Synergism in the Oviposition Behavior of *Plutella xylostella*: Sinigrin and Wax Compounds

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Diamondback moth, *Plutella xylostella*, host examining and oviposition behaviors were measured in response to sinigrin dosages (10⁻³, 10⁻⁴, and 10⁻⁵ M) and controls with and without the addition of n-alkanes. Individual females were presented with a treatment and videotaped while an observer documented specific behaviors during 5-min observation periods. Behavior in response to sinigrin alone was not significantly different from that in response to controls. Alkane alone significantly reduced movement rate during treatment contact, but did not significantly affect other behaviors. Sinigrin concentrations combined with alkane significantly slowed the rate of insect movement, increased turning, and led to significantly longer treatment encounter durations. Behavior changes in response to sinigrin + alkane increased insect exposure to the sinigrin concentrations and led to greater oviposition compared to that in response to sinigrin treatments alone. The synergistic effect that mixing sinigrin and alkane has on *P. xylostella* behavior arises because the additional time females spend in contact with the treatment increases the rate at which they experience the available stimuli. Involvement of the antennae during examining of a treatment, referred to as “swabbing,” was usually associated with oviposition on alkane-coated sinigrin treatments. The presence of alkane may alter the way sinigrin is perceived. Oviposition in response to the treatment combinations was also tested in overnight bioassays. The pattern of oviposition in response to treatments during bioassays differed from that established during observations. The value of direct observations and the mechanistic interpretations they allow are emphasized.

**KEY WORDS:** *Plutella xylostella*; diamondback moth; arrestment; excitation; n-alkanes; oviposition; sinigrin; synergy; wax.

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INTRODUCTION

An insect herbivore, having alighted on a host, is exposed to a variety of stimuli associated with a plant’s visual, physical, and chemical features. Nonetheless, host selection for many insects is thought to be mediated by appropriate concentrations of “key” host plant chemicals (Renwick and Radke, 1983; Städdler, 1992; Honda, 1995). Among crucifer specialists, for example, glucosinolates and their breakdown products are primary feeding and oviposition stimulants (Gupta and Thorsteinson, 1960; Renwick and Radke, 1983; Talekar and Shelton, 1993). However, as studies with artificial hosts have demonstrated, the effectiveness of “key” host cues can be greatly enhanced or reduced in the presence or absence of presumed subordinate cues (Harris and Miller, 1988; Harris and Rose, 1990; Roessingh and Städdler, 1990; Spencer, 1996). Furthermore, mixtures of multiple host plant constituents are often more stimulatory than even so-called “key” stimuli (Renwick and Radke, 1983; Woodhead, 1983; Schöni et al., 1987; Visser and Jong, 1988, Roessingh et al., 1992; Pivnick et al., 1994), and in some cases mixtures yield more than additive increases in response (Lampman and Metcalf, 1987; Feeny et al., 1988; Renwick and Chew, 1994).

When a response to a stimulus mixture is greater than the sum of responses to the individual components, the interaction is said to be a synergistic one. In insects, synergistic responses to combinations of stimuli from multiple sensory modalities (olfactory/gustatory, visual, and tactile) or those within a single modality are well documented (Hamilton et al., 1979; Harris and Miller, 1982; 1988; Feeny et al., 1988; Harris and Rose, 1990; Foster and Harris, 1992; Beehler et al., 1993; Kostal, 1993; Renwick and Chew, 1994; Spencer, 1996). Unfortunately, evidence of synergistic interactions between stimuli often comes from end-result bioassays, few of which address behavior directly, and consequently mechanisms underlying a synergy are left largely unexplored. Direct observations can return the same end results and provide a mechanistic understanding of phenomena such as synergy (Eigenbrode et al., 1995; Eigenbrode and Bernays, 1997).

Observations and bioassays with the diamondback moth, Plutella xylostella, a worldwide pest of cruciferous crops, revealed that the combination of alkane waxes with host-specific chemical cues had a synergistic affect on oviposition (Spencer, 1996). Oviposition in response to a combination of host-specific chemical cues (sinigrin or aqueous cabbage leaf homogenates) and wax (paraffin or a mixture of \(n\)-alkanes including those occurring on host plant surfaces) was 3–20 times greater than that on substrates treated with only host-specific cues (Spencer, 1996). Wax alone does not stimulate \(P.\ xylostella\) oviposition, however, it can synergize a female’s response to sinigrin leading to greatly increased oviposition. In this study we address the behavioral mechanisms behind synergistic interactions between alkane and sinigrin, by focusing on \(P.\ xylostella\) behavior and oviposition on sinigrin-treated substrates with or without alkane.
MATERIALS AND METHODS

Insects. Adult *P. xylostella* (1988 Geneva, NY, strain) were maintained on a 16L:8D photoperiod at 28°C in 30 x 30 x 30-cm screened cages and provided ad libitum with a 10% sucrose solution. Spencer (1996) provides details of the egg collection, larval diet, and rearing process. The insects used in these experiments were 2- to 4-day-old reproductively mature females and were tested within 30 min of their collection from culture cages.

Oviposition Substrate Preparation. Substrates were made from Reynolds 656 Standard aluminum foil. Because physical features can influence *P. xylostella* oviposition (Gupta and Thorsteinson, 1960), a standard 1-cm-diameter pattern of surface ridges was embossed in the center of each 5 x 7-cm oviposition substrate with a circular plastic die bearing a set of six approximately parallel grooves. A raised circular rim was added around the ridges by pressing the open end of a shell vial (1.4-cm diameter) into the foil. The rim circumscribed the pattern of ridges, defining the boundaries of a treatment well, and confined sinigrin and alkane solutions to the embossed region during substrate preparation.

**Sinigrin.** Sinigrin [as sinigrin monohydrate (C\textsubscript{10}H\textsubscript{10}NO\textsubscript{9}S\textsubscript{2}K\textsubscript{2}O) (Sigma Chemical Company)], a known glucosinolate oviposition stimulant found in many hosts of *P. xylostella*, was prepared at 10\textsuperscript{-3}, 10\textsuperscript{-4}, and 10\textsuperscript{-5}M in 70% MeOH and stored at 4°C. One 0.015-ml drop of sinigrin solution was dispensed to the treatment well of each sinigrin treatment substrate, and the solvent was evaporated at 34°C for about 10-20 min before proceeding. A drop of 70% MeOH dispensed into an untreated well and allowed to evaporate at 34°C was used as a solvent control (hereafter referred to as “MeOH”). The untreated control was an embossed oviposition substrate with no other treatment.

**Alkane Wax.** Pure n-alkanes of different chain lengths (hereafter “alkane”) (C\textsubscript{16}, C\textsubscript{17}, C\textsubscript{18}, C\textsubscript{19}, C\textsubscript{20}, C\textsubscript{21}, C\textsubscript{23}, C\textsubscript{25}, C\textsubscript{28}, and C\textsubscript{29}; Aldrich Chemical Co.) were prepared individually at 0.01 g/ml in hexane. Equal volumes of each single alkane solution were combined to make a 10-alkane mixture with a total wax concentration of 0.01 g/ml. To prepare a treatment with alkane, a 0.033-ml drop of the alkane mixture was dispensed in a substrate well, completely covering the previously applied and dried sinigrin or MeOH treatment. The treated foil was set aside and the hexane allowed to evaporate before use. Fresh substrates were prepared each day. At 0.01 g/ml, the alkane load in a treatment well was 21 μg alkane/m\textsuperscript{2}. This falls within the range of epicuticular wax loads (10 to 75 μg/cm\textsuperscript{2}) reported on the surface of susceptible cabbage varieties (Eigenbrode *et al.*, 1991). Previous experiments indicated that oviposition on sinigrin was greatest at alkane loads between 2.1 and 21 μg/cm\textsuperscript{2} (Spencer, unpublished).

Experiment. One replicate consisted of 10 treatment combinations: five basic treatments (sinigrin at 10\textsuperscript{-3}, 10\textsuperscript{-4}, and 10\textsuperscript{-5}M and the MeOH control, tested
with or without addition of the alkane mixture, and an untreated control, which was always tested without alkane). A given replicate was completed over a 2-day period: on the first day, all five basic treatments were tested without alkane; on the following day, the sinigrin and MeOH treatments were prepared with alkane (as described above) and tested along with the untreated control. A fresh insect and substrate were used for each test.

Individual female *P. xylostella* were observed as they interacted with one treatment combination. The observer, given only numbered treatments to test, was unaware of which sinigrin treatment was being tested; the presence or absence of alkane could not be concealed. Approximately 1 h was required to complete observations with a set of five treatments. To avoid observer fatigue, no more than three sets of five treatments (with or without alkane) were tested per day. Sixteen replicates of each combination were conducted (160 individual observations, each about 5–8 min in duration).

**Observations.** The behaviors of individual *P. xylostella* were observed while insects were in an observational arena under subdued (96-lux) fluorescent lighting. The arena, constructed from the top of a 50-mm-diameter petri dish, was centered over an oviposition substrate. Insects entered the arena through a 1-cm-diameter port glued into a hole in the arena’s side. A 2-ml Eppendorf tube, containing a *P. xylostella* female, was slipped over the end of the port. Most insects entered the arena within a few seconds; if a female did not immediately enter, the insect was coaxed into the arena by advancing a cotton-tipped plunger from the bottom of the tube.

In the arena, females were given 3 min to encounter the treatment well; otherwise they were discarded and another observation was initiated with a new female and substrate. Once a female encountered the treatment, she was observed for an additional 5 min, after which the observation ended. The behavior of each female was recorded on a Panasonic AG1970 VCR through an NEC NX 18AS CCD Color Television Camera (NEC Corporation, Tokyo) equipped with a Navitar Zoom 7000 macro lens (Navitar Inc., 200 Commerce Dr., Rochester, NY 14623) mounted 60 cm above the arena. Specific details of movement, location, and oviposition were simultaneously recorded using Observer 3.0 event recording software (Noldus Information Technology, Wageningen, The Netherlands).

Track angles, rate of displacement, and turning data for females on and near a treatment were obtained by later making tracings onto acetate and digitizing the insect’s position and body-axis orientation during its initial encounter with the treatment well for each of the 160 video records. Insect position was plotted at 20 frame intervals (resolution was 30 frames/s) for 3 s (five 20-frame bins) preceding the initial contact and following the last physical contact with the treated region. While in contact with a treatment, insect paths were traced for up to 5 s (eight 20-frame bins). Path deviation was measured as the degree change in direction of movement (heading) between the point of initial contact (designated
and the point of last contact with a treatment well. Insects moving over the treatment without turning would generate a 0° path deviation angle; one turning completely around before exiting would generate a 180° path deviation angle. To simplify data collection, cumulative turning (total degrees turned per female during the first encounter with a treatment) was classified as being either <90, >90 but <360, or >360°.

Supplemental Bioassay. An overnight oviposition bioassay was conducted using the same treatment combinations as tested above to compare oviposition patterns between observations and a long-term bioassay. Using the methods of Spencer (1996), a female was held with a single treatment and allowed to oviposit until the following morning (ca. 15 h later) when eggs laid on the treatments were counted. Each of the 10 treatment combinations were replicated 12 times.

Analysis. The observational experiment and the supplemental bioassay were both conducted as a two-factor factorial, with sinigrin and alkane treatment as factors with five and two levels, respectively. For each measured quantity, the degrees of freedom, F ratios, and P values relating the effects of sinigrin alone, alkane alone, and sinigrin × alkane interactions are presented in Table 1. When ANOVA indicated significant sinigrin × alkane interactions, mean separation using Fisher’s protected LSD procedure, with $\alpha = 0.05$, was carried out on all 10 sinigrin × alkane combinations. When ANOVA indicated significant effects without interactions between sinigrin and alkane treatment, the x-axis in the figures is broken and multiple-comparison results (Fisher’s protected LSD) indicate significance between sinigrin treatments within alkane treatment. Cumulative turning distributions were analyzed with chi-square tests. Paired t tests ($\alpha = 0.05$) were used to compare movement rates between the intervals before, during, and after contact with treatments.

RESULTS

Arrestment

Sinigrin Dosages Combined with Alkane Promoted Arrestment on Contact. P. xylostella movement rate was significantly affected by sinigrin + alkane treatments during treatment contact, however, there were no significant differences in movement rate among treatments without alkane before, during, or after treatment contact (Fig. 1). Because there were no significant differences in movement rate among the sinigrin concentrations (with or without alkane), those treatment means were pooled for this analysis. The lack of any significant differences in P. xylostella movement rate before treatment contact suggests that the treatments were not significant sources of stimulatory olfactory cues (Fig. 1A). During similar observations, antennectomy did not affect the elapsed time to contact the same treatments compared to that for intact P. xylostella (Spencer, unpublished).
Table I. Significance of Alkane and Sinigrin Effects and Their Interactions on the Behavior of *P. xylostella* Observed While on Oviposition Substrates Bearing Sinigrin Concentrations With or Without Alkane

<table>
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<td>Sinigrin</td>
<td>Interaction</td>
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<td>P</td>
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<td>2</td>
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Sinigrin and Alkane Synergy

Fig. 1. Mean (±SE) \( P. xylostella \) movement rates (cm/s) (A) before, (B) during, and (C) after losing contact with a treatment. \( n = 16 \) for untreated and MeOH treatments; \( n = 48 \) for the pooled sinigrin \((10^{-3} - 10^{-5} \text{ M})\) concentrations. Data were analyzed by two-factor ANOVA, followed by Fisher’s protected LSD procedure. Treatments bearing the same letter are not significantly different at \( \alpha = 0.05 \). The broken x-axis indicates that there were no significant interactions between sinigrin and alkane and that multiple-comparison results are for sinigrin concentrations within alkane treatment. See Table I for additional ANOVA results.

During contact, movement rates were significantly slower for insects on treatments with alkane (Fig. 1B); insects on sinigrin treatments + alkane moved the slowest of all. Compared to the rates before contact (Fig. 1A), movement was significantly increased when treatments (including untreated) without alkane
were contacted (Fig. 1B) \( (P = 0.04, t = 2.3, \text{df} = 14) \). Movement rates decreased significantly when sinigrin treatments + alkane were contacted \( (P < 0.0001, t = 4.5, \text{df} = 45, \text{paired} \, t \text{test}) \). Movement rates for females on MeOH + alkane did not change significantly \( (P = 0.11, t = 1.7, \text{df} = 15) \) once treatment contact occurred.

After loss of treatment contact, movement rates declined significantly for treatments without alkane \( (P < 0.0001, t = 5.9, \text{df} = 92) \). Movement rates for females on sinigrin and MeOH treatments + alkane did not change significantly \( (P = 0.07, t = 1.9, \text{df} = 39, \text{and} \, P = 0.11, t = 1.7, \text{df} = 15, \text{respectively}) \), however the movement rate on sinigrin + alkane remained significantly lower than that on MeOH + alkane (Fig. 1C). After loss of treatment contact, rates of movement for insects were not significantly different from those before contacting treatments with alkane \( (P = 0.88, t = 0.146, \text{df} = 92) \), movement rates before and after treatment contact were significantly different only for females on sinigrin and MeOH treatments + alkane \( (P = 0.05, t = 2.0, \text{df} = 37, \text{and} \, P = 0.01, t = 3.0, \text{df} = 15, \text{respectively}) \).

The Addition of Alkane to Sinigrin Significantly Increased the Time in Contact with Treatments. The total duration of contact with a treatment is a function of the visitation frequency and the per-visit encounter duration. Treatment visitation frequency was significantly affected by sinigrin treatment \( (F = 2.46, \text{df} = 4, P = 0.048) \); it ranged from 10.3 visits per female on \( 10^{-3} \text{M} \) sinigrin without alkane to 5.1 visits per female on untreated. There was no significant effect of alkane on visit frequency \( (F = 1.0, \text{df} = 1, P = 0.32) \). First encounter durations varied across treatments by more than 100-fold (Fig. 2). Mean encounter durations paralleled first encounter durations, ranging over 80-fold, from 1.5 s for untreated to 115.9 s for \( 10^{-3} \text{M} \) sinigrin + alkane. Mean encounter duration overwhelms the effect of visit frequency differences, contributing disproportionately to the pattern of total encounter (Fig. 3).

Excitation

Path Deviation Was Increased After Encountering Sinigrin with Alkane. Sinigrin combined with alkane significantly affected \textit{P. xylostella} path deviation (Fig. 4). The paths of most females deviated only slightly from a straight line when crossing alkane-free treatments. Because there were no significant differences in path deviation among the three sinigrin concentrations (with or without alkane), those treatment means were pooled for this analysis. In the presence of alkane, path deviation was significantly increased on sinigrin treatments (Fig. 4). Overall, path deviation was also significantly greater in the presence of alkane \( (x = 79.1\pm9.7^\circ; \text{mean} \pm \text{SE}) \) than without it \( (x = 43.2\pm6.1^\circ) \). Contrast between sinigrin treatments with or without alkane, \( F = 13.2, P = 0.0004, \text{df} = 1 \). There was no difference in path deviation between control treatments with and those with-
Sinigrin and Alkane Synergy

Fig. 2. Mean (±SE) first encounter durations for female *P. xylostella* contacting sinigrin concentrations with or without alkane. Encounter duration was measured from beginning to end of a female’s first encounter with a treatment. Data analysis is described in the legend to Fig. 1. *n* = 16 for each treatment combination. See Table I for additional ANOVA results.

Sinigrin concentration (M)

![Graph showing mean first encounter durations](image)

**Fig. 2.** Mean (±SE) first encounter durations for female *P. xylostella* contacting sinigrin concentrations with or without alkane. Encounter duration was measured from beginning to end of a female’s first encounter with a treatment. Data analysis is described in the legend to Fig. 1. *n* = 16 for each treatment combination. See Table I for additional ANOVA results.

Out alkane (*x* = 36.5 ± 6.4° and *x* = 30.0 ± 4.8°, respectively; contrast between untreated and MeOH without alkane vs MeOH + alkane, *F* = 0.35, *P* = 0.55, df = 1). Cumulative turning while contacting sinigrin with or without alkane was also significantly greater than that on non-sinigrin-containing controls (*χ²* = 31.5, *P* < 0.001, df = 2, and *χ²* = 10.0, *P* < 0.01, df = 2, respectively). Cumulative turning was significantly greater in the presence of alkane (*χ²* = 32.0, *P* < 0.001, df = 2).

In addition to turning more, a significantly greater proportion of females on sinigrin + alkane returned to the treatment within 3 s after leaving it than did those on sinigrin without alkane (0.33 vs 0.13; *χ²* = 4.78, *P* < 0.05, 1 df). Notably, 6 of 48 females on a sinigrin + alkane treatment never left them after first contact; no females remained continually on any treatment lacking alkane.

**Sinigrin with Alkane Increased the Likelihood of Antennal Swabbing.** Swabbing is a behavior where the distal half of each antenna is rubbed over the treatment surface while the insect moves forward, pivoting slowly side to side. Swabbing was significantly more likely among females (20 of 27 females...
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Fig. 3. Total time in contact with a treatment (±SE) (total encounter duration) of a possible 300 s. Data analysis is described in the legend to Fig. 1. \( n = 16 \) for each treatment combination. See Table 1 for additional ANOVA results.

= 74%) when oviposition was observed than when no eggs were laid (21 of 131 females = 16%) (\( \chi^2 = 36.3, P < 0.0001, \text{df} = 1 \)). Swabbing was also significantly more frequent when sinigrin was treated with alkane (33 of 47 females = 70.2%) than when it was not (7 of 48 females = 14.6%) (\( \chi^2 = 27.9, P < 0.0001, \text{df} = 1 \)). In 95% (20/21) of the records where swabbing was observed but eggs were not laid, the treatment was one with sinigrin; 14 of those females (70%) were on a sinigrin + alkane treatment. When egg-laying occurred during the first treatment encounter, it was exclusively on a sinigrin + alkane treatment and was always preceded by swabbing (\( n = 13 \)). Overall, 41 females swabbed treatment surfaces. In 40 of these occurrences individuals were on a sinigrin-bearing treatment (33 on sinigrin + alkane and 7 on sinigrin alone).

Oviposition

*Sinigrin with Alkane Was Favored for Oviposition.* Forty percent of females (19/48) on a sinigrin + alkane treatment laid one egg or more during their
first encounter with a treatment. No eggs were laid during any first encounter with a sinigrin treatment without alkane (Fig. 5). Oviposition rate (total eggs laid on a treatment/total time in contact with a treatment) increased significantly with sinigrin concentration in the presence of alkane (Fig. 6).

**Test Duration Affected the Pattern of Response to Treatments.** Unlike the observational results for total eggs laid per female (Fig. 7A), significant numbers of eggs were deposited on sinigrin treatments lacking alkane during overnight no-choice bioassays (Fig. 7B). Significant effects of sinigrin dosage on the distribution of eggs among sinigrin + alkane treatments were also not found during overnight bioassays (Fig. 7B).

**DISCUSSION**

**Effect of Sinigrin and Physical Features**

Sinigrin and other glucosinolates are known to have excitatory effects on *P. xylostella* (Gupta and Thorsteinson, 1960; Talekar and Shelton, 1993; Spencer, 1996). During our observations, the *P. xylostella* response to sinigrin without alkane was modest at best. However, sinigrin dosages that did not stimulate significant oviposition or behavior responses during observations received signifi-
Fig. 5. Mean eggs laid per female (±SE) during her first encounter with a sinigrin concentration. Data analysis is described in the legend to Fig. 1. n = 16 for each treatment combination. See Table I for additional ANOVA results.

significantly more eggs than controls during overnight bioassays. A longer period of exposure may be required before sinigrin alone can evoke responses like those observed to follow quickly after contact with sinigrin + alkane. Sinigrin alone is stimulatory, but the rate at which insects respond to its stimulation is slow compared to that for sinigrin + alkane.

Physical features of the test substrate appear to have caused excitation without arrestment. The ridges embossed in the treatment wells are the only shared feature among the treatments where movement rate increased during contact, suggesting that encountering just the ridges may be mildly excitatory (Figs. 1A and B). The increased movement cannot be due to the presence of sinigrin. Gupta and Thorsteinson (1960) demonstrated that physical features (“rugosity”) could affect *P. xylostella* oviposition. Perhaps the ridges constitute a minimal leaf recognition cue, stimulating insects to increase their movement rate? If correct, insects encountering sinigrin or MeOH with alkane would also have experienced a similar ridge-induced excitation. However, where sinigrin and alkane were together, a significant reduction in movement rate occurred, suggesting an arrestment-promoting role for the combination that countered or masked the increase in rate attributable to the ridges.
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Fig. 6. Egg-laying rate (eggs/min ± SE) per female while contacting sinigrin concentrations. Data analysis is described in the legend to Fig. 1. n = 16 for each treatment combination. See Table I for additional ANOVA results.

**n-Alkanes**

Previously, the mixture of 10 n-alkanes used in this experiment was demonstrated to have no significant independent effects on the behavior of *P. xylostella* (Spencer, 1996). The major effects of alkane [and other waxes previously tested (Spencer, 1996)] on examining and oviposition behavior seem to be dependent upon combination with a host-specific chemical such as sinigrin or sinigrin-containing host-plant extracts (Spencer, 1996).

In the current experiment, however, a significant arrestment-promoting effect of alkane is suggested by the reduced movement rate of females on MeOH + alkane compared to that of untreated females (Fig. 1B). In addition, the lack of a drop in movement rate after losing contact with MeOH + alkane, combined with significant rate reductions on treatments without alkane, suggests that there may be a short-term lingering effect of alkane exposure on *P. xylostella* movement.
Sinigrin and Alkane

The addition of alkane to sinigrin dramatically changed the behavior of *P. xylostella*. Females in contact with sinigrin + alkane treatments displayed movement patterns that increased their exposure to a treatment. Increased turning upon encountering a stimulus is an effective way to remain arrested in its vicinity, although it needs to be accompanied by a reduction in movement rate to have
a major effect (Kennedy, 1978). Reduced movement rates, absent when sinigrin was presented alone, along with increased path deviation and greater cumulative turning promoted sustained arrestment in the vicinity of a sinigrin + alkane treatment. At the highest sinigrin concentrations + alkane, insects were in contact with the treatment well for more than 80% of the available observation period. Contact with sinigrin + alkane led to rapid oviposition. The number of eggs laid was positively associated with the total encounter duration on a treatment ($F = 206.4, P < 0.0001, r^2 = 0.56$). The oviposition rate of *P. xylostella* on sinigrin + alkane also was greater on high sinigrin concentrations when alkane was present. From these data, we may hypothesize the existence of a stimulation threshold that must be exceeded before eggs are laid. How rapidly the threshold is exceeded may be a function of the stimulus concentration and the cumulative exposure time.

With equivalent exposure times, *P. xylostella* on a high sinigrin concentration + alkane treatment are more rapidly (or more frequently) stimulated beyond the threshold for oviposition than are individuals on treatments with lower sinigrin concentrations. We presume that more eggs were laid on higher sinigrin concentrations + alkane during observations because less exposure time was required to exceed the stimulation threshold. Some oviposition by females encountering sinigrin without alkane indicates that they are stimulated by sinigrin, but because their total encounter durations were low, an observation ended before most accumulated sufficient treatment exposure to exceed the threshold for oviposition.

Unlike 5-min observations, an overnight bioassay provides a long period for treatment exposure so that even on less stimulatory treatments the threshold is eventually exceeded and are eggs laid. When time is not limiting, similar egg counts on a range of stimulatory treatment combinations likely reflect similar egg loads rather than an absent differential response to stimulus concentration. Dissimilar patterns of response to sinigrin concentrations + alkane during observations versus overnight bioassays illustrate how extended exposure can obscure differences between treatments (Fig. 7).

When rates of response to different stimuli vary (e.g., egg-laying rates), but the capacity to respond is finite (e.g., eggload), a long duration assay will compromise the test’s ability to detect real differences between treatments (i.e., Type II errors will be more likely). The likelihood of Type II error can also increase if slow, but significant, responses to treatments are investigated over too short a time interval because differential responses to stimuli will have insufficient time to develop. However, when response rates are very low (e.g., oviposition on sinigrin concentrations without alkane), an overnight bioassay proves to be an efficient way to identify differences. Since these Type II errors arise when the response rate is poorly matched to the duration of the assay, increased replication cannot decrease the error rate.
Combining sinigrin with alkanes changes *P. xylostella* behavior in such a way that insects respond rapidly to a host-specific stimulus. The presence of alkane may alter the way sinigrin is perceived. *P. xylostella* normally wave their antennae continually through the air and occasionally tap them on the substrate surface while walking over potential hosts. However, during exposure to sinigrin + alkane-treated substrates, *P. xylostella* use their antennae to swab over the treatment surface. This behavior was initiated upon contact with sinigrin + alkane and was associated with imminent oviposition. The first response to a stimulatory treatment occurred when either tarsi or antennae contacted the stimuli in a treatment well and generally consisted of a turn toward a stimulatory site and brief arrestment. Swabbing, changes in movement rate, turning frequency, and other behaviors were usually observed immediately after the insect resumed movement.

The ovipositor tip, which also features prominently in *P. xylostella* host examining (Justus, 1996), probably does not play as major a role as the antennae in the interaction between sinigrin and alkane. Given a choice, antennectomized females do not discriminate between alkane-treated and untreated sinigrin when ovipositing; however, females with at least one intact antenna concentrate their oviposition on sinigrin + alkane (Spencer, unpublished).

A comparison between the patterns of oviposition on sinigrin treatments during observations and those during overnight bioassays suggests that longer exposure is the key to significant oviposition on less stimulatory treatments and implies that the rate of stimulation may be less on treatments like sinigrin without alkane. If the *P. xylostella* decision to deposit an egg depends on exceeding a fixed stimulation threshold, then the elapsed time in contact with potential hosts becomes an important variable in host selection. Lack of sustained arrestment around even a highly stimulatory site may greatly delay or prevent acceptance of a host because stimulation is incremented too slowly. A similar response in the field could result in movement away from a potential host after only brief contact or greatly increased examining before investment. Manipulation of plant characteristics that most significantly affect arrestment could slow the rate at which pests inflict damage to plants.

Our results illustrate how small changes in simple movement parameters, turn angles, and movement rate can lead to divergent patterns of behavior. Without sustained arrestment, promoted by alkane on sinigrin, the impact of sinigrin as an oviposition stimulant was greatly diminished. Perhaps alkanes, and waxes in general, contribute a general plant recognition cue which “focuses attention” (sensu Bernays, 1996) on relevant host cues and releases behaviors not seen at the same concentration without benefit of attention.
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