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Pheromone-Mediated Flight by Male *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)[1]

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ABSTRACT Flights and captures of male *Plodia interpunctella* (Hübner) were observed in a warehouse room containing pheromone traps baited with different doses and blends of (*Z,E*)-9,12-tetradecadien-1-ol (ZETOH) and (*Z,E*)-9,12-tetradecadien-1-ol acetate (ZETA). Flights were divided into two categories: preapproach and approach flight, the approach being differentiated from preapproach flight by the simultaneous occurrence of increased frequency and angle of turning, decreased net velocity, and net movement toward a trap. Approaches and captures were analyzed with respect to dose and blend. Alone, ZETOH failed to elicit responses significantly different from the control (zero dose), whereas ZETA elicited approaches and landings in traps. Significantly more landings and approaches occurred with blends of ZETOH:ZETA in a 2:8 ratio than with 4:6 or 6:4 blends, but the ratio of captures to approaches did not vary among blends. The frequency of approaches and captures increased with dose but the ratio of captures to approaches decreased when trap release rates exceeded 10 ng/h. Practical and theoretical implications of the results are discussed.

The orientation of insects to sex pheromone sources is strongly affected by the airborne concentration and blend of pheromone components (Linn and Gaston 1981, Baker et al. 1981). Prediction of the most effective doses and blends as baits for pheromone traps requires an understanding of how an insect directs its movement in a pheromone plume or gradient and how changes in blend and concentration alter internally generated motor output patterns (Bell and Tobin 1981). Populations of *Plodia interpunctella* (Hübner) and other phycitine pests of stored products frequently are monitored by traps baited with ZETA [(*Z,E*)-9,12-tetradecadien-1-ol acetate] (Burkholder 1981). Other pheromone components have been identified for some of these insects, but their effects on orientation have not been studied extensively. Soderstrom et al. (1980) recently demonstrated that captures of male *P. interpunctella* by pheromone traps increase when ZETOH [(*Z,E*)-9,12-tetradecadien-1-ol] is added to the trap bait. To determine and interpret the changes that ZETOH induced in orientation, particularly those that resulted in increased trap captures, we examined the orientation of *P. interpunctella* males in a warehouse room to traps baited with different doses and blends of ZETA and ZETOH.

Materials and Methods

Pheromone Emission Rate Calibration

The ZETA was purified as described in Vick et al. (1979), and ZETOH was obtained by saponification of the ZETA. Each compound was determined to be >98% pure by gas-chromatographic analysis (GLC) (Coffelt et al. 1978). Pheromone components were applied in 10-μl aliquots (neat or in hexane, depending on dose) to dispensers made from two-layered pieces of 1-cm² fiber glass-coated window screen. Pheromone emission rates were determined for dispensers dosed with 0.1, 1, or 10 mg, aged at 25 ± 0.5°C for 1, 4, and 7 days, by GLC analysis of the quantities recovered from rinses of stoppered flasks into which dispensers had been placed for 1 h. The calibration methods are described in greater detail by Vick et al. (1978). The dispensers were suspended in the center of Pherocon 1C traps and used for 4 consecutive days beginning 2 days after pheromone application.

The emission rates of pheromone components from the screen dispensers depended on the dose, the blend, and the time after application of the dose as shown in Fig. 1. The model relationship that we chose for the regression analysis was exponential on time and a power function on dose:

$$E = a\,D^{b}\exp(-t/Y) \qquad (1)$$

where E is the emission rate in ng/h, a is a regression constant whose magnitude depends on the chemical and the units of measurement, b is a unitless regression constant, dependent on the adsorptive forces between the screen and the pheromone components, D is the dose in mg, t is the time from loading in days, Y is a regression constant (Y/ln2 is the half-life), exp refers to the exponential function, and ln is the logarithm to the base e. We fitted the results of the calibration tests by computer to equation 1, using a least-squares analysis. The logarithmic transformation of equation 1

$$\ln(E) = \ln(a) + b\ln(D) - t/Y \qquad (2)$$

was used for the regression analysis instead of equation 1 because the values of ln (E) satisfied more closely than E the assumption that variances must be equal across dose. The regression constants ± SEs were ln (a) = 5.895 ± 0.197, b = 0.933 ± 0.054, and 1/Y = 0.156 ± 0.042. The coefficient of determination was $r^2 = 0.98$ with F = 151 for 8 df. The regression is plotted in Fig. 1. By this analysis, the half-life, Y/ln2, was about 4.4 days. It should be noted that, although the blend in the applied dose was 6:4 of ZETOH to ZETA, the corrected volatile collections were in the ratio 7:3, indicating the

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alcohol evaporated at a rate 1.17-fold the rate of the acetate. Also, because emission in the open air is faster than evaporation in a closed flask, the rates determined here may slightly underestimate the actual emission rates in the tests.

Insect Behavior Tests

Test insects were 2 to 4 days posteclosion and were reared as described in Mankin et al. (1980). Four traps were suspended 1.5 m from the ceiling of an empty room (6.1 by 6.1 by 2.4 m). Traps were spaced 2.6 m apart and 1.5 m from the walls. A photoperiod of LD 14:10 was maintained in the rooms, with eight 40-W fluorescent bulbs providing light during the photophase and one 15-W incandescent bulb providing light during the scotophase (ca. 200 and 40 lux, respectively, at floor level). Room temperature ranged from 22 to 33°C during the course of the experiments.

In each replication of a test, 50 to 75 males were released into the center of the room 15 min before the scotophase, and the frequency of approach flights and captures during the next 2 h was recorded. Approach flight was distinguished from other types of flight by the simultaneous occurrence of a sustained increase in the frequency and angle of turns (casting motion), a decrease in the net velocity, and movement toward the trap. One replication was run each day. Traps were rotated one position after each replication.

The following tests were conducted.

(1) Comparison test. One trap had an untreated screen as a control; the others had screens treated with either 10 mg of ZETA, 10 mg of ZETOH, or 5 mg each of both compounds (five replications).

(2) Dosage-ratio response test. One trap had an untreated screen as a control; the three others were treated with mixtures of 2:8, 4:6, and 6:4 ZETOH:ZETA in amounts of 0.01 mg (16 replications), 0.1 μg (7 replications), and 1.0 μg (8 replications). The specific choices of blend were chosen so that the release ratios bracketed the 67:33 ratio of ZETOH:ZETA that Sower and Fish (1975) reported for *P. interpunctella*.

(3) Female comparison test. One trap contained an untreated screen for a control and another contained three 2-day-old females inside a tetrahedral screen cage (5 by 5 cm) (four replications).

Results

Comparison Test

In the test comparing separate components to the 50:50 blend and the control, the moths approached traps baited with the blend or with ZETA alone more frequently than they approached the traps baited with ZETOH or the control (Table 1). There was no statistical difference in this test between the rate of approaches to the blend and to the ZETA alone. Males were captured in traps baited with the blend more frequently than with the other treatments. Of particular interest was the observation that approaches to the blend were greater than to the ZETA. This observation prompted the next test, which considered how approaches, captures, and the ratio of captures to approaches varied with changes in the blend ratio.

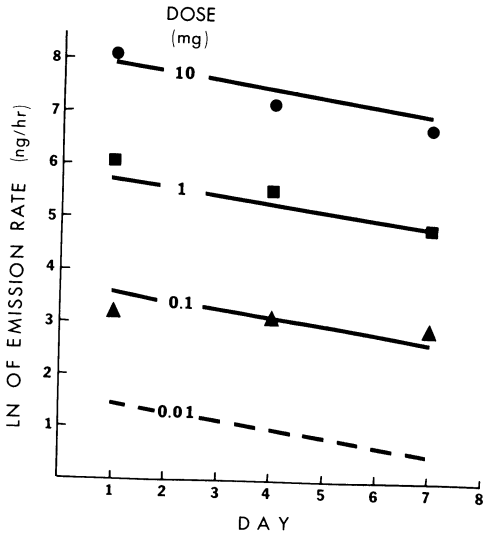

FIG. 1. Emission rate over a 7-day period from screens dosed with 0.1 mg (▲), 1 mg (■), and 10 mg (●) of a 6:4 blend, by weight, of ZETOH and ZETA. Emission ratio blend was 7:3 ZETOH:ZETA.

Table 1. Mean captures and approaches per release of 50 male *P. interpunctella* to traps baited with 5 mg each of ZETA and ZETOH, 10 mg of ZETA, and 10 mg of ZETOH (five replicates)[a]

Dose	Mean approaches (A)	Mean captures (C)	Ratio (C/A)
AC:OH	35.4a	11.2c	0.32
AC	29.4a	2.2d	0.07
OH	8.2b	1.0d	0.12
Control	3.8b	0d	—

[a]Means followed by the same letter are not statistically different at the 95% confidence level, by Duncan's multiple range test.

Dosage-Ratio Response Test

In general, the male *P. interpunctella* released in the test comparing doses and blends approached and landed more frequently in traps baited with the 2:8 ZETOH:ZETA blend than in traps baited with the other blends, the frequency of both approaches and captures increasing with dose. The ratio of captures to approaches did not vary among blends, and it decreased with dose. We will consider first the dose-response relationship and then the ratio of captures to approaches.

Emission Rate-Response Relationship.—The differences in the frequencies of approaches of moths to traps baited with different blends and doses are shown in Fig. 2. The three lines are the regressions of best fit for each blend fitted by computer to the equation

$$\log (R) = \log (k) + \phi \log (E). \quad (3)$$

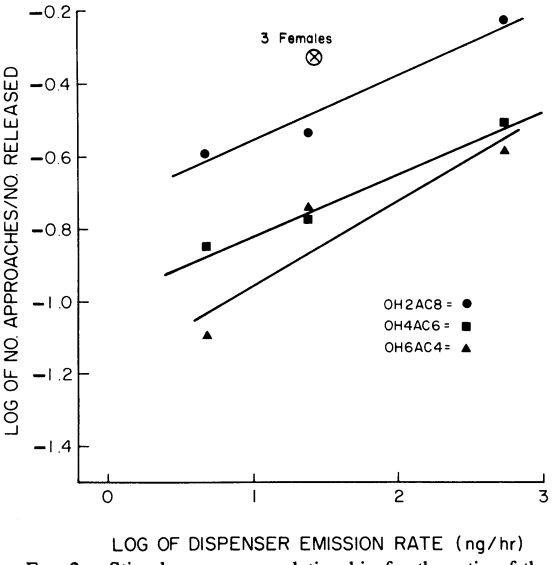

FIG. 2. Stimulus response relationship for the ratio of the number of male *P. interpunctella* approaching a trap over a 2-h period to the number released. Also shown is the response to a trap with three calling females.

Table 2. Statistics for the regressions of approaches on emission rate in Fig. 2 (see equation 3)[a]

Parameter	Estimate	$P > [T]$	SE
Log (K)			
OH2AC8	0.734	0.001	0.051
OH4AC6	−1.186		
Δ	−0.452	0.001	0.071
OH6AC4	−0.979		
Δ	−0.245	0.005	0.071
ϕ			
OH2AC8	0.179	0.001	0.028
OH4AC6	0.232		
Δ	0.053	0.203	0.039
OH6AC4	0.166		
Δ	−0.013	0.748	0.039

[a]The Δ parameters are the differences between the parameter estimates for the 4:6 or 6:4 blends and the 2:8 blend. $P > [T]$ is the probability of obtaining the estimate of the parameter by chance when the parameter is actually 0.

where R is the response (defined in this bioassay as either the number of approaches or the number of captures in 2 h divided by the total number of males released), k and ϕ are regression constants, and E is the dispenser emission rate in ng/h. The rationale for this choice of regression equation is considered in the discussion.

The regression of approaches on emission rate for the data in Fig. 2 yielded $r^2 = 0.96$ and $F = 53.05$ for 8 df. Estimates for log (k) and ϕ are listed in Table 2 for each blend. The values of log (k) for the 4:6 and 6:4 blends are significantly lower than for the 2:8 blend, with all values of log (k) being significantly different from 0. By contrast, the values of ϕ do not differ among blends. These results for log (k) and ϕ indicate that the observed increase in the frequency of approaches to traps baited with the 2:8 blend over approaches to traps baited with other blends is statistically significant at all doses. Other implications of these results are considered in the discussion below.

The attempt to calculate for each blend the regression equation of captures on emission rate was complicated by a qualitative change that occurred in the approach flight patterns at the 1-mg dose. The result was a decrease in the ratio of captures to approaches, which is discussed in the next section. Because of this decreasing ratio, the stimulus-response regression of captures on emission rate was not significant ($F = 2.59$, 9 degrees of freedom, $P > F = 0.10$), by contrast to the regression of approaches on emission rate.

Ratio of Captures to Approaches.—In the dose-ratio response test, the ratio of captures to approaches varied significantly at the 95% confidence level for blend and at the 99% level for dose. Duncan's test indicated that the ratio was higher for the 2:8 and 4:6 blends of ZETOH:ZETA than for the 6:4 blend and the control (Table 3). Duncan's test for dose indicated that the ratio was significantly lower for the 1-mg dose than the 0.01- and 0.1-mg doses, which explains why the regression of captures on emission rate was not significant.

The reason for the decreasing capture-approach ratio was clear from observations of the flight patterns. Although moths frequently initiated approach flights when preapproach flights brought them to within ca. 1 m of a trap baited with 1 mg of any blend, the flight patterns appeared to be qualitatively different from the patterns at lower doses. The frequency of turning appeared to increase, whereas the net movement decreased. Often it was observed that a male would remain within a 0.75- to 0.4-m radius of a trap for several minutes and then begin preapproach flight again. Similar qualitative changes in behavior at higher pheromone concentrations also have been reported for other insects (Baker and Roelofs 1981, Mankin et al. 1980).

Female Test

The bioassays in which a trap was baited with three females in a screen cage obtained higher rates of approach flights and captures than any of the blends tested excepting the 2:8 blend at the 1.0-mg dose (see Fig. 2). It should be noted, however, that the tests were not strictly comparable, because only two traps were present in the female test. The position of the female test results on the emission rate axis in Fig. 2 is based on the measurement by Sower and Fish (1975) of the release rates of ZETA and ZETOH from calling females which were 3 and 6 ng/h per female, respectively. The mean ratio of the number of approaches to the number of males released was 0.481 ± 0.114. The mean ratio of the number of captures to the number of males released was 0.297 ± 0.094. The ratio of captures to approaches was 0.617, which, from Table 3, appears to be significantly higher than the capture-approach ratio for the blends. This result supports observations that males initiating

Table 3. Effect of pheromone blend and dose on ratio of captures vs. approaches by *P. interpunctella* males

Parameter	Mean ratio of captures to approaches[a]
Blend ZETOH:ZETA	
2:8	0.3669a
4:6	0.3623a
6:4	0.2773ab
Control	0.1713b
Dose (mg)	
0.01	0.3641c
0.1	0.3352c
1.0	0.1537d

[a]Means followed by the same letter are not statistically different at the 95% confidence level, by Duncan's multiple range test.

approach flights toward female-baited traps appeared to have a greater tendency to land upon reaching the trap surfaces than when they approached synthetic pheromone-baited traps.

Discussion

The subtle nature of the effects of ZETOH on flight patterns of male *P. interpunctella* illustrates some of the difficulties involved in attempts to describe and interpret pheromone-mediated orientation behavior. Although ZETOH was identified in 1974 as a chemical released by female *P. interpunctella*, its effect on orientation behavior was not clear in olfactometer tube bioassays (Sower et al. 1974). Trapping studies (Soderstrom et al. 1980) indicated that ZETOH was involved in the orientation process, but its mechanism of action remained unknown. In the studies reported here it was determined that the addition of ZETOH to a bait containing ZETA increases the ratio of captures to approaches and that a 2:8 blend of ZETOH:ZETA elicits more approaches and landings than 4:6 or 6:4 blends. Beyond these findings, the tests provided little additional quantitative information about the behavioral processes that resulted in increased trap captures. Apparently, the addition of ZETOH to the bait did not cause a decrease in the threshold of response but an increase in the frequency and angle of turning. Such effects cannot be quantified easily, and a complete analysis of the effects of ZETOH on orientation behavior will require two- or three-dimensional video analysis of flight patterns.

Nonetheless, the results of these orientation studies do have some practical and theoretical significance. In this section, we will discuss the results briefly from two perspectives, one which considers the choice of pheromone blend for trapping applications, and another which considers the psychophysical implications of the dose-response relationship.

Use of ZETOH in Pheromone Trap Baits

Although the 2:8 blend of ZETOH:ZETA elicited the greatest frequency of approaches and trap captures, this blend is not necessarily the best choice for surveying or monitoring traps. There would be no practical differences among captures by traps baited with any of the blends (or with ZETA alone), unless the *P. interpunctella* population was extremely low; i.e., if a control program was set up on the basis of a criterion level of trap captures with ZETA baits, the use of a ZETOH:ZETA blend would only change the criterion level. Consequently, the exact blend used for pheromone trap baits can be chosen on the basis of availability of materials rather than optimization of behavioral response, unless the population is expected to be so low that the probability of a group of traps capturing one or more moths is small.

Another consideration is that ZETA is a major pheromone component not only for *P. interpunctella* but also for *Ephestia cautella* (Walker), *Anagasta kuehniella* (Zeller), *Ephestia figulilella* (Gregson), and *Ephestia elutella* (Hübner) (see review by Mayer and McLaughlin [1976]). A trap to survey all of these insects should not contain ZETOH because this chemical inhibits captures of *E. cautella* (Read and Haines 1976).

Psychophysical Correlates of the Dose-Response Relationship

The model relationship chosen for testing the regression of dispenser emission rate on response was different from the customary probit dose-response function because we wanted to emphasize some apparent psychophysical correlations. A number of standard statistical analyses could be applied to show that the moths responded differently to the blends. The use of a power function analysis, however, also allows some important similarities among the responses to be detected.

Frequently the intensity of perception of a stimulus can be represented by a power function (Stevens 1975):

$$R = kE\,\phi \qquad (4)$$

where R is a measure of the intensity of response, k and ϕ are constants, and E is a measure of the intensity of the stimulus. Stevens hypothesized that this relationship may reflect the responses of peripheral sensory cells because the responses are power functions of the stimulus intensity over a large part of the stimulus range. Mankin and Mayer (1983) derived a model for the specific case of olfactory sensation which extends Steven's concepts further to the electroantennogram and orientation behavior of single insects.

In the tests reported here, it was not possible to measure directly the perceived intensity of the pheromone stimulus or to measure the intensity of response of a single insect in its approach flight. It was possible, however, to measure the proportion of insects in a bioassay that initiated approach flight or were captured. In choosing the model equation for the regression analysis, we hypothesized that the proportion of insects responding could be considered a generalized correlate of responsiveness and consequently could be described by a standard psychophysical dose-response regression.

The importance of the use of equation 3 for analysis of the data in Fig. 1 can be seen by examining the calculated regression constants in terms of psychophys-

ical theory. The constant, k, is considered to be a measure of the response gain, and ϕ is considered to be primarily a measure of sensory transduction characteristics (Stevens 1975). The values of log (k) in Table 2 differ significantly among blends, indicating that the moths respond differently to the blends. Further, the values for ϕ are not significantly different from each other. This result is not a surprise, since both components are present in all three blends. If the same receptor cells with specialized transduction mechanisms for detection of one or both components are stimulated in each case, then the transduction mechanisms probably are identical, irrespective of changes in the absolute or relative rates of generation of action potentials. It follows that ϕ, which is indicative of the transduction mechanism, would be constant.

Indeed, as long as a chosen measure of the intensity of response is an accurate measure of the intensity of perception, psychophysical theory predicts that the regression constant ϕ will be independent of the response measure chosen. A wing flutter bioassay, for example, would be predicted to yield a ϕ similar to the ϕ obtained from analysis of a flight bioassay, an approach bioassay, a trap capture bioassay, or even an EAG bioassay. This prediction is corroborated for flight and approach bioassays by the results of Mankin et al. (1980). If the anemotactic responses to ZETA in a wind tunnel are reanalyzed in terms of equation 4, $\phi = 0.22 \pm 0.09$ for tests run at 23°C and $\phi = 0.14 \pm 0.03$ for tests run at 34°C. These results bear a striking resemblance to the range (0.17–0.23) in the approach bioassay. The finding that the regression of captures on emission rate was not significant suggests that the rate of capture in a trap is influenced by many factors other than time-averaged pheromone concentration. It thus can be seen that a psychophysical approach to the analysis of bioassay data can provide a useful framework within which to interpret bioassay results.

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