revival of integrated pest management (IPM) strategies.

The sterile insect technique (SIT) is regarded as an important complementary tool in area-wide integrated pest management (AW-IPM) against a selected number of key insect pests (Dyck et al. 2005). In AW-IPM programs with a SIT component against lepidopteran pests, sex pheromone-baited traps or live female-baited traps are often used to attract and kill the male moths for population suppression prior to the release of sterile males, as well as for monitoring the effectiveness of a program with a SIT component by assessing the sterile to wild male ratio (Dyck et al. 2005; Suckling et al. 2005). However, female moths, which release the sex pheromone to attract male moths and whose progeny are responsible for crop damage, cannot be trapped to allow accurate assessment of the direct effect of the release of sterile males, i.e., to determine if the trapped females are inseminated by wild or sterile males, and as a direct measurement of the overall quality and sexual competitiveness of sterile males compared with wild males. Hence, the development of female attractants would be very useful to enhance the management and monitoring of AW-IPM programs for lepidopteran pests that have a sterile male release component.

In general, insects depend on host-related olfactory cues for food, and mating and oviposition site selection (Feeny et al. 1989; Pittendrigh & Pivnick 1993; Pivnick et al. 1994; Justus & Mitchell 1996; Landolt & Philips 1997; Couty et al. 2006; Anton et al. 2007; Masante-Roca et al. 2007; Silva & Furlong 2012).). Thus, understanding the response of *P. xylostella* to host plant volatiles appears essential for the discovery and development of an effective female attractant. When tested in a no-choice experimental set-up, volatiles emanating from intact uninfested and conspecific larvae-infested brassica hosts were attractive to gravid female *P. xylostella* (Reddy & Guerrero 2000; Reddy et al. 2004; Couty et al. 2006) with the larvae-infested hosts being more attractive than the intact uninfested hosts.

Most insect species exhibit a repertoire of post-mating behavioral and physiological changes that can be observed in dietary preference, stimulation of ovulation and reduction of courtship receptivity (Wolfner 1997; Jin & Gong 2001; Jang 2002; Tsukamoto et al. 2014). Generally, gravid females were found to be more sensitive to host-derived volatiles because they were ready to deposit eggs (Mechaber et al. 2002) although there were also exceptions (Martel et al. 2009). It is not known, however, when given a choice, how mating status will affect the behavioral response to host odor of a specialist pest like the diamondback moth. Another question that remains unanswered is how the status of host plant physiology (intact uninfested versus larvae infested) influences the host searching behavior and the female's final decision to deposit their eggs.

Amongst the brassica hosts, cabbage, *Brassica oleracea* L. (capitata group; Brassicales: Brassicaceae), was reported to be the preferred host of *P. xylostella* (Sant et al. 1982; Reddy et al. 2004). This study aimed to determine how physiological changes in female moths related to their mating status and conspecific larval damage of the host plant would affect the female's olfactory perception in relation to host searching, recognition and acceptance in no-choice and dual-choice bioassays. Such information would allow a better understanding of insect-host interactions and the female decision on whether or not to oviposit. It is hoped that the findings will contribute to the development of a plant-derived female diamondback moth attractant for use in novel IPM-based strategies against *P. xylostella* as well as to improve the current assessment technique used in lepidopteran SIT programs.

Materials and Methods

SOURCE OF INSECTS

A diamondback moth culture was sourced from a colony maintained at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia. The laboratory stock had been maintained for 68 generations. This stock had originated from local brassica farms and was reinvigorated regularly with addition of wild moths. Eggs were collected on corrugated aluminum foil, and larvae were reared on an artificial diet held in foam cups. The sexes were separated during the late instar larval stage using the male gonads at the 8–9 abdominal segments as the discriminating trait. Pupae were kept individually in plastic containers until adult emergence. Newly emerged female moths were separated randomly into 2 groups designated to be either virgin or mated.

To prepare mated females, each virgin female moth was paired with 2 virgin males in a 15 mL specimen container, covered with mesh cloth, and observed for mating during the late evening. Females that had mated were used as gravid females 1 d after the first mating whereas females of the same age deprived from mating, were used as virgin controls. All moths were used only once and then returned to the colony for subsequent egg production. All adults were provided with a 10% honey solution and maintained under a 12:12 h L:D cycle at 25–29 °C and 85–90% RH. Moths were used in the behavioral bioassays within the first 4 h after the onset of the scotophase and usually between 20:00 and 22:00 h.

CABBAGE PLANTS

Cabbage seeds were purchased from commercial markets, and cabbage plants were planted in plastic pots in a screened greenhouse and kept under a fine mesh cloth tent for protection from aphids or other insects. All cabbage plants were 6–8 weeks old and had approximately 9–11 leaves. Larvae-infested cabbage plants were prepared by placing 10 second or third instar larvae on each plant at a density of 1 or 2 larvae per leaf for 48 h. Just before the start of each experiment, all larvae were removed from the infested plant.

FEMALE MOTH RESPONSES TO INTACT UNINFESTED AND LARVAE-INFESTED CABBAGE IN WIND TUNNEL BIOASSAYS

The attractiveness of either larvae-infested or uninfested cabbage plants to female *P. xylostella* was assessed separately in a wind tunnel. Observations under dimmed red lights (ca. 5 lux) were made on the flight behavior of female moths responding to the volatiles emanating from a potted infested cabbage plant, placed 15 cm from the upwind end of a wind tunnel. The wind tunnel (30 cm diam × 200 cm long) was made of plastic acetate sheets that were replaced regularly to avoid possible contamination. A continuous flow of outdoor air, regulated by voltage regulators and electric exhaust fans placed at the upwind and downwind ends, was filtered through a laminated honey comb struc-

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ture and blown into the tunnel at a constant speed of 25–30 cm/s. A 13-cm high release platform and the source of attraction were placed approximately 15 cm away from the downwind and upwind ends of the wind tunnel, respectively. The total distance between the release platform and the attractant source was 170 cm.

Two to 3 mated females, housed in an open-ended rolled acetate sheet, which was supported by the release platform, were released at the downwind end of the wind tunnel. A response was recorded as positive when the moths carried out odor-modulated flights within 10 min of the start of the bioassay by flying upwind to the odor source placed at the upwind end. The odor-modulated flight (also known as an anemotaxis flight) was first described by Kennedy & Marsh (1974) as a directional flight response against the air flow that is guided and motivated by chemical stimuli. The responding females of *P. xylostella* often performed several short flights intermittently with walking—as they are not strong flyers—and eventually landed at the upwind end either on the wall of the tunnel or on the source of attraction. In addition, the total time taken for each moth to arrive at the upwind end was recorded. Gravid females were tested separately for their response to both intact uninfested and larvae-infested cabbage plants on different days. There were about 3 to 8 trials each night, depending on insect availability. Between each trial, the wind tunnel was cleaned with 70% ethanol. Experiments were replicated 20–25 times using 65 and 55 test females for the intact uninfested and infested cabbages, respectively.

MATING STATUS OF FEMALE MOTHS AND THEIR RESPONSES TO LARVAE-INFESTED CABBAGE IN WIND TUNNEL BIOASSAYS

The effect of mating status on the responses of female *P. xylostella* to host volatiles was assessed using the wind tunnel protocol as described above with the exception that the responses of virgin and mated females towards infested cabbage plants were assayed separately on different nights. Each treatment was replicated 33 times.

FEMALE OVIPOSITION BEHAVIOR IN NO-CHOICE BIOASSAYS IN SCREENED CAGES

In no-choice bioassays, 2 mated females were released into a screened observation cage (40 × 40 × 40 cm; mesh size: 0.1 × 0.1 cm) containing either an intact uninfested or a larvae-infested cabbage plant. Female behavior was observed for 90 min under dimmed red light after the start of the scotophase. The behavior of the female moths was divided into 3 major phases in relation to the host plant: (a) host location—behavior before or during host searching, i.e., quiescent, antennal grooming, walking/flying, antennal rotation (after Justus & Mitchell 1996), and landing attempts on host, (b) host recognition—behavior after landing on a host plant, i.e., quiescent, walking/flying, antennal preening, antennae rotation, palpitation with the antennae and probing with the ovipositor on the host plant, and (c) host acceptance—a final decision made by a female to deposit her eggs after finding a suitable oviposition site.

During this experiment, moth behavior was observed continuously, and the frequency of each behavior was scored. At the end of the 90 min observation period, the females were left in the screened observation cage throughout the scotophase for further oviposition activity. The number of eggs oviposited during the scotophase and their distribution on the plant, i.e., leaf petiole, stem, either upper or lower leaf surfaces, was assessed under a stereomicroscope on the following morning. In addition, for eggs oviposited on leaves, the leaves were further differentiated by their maturity/position within the plant starting from the most mature/bottom leaves as leaf-1, followed by leaf-2, etc. A total of 10 replicates were carried out for each treatment (intact uninfested and larvae-infested cabbage).

FEMALE OVIPOSITION BEHAVIOR IN DUAL-CHOICE BIOASSAYS IN SCREENED CAGES

Similar experimental procedures were followed as in the no-choice experiment, except that in the dual choice bioassay a larger arena was used (65 × 65 × 65 cm) and both intact uninfested and larval-infested cabbage plants were presented simultaneously to the test moths. Behavioral data of the moths as well as egg distribution on different parts of the intact uninfested and larvae-infested plants were collected in the same manner as in the no-choice bioassays, except that the host landing behavior in the host location phase was further differentiated between a landing on an infested or an intact uninfested cabbage plant.

STATISTICAL ANALYSIS

Data of moth responses from wind tunnel bioassays were arcsine square root transformed (Anscombe 1948) and further analyzed using a Shapiro-Wilk Normality Test ($P = 0.05$). A one-way ANOVA (for normally distributed data) or a Kruskal-Wallis ANOVA (for non-normally distributed data) were used for, followed by Tukey's test for multiple mean comparison. For comparison of the means between intact uninfested and infested cabbage plants for each behavioral category, a Student's *t*-test (for normally distributed data) or a Mann-Whitney U test (for non-normally distributed data) were used ($P = 0.05$). SigmaPlot 12.0 software was used for all analyses.

Results

FEMALE MOTH RESPONSES TO INTACT UNINFESTED AND LARVAE-INFESTED CABBAGE IN WIND TUNNEL BIOASSAYS

In the wind tunnel bioassays, *P. xylostella* females responded more strongly to the volatiles emanating from larvae-infested (37.5 ± 4.0%) than from intact uninfested (18.0 ± 4.0%) cabbage plants with significantly more moths engaging in odor-modulated flights (Mann-Whitney U = 125.5; $n_1 = 25$; $n_2 = 20$; $P = 0.003$). The female moths also made significantly shorter flights to reach the infested cabbage (3.71 ± 0.23 min, $n = 21$) than the intact uninfested (5.36 ± 0.28 min, $n = 27$) cabbage plants ($t = 4.39$; df = 46; $P < 0.0001$).

MATING STATUS OF FEMALE MOTHS AND THEIR RESPONSES TO LARVAE-INFESTED CABBAGE IN WIND TUNNEL BIOASSAYS

There was no significant difference in the percentage of odor-modulated flights between virgin (38.4 ± 4.7%) and mated (34.6 ± 5.0%) female *P. xylostella* toward larvae-infested cabbage (Mann-Whitney U = 446.0; $n_1 = n_2 = 33$; $P > 0.05$). There was also no significant difference in the mean flight duration of virgin (3.71 ± 0.23 min, $n = 21$) and mated (3.93 ± 0.25 min, $n = 24$) female moths from the release point and to the cabbage plant at the upwind end of the wind tunnel ($t = 0.636$; df = 43; $P > 0.05$).

FEMALE OVIPOSITION BEHAVIOR IN NO-CHOICE BIOASSAYS IN SCREENED CAGES

During the host location phase, gravid females in both the intact uninfested and infested cabbage treatments were engaged in similar behavioral activities, i.e., antennal rotation > flying/walking locomotion > quiescent > antennae grooming > host landing in decreasing order of frequency, throughout the observation period from 18:30 to 20:00 h ($H_{\text{intact uninfested}} = 38.34$; df = 4; $P < 0.001$; $H_{\text{infested}} = 28.36$; df = 4; $P < 0.001$) (Table 1). During this phase and for both treatments, females

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Table 1. Mean frequencies (± SE) of various behaviors shown by diamondback moth females towards intact uninfested or infested cabbage plants in 3 different behavioral phases, i.e., host location, host recognition and host acceptance, in a no-choice experiment conducted during the first 90 min of the scotophase.

Phases	Behavior	Mean frequency[a]		P^b
		Intact uninfested	Larvae-infested	
I. Host location	Quiescent	27.8 ± 2.0bc	18.3 ± 2.1abc	0.003
	Antennal grooming	3.4 ± 0.7ab	4.4 ± 1.5ab	0.878
	Flying/walking	46.6 ± 9.3c	31.9 ± 5.7c	1.960
	Antennal rotation	52.0 ± 9.1c	39.6 ± 7.6c	0.311
	Landing on host landing	1.0 ± 0.5a	3.5 ± 0.8a	0.018
II. Host recognition	Quiescent	0.2 ± 0.2a	4.2 ± 1.1ab	0.002
	Antennal grooming	0 ± 0 a	0.2 ± 0.2a	0.368
	Flying/walking	1.8 ± 0.9a	8.1 ± 2.1b	0.006
	Antennal rotation	1.6 ± 0.8a	8.2 ± 2.5b	0.012
	Antennal palpitation	0 ± 0a	1.0 ± 0.8a	0.168
	Probing with Ovipositor	1.8 ± 0.9a	11.4 ± 2.6b	0.003
III. Host acceptance	Egg laying	1.0 ± 0.5	3.4 ± 0.9	0.033

[a]Means within the same column of the same designated phase followed by the same letter are not significantly different at $P = 0.05$ (Tukey's test, $N = 10$).
[b]Probability of significance for means within the same row, i.e., between intact uninfested and infested cabbage plants, as tested by Student's *t*-test or Mann-Whitney U-test.

spent significantly more time with antennal rotation and flying/walking than all other activities. Nevertheless, there was no significant difference in female behavior between both treatments for any of the activities, except for quiescent ($t = 3.359$; df = 18; $P = 0.003$) and host landing attempts ($t = 2.611$; df = 18; $P = 0.018$) (Table 1). Infested plants elicited more frequent host landing attempts by the gravid females as compared with intact uninfested plants and the affected females were more frequently engaged in host location activity by having fewer rests.

After landing on the cabbage, females were more active on infested plants, with more flying/walking attempts on the host (Mann-Whitney U = 14.0, $n_1 = n_2 = 10$; $P = 0.006$), more antennal rotation (Mann-Whitney U = 17.0; $n_1 = n_2 = 10$; $P= 0.012$) as well as probing the cabbage leaf surface with her ovipositor (Mann-Whitney U = 10.5; $n_1 = n_2 = 10$; $P = 0.003$) as compared with females that had landed on intact uninfested hosts (Table 1). Accordingly, egg laying activity was significantly greater on infested than on intact uninfested hosts ($t = 2.312$; df = 18; $P = 0.033$) (Table 1).

At the end of the no-choice experiment, mated females had oviposited significantly more eggs on larvae-infested cabbage (31.2 ± 5.7 eggs per replicate) than on intact uninfested cabbage plants (9.0 ± 3.3 eggs per replicate) ($t = 3.36$; df = 18; $P = 0.0035$). There was no significant difference in the distribution of the eggs on the different parts of the intact cabbage plant ($F = 2.892$; df = 3,36; $P = 0.05$). However, mated females oviposited significantly more eggs on the leaves than any other parts of larval-infested cabbage plants ($F = 9.535$; df = 3,36; $P < 0.001$) (Table 2). Nevertheless, an equal number of eggs were deposited on the upper and lower surface of leaves (Table 2; $P > 0.05$).

Table 2. Numbers of eggs (mean ± SE) oviposited by newly mated diamondback moth females and their distribution on various plant parts of intact uninfested or larvae-infested cabbage in no-choice experiments.

Location	Eggs oviposited[a]	
	Intact uninfested	Larvae-infested
Lower leaf surface (abaxial)	4.4 ± 2.2 a	15.7 ± 4.0 a
Upper leaf surface (adaxial)	3.8 ± 1.7 a	12.5 ± 3.8 a
Stem	0.8 ± 0.5 a	1.9 ± 0.9 b
Leaf petiole	0 a	1.1 ± 0.5 b

[a]Means within the same column followed by the same letter are not significantly different at $P = 0.05$ (Tukey's test, $N = 10$).

In addition, females did not show any preference for young or mature leaves for both intact uninfested ($H = 12.138$; df = 9; $P = 0.206$) and larval-infested ($H = 15.441$; df = 9; $P = 0.08$) cabbage plants (Kruskal Wallis ANOVA on ranks).

FEMALE OVIPOSITION BEHAVIOR IN DUAL-CHOICE BIOASSAYS IN SCREENED CAGES

In dual choice tests, female behavior in response to host odor was similar to that observed in the no-choice experiment during the host location phase, with females significantly more engaged in antennal rotation, and flying/walking, although some females remained quiescent when first introduced to both intact uninfested and infested cabbage plants (Table 3). During the host recognition phase after making contact with either intact uninfested or larvae-infested cabbage plants, the females were more active on larvae-infested cabbage ($H = 27.18$; df = 5; $P < 0.001$) than on intact uninfested cabbage ($H = 4.57$; df = 5; $P > 0.05$) (Table 3). Females spent significantly more time probing with their ovipositors on infested than intact uninfested cabbage hosts, followed by antennal rotation and flying/walking. The frequency of egg laying activity was greater on infested than on intact uninfested cabbage plants, but the differences were statistically not significant ($P > 0.05$).

In the dual choice tests, females deposited significantly more eggs on larvae-infested (33.4 ± 5.2 eggs) than on intact uninfested (5.6 ± 1.8 eggs) cabbage plants ($t = 5.58$; df = 18; $P < 0.001$). There was also a significant difference in the selection of oviposition sites by females in the dual-choice test ($H = 59.591$; df = 7; $P < 0.001$) (Table 4). More eggs were oviposited on the leaves of infested cabbage than of intact uninfested cabbage but there was not a single egg deposited on any other parts of the plants (Tukey's test; $P < 0.05$) (Table 4).

Discussion

In many lepidopteran species, mating induce or enhances the response of the females to plant odors (Phellan & Baker 1987; Landolt 1989; Mechaber et al. 2002; Masante-Roca et al. 2007). This is related to physiological changes in female moths after mating that prompt the gravid females to become more sensitive to host plant odors because they need to deposit their eggs (Anton et al. 2007). However, cases have

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Table 3. Mean frequencies (± SE) of various behaviors shown by diamondback moth females towards intact uninfested and infested cabbage plants in 3 different phases designated as host location, host recognition and host acceptance in a dual-choice experiment conducted for the first 90 min of the scotophase.

Phases	Behavior	Mean frequency[a]		P^b
		Intact uninfested	Infested	
I. Host location	Quiescent[†]	11.7 ± 0.9b		
	Antennal grooming[†]	0.8 ± 0.3a		
	Flying/walking[†]	14.3 ± 1.8b		
	Antennal rotation[†]	18.5 ± 2.0b		
	Host landing	0.2 ± 0.1	1.2 ± 0.3	> 0.05
II. Host recognition	Quiescent	0.2 ± 0.1a	0.6 ± 0.5ab	> 0.05
	Antennal grooming	0a	0a	
	Flying/walking	0.8 ± 0.6a	3.5 ± 1.5ab	> 0.05
	Antennal rotation	0.8 ± 0.5a	3.5 ± 1.5ab	> 0.05
	Antennal palpitation	0a	0a	
	Ovipositor probing	0.6 ± 0.4a	4.0 ± 1.7b	< 0.05
III. Host acceptance	Egg laying	0.3 ± 0.2	2.2 ± 0.9	> 0.05

[†]Behavior of female moth before making a choice on whether to land either on an intact uninfested or on an infested cabbage plant.
[a]Means within the same column in different phases followed by the same letter are not significantly different at $P = 0.05$ (Tukey's test, $N = 10$).
[b]Probability of significance for means within the same row, i.e., between intact uninfested and infested cabbage plants, as tested by Student's *t*-test or Mann-Whitney U-test ($P = 0.05$).

been reported where virgin females were more sensitive to host odor than mated females, as in the case of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Martel et al. 2009). In addition, some extreme examples are known such as the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera : Pyralidae) and the cabbage moth, *Mamestra brassicae* (L.) (Lepidoptera : Noctuidae), where mated females respond to host odors, but virgin females do not (Phellan & Baker 1987; Rojas 1999). Our results with *P. xylostella* did not conform to any of the results mentioned above. In our experiment similar proportions of virgin and mated females responded to the cabbage host, and both types of females engaged in flights of similar duration to arrive at the cabbage host in wind tunnel bioassays. This suggests that mating status had little effect on the response of *P. xylostella* females towards host-related volatiles. This result corroborates those of Reddy et al. (2004) where both virgin and mated female *P. xylostella* were attracted to host plant volatiles. Hence, it is not possible to generalize the manner in which mating affects the female's olfactory perception of host plant volatiles.

Our findings that both virgin and mated females showed equal sensitivity towards the cabbage-derived volatiles are not surprising. Being a brassica specialist, the life history of *P. xylostella* largely revolves around its host plant. Mating of *P. xylostella* often occurs on brassica host plants on the same day as adult emergence (Talekar & Shelton 1993; Lee et al. 1995). During courtship, the virgin females call in proximity of their brassica hosts and release the sex pheromone to attract male moths for mating (Talekar & Shelton 1993; Lee et al. 1995). Olfactory cues from the brassica host plant were reported to affect the mating behavior of *P. xylostella* so that females started to call when they were younger, called earlier in the scotophase, and showed a tendency to extend the calling period (Pittendrigh & Pivnick 1993). Host cues also have a positive impact on the reproductive physiology of *P. xylostella* by accelerating egg maturation, increase mating incidence and the onset of oviposition behavior (Hillyer & Thorsteinson 1971; Pivnick et al. 1990). Since most lepidopteran neonates have limited dispersal possibilities and generally feed on nearby tissue following egg hatch, they largely depend on their mother for host selection during oviposition (Renwick & Radke 1990). Therefore, it appears likely that both virgin and gravid females of *P. xylostella* rely heavily on host plant volatiles for mating and oviposition.

The diamondback moth is a nocturnal insect, and our wind tunnel results indicate that the moth relies more on olfactory cues for host finding, selection and oviposition than on any other cues, e.g., visual ones. Mated female diamondback moths were more attracted to odors that emanated from the larvae-infested cabbage plant than those from intact uninfested cabbage plants—as measured by arrival rates in the wind tunnel. These results were consistently similar in both the no-choice and dual-choice tests in the screened cages. In the screened observation cage, more landings were attempted and more eggs were oviposited by gravid females on infested cabbage plants as compared with intact uninfested cabbage plants in both the no-choice and choice experiments. *Plutella xylostella* larvae that are feeding on the cabbage plant induce the plant to produce volatile isothiocyanates, which are highly attractive olfactory cues for female *P. xylostella* (Reed et al. 1989; Reddy & Guerero 2000; Renwick et al. 1990, 2006). Although this study did not directly demonstrate the qualitative or quantitative nature of the chemical stimuli released from the cabbage plant, it is evident that *P. xylostella* perceived infested cabbage plants as more attractive and more suitable as oviposition sites than the intact uninfested plants by making shorter flights with more direct flight paths toward infested plants than to intact uninfested plants both in wind tunnel and screen

Table 4. Numbers of eggs (mean ± SE) oviposited by mated diamondback moths and their distribution on intact uninfested and larvae-infested cabbage in dual-choice experiments.

Treatment	Plant part on which eggs were oviposited[a]			
	Leaf petiole	Stem	Upper leaf surface (adaxial)	Lower leaf surface (abaxial)
Intact uninfested	0 ± 0 a	0 ± 0 a	3.9 ± 1.4 ab	1.7 ± 1.1 ab
Larval-infested	0 ± 0 a	0 ± 0 a	15.8 ± 4.2 b	17.6 ± 2.7 b

[a]Means followed by the same letter are not significantly different at $P = 0.05$ (Tukey's test, $N = 10$).

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cage assays. The behaviors of gravid females during the host location phase, i.e., antennal rotation and flying/walking, were consistently and frequently observed regardless of whether the host was intact uninfested or infested and damaged. Insects often engage in antennal grooming to remove foreign particles from the antennae in order to maintain the olfactory acuity of the antennae (Böröczky et al. 2013). Antennal rotation on the other hand, is thought to help screening and possibly enhancing the probability of detection of odor molecules in the surrounding air by the multiporous sensilla on the antennae (Justus & Mitchell 1996). This behavior was observed in Lepidoptera and Hemiptera and has been linked to host detection (Chow et al. 1984; Justus & Mitchell 1996; Ventura & Panizzi 2000). In the observation cage, female moths were mostly engaged in the antennal rotation activity during the host location phase, and the frequency of moths being engaged in antennae rotation activity was significantly greater when the females were exposed to intact uninfested plants than to larvae-infested plants. Larvae-infested plants release higher quantities of host volatiles than intact plants (Geervliet et al. 1997), and this reduced the need of air sampling by the moths; hence, a lower frequency of antennal rotation activity as the odors were present in higher concentrations when the moths were placed in an environment with an infested plant. Similar observations were documented by Justus & Mitchell (1996) who observed that *P. xylostella* females spent significantly more time engaged in antennal rotation activity when host volatiles were absent. In the screened cage, females were frequently engaged in flying and walking during the host location phase in an effort to increase the chances of locating a host, as well as in directional movement following host location. Upon successfully locating the host, flying and walking was very much reduced in the host recognition phase.

Both olfactory and gustatory cues are critical stimuli for subsequent host acceptance and associated oviposition behavior (Klijnstra 1985). After landing on the plant and making first contact with the plant substrate, females rely on post-alighting gustatory perception of non-volatile glucosinolates by palpitation of the surface with their antennae and probing with the ovipositor to assess suitability of oviposition sites (Justus & Mitchell 1996). This was evidenced by a decrease in antennal rotation and increased of ovipositor probing in the host recognition phase as compared with the host location phase. Ovipositor probing is also known as abdomen curling in some studies, and is regarded as a precursor to oviposition in lepidopteran females (Matsuda 1976; Eaton 1986; Mechaber et al. 2002). In this study, diamondback moth females never oviposited a single egg without first engaging in ovipositor probing. Further observations revealed that the frequency of ovipositor probing was significantly greater on larvae-infested than on intact uninfested cabbage plants, and hence the females oviposited more eggs on larvae-infested plants. This finding is in agreement with that of Mechaber et al. (2012) on *M. sexta.* Justus & Mitchell (1996) described the act of antennation or antennae palpitation on plant substrates by *P. xylostella* as analogous to tarsal drumming by the cabbage butterfly, *Pieris* spp. (Lepidoptera : Pieridae). Such antennation is considered important for 'tasting' a potential oviposition substrate via the olfactory sensilla on the distal segments of the antennae (Chow et al. 1984). Chow et al. (1984) further emphasized that antenna palpitation is a primer to ovipositor probing because ovipositor probing does not occur unless preceded by antennal palpitation. Our results showed that the frequency of antennal palpitation was rather low or non-existent for moths that had landed on intact plants, but ovipositor probing was a prerequisite for oviposition. This might be related to the additional use of chemosensilla on the legs of *P. xylostella* that are always in close contact with the host plants when the female lands. Female diamondback moths were often observed to walk on the plant thus making contact with the tarsi. Gravid female lepidopterans have often been shown to use tarsal contact to determine potential oviposition sites on host and non-host plants, apart from antennal perception (Ramaswamy et al. 1987; Feeny et al. 1989).

Previous studies suggested that *P. xylostella* females would select the upper (Harcourt 1957; Justus et al. 2000) or the lower surface and stem of leaves (Marazzi et al. 2004; Reddy et al. 2004; Silva & Furlong 2012) as preferred oviposition sites. Our results tend to be in agreement with those of Badenes-Pérez et al. (2011) in that no clear preference was observed for oviposition on either the upper or lower surface of a leaf. No eggs were deposited on the stem or leaf petiole and most of the eggs were deposited on leaves irrespective whether the plant was intact uninfested or larvae-infested. Similarly, no clear preference for young or old leaves within the same host plant was observed in our study. The presence of uneven surfaces or crevices adjacent to the veins of the leaves may be the explanation for this choice. Preliminary scanning electron microscope investigations revealed the presence of dense and long non-porous sensilla on the ovipositor of the moth (unpublished data). These long sensilla are believed to play a role in the 'screening' for potential oviposition sites by detecting crevices on the host surface as potential oviposition sites.

The combined knowledge gained on the sex (pheromone-mediated) and brassica host-insect (kairomone-mediated) communication systems of *P. xylostella* may be important for manipulating the behavior of *P. xylostella* for control and management purposes. The incorporation of some host plant volatiles particularly those from the green leaves might have the potential to enhance the performance and efficacy of pheromone-baited traps (Landolt & Philips 1997; Li et al. 2000; Reddy & Guerrero 2000; Dai et al. 2008). The results obtained here are encouraging and show the potential of developing a host-derived kairomone that may target both virgin and mated *P. xylostella* females. Therefore, it is hoped that this knowledge may provide brassica growers with additional and highly advantageous options for improved integrated management of the pest leading to reductions in insecticide use, and to steer them away from the conventional preventive application of broad spectrum insecticides.

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