Factors affecting oviposition and orientation by female *Plodia interpunctella*

T.W. Phillips [*†] and M.R. Strand[†]

Abstract

Female Indianmeal moths, *Plodia interpunctella*, are attracted to food volatiles, and oviposit in response to secretions by conspecific larvae. Female moths oviposited on dishes containing larval rearing medium (food), and cornmeal was found to be the active component of this mixture. Cheesecloth contaminated by different densities of fifth instar larvae elicited more oviposition than untreated cheesecloth or dishes with food. The combination of larval contamination and food was preferred over food only or larval contamination only in both two- and four-choice experiments. The factor(s) in larval contamination responsible for eliciting oviposition was extracted in hexane, confirming that organic semiochemicals are responsible for the effect. In two-choice wind tunnel bioassays, female moths initiated flight only when food was present in one of the treatments, and they displayed the highest landing responses to a combination of larval contamination and food. Earlier work on *P. interpunctella* and related pyralid species found that secretions from the mandibular glands of larvae acted as both a spacing pheromone for wandering larvae and as a kairomone for host-seeking parasitoid wasps. The present study suggests that a similar secretion acts as an oviposition eliciting pheromone for conspecific females, and that there is a distinct interaction between food and larval secretion.

Introduction

The Indianmeal moth, *Plodia interpunctella* (Hübner) feeds on stored grains, legumes, nuts, dried fruits and other food products maintained in storage. Research on the chemical ecology of *P. interpunctella* has dealt primarily with identification and activity of female sex pheromones (e.g. Brady et al. 1971; Mankin et al. 1983). Although subtle differences in sex pheromone systems occur, at least four species of the stored-product Phycitinae share a major pheromone component that is responsible in part for cross-attraction (e.g. Phelan and Baker 1986). Synthetic pheromone-baited traps are commercially available to monitor activity of males of *P. interpunctella*. However, little is known about the orientation of *P. interpunctella* females to stored products, or whether specific oviposition stimuli exist.

Research on other species of Phycitinae suggest probable mechanisms that mediate host orientation and oviposition in *P. interpunctella*. *Ephestia kuehniella* (Walker) laid more eggs on substrates contaminated by conspecific wandering fifth instar larvae than on untreated surfaces (Corbet 1973). An oviposition stimulant was found in secretions from the larval mandibular glands, but no compounds were identified, nor was the activity of any larval food investigated (Corbet 1973). *Amyelois transitella* (Walker) preferentially oviposited in the vicinity of host fruits infested with conspecific larvae over uninfested fruits (Andrews and Barnes 1982). Barrer (1977) found that *Cadra* (=*Ephestia*) *cautella* (Walker) oviposited in response to volatiles from a grain-based rearing medium. Phelan et al. (1991) recently identified five fatty acids from almond oil that elicited upwind orientation of *A. transitella*. The studies above suggest that chemical factors associated with both conspecific larvae and larval food may influence female host selection behaviour.

Here we review our previous findings (Phillips and Strand 1994) and discuss additional experiments designed to determine the relative importance and interaction of food and larval secretions in oviposition and flight behaviour of female *P. interpunctella*. Our studies found that larval contamination elicited stronger close-range oviposition responses than food, but that food was required to elicit upwind flight orientation from a distance. The combination of food and larval secretions, however, elicited stronger responses than either component alone in both close-range and long-range orientation.

Methods

P. interpunctella were reared on a standardised diet of corn meal, chick starter mash, and glycerine 1: 1: 1 (v/v/v). Ten gravid females were placed in ventilated 500 mL jars containing 400 g of diet and maintained at 27°C with a 16: 8 (L: D) photoperiod. Larvae used in experiments were in the wandering phase of the fifth instar. Newly emerged adult moths were collected daily and held in plastic boxes (10 × 22 × 30 cm) at 27°C and a 16: 8 (L: D) photoperiod. Females usually mated during the first or second day after emergence and started egg laying during scotophase of day four. Thus, four-day-old mated females were used in all experiments.

The influence of food and larval contamination on *P. interpunctella* oviposition was examined in two and four-choice bioassays conducted during scotophase under the rearing conditions described above. In two-choice assays, a single female was introduced into a 10 × 22 × 30 cm plastic box that contained two potential oviposition sites. These sites consisted of 60 × 15 mm petri dishes overlaid with a circular piece of cheesecloth, about 70 mm in diameter, placed about 10 cm apart on the floor of the box. Moths generally laid eggs directly on the cheesecloth patch when the appropriate stimulus was present in the dish or on the cheesecloth. The influence of larval food was tested by filling petri dishes with 10 g of our standard rearing diet (see earlier) or with components of the diet. The effects of different levels of larval contamination were examined by allowing 5, 20 or 50 larvae to crawl on the cheesecloth patches for 24 hours prior to the experiment. Petri dishes were filled with food and/or overlaid with cheesecloth

* USDA ARS Stored-Product Insect Research Unit., Madison, WI USA 53706

† Department of Entomology, 237 Russell Laboratories, University of Wisconsin, Madison, WI, USA, 53706.

immediately prior to introducing a female. In all oviposition experiments a single moth was introduced into the bioassay arena 1 hour prior to scotophase and the number of eggs laid on the cheesecloth pieces or within 1 cm beyond the edge of a dish was counted the following day. Females that laid five or fewer eggs were assumed to be unmated and were omitted from the analysis. The following series of two-choice experiments were conducted: 1) food vs. an empty dish; 2) the three components of the rearing diet (cornmeal, chick feed, or glycerine) vs. an empty dish; 3) larval contamination vs. an empty dish; 4) larval contamination vs. food; 5) a combination of larval contamination with food vs. food; and 6) a combination of larval contamination with food vs. larval contamination.

Extracts of larval contamination were made to determine if the biological activity of these depositions was chemical in nature. One, 5, and 20, larvae were allowed to wander for 24 hours on cheesecloth patches overlaid with a clean glass petri dish (60x15 mm) as described previously. The contaminated cheesecloth and the inside surfaces of the glass dish were extracted with a total of 5.0 mL of hexane (HPLC grade); ten dish/patch preparations were extracted separately for each larval density. Uncontaminated patches were extracted as controls. Extracts were concentrated under a stream of nitrogen to a volume of 500 µL. The entire 500 µL extract from a dish/patch preparation was applied to a 2 cm × 2 cm piece of filter paper. Two-choice oviposition experiments were conducted by placing in one dish a filter paper treated with extract from a larval contaminated preparation and in the second dish a filter paper with the control extract from an uncontaminated preparation. Petri dishes containing the filter paper were overlaid with clean cheesecloth and then assayed as described above.

Four-choice assays were conducted in 38 × 30 × 14 cm ventilated plastic boxes with the petri dishes placed 12 cm apart in the centre of the box. Females were introduced into the bioassay arena as described previously and simultaneously presented the choices of food, larval contaminated cheesecloth, food plus larval contaminated cheesecloth or an empty dish. The placement of the four oviposition sites in the box was randomised for each replicate. The numbers of eggs a female laid on the cheesecloth-covered petri dishes were then counted the following day. Three separate four-choice experiments were conducted in which larval densities of 1, 5, and 10 larvae were examined.

Flight responses of *P. interpunctella* to food and/or larval odours were examined in wind tunnel bioassays. The working portion of the tunnel measured 127 × 62 × 61 cm and was constructed of 6 mm Plexiglas. Wind speed was maintained at 25 cm/sec using an electric fan mounted upwind; the bioassay room was kept at 28°C and 50% r.h. Bioassays were run under dim red light within the first 2–3 hours of scotophase because this is the time females are most likely to fly and oviposit. Females were acclimatised to experimental conditions by holding them in a ventilated chamber in the wind without odours for 15 min prior to testing. Two petri dishes containing odour sources were placed on small platforms 30 cm apart and 45 cm above the floor at the upwind end of the tunnel. Five females were then released from a 5.5 × 10 cm screen release cylinder placed on a 45 cm platform at the downwind end of the tunnel. Treatments of food (10 g of diet), contamination by 10 larvae, and food plus larval contamination were prepared as described for the oviposition experiments with each tested against a blank dish in a series of two-choice experiments. Three categories of female behaviour were recorded during a five-minute test period: initiation of flight, upwind flight within the plume, and landing on the source. The percentages of test insects in a group exhibiting flight behaviours and landing on either the treatment or control were calculated for each replicate.

Results

Female *P. interpunctella* laid more eggs on cheesecloth overlaying dishes containing food than on those overlaying empty dishes. A mean of 15.5 eggs (±3.1 SE) were laid on dishes containing 10 g of laboratory rearing diet compared to a mean of 7.5 (±2.4 SE) on empty dishes ($P<0.01$, N=10, Student's t-test). When the components of the larval rearing diet were examined, only cornmeal elicited a significant oviposition response compared to empty dishes (Fig. 1). In choice tests between increasing levels of larval contamination versus blank patches (no food in either dish), females consistently laid more eggs on larval-contaminated patches (Fig. 2a). *P. interpunctella* females laid more eggs on cheesecloth with larval contamination alone than on untreated cheesecloth overlaying food (larval rearing diet), but this preference was significant only at contamination levels of 5 and 20 larvae (Fig 2b)). Preference for substrates containing larval contamination was observed again in the next experiment (Fig. 2c), in which a combination of larval contamination and food was preferred over food only. The last set of two-choice experiments showed that the combination of food with larval contamination elicited significantly more oviposition than larval contamination only (Fig. 2d). Four-choice oviposition experiments that used contamination by 1 larva and 5 larvae found that the combination of food with larval contamination elicited the highest oviposition responses relative to larval contamination alone, food alone, or a blank patch (Table 1, Experiments I and II). However, when cheesecloth patches were contaminated by 10 larvae (Table 1, Experiment III), more eggs were laid on dishes with food only, while those with larval contamination received eggs at low levels comparable to those of the blank controls.

The oviposition-eliciting activity of larval contamination was recovered in hexane extracts from all larval densities sampled. In a series of two-choice experiments, extracts from contamination by one larva elicited a mean of 42.0 (±5.2 SE)

Table 1. Oviposition by single female *P. interpunctella* in three different four-choice experiments.

Experiment no.	Treatment[a]	Mean no. eggs per dish (+/- SE)[b]
I	Empty dish	8.3 (3.3) a
	Food only	21.6 (5.3) b
	1 larva	12.0 (3.8) ab
	Food + 1 larva	32.4 (5.9)c
II	Empty dish	4.2 (1.7) a
	Food only	20.2 (4.7) b
	5 larvae	33.8 (8.2) bc
	Food + 5 larvae	49.3 (6.7) c
III	Empty dish	6.8 (1.9) a
	Food only	20.9 (3.9) b
	10 larvae	7.5 (2.6) a
	Food + 10 larvae	13.7 (2.8) a

[a]Food=10 g of laboratory rearing diet overlaid with uncontaminated cheesecloth; the number of larvae refers to a cheesecloth piece contaminated by wandering fifth instar *P. interpunctella* larvae for 24 hours.

[b]Means within an experiment followed by different letters are significantly different (ANOVA $P<0.05$, Fisher's PLSD test, N=10).

eggs compared to 8.2 (±2.6 SE) for the hexane control; extracts from contamination by five larvae yielded a mean of 56.9 (±12.2) eggs compared to 2.8 (±0.8 SE) for the control; and extracts from contamination by 20 larvae yielded a mean of 66.0 (±13.8 SE) eggs compared to 9.0 (2.9) for the control. All differences between treatments and controls were statistically significant ($P<0.05$, $N=10$, Student's t-test).

Female *P. interpunctella* flew upwind in our two-choice wind tunnel bioassays only when food was included with one of the treatments. When larval contamination was compared with uncontaminated controls, no moths took flight. When food only was compared to an empty dish, 34.0 (±6.0 SE)% of the moths took flight and contacted the dish containing food. The combination of food with larval contamination elicited flight and contact by 52.0 (±5.9 SE)% of the moths tested and none contacted the empty dish. All moths that ultimately landed on a source had preceded that behaviour by initiation of flight and upwind flight within the odour plume characterised by a side-to-side movement on approach to the source. Once a female moth contacted and landed on a treatment (either food only or food+larval contamination) she would remain on the patch for the duration of the trial. Females walked rapidly around the substrate and some laid single eggs during brief stops.

Discussion

This study clearly shows that female *P. interpunctella* orient to odours from food sources for oviposition, and that hexane-extractable semiochemicals associated with secretions from wandering fifth instar larvae elicit oviposition. Our results with responses to food are consistent with earlier work on related species of moths (Barrer 1977; Barrer and Jay 1980; Phelan et al. 1991). The behavioural activity of larval secretions in *P. interpunctella* is similar to that found for *Ephestia kuehniella* by Corbet (1973). In that study, Corbet (1973) showed the paired mandibular glands of larvae harboured a secretion responsible for eliciting oviposition. We are currently investigating the source of oviposition stimulants from *P. interpunctella* larvae. Corbet (1973) did not identify conspecific oviposition stimulants from mandibular glands of *E. kuehniella* , but compounds were subsequently identified that acted as kairomones for the parasitic wasps *Venturia* (=*Nemeritis*) *canescens* (Gravenhorst) (Mudd and Corbet 1973; Mossadegh 1980; Mudd and Corbet 1982) and *Bracon hebetor* (Say) (Strand et al. 1989). Larvae from several species of phycitines, including *P. interpunctella*, possess a similar blend of compounds in their mandibular gland secretions that are kairomonally active for *V. canescens* (Mudd and Corbet 1973; Nemoto et al. 1987). Since we found that the same type of secretions used in kairomone studies elicit oviposition by conspecific females, it is possible that kairomonal factors from the mandibular glands may also serve as pheromones for oviposition.

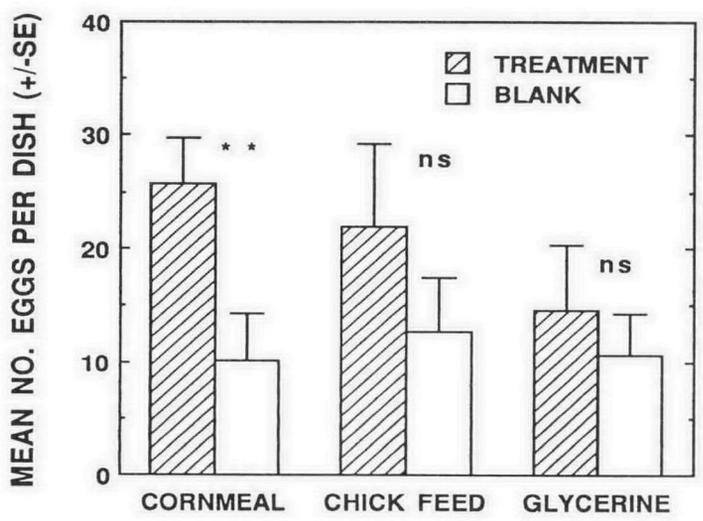

Fig. 1. Oviposition of *P. interpunctella* in two-choice bioassays in which cheesecloth overlaid dishes containing components of the laboratory diet (t-test, $P<0.05$, $N=10$ for each experiment).

Larval contamination apparently signals an oviposition site to female *P. interpunctella*, eliciting different levels of oviposition as a function of the density of larvae producing the contamination and the quality of alternative oviposition sites available to a female (Fig. 2, Table 1). The same mandibular gland secretion that elicited oviposition in *E. kuehniella* also acted as a spacing (epideictic) pheromone for larvae (Corbet 1973). Our data show that distribution of eggs by female *P. interpunctella* is affected by contaminations resulting from high numbers of larvae, and that a priority system for oviposition based on the suitability of the available oviposition sites may be operating. If larval contamination in the absence of food represents the only oviposition site, then it might be preferred for oviposition over a blank patch, regardless of larval density (Fig. 2a). However, when uncontaminated food occurs as an alternative to larval contamination, low densities of larval contamination were preferred for oviposition, but higher densities, perhaps signalling an overcrowded resource, caused a redistribution and reduction of eggs among treatments (Fig. 2, Table 1).

Our experiments show an interactive effect of food and larval secretions on orientation of female *P. interpunctella*. The combination of food and larval secretions elicited the highest oviposition responses in two- and four-choice experiments (Fig. 2, Table 1), and more females flew upwind and contacted the source when food and larval secretions were presented together. Oviposition experiments in small chambers could not differentiate long-distance response to volatiles from contact chemoreception of ovipositional substrates. Wind tunnel experiments, however, confirm that *P. interpunctella* fly upwind in response to volatiles from food. Since larval secretions alone did not elicit flight in the wind tunnel, we suspect that higher landing rates on food with larval secretions resulted from the less volatile components of those secretions being perceived at closer range, prior to contact, and acting to enhance the activity of food odours. Previous work with *E. kuehniella* (Corbet 1973) and *C. cautella* (Barrer 1977; Barrer and Jay 1980) did not address orientation to combinations of larval secretions with food. Interactive effects between host plant and larvae were demonstrated in *Amyelois transitella*, which laid more eggs on mummy almonds infested with conspecific larvae than on uninfested mummies (Andrews and Barnes 1982). Curtis and Clark (1979) found that polar extracts from larval frass of *A. transitella* elicited oviposition by conspecific females in laboratory tests. Although Phelan et al. (1991) identified components from crude almond oil that induced upwind flight and landing by *A. transitella*, larval secretions were not analysed and no studies of flight orientation to combinations of almond oil and larval secretions were reported. The role of plant volatiles in long-range orientation by females from several insect species is gaining more research attention (e.g. Landolt 1989; Dickens et al. 1990; Phelan et al. 1991). The *P. interpunctella* system described here has both host (food) and pheromone components (oviposition and spacing pheromone from larval secretions) operating in concert to affect orientation of females. As suggested by this study and others (e.g. Dickens et al. 1990; Landolt and Heath 1990; Raina et al. 1992), interac-

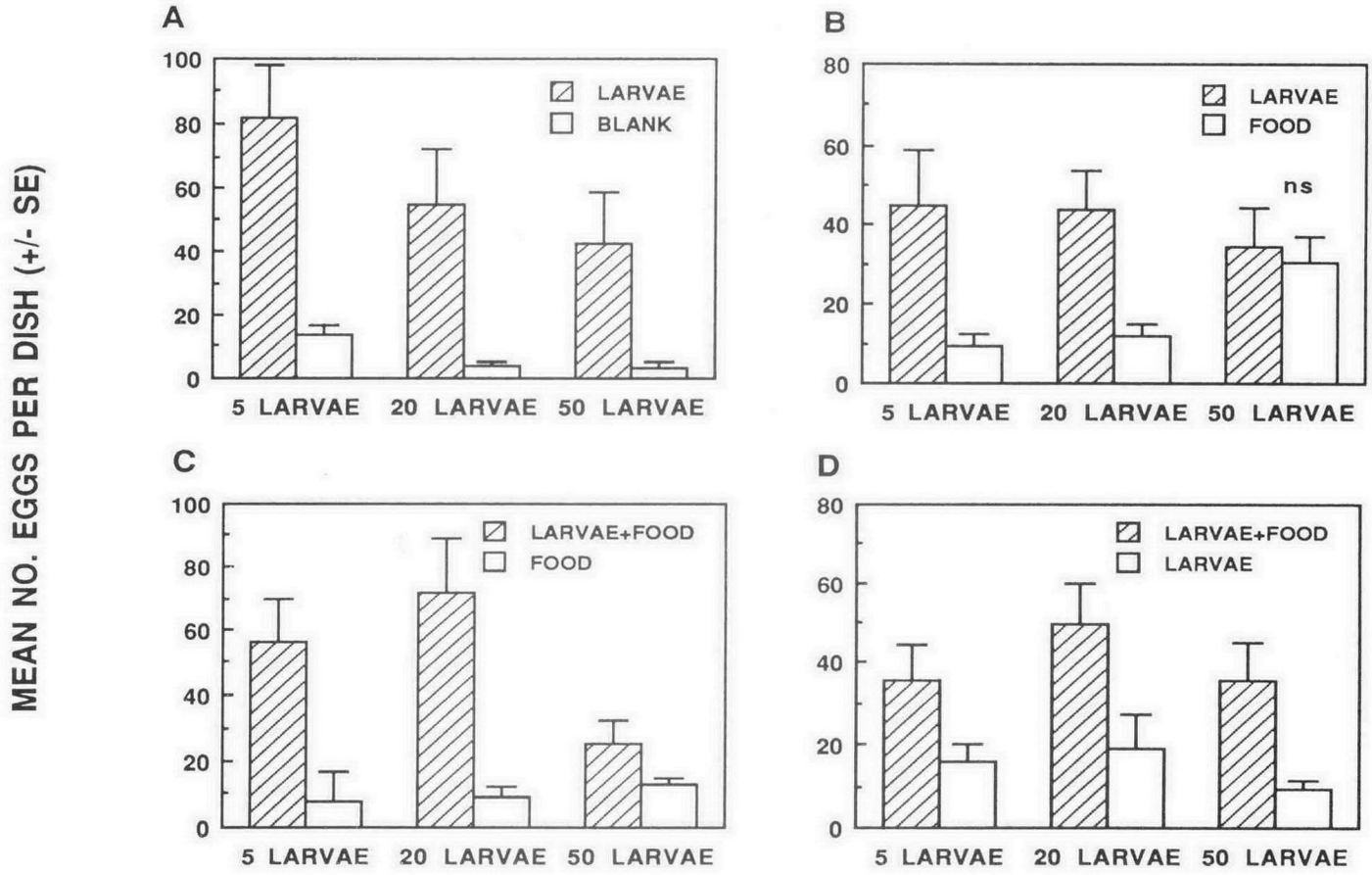

Fig. 2. Oviposition of *P. interpunctella* in four series of two-choice experiments. BLANK=an uncontaminated cheesecloth piece over an empty dish, FOOD=10 g of laboratory rearing diet, LARVAE=cheesecloth contaminated by either five, 20, or 50 wandering fifth instar larvae for 24 hours. All two-way comparisons were significantly different ($P<0.05$, $N=10$, Student's t-test) except where indicated by 'ns'.

tions of insect pheromones with host kairomones may be a common theme in the orientation of insects to host plants.

Acknowledgments

We appreciate the technical assistance of Amy Schermacher and Miel Barman. P. G. Fields and M. N. Parajulee provided valuable critical reviews of the manuscript. This work was partially supported by funding from USDA competitive grant no. 9202382 and through a cooperative agreement between the USDA Agricultural Research Service and the University of Wisconsin-Madison. All programs and services of the U. S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, colour, national origin, religion, sex, age, marital status, or handicap. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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