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Oviposition Site Selection by the Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Although Plutella xylostella *(L.) is a worldwide pest of cruciferous crops, relatively little is known about its oviposition behavior. This study was undertaken to provide necessary information about mechanisms involved in* P. xylostella *host selection. Four oviposition behaviors were described. Moths were given artificial substrates treated with water, sinigrin, or* Brassica napus *(cv. Westar) squashes, combined with* B. napus *volatiles and/or grooves in the substrate. No eggs were deposited in the absence of olfactory and gustatory stimuli. Moths given gustatory but not olfactory stimuli deposited similar numbers of eggs but spent significantly more time performing olfactory-related behaviors. Conversely, moths given olfactory but not gustatory stimuli did not oviposit. Substrate grooves did not influence egg numbers but appeared to influence egg location. The order in which oviposition behaviors occur and the relative importance of stimuli type are discussed.*

KEY WORDS: oviposition behavior; insect-plant interactions; Cruciferae.

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), is a major cosmopolitan pest of cruciferous crops. Cabbage and rapeseed crops can be devastated by *P. xylostella* larvae feeding on the leaves, stems, vegetable curds, and seed pods. A number of investigators have examined the attraction of secondary compounds of crucifers (i.e., glucosinolates and volatile mustard oils) to ovipositing dia-

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mondback moths (see Gupta and Thorsteinson, 1960; Reed *et al.*, 1989; Renwick and Radke, 1990). However, details about the nature of such attractants and their importance to ovipositing *P. xylostella* are virtually unknown. In contrast, investigations of other cruciferous pests, such as *Delia radicum* and *Pieris rapae*, have demonstrated factors involved in host plant location (Nottingham, 1988; Renwick and Radke, 1988) and critical stimuli important for host acceptance and associated ovipositional behaviors (Klijnstra, 1985).

Chemical and microbial controls are no longer effective against some populations of diamondback moth, and alternative control strategies are urgently required. While life history knowledge is crucial for successful IPM strategies, behavioral information is also essential for understanding plant–insect interactions (Opp and Prokopy, 1986). Such information could be crucial for the development of novel control strategies of this moth.

This report of mechanisms of host selection by *P. xylostella* is intended to provide the necessary foundation for the development of management strategies. It is also the first step in developing a better understanding of the role of environmental features (such as chemical and mechanical stimuli from the host plant) in oviposition behavior.

MATERIALS AND METHODS

Moths

A colony of *P. xylostella* was reared in the laboratory on *Brassica napus* cv. Westar (27°C, 70% RH, 16:8 L:D), and adults were provided with a mixture of honey and water. Pupae were held singly in corked glass vials (8 cm × 2.5-cm diameter) until emergence. Newly emerged adults were sexed and then placed in a glass cage (2.7 × 17.3 × 29.6 cm) as a group (four to eight) at an approximate 50:50 male:female ratio. Honey–water and a freshly cut stem of *B. napus* in water were also placed in this "mating cage." Moths were allowed to interact freely for a 3-day period. On the fourth day, females were removed from the mating cage and placed into individual oviposition arenas (8-cm × 2.5-cm-diameter glass vials) that contained one of the treated substrates.

Oviposition Arenas

Lunar Blue 60-lb bond paper (Smead) was cut into 1.2 × 10-cm strips. Prior to each experiment, a 1.2 × 2.0-cm area at one end of the strip was treated in one of the following ways: painted with distilled H_2O and allowed to dry (H2O), painted with 0.5 m*M* sinigrin (AldrichChemical Co.) dissolved in H_2O and allowed to dry (SIN), or rubbed with a squashed *B. napus* leaf (NAP). Grooves (GRV) were made in the paper (at the treated area) using the blunt side of a one-sided razor blade. The treated strip was then placed vertically in an oviposition arena containing a female moth and held in place by a cork. For assays involving host volatiles only (VOL), arenas containing an H2O-treated substrate were sealed with fiberglass screening (1-mm mesh) and held approximately 3 cm above fresh *B. napus* homogenate so that odors should have been detectable but contact with the homogenate impossible. (See Table II for combinations of treatments and sample sizes.)

Observations

A single moth was introduced to an oviposition arena at the onset of the scotophase and observed with the aid of a red safe light (General Electric Model 810-S, Kodak filter series 1A). Continuous focal sampling was recorded on audiocassette tapes, and the information transferred to Observer 2.0 software (Noldus Information Technology, 1989). Locations and numbers of eggs were noted during the observation period and immediately following the 8-h scotophase.

Data Analysis

First-order transition sequences were illustrated in transition matrices (preceding behavior × succeeding behavior) for each moth, and *G* tests used to test for similarities between moths. Cells on the descending diagonal of matrices held structural zeros since a behavior could not be followed by itself [i.e., considered to be a continuation of the behavior (Slater, 1973)]. Expected values of behavior transitions were calculated using Stephen–Denning iterative proportional fitting (Jobson, 1992). Observed and expected transitions were tested for significance using chi-square goodness of fit. Kruskal Wallis nonparametric ANOVAS were used for egg count and behavior duration data, and significant differences were identified using Mann–Whitney *U* tests.

RESULTS

Descriptions of behaviors are given in Table I and selected behaviors are depicted in Figs. 1a–g.

Rates at which the antennae struck the substrate during antennation were relatively constant, at 2.5 ± 0.3 strikes per s ($\bar{x} \pm$ SD), for all moths regardless of substrate. Rate of OS was slower when OS led to egg deposition (Fig. 2), which was attributed to a decrease in OS rate just prior to OV (Fig. 3). This decrease in OS rate, combined with cessation of walking, was labeled the OS determinant phase (OS^{dt}). A decrease in OS rate was not observed when OS did not lead to OV.

Table I. Ethogram: Behaviors Performed by Ovipositing *P. xylostella*

*Still (ST)[a]	No visible movement. Moth is standing. Antennae are forward. Posterior end of wings nearly touch the substrate (Fig. 1a).
Antennal rotation (AR)	Antennae are independently rotated posteriorly, laterally, and then anteriorly to the resting position. Antennae may rotate in unison or separately, not necessarily alternating. The arc of rotation varies greatly within individuals (Figs. 1b, c). AR can occur while insect is walking or standing.
Antennation (AN)	Antennae simultaneously and repeatedly strike the surface of the substrate with the most distal segment(s). Angle of the antennae decreases with respect to the head via muscles of the scape and pedicel, and angle of the head with respect to the body diminishes by bending at the head-thorax junction (Fig. 1d). Usually occurs while insect is walking but can occur while standing.
Ovipositor sweep (OS)	Distal abdominal segments bend, lifting the wings and lowering the ovipositor. Ovipositor touches substrate at a point lateral to the longitudinal axis of the moth (Fig. 1e). Ovipositor is swept mediad from the lateral point (Figs. 1f, g). Sweep usually alternates sides but can occur on the same side twice before changing sides. OS occurs while insect is walking but can continue if walking ceases (see results Fig. 3). OS occurs at relatively fast rate while moth is walking (termed OS search phase) and slows after walking ceases (termed OS determinant phase).
Oviposition (OV)	A single egg is discharged through the ovipositor as the abdomen moves side to side slightly. Egg is glued on its side to the substrate.
*Proboscis extension (PE)	Proboscis is extended to touch substrate. (Not performed by all individuals; can occur in conjunction with AR.)
*Grooming (GR)	Foreleg is placed at base of pedicel of antenna and slid down the length of antenna in a combing fashion. Moth is standing.

[a] Behaviors marked with an asterisk are grouped as "other" in the kinematic diagram (Fig. 5).

First-order behavior transitions did not differ significantly between moths that oviposited and were combined in one matrix. Observed behavior transitions significantly differed from expected ($P < 0.0001$) (Fig. 4). Probabilities of behavior transitions were calculated from 27 ovipositing moths (see Table II) and are given in a kinematic diagram (Fig. 5). Ovipositing moths followed a linear sequence ($\mathrm{AR} \rightarrow \mathrm{AN} \rightarrow \mathrm{OS}^{sr} \rightarrow \mathrm{OS}^{dt} \rightarrow \mathrm{OV}$) but did not necessarily complete each attempt. Also, moths could bypass AN and enter OS directly from AR only if they had already performed AN but had not yet entered OV or "other" states. Any attempt could be aborted, in which case a moth would return to a previous behavior. The sequence was not usually aborted once OS^{dt} occurred (Fig. 3). A complete oviposition sequence resulted in the deposition of one egg. Only 13.6% of oviposition attempts resulted in egg deposition.

Time between oviposition events was highly variable (range, 73.8–1068.1

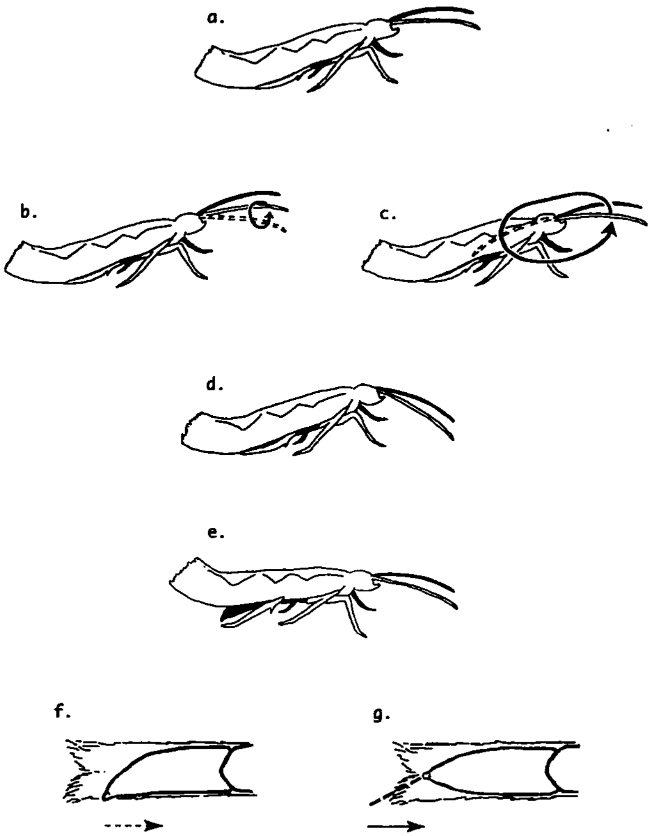

Fig. 1. Oviposition behaviors of *P. xylostella:* (a) resting position; (b) antennal rotation—acute arc; (c) antennal rotation—obtuse arc; (d) antennation—head is lowered and antennae strike surface of substrate; (e) ovipositor sweep; (f) ovipositor sweep (ventral view)—starting point of one sweep (dashed arrow = direction and distance moth will travel); (g) ovipositor sweep (ventral view)—end point of sweep (dashed line = ovipositor dragged on substrate; arrow = direction and distance traveled by moth).

s). However, when each oviposition event was partitioned into durations of AR, AN, and OS, moths given substrates that included volatiles (i.e., SIN+VOL, NAP, and NAP+GRV) spent significantly less time performing AR than moths given nonvolatile substrates (i.e., SIN and SIN+GRV) ($P < 0.0001$; Table III). (Note that H2O+VOL was not included since this did not result in oviposition.) Time spent performing AN and OS did not differ between moths regardless of substrate treatment (Table III).

Mean number of eggs deposited during the scotophase was not significantly

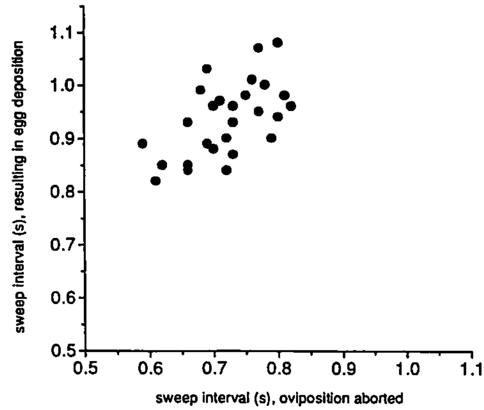

Fig. 2. Ovipositor sweep intervals of 27 moths. Each data point represents the mean sweep interval when egg deposition occurred (*y* axis) and the mean sweep interval when the oviposition sequence was aborted (*x* axis) of each moth.

different between treatments (Table IV), except for treatments lacking contact stimuli which received no eggs (Table II). However, a proportionately greater number of eggs were deposited in the first hour of the scotophase when moths were given both volatile and contact stimuli (Table IV).

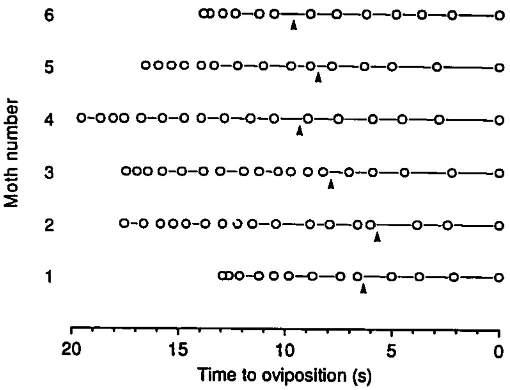

Fig. 3. Diagrammatic representation of OS intervals prior to one oviposition event by six moths. O represents one sweep. Time 0 is egg deposition. Arrowheads indicate cessation of walking.

OBSERVED TRANSITIONS:

preceding behavior	succeeding behavior				
	AR	AN	OS	OV	TTL
AR	—	519	278	0	797
AN	388	—	301	0	689
OS	267	170	—	142	579
OV	129	0	0	—	129
TTL	784	689	579	142	2194

EXPECTED TRANSITIONS:

	succeeding behavior				
	AR	AN	OS	OV	TTL
AR	—	418.2	317.1	61.7	797
AN	407.8	—	235.4	45.9	689.1
OS	309	235.3	—	34.7	579
OV	55.1	42	31.9	—	129
TTL	771.9	695.5	584.4	142.3	2194.

Fig. 4. Behavioral transition matrices: observed (left) and expected (right) transitions calculated using Stephen–Denning iterative proportional fitting. AR, antennal rotation; AN, antennation; OS, ovipositor sweep; OV, oviposition; TTL, total.

DISCUSSION

Regardless of substrate, first order transition probabilities were similar between all moths that oviposited. Also, transition probabilities differed from random, suggesting that oviposition is a structured program. Three of the seven behaviors identified in ovipositing moths (PE, GR, and ST) were considered ancillary behaviors because they were not observed in all ovipositing moths, did not occur with any regular periodicity, and have also been observed in males and virgin females (personal observation). *Plutella xylostella* exhibits four oviposition behaviors that appear to be organized as a linear sequence, with each subsequent behavior depending on the previous one. However, this sequence is probabilistic rather than deterministic because the probability of a complete sequence is less than 100%. While there appears to be no regular periodicity to egg deposition, several external factors influence ovipositing *P. xylostella*, and

Table II. Behaviors and Egg Placement by *P. xylostella* Presented with Treated Substrates

Treatment	*n*	Behaviors completed	Egg placement
H_2O	4	AR & ST	No eggs
H_2O+VOL	5	AR, AN, & other[a]	No eggs
SIN	4	AR, AN, OS, OV, & other	100% on substrate
SIN+GRV	5	AR, AN, OS, OV, & other	96% on substrate 62% in grooves
SIN+VOL	4	AR, AN, OS, OV, & other	92% on substrate
NAP	7	AR, AN, OS, OV, & other	93% on substrate
NAP+GRV	7	AR, AN, OS, OV, & other	95% on substrate 85% in grooves

[a] "Others" are ST, PE, and GR, but not all moths exhibited these behaviors.

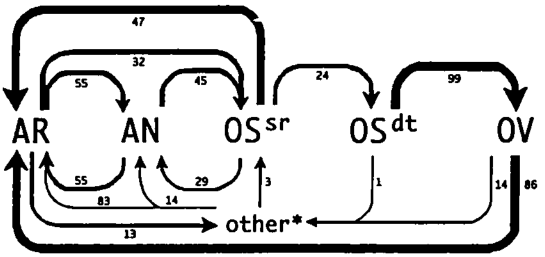

Fig. 5. Kinematic diagram (n = 27 moths). Thickness of lines represent likelihood of occurrence. Numbers are percentage probability of transition. AR, antennal rotation; AN, antennation; OS^{sr}, ovipositor sweep search phase; OS^{dt}, ovipositor sweep determinant phase; OV, oviposition. Other* includes grooming, proboscis extension, and still; see Table I.

the probability of oviposition depends to some degree on the perception of those factors.

We suspect AR to be involved in host detection because of the presence of multiporous sensilla on the antennae (Chow *et al.*, 1984; Justus and Mitchell, in preparation). Such movements by antennae allow moths to sample larger volumes of air and create wind vortices around the antennae, both of which would facilitate sensillar contact with odorant molecules. This is analogous to "sniffing" in mammals and antennule flicking in lobsters. Although this does not presume contact with a receptive surface, it should enhance that probability. Palaniswamy *et al.* (1986) noted that both male and female *P. xylostella* respond to host plant volatiles, and Gupta and Thorsteinson (1960) reported that allyl isothiocyanate (the volatile produced when sinigrin is hydrolyzed), enhanced

Table III. Time Spent Performing Antennal Rotation (AR), Antennation (AN), and Ovipositor Sweeping (OS) During Individual Oviposition Sequences (Expressed as Range of % Total Time for Each Oviposition Event) for Moths Given Substrates Treated with Sinigrin (SIN), Plant Juice (NAP), Volatiles (VOL), and Grooves (GRV)

Substrate	n	% AR*	% AN[a]	% OS[a]
SIN	4	63.9-79.9^{a}	2.7-6.3	7.9-67.6
SIN+GRV	5	62.1-80.4^{a}	5.5-12.9	5.6-23.7
SIN+VOL	4	27.2-54.1^{b}	7.0-27.2	4.1-40.0
NAP	7	29.6-55.2^{b}	2.2-31.3	15.3-73.6
NAP+GRV	7	21.9-57.9^{b}	2.1-24.6	26.8-75.7

[a] No significant difference between treatments.

*Scores followed by different superscript letters are significantly different between treatments ($P <$ 0.0001; Mann-Whitney U test).

Table IV. Oviposition Rate: Number of Eggs Oviposited During the First Hour of the Scotophase and During the Entire (8-h) Scotophase

		Mean number of eggs ± SD	
Substrate	n	First hour*	Scotophase[a]
SIN	4	3.25 ± 0.5^a	27.25 ± 6.7
SIN+GRV	5	5.20 ± 1.9^a	24.40 ± 7.6
SIN+VOL	4	11.00 ± 5.4^b	26.00 ± 7.5
NAP	7	18.40 ± 10.3^b	27.80 ± 17.4
NAP+GRV	7	14.70 ± 7.9^b	31.30 ± 15.5

[a] No significant difference between treatments.

*Scores followed by different superscript letters are significantly different between treatments ($P <$ 0.0001; Mann-Whitney U test).

oviposition. Our study showed that moths given both volatile and contact stimuli deposit significantly more eggs during the first hour of the scotophase than those without volatiles. In addition, moths spent significantly more time involved in AR when volatiles were absent than when volatiles were present. We consider AR to be involved in host location, but caution that it may be of secondary importance in such small enclosures since moths will explore and oviposit even in the absence of olfactory cues.

Presumably, AR has some effect on AN because moths not given volatiles spend a larger proportion of time in AR before making the transition to AN. The precise nature of the relationship between AR and AN is not understood and requires further study; some threshold of volatile may trigger the switch to AN, though there is sufficient motivation to investigate by AN inspite of a lack of olfactory input.

AN is likely the first mechanism by which moths "taste" a potential oviposition substrate via uniporous sensilla present on the distal segments of the antennae (Chow *et al.*, 1984; Justus and Mitchell, in preparation). Faucheux (1991) described uniporous sensilla on the antennae of *Homoeosoma nebulella*, as did Cuperus (1986) for three species of Yponomeutidae (*Yponomeuta vigintipunctatus*, *Y. cagnagellus*, and *Adoxophyes orana*), but neither study noted whether antennae were involved in oviposition behavior. Conversely, Chadha and Roome (1980) observed similar AN behavior in *Chilo partellus* but did not describe antennal sensilla.

AN is analogous to the tarsal drumming of *Pieris* sp. and appears to be a primer for OS, since AN only proceeds to OS (assuming input is positive/sufficient) or returns to AR (assuming input is negative/insufficient), and OS does not occur if AN does not occur.

OS appears to include a "search phase" and a "determinant phase." The search phase is characterized by a relatively fast rate of sweeping and is the period in which gustatory and tactile information seem to be most important in oviposition site selection. It is during the OS search phase that moths may abort the sequence and begin again at either AR or AN. Four to six uniporous sensilla are present on the anal papillae of the ovipositor and are surrounded by many long, aporous sensilla (Justus and Mitchell, in preparation) that we believe are mechanosensory in function. Thus, OS probably has two functions—allowing the moth both to "taste" and to "feel" a potential egg deposition site—and these two mechanisms of site selection probably act in tandem.

Although contact chemosensilla on the ovipositor may initially seem to be redundant to those of the antennae, we do not know if the sensilla of the antennae and ovipositor respond to the same stimuli. There is some speculation in the literature that antennal taste sensilla are used for conspecific identification via contact pheromones. However, if these receptors do respond to host plant chemicals, OS may act as a "safety check" for depositing an egg at a specific site, since there is a relatively large distance between antennal tip and ovipositor; the antennae may identify a site that is not contiguous to the site of egg deposition. Egg location is important for larval survival because first-instar larvae are leaf miners and desiccate very quickly if prevented from burrowing under the protective plant cuticle (personal observation).

Chilo partellus and *Ostrinia nubilalis* have similar arrangements of contact chemo- and mechanosensilla (Chadha and Roome, 1980; Marion-Poll *et al.*, 1992). Both species appear to use these sensory hairs for detecting and selecting an oviposition site. In particular, mechanosensilla are used for arranging eggs in orderly rows. Because *P. xylostella* do not lay eggs in batches and prefer to deposit eggs in crevices, we believe that the long aporous sensilla are the means by which such crevices are located. Crevices may offer protection to eggs or, perhaps, allow larvae easier access to parenchymatous tissues. We hypothesized that the absence of grooves in the substrate would lead to a higher proportion of time spent performing OS. That was not the case and we presume that while grooves are preferred, and mechanosensory hairs facilitate the perception of such grooves, they are not a required stimulus for egg release but are involved in egg placement.

The OS determinant phase is denoted by a marked decrease in OS rate and by the cessation of walking. Once this phase begins, it nearly always leads to egg deposition. Because OS^{dt} and OV are so closely linked, we suspect that OS^{dt} facilitates abdominal muscle contractions and the movement of the egg through the ovipositor, as in *Bombyx mori* (Yamaoka *et al.*, 1971).

Renwick and Radke (1990) demonstrated that glucosinolates are important to ovipositing *P. xylostella*. We suggest that the most important influence on host selection by *P. xylostella*, is the presence of contact stimuli (such as sinigrin) that may be perceived via contact chemosensilla on the antennae, tarsi, and ovipositor. Host plant volatiles are likely involved in host location as discussed by Palaniswamy *et al.* (1986) and, also, enhance egg deposition rate. The presence of grooves does not appear to induce oviposition by *P. xylostella* but does bias egg placement.

Further investigations are required to understand better the oviposition behavior of *P. xylostella*. For example, are the antennal and ovipositor chemosensilla stimulated by the same chemicals? Are tarsal sensilla active in substrate selection? What governs the transition from AR to AN or from AN to OS? If the oviposition sequence is not completed, does a moth leave a potential host to search out another? We are currently exploring the first of these questions through electrophysiological investigations of the antennal, tarsal, and ovipositor contact chemosensilla.

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