ANEMOTACTIC RESPONSE THRESHOLD OF THE INDIAN MEAL MOTH, *Plodia interpunctella* (HÜBNER) (LEPIDOPTERA: PYRALIDAE), TO ITS SEX PHEROMONE

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Abstract—The effect of different concentrations of the sex pheromone (Z,E)-9,12-tetradecadien-1-ol acetate on the upwind anemotactic behavior of the male *Plodia interpunctella* (Hübner) was measured at 23 ± 1°C and 34 ± 1°C. The stimulus–response regression lines were analyzed by a new procedure that accounts both for control responses in the absence of pheromone and also for peak responses below 100% in the presence of concentrations considerably above the normal physiological levels. From the regression line for each temperature, the upwind anemotactic thresholds were calculated to be 1.34 × 10⁶ molecules/cm² at 23°C and 1.65 × 10⁶ molecules/cm² at 34°C, similar to other thresholds reported in the literature. Since departures from the two lines occurred at the highest concentrations tested, near 10⁸ molecules/cm³, the upwind anemotactic behavior may change qualitatively above an altered-behavior threshold that is about two orders of magnitude higher than the upwind anemotactic threshold. The lower response at 23°C suggests that cool temperatures inhibit flight in response to pheromonal stimulation.

Key Words—Sex pheromone, anemotaxis, *Plodia interpunctella*, Lepidoptera, Pyralidae, behavioral threshold, behavioral alteration threshold, (Z,E)-9,12-tetradecadien-1-ol acetate, Indian meal moth.

INTRODUCTION

The behavioral threshold of an insect to sex pheromone is an important stimulus–response parameter in olfactory physiology (Kaisling, 1971) and

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applied entomology (Mankin et al., 1980). The sex pheromone and the behavioral response of the Indian meal moth (IMM), *Plodia interpunctella* (Hübner), a widespread postharvest pest, have been studied extensively but the threshold has not been quantified precisely. Because IMM populations are now being monitored by sex pheromone traps (Vick et al., 1979), there is a need to incorporate an exactly quantified response threshold for IMM into a mathematical trapping model. The most appropriate threshold for a trapping model is the threshold for upwind anemotaxis, because an insect stimulated to fly has a greater likelihood of being captured by a trap than an insect stimulated merely to respond orthokinetically (by wing flutter or antennal vibration). We therefore measured the upwind anemotactic response of the male IMM at 23 and 34° C to compare it with that of other insects and to determine a representative threshold for use in postharvest pest trapping models.

**METHODS AND MATERIALS**

_Insects._ Male pupae were transferred at 3-day intervals from a laboratory colony (Silhacek and Miller, 1972) to an environmental chamber held at 27° C and 60% relative humidity with a 16:8 L:D cycle (20-W GE #F201-T2-CW light source in photophase, <0.05 lux in scotophase). During the photophase, groups of 50–75 newly emerged adults were placed into plastic boxes (20 × 10 × 10 cm) with screen lids, where they remained without food or water until testing began 3–5 days later.

_Pheromone._ The IMM pheromone, (Z,E)-9,12-tetradecadien-1-ol acetate (Z9,E12-14: AC), purchased from Story Chemical Co., Willoughby, Ohio, was purified twice before use by elution with benzene through a column of 25% AgNO3 on silicic acid. Gas and thin-layer chromatographic analyses indicated that the product was at least 99% pure. Between tests the pheromone was stored at −25° C.

The pheromone dispenser was a glass tube made by reducing the unground ends of a 14-cm-long by 2-cm ID assembly of 24/40 ground-glass joints to 0.5 cm ID (Mayer, 1973). About 5 min before a test the inside of the assembly was coated evenly with a 0.5-ml aliquot of pheromone in diethyl ether. (Levels ranged from 0 control to 10⁵ ng.) After the ether evaporated, the assembled tube was stoppered until the test began.

_Olfactometers._ The bioassay was done in three olfactometer tunnels (0.3 × 0.3 × 3.5 m) described previously by Mayer (1973). Each tunnel had four closable sections designated S1 to S4 in upwind to downwind order (Figure 1). S1, S2, and S4 were all 56 cm long, and S3 was 140 cm long. The pheromone dispensers were placed near the center of S2 at point PD, and the moths were released near the center of S4 at point IR, 3 m downwind from
Fig. 1. Diagram of olfactometer used in bioassay: PD is the pheromone dispensing point, IR is the point of insect release, and S1-S4 are closable compartments.

PD. The tunnels were supplied with filtered air (55-60% relative humidity, \(3.6 \times 10^4\) cm\(^3\)/sec flow rate, 35-50 cm/sec velocity) and maintained within one of two temperature ranges: 22-24 or 33-35°C. Light was provided by eight rheostat-dimmed, 60-W tungsten bulbs placed separately inside diffuser boxes spaced uniformly around the perimeter of the room at the ceiling. The average light intensity inside the tunnels was 1 lux (1.5 mW/m\(^2\)).

The air-flow pattern in the tunnels was observed by passing humidified air through a dispenser tube dosed with TiCl\(_4\) at PD or by placing a smoke generator (TEM Eng., Ltd., Crawley, England) at PD. Both methods generated smoke plumes that quickly dispersed into less and less distinct filaments. Within 2 m downwind from PD, there was no more than a 4:1 variation in the observed smoke density across the cross-section of the tunnel. Because pheromone molecules disperse at least as fast as smoke particles (Miller and Roelofs, 1978), the average pheromone concentration at IR was estimated to be the ratio of dispenser emission rate to tunnel airflow.

Bioassay Procedure. After preliminary experimentation the following 5-step protocol was adopted: (1) during the first 2-5 hr of the scotophase, 40-50 male IMM were loaded into a screen cage (17.5 \(\times\) 20.3 \(\times\) 25.4 cm) and placed at point IR in a tunnel. (2) A period of 0.5-3 hr ensued to allow acclimation of the insects. (3) A dosed, stoppered dispenser tube was placed at PD; the stoppers were removed, and filtered air (22°C, 60 ml/min) was passed through the tube. Since the dispenser air was kept at a constant temperature, the pheromone concentration in the tunnel was unaffected by changes in tunnel temperature. (4) Immediately (within 10 sec) the holding cage was opened so the IMM were free to move within the tunnel. Sixty seconds later the sections were closed, and the IMM in each section were counted and removed. The upwind anemotactic response was estimated by the fractional response, Fr, the number in S2 and S3 divided by the total number in S1-S4. (5) The dispenser tubes and the holding cages were washed and baked for 12 hr.
at 200° C after each use, and the tunnels were cleaned with ethanol twice a week to minimize the possibility of pheromonal contamination.

The bioassay had an incomplete block design. A treatment was one of 20 combinations of two temperatures (23, 34° C) and ten tube doses (blank control or doses of 0.3, 1, 10, 10², 10³, 3 x 10³, 10⁴, 3 x 10⁴, or 10⁵ ng). Tests were also done at 17° C with 10⁴ ng doses, but these were discontinued because of the negligible response. A block was a set of six treatments tested on a given day at a given temperature. One of the six was always a blank placed randomly within the block; and at least three other treatments were randomized within the remainder of the block to account for day-to-day variation in the IMM response.

Response Analysis. The standard procedure for calculating a behavioral threshold is to adjust the fractional response for the control (Abbot, 1925) and then to analyze the regression of the probit-adjusted response on dose (Finney, 1971). However, the stringency of the upwind anemotactic response criterion kept the maximum response in this bioassay well below 100%, which is contrary to the normal distribution of response frequency assumed in probit analysis. With a normally distributed response frequency there would be a concentration above which the response would approach 100%, just as there would be a concentration below which the response would approach 0%. The standard Abbot’s correction accounts only for deviations from the normal distribution at the 0% limit. Thus, we used the following procedure to account for deviations at both the 0 and the 100% limits, a method that is more generally applicable to quantal bioassays.

First, the response was transformed to probit coordinates by the normalizing equation (Box et al., 1978):

\[
 Nr = \text{Prob} \left( \frac{100(Fr - Frc)}{Frm - Frc} \right)
\]

(1)

where: Nr is the normalized response, in units of probits; Fr is the uncorrected fractional response; Frc is the average fractional response in the control; Frm is the maximum average fractional response at the given temperature (the standard Abbot’s correction is obtained by setting Frm = 1); Prob is the integral probit operator (Finney, 1971).

Next, the normalized responses from equation 1 were fitted by the standard probit analysis to the equation:

\[
 Nr = I + S \log (DD)
\]

(2)

where: I is the temperature-dependent intercept, in units of probits; S is the temperature-dependent slope, probits/μg; DD is the dispenser dose, μg.

Finally the Nr-DD regression line, equation 2, was converted to Abbot’s
corrected coordinates by the inverse transformation:

$$Pr = \frac{Frm - Frc}{1 - Frc} \text{Prob}^{-1} (Nr)$$ (3)

where: Pr is the Abbot's corrected % response (Abbot, 1925); \text{Prob}^{-1} is the inverse probit operator.

**Calibration of Dispenser.** Because a stimulus quantified in terms of micrograms dose conveys little information about the actual quantity of pheromone released into the tunnel, it is necessary to determine the relationship between dispenser dose and emission rate. Previous studies with a different pheromone, \((Z)-7\)-dodecen-1-ol acetate \((Z7-12: \text{AC})\), indicated that the dispenser emission rate was a constant proportion of dose over the range 1–175 \(\mu g\) for emission periods of 15–180 sec (Mayer and Mankin, 1980). The \(Z7-12: \text{AC}\) emissions were collected in capillary tubes \((1 \text{ mm ID} \times 30 \text{ cm glass}, \text{or} 1 \text{ mm ID} \times 70 \text{ cm stainless steel})\) by the method of Brownlee and Silverstein (1968) and quantified by standard gas chromatographic procedures. We assumed that \(Z9,E12-14: \text{AC}\) was emitted in similar fashion and estimated \(Ke\), the constant of proportionality between the dose and the emission rate of \(Z9,E12-14: \text{AC}\), by calibration at a single dose.

The method of calibration was a separate bioassay making use of a polyethylene cap that emitted \(Z9,E12-14: \text{AC}\) at the rate of 0.13 \(\pm 0.07\) SE ng/sec, as calibrated by the method of Vick et al. (1978). The bioassay protocol was identical to that described above for the dispenser tube tests at 23\(^\circ\) C, except that the tube system was replaced by the calibrated cap.

The emission constant was

$$Ke = \frac{Q}{\text{DDC}}$$ (4)

where: \(Q\) is the emission rate of the cap, ng/sec; \(\text{DDC}\) is the tube dose evoking the same mean bioassay response as the cap, ng.

**RESULTS**

The fraction of tested IMM attracted to a dispenser was sigmoidally proportional to the logarithm of the dose, as shown in Figure 2, and was uniformly higher at 34 than at 23\(^\circ\) C. The slope and intercept of each regression line are listed in Table 1. Both the slopes and the intercepts of the two lines are statistically different \((t = 4.9, P < 0.01; \text{and} t = 57.3, P < 0.001, \text{respectively})\). The good fit of the regression lines to the data, indicated by the low \(\chi^2\) values \((P > 0.5 \text{ at } 23^\circ \text{C} \text{ and } P > 0.75 \text{ at } 34^\circ \text{C})\), supports the use of the modified probit analysis for interpreting pheromonal stimulus–response relationships.

The pheromone concentration in an olfactometer at the point of insect
release, IR, was estimated by assuming that the dispenser output disperses uniformly in the $3.6 \times 10^4$ cm$^3$/sec airflow (Methods; olfactometer section). The output rate was $DD \times Ke$, with $Ke$ given by point CP (Figure 2) and equation 4. Thus, the pheromone concentration at IR was

$$C = (0.13 \text{ ng}/2500 \text{ ng}/36 \times 10^4 \text{ cm}^3) \cdot DD$$

$$= 1.44 \times 10^{-9} \text{ DD/cm}^3$$

(5)

The threshold of upwind anemotaxis for a male IMM, i.e., the

**Table 1. Analysis of Dose–Response Regression Lines in Figure 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23</td>
</tr>
<tr>
<td>$I$ (probits)</td>
<td>5.38</td>
</tr>
<tr>
<td>$I$ Standard error</td>
<td>0.114</td>
</tr>
<tr>
<td>$S$ (probits/μg)</td>
<td>0.405</td>
</tr>
<tr>
<td>$S$ Standard error</td>
<td>0.048</td>
</tr>
<tr>
<td>$DD$ for $Nr = 5$ (μg)</td>
<td>3.88</td>
</tr>
<tr>
<td>Upper limit $DD$ (95%)</td>
<td>13.5</td>
</tr>
<tr>
<td>Lower limit $DD$ (95%)</td>
<td>0.054</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>3</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>1.66 ($P &gt; 0.5$)</td>
</tr>
</tbody>
</table>

*Symbols $I, S, Nr,$ and $DD$ are defined in equations 1 and 2.*
concentration at which the normalized fraction of response was 0.5 (5 probits), was calculated from Table 1 and equation 5. At 23 and 34°C, the thresholds were, respectively, 1.34 × 10^6 and 1.65 × 10^4 molecules/cm^3 (2.22 × 10^{-15} and 2.74 × 10^{-17} molar). These thresholds are compared in Table 2 with those reported for other insects.

DISCUSSION

The results of the bioassay suggest that the upwind anemotaxis threshold of the male IMM for its sex pheromone is similar to the thresholds of other insects for their respective sex pheromones (Table 2). Specifically, the IMM 34°C upwind anemotactic threshold was similar to the orthokinetic thresholds of Bombyx mori (L.) and Trichoplusia ni (Hübner); and 23°C threshold is closer to those of Trogodermaglabrum (Herbst) and Lymantria dispar (L.). By contrast, human olfactory thresholds generally range from 10^8 to 10^{11} molecules/cm^3, and the theoretical limit of perception is about 200 molecules/cm^3 (Kaisissing, 1971). Because the four lowest thresholds listed in Table 2 approach this lower limit, we assume that they approximate the corresponding perceptual thresholds. In addition, all the reported sex pheromone behavioral thresholds are within four orders of magnitude of this lower limit. Thus, if a trapping model, e.g., that of Mankin et al. (1980), is applied to an insect with an unknown threshold for attraction to sex

<table>
<thead>
<tr>
<th>Insect</th>
<th>Pheromone</th>
<th>Threshold concentration (10^3 molecules/cm^3)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombyx mori</td>
<td>17°C (E,Z)-10,12-hexadecadien-1-ol</td>
<td>20</td>
<td>Kaisissing and Priesner, 1970</td>
</tr>
<tr>
<td></td>
<td>21°C (same)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Trichoplusia ni</td>
<td>24°C (Z)-7-dodecen-1-ol acetate</td>
<td>8</td>
<td>Sower et al., 1971</td>
</tr>
<tr>
<td>Plodia interpunctella</td>
<td>34°C (Z,E)-9,12-tetradecadien-1-ol acetate</td>
<td>16.5</td>
<td>This paper</td>
</tr>
<tr>
<td></td>
<td>23°C (same)</td>
<td>1340</td>
<td></td>
</tr>
<tr>
<td>Trogodermaglabrum</td>
<td>27°C (-)-14-methyl-(Z)-8-hexadecenal</td>
<td>2300</td>
<td>Shapas and Burkholder, 1978</td>
</tr>
<tr>
<td>Lymantria dispar</td>
<td>25°C cis-7,8-epoxy-2-methyloctadecane</td>
<td>115</td>
<td>Aylor et al., 1976</td>
</tr>
</tbody>
</table>

*Other thresholds are reported in Kaisissing (1971). The behavioral response criterion was upwind anemotaxis for the IMM and orthokinesis for the other insects.*
pheromone, the threshold can be estimated to lie in the range of \(10^3\) to \(10^6\) molecules/cm\(^3\).

**Dose Dependence of Attraction Response.** In most respects, the attraction responses diagrammed in Figure 2 follow the standard sigmoidal relationship obtained with many other insects (Schneider et al., 1967; Kaisling and Priesner, 1970; Sower et al., 1971; Mayer, 1973; Shapas and Burkholder, 1978). In particular, the change from 0 to 100% normalized response occurs within about four concentration decades. However, apparent departures from the sigmoidal relationship occurred at the highest tested doses. For example, at 34\(^\circ\)C the response to 10 \(\mu\)g is lower than the response to 1 \(\mu\)g, and at 23\(^\circ\)C the response to 100 \(\mu\)g is lower than the response to 30 \(\mu\)g. Neither of these decreases are statistically significant, but they are systematic. Similar decreases in response to increasing dose have been reported in olfactometer studies of other insects (Mayer, 1973; Fuyama, 1976; Hawkins, 1978), and decreases in trap catch with increasing dose have been reported in field trapping studies (Wolf et al., 1967; Shorey et al., 1967; Gaston et al., 1971; Vick et al., 1979). This suggests that the observed decreases are not statistical irregularities.

One explanation for the response decrement is contamination of the pheromone with a detectable quantity of \((Z,Z)-9,12\)-tetradecadien-1-ol acetate, which is known to act as an inhibitor of attraction behavior (Vick and Sower, 1973). Such contamination is unlikely, however, because the purified pheromone was found to be at least 99% pure by gas- and thin-layer chromatography. More likely, the observed decrease is the effect of an altered-behavior threshold (Roelofs, 1978). The relationship between the upwind anemotactic threshold and the concentration at which the response begins to depart from the sigmoid supports the latter explanation. According to Roelofs (1978), the altered-behavior threshold of an insect is typically about three orders of magnitude above the orthokinetic threshold, which is somewhat lower than the upwind anemotactic threshold. As predicted, observed departure occurred near \(10^{-5}\) ng/cm\(^3\) 2–3 orders of magnitude above the upwind anemotactic threshold.

**Temperature Dependence of Attraction Response.** The effect of temperature on the IMM upwind anemotactic behavioral threshold is similar to that reported previously for the orthokinetic threshold of *B. mori* (see Table 2). The *B. mori* threshold shifted 0.13 log units/\(^\circ\)C, the IMM threshold shifted 0.19 log units/\(^\circ\)C. A related effect on the percentage mating of *T. ni* was reported by Shorey (1966). The most likely hypothesis for the observed increase in threshold with decreasing temperature is that the lower temperatures tend to inhibit flight in response to pheromone. Indeed, in several preliminary assays done at 17\(^\circ\)C, the IMM exhibited no response to 10 \(\mu\)g doses that were highly stimulatory at 23\(^\circ\)C. Low-temperature effects on flying
activity have been reported in a variety of other insects (Taylor, 1963; Bursell, 1964; Sanders et al., 1978; and references therein).

The findings regarding temperature effects suggest that sex pheromone traps may capture more IMM at warm than at cool temperatures. Such effects of temperature on the pheromone trapping of insects have been reported by Showers et al. (1974), Marks (1977), and Coster et al. (1978). However, the effects of temperature on trapping are more complicated than the effects on attraction behavior because the temperature also affects the rate of pheromonal emission from the trap and because temperature stratification can affect the pheromonal dispersal pattern (Mankin et al., 1980). In addition temperature variation may have a greater influence on the behavior of some insects than average temperature (Showers et al., 1974).

REFERENCES


