Journal of Insect Behavior, Vol. 5, No. 3, 1992

Reduction of the Response to Sex Pheromone in the Oriental Fruit Moth, *Grapholita molesta* (Lepidoptera: Tortricidae) Following Successive Pheromonal Exposures

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Accepted May 7, 1991; revised November 21, 1991

The effects of prior pheromonal experience upon the pheromone-mediated upwind flight response was examined in the oriental fruit moth, Grapholita molesta *(Busck) (Lepidoptera: Tortricidae). Adult male* G. molesta *were subjected to a parallel series of staggered and repeated pheromonal exposures in a sustained-flight wind tunnel. Levels of response to pheromone in male* G. molesta *significantly decreased in a (a) rectilinear function with increased ages of individuals, (b) logarithmic function of successive trials, and (c) steeper logarithmic function of successive trials with increased dosage of sex pheromone. The baseline levels of responding were not affected by either the (a) dosage of sex pheromone, (b) posteclosion ages of individuals for their initial exposures once the main effect of age itself was estimated, (c) elapsed time in hours between trials, or (d) discrete days of testing as integral intervals, disregarding hours within days.*

KEY WORDS: oriental fruit moth; *Grapholita molesta;* sex pheromone; habituation; spontaneous recovery; aging.

INTRODUCTION

The role of long-term learning as a possible factor in the ethology and evolution of lepidopteran sex pheromones has been virtually ignored (Baker and Cardé, 1984). All responses by male moths have been considered genetically prepro-

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0892-7553/92/0500-0347$06.50/0

grammed and relatively inflexible. Any variation in the specificity of the response a particular blend of pheromone components, therefore, has been ascribed to relatively short-term effects such as sensory adaptation or habituation (Linn *et al.*, 1988; Flint and Merkle, 1984). There is, however, a considerable amount of variability reported in the responses of moths to conspecific sex pheromone, with regard both to shifts in blend ratios and to the same blend and dosage presented at different times. Some sources of this variance are now well understood, more have been theorized, and most are probably still unidentified.

For example, the ability to locate a source of sex pheromone is known to vary with respect to certain physical environmental factors that affect the ability to initiate and control upwind flight. Of these factors, wind velocity and changes in direction may be the most important, as optomotor anemotaxis is necessary for successful orientation by flying males to conspecific females (Kennedy and Marsh, 1974; Marsh *et al.*, 1978; Baker, 1984, 1986; Cardé, 1984; David, 1986). Indirectly, therefore, related environmental parameters such as the density and distribution of surrounding vegetation and geological topography may affect pheromonal response in the field through their impact upon natural airflow (David *et al.*, 1983; Elkinton *et al.*, 1987).

Other physical factors, such as ambient temperatures and humidities, circadian photoperiodicity, and seasonal and other circannual cycles, including those of conspecific and certain allospecific population densities, are known to modify significantly the upwind flight propensity of moths in response to pheromone (e.g., Baker and Cardé, 1979b). For example, elevated temperatures have been shown to mimic the effect of excessive dosages of pheromone in lowering response specificity to pheromone blends. This influence is mediated by the modulation of olfactory perception of pheromone and not by the enhancement of the rates of either motor activity or pheromone release (Linn *et al.*, 1988; Baker *et al.*, 1988, 1989). In addition, variation in response to blends and dosages within a population under constant physical conditions has been examined using a single exposure and explained as being due to relatively nonspecific tuning in the sensory pathways of individual males (Haynes and Baker, 1987; Cardé *et al.*, 1976).

With regard to successive exposures to pheromone, there has been considerable experimentation due to the possible application of this knowledge to mating disruption for pest management. These response decrements have been examined following brief preexposure to synthetic pheromones (Sanders, 1985) and geometrical isomers (Bartell and Roelofs, 1973), pulsed (Bartell and Lawrence, 1976c) and nonpulsed (Linn and Roelofs, 1981) single pheromone components, pulsed (Bartell and Lawrence, 1976b) and nonpulsed (Bartell and Lawrence, 1973) pheromone blend, and different dosages of pheromone blend (Bartell and Lawrence, 1976a). As a result of these and supporting electroantennogram (EAG) studies, which showed no corresponding decrement in

responses from the olfactory receptors (Kuenen and Baker, 1981), the observed reduction in response to pheromone has been attributed to the central process of habituation instead of any peripheral process of sensory adaptation.

These prior studies support the existence of at least short-term habituation to pheromone in moths, from several minutes to several hours in duration. To establish that a response decrement is attributable to the inferential process of habituation and not some other behavioral mechanism, however, certain defining parameters need to be subjected to experimental scrutiny (Peeke and Petrinovich, 1984; Petrinovich, 1984). Long-term temporal persistence, spontaneous recovery, stimulus specificity, context specificity, dishabituation, and sensitization (as a concurrent opponent process) have yet to be investigated.

In summary, although some excellent progress has been made, much further work remains to be done before the status of these reported decreases in response to pheromone is fully understood. Aside from the rather conclusive rejection of sensory adaptation as an alternative explanation for this phenomenon, little more can be asserted with confidence about the type of olfactory conditioning that this represents or the level of learning ability that it indicates (Baker and Cardé, 1984). This report presents the first in a planned series of studies aimed at specifying the behavioral parameters of pheromonal conditioning in moths. The present paper reports the results of a parallel series of staggered and repeated habituation trials on different individuals spanning up to two consecutive weeks, to determine whether long-term habituation and spontaneous recovery of response to pheromone occur in oriental fruit moths.

METHODS

Subjects

All the insects used in these experiments were adult male oriental fruit moths, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae). These were reared in captivity at the University of California, Riverside, and shipped as pupae to the University of Arizona, Tucson, for posteclosion testing. The larvae were reared continuously under controlled conditions (LD 16:8; 25°C), on little green apples. Pupae were separated according to sex and males allowed to emerge in isolation from females. Adult males are further separated daily according to age.

All males were the following ages, plus or minus 1 day, at the start of each experiment: Group A and C moths were 3 days posteclosion, and Group B and D moths were 10 days posteclosion. Subjects were allowed to feed freely on sugar water from a saturated plastic foam stopper, refreshed as needed every other day. Before and between trials, males were kept in a laboratory incubator at LD 16:8, 20 ± 2°C, and 80 ± 10% RH. All testing was carried out starting during the last hour of the photoperiod and continuing for 1 h thereafter (with

lights on) to ensure maximal response to sex pheromone (Baker and Cardé, 1979b).

Experimental Groups A, B, C, and D had 10 moths each. These 40 moths were subjected to a total of 360 repeated trials. The identity of each male was maintained throughout the experiment by assigning each a code number. Inter-individual variation in response could then be measured and factored out as a known source of variance. Control Groups for A, B, C, and D had 10 moths each per each of the successive days of testing. These totaled 60 moths for A, 30 moths for B, 60 moths for C, and 30 moths for D, or a sum of 180 additional insects each subjected to a staggered single trial. No males were prescreened for response to pheromone due to the danger of statistical "regression toward the mean" with repeated measurement (Smith, 1991a,b).

Apparatus

Males were flown in a large sustained-flight wind tunnel of clear polycarbonate plastic. The tunnel has a working section 2.2 m long, 1 m high at maximum, and 1 m wide at floor level. A ground pattern consisting of 10-cm-diameter solid red circles randomly located (about 20–30 cm apart) on a white cloth background was positioned 9 cm below the 6-mm-thick clear acrylic plastic floor. All males were tested at a windspeed of 0.5 m/s. They were released from an open conical screen cage suspended 15 cm above the wind tunnel floor and 40 cm upwind from the exhaust by a metal laboratory ring stand. A rubber septum (A. H. Thomas Co. No. 8753-D22, sleeve type, 5 × 9 mm), impregnated with the natural blend of three components used by *G. molesta* for sexual communication [5% (*E*)-8-dodecenyl acetate plus 3% (*Z*)-8-dodecenyl alcohol in (*Z*)-8-dococenyl acetate], was placed in the center of a 15 × 15 × 0.05-cm galvanized sheet metal plate on a sheet metal stand positioned 15 cm above the center of the floor and 15 cm from the upwind end of the working section of the tunnel. Two dosages of synthetic sex pheromone were used: 10 μg for Groups A and B and 30 μg for Groups C and D.

Procedure

Males were exposed to a source of their natural blend of pheromone components and scored for flying upwind all the way to that source. Twice a day for several days following their first exposure, individual males were repeatedly released in the wind tunnel into a plume of the same natural blend of pheromone components. Daily control groups were subjected to single staggered pheromone trials matching (i.e., "yoked" to) the schedules of all the repeated exposure groups. Moths were randomly assigned an order of testing but that fixed order was preserved through all the trials to maintain consistent intertrial intervals. The repeated tests were carried out twice a day, 1 h apart, 3 consecutive days

a week, for up to 2 weeks. An initial interval of 60 s was allowed before terminating any given trial as nonresponsive. A subsequent full stop of 10 s or more was deemed as terminating a previously responsive trial. The somewhat arbitrary cutoffs were informally validated by observing several unresponsive moths for greater intervals and detecting no changes in progress. Immediately following their random assignment to either the experimental or the control groups, starting at either 3 or 10 days posteclosion, and between any repeated trials thereafter, all moths were separately housed in identical and individually labeled plastic culture vials, provided with cotton plugs soaked in sugar water.

Scoring

The type of behavioral assay technique used has been well documented and empirically validated elsewhere (e.g., Baker and Cardé, 1979a). The level of response to pheromone elicited (L) was measured as follows on a six-point ordinal scale:

(0) no pheromonal response,
(1) wing fanning while stationary or while walking in the release cage,
(2) takeoff and at least momentary airborne flight in any direction,
(3) locking onto the pheromone plume and zigzagging upwind flight,
(4) close approach (within 10 cm to metal table), and
(5) landing and wing fanning near the pheromone source.

For instance, a score of 4 on this ordinal scale indicates that the moth has performed stages 1, 2, 3, and 4 of this species-typical sequence of male response to sex pheromone but not progressed to stage 5. A perfect score of 5 indicates completion of the entire behavioral sequence, up to and including landing and wing fanning near the pheromone source.

The three independent variables that were directly manipulated were scored as follows: (T) the repeated pheromonal exposures, or "trials," numbered 1–12; (K) the dummy variable representing dosage of sex pheromone, defined as "0" at 10 μg (for Groups A and B) and "1" at 30 μg (for Groups C and D); and (R) the dummy variable representing the Group B and D constructive replication, defined as "0" for moths starting at 3 days (Groups A and C) and "1" for moths starting at 10 days (Groups B and D) posteclosion. Three additional predictors that were not directly manipulated, but recorded and analyzed, were scored as follows: (H) the elapsed time of consecutive trials, measured continuously as integral hours; (D) the discrete days of repeated testing, measured categorically using six levels; and (A) the actual posteclosion age of the moth at each trial, measured continuously as integral days. Several algebraic functions of the above were also computed and analyzed as described below. These were (LNT) the natural logarithmic function of T; (T1) the linear function

of T, where T is treated as a class variable; (LNA) the natural logarithmic function of A; (A1) the linear function of A, where A is treated as a class variable; and (CSA1) the "criterion scaled" expected value of L, based upon the least-squares linear regression estimates described below.

Statistical Analyses

The data were subjected to a series of multiple regressions using the SAS General Linear Models procedure (SAS Institute, 1985). The control groups were analyzed separately in a set of pure between-subjects GLM designs since they involved no repeated measures. The experimental groups were analyzed in a set of mixed, or "split-plot," GLM designs since they involved both between-subjects and within-subjects variables. The power of testing individually identified and labeled males repeatedly and factoring out interindividual variation in response was fully realized with this design (cf. Winer *et al.*, 1991). All significance tests were performed hierarchically and all regression weights were obtained simultaneously from the significant predictors. Although the designs of these experiments were relatively simple, the data-analytic strategy necessary for an adequate understanding of their results was not. This somewhat involved data-analytic strategy was nonetheless designed to achieve four major objectives: to estimate separately the parametric effects of the following four predictors upon the levels of response to pheromone elicited during repeated testing:

(1) posteclosion age of individual males,
(2) repeated exposure to sex pheromones,
(3) dosage of sex pheromones used, and
(4) variable intertrial intervals used.

To accomplish these four major objectives, however, certain minor steps were also necessary. For the first major objective, for example, it was necessary to recognize that the effects of increasing age in the experimental groups were necessarily confounded with those of repeated exposures, due to the inevitable passage of time between exposures. Thus, the best regression model for the effects of male moth posteclosion age was first obtained from the control groups, which did not experience repeated exposures. CSA1, a "criterion scaled" variable (Pedhazur, 1982) equal to the expected value of level of response to pheromone for each age, was then computed from the regression equation obtained from the control groups and assigned to all the moths of corresponding age in the experimental groups. To control for the effect of age in the experimental groups, this variable was used as a predictor in the regression models for the experimental groups and estimated hierarchically before the effects of repeated exposures. As an added experimental control on this procedure, a constructive replication was performed, using an initially older cohort of males,

to confirm that any confounding of increasing age with repeated exposures had been completely removed by this application of statistical control. This hypothesis was supported by the results.

To accomplish all four major objectives, it was also necessary to determine the correct function forms of the effects of both increasing ages *and* repeated exposures. For the fourth major objective, for example, regression discontinuity analyses (Cook and Campbell, 1979) were performed to test for any evidence of "spontaneous recovery" from habituation. This highly sensitive procedure tests for any significant point displacements or "discontinuities" along the regression line associated with discrete events and must be applied only after appropriate function forms have been fitted to avoid spurious results (Cook and Campbell, 1979; Petrinovich and Widaman, 1984). Thus, the best regression model was first obtained for the effects of repeated pheromonal exposure in the experimental groups. Regression discontinuity analyses were then done by entering the passive "elapsed time" variables (either H or D) hierarchically after the best-fitting significant functions of the main effects and interactions of A, K, and T were estimated.

The best-fitting function forms were determined empirically as follows. For both the aging and the habituation functions (the effects of A and T), formal significance tests for "deviations from linearity" (Cohen and Cohen, 1983) were conducted. This was done by entering the single degree-of-freedom continuous form (either A1 or T1) hierarchically before the multiple degree-of-freedom categorical form (either A or T treated as a "class" variable) of the predictors. Since we expected negatively accelerated habituation functions, similar tests were conducted for such "deviations" from logarithmic curvilinearity. These were done by entering the logarithmic transformation (either LNA or LNT) hierarchically after the rectilinear term (A1 or T1) but before the fully categorical term (A or T as a class variable) for these predictors. These procedures test for the significance of the "residual" variance of the multiple degree-of-freedom variables, once the variance attributable to the hypothesized single degree-of-freedom function forms have been accounted for (Cohen and Cohen, 1983). The magnitudes of these residuals thus indicate whether the hypothesized algebraic functions estimated hierarchically before them are statistically adequate to account for the empirical data or whether more complex algorithms are required.

RESULTS

Figures 1 and 2 display the mean levels of response to pheromone for all groups, as operationalized by the ordinal scale defined above. Results for the experimental groups are plotted in both Fig. 1 and Fig. 2 by the repeated exposures of individuals to pheromone, in successive trials. Results for the control groups are plotted in both Fig. 1 and Fig. 2 by the increasing ages of

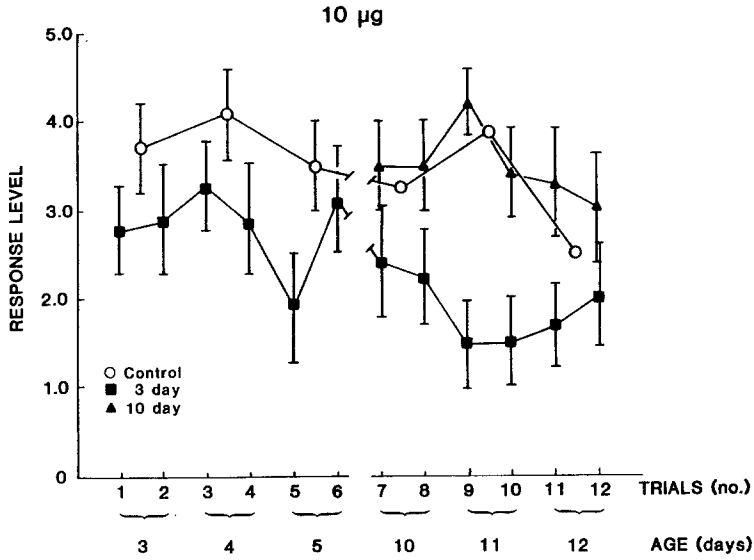

Fig. 1. The mean ordinal levels of response by male oriental fruit moths to the 10-µg dosage of sex pheromone, plotted by the repeated exposures of individuals to sex pheromone, as successive trials, for the experimental groups and plotted by the increasing ages of the males, as days posteclosion, for the control groups.

the males, as days posteclosion. These descriptive statistics should be interpreted with great caution, however, because reductions in response to pheromone plotted according to the repeated exposures to pheromone of experimental individuals over time are necessarily confounded with reductions in response to pheromone attributable to the increasing ages of those individuals over that time.

In addition, multiple regression techniques, described under Methods, were used to determine the best-fitting function forms for both the aging and the habituation functions. As reported in detail below, these best-fitting function forms were linear for aging and logarithmic for habituation, and any residual deviations from these respective functions were found to be statistically nonsignificant. The traditional presentation format, followed in both Fig. 1 and Fig. 2, showing a series of straight-line segments connecting the successive sample means, is potentially deceptive in that it encourages the overinterpretation of "sawtooth" effects that have been shown to consist primarily of sampling error. Since the basic function of statistical analysis is the discrimination of significant treatment effects both from the sampling errors and from the confounding effects of significant covariates (i.e., the "signal" from the "noise"), the regression models developed provide a more interpretable representation of the causal processes involved than a scatterplot of the raw data. For that reason, Tables I and

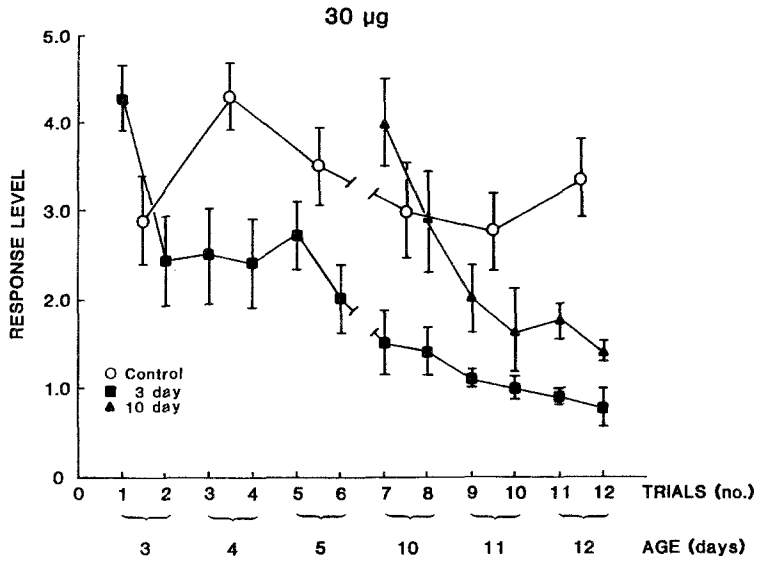

Fig. 2. The mean ordinal levels of response by male oriental fruit moths to the 30-μg dosage of sex pheromone, plotted by the repeated exposures of individuals to sex pheromone, as successive trials, for the experimental groups and plotted by the increasing ages of the males, as days posteclosion, for the control groups.

Table I. Hypothesis Tests for Between-Subjects (BSS) and Within-Subjects (WSS) Predictors

Type	Predictor	F ratio	NDF, DDF	$P(H_0)$
BSS	A1	4.54	1, 155	0.0347
BSS	LNA|A1	1.53	1, 155	0.2181
BSS	A|A1 LNA	1.31	3, 155	0.2730
BSS	K	0.72	1, 155	0.3990
BSS	R	2.94	1, 155	0.0884
BSS	K*A	1.95	5, 155	0.0886
BSS	R*A	1.60	2, 155	0.2046
BSS	K	5.54	1, 37	0.0241
BSS	R	5.67	1, 37	0.0225
WSS	CSA1	38.48	1, 37	0.0001
WSS	T1	8.89	1, 37	0.0050
WSS	K*T1	9.63	1, 37	0.0037
WSS	R*T	1.44	1, 37	0.2281
WSS	LNT|T1	1.50	1, 36	0.2284
WSS	K*LNT|K*T1	7.89	1, 36	0.0080
WSS	T|T1 LNT	0.96	8, 166	0.4721
WSS	K*T|K*T1 K*LNT	1.57	9, 166	0.1285
WSS	H|BEST MODEL	0.98	1, 38	0.3291
WSS	D|BEST MODEL	1.24	4, 89	0.3018

II present the results of a series of multiple regressions in which these two distinct effects and the residual error variance are separately estimated.

Table I summarizes the results of the various hypothesis tests performed. The corresponding verbal descriptions and statistical results are individually coded in both tables and text (in parentheses) for cross reference. Table II presents the squared multiple correlations (SMC), unstandardized least-squares regression weights (ULS), and standard errors (SE) obtained for the most parsimonious regression models, containing only the predictors found significant by the appropriate Table I hypothesis tests. Both tables combine results from several analyses. The order in which the predictors are listed in Table I reflects the order in which they were actually entered into the regression model. The order in which they are described verbally in the text below has been reorganized by topic. Because of this verbal reordering of the predictors, the logical symbol "|" is used to indicate explicitly that one term was statistically controlled for one or more others: "A|A1 LNA" is thus read "A 'given' both A1 and LNA." Statistically nonsignificant results are described when parametric hypotheses of theoretical importance, such as those detailed under Statistical Analyses, are rejected. A few moths died, apparently of natural causes, between the last several pheromone trials during repeated testing: their otherwise normal results were included in the regression analyses for all trials prior to their various different times of death.

Control Groups

Ages. Initial levels of response to pheromone declined with the increasing ages of the males. The main effect of age (Control A1) was rectilinear and negative, indicating a spontaneous but regular and predictable decrease in response at first pheromonal exposure according to the age (posteclosion) of the males. The decline in initial levels of response to pheromone according to the ages of the males showed no significant deviations from linearity (Control

Table II. Unstandardized Least-Squares Regression Weights and Standard Errors for Best Regression Models

SMC	Predictor	ULS	SE
0.0250	Intercept	3.950	0.314
	A1	−0.070	0.034
0.0799	Intercept	2.502	0.144
	K	−0.690	0.180
	R	+0.734	0.190
0.5136	CSA1	1.262	0.588
	LNT	−0.188	0.207
	K*LNT	−0.842	0.204

LNA|A1 and Control A|A1 LNA), logarithmic or otherwise, after the rectilinear term (Control A1) was estimated.

Doses. There was no significant effect of the pheromone dosage (Control K) upon initial levels of response to pheromone. Since the dosages of sex pheromone used were both within the broad optimal range (Baker *et al.*, 1981; Linn *et al.*, 1981), it is not surprising that both dosages yielded close to peak response to pheromone and therefore did not initially differ in effect. Furthermore, the systematic decrease in initial levels of response to pheromone as the males aged over time was statistically equivalent for both dosages of sex pheromone, as indicated by the lack of significant interactions (Control K * A) between the age of males and the pheromone dosage upon the response to pheromone.

Replications. There was also no significant effect of the constructive replication (Control R) upon initial levels of response to pheromone. Thus, there were no initial differences in response to pheromone either between Group A and Group B (10 μg, 3 versus 10 days old) or between Group C and Group D (30 μg, 3 versus 10 days old), respectively, other than those attributed above to age (Control A1). The systematic decrease in initial levels of response to pheromone with the increasing ages of the males also did not differ statistically either between Group A and Group B or between Group C and Group D, respectively, as indicated by the lack of significant interactions (R * A) between the ages of the males and the constructive replication upon initial levels of response to pheromone.

Experimental Groups

Doses. Despite the lack of significant effects upon initial response levels when males were exposed to 10 versus 30 μg, the pheromone dosage did significantly affect the *successive* levels of response to pheromone when repeated exposures to pheromone were administered to individual males. The regression weight for this main effect of pheromone dosage (Experimental K) was negative, indicating that the mean level of response to successive pheromone exposures was lower for the 30- than for the 10-μg conditions. Since the control group described above (Control K) showed no initial differences in response between the two pheromone dosages, this main effect was thus wholly attributable to the repeated exposures of individuals (Experimental K * T1, Experimental LNT|T1, and Experimental K * LNT|K * T1 described below).

Ages. The mean level of response to successive pheromone exposures was lower for the moths starting their initial exposures at 3 days posteclosion than for those starting their initial exposures at 10 days posteclosion, as indicated by a significant main effect (Experimental R) of the constructive replication upon repeated levels of response to pheromone. The regression weight for this term

was therefore positive. Since there was no residual effect of the constructive replication (Control R) after the main effect of age (Control A1) had been estimated, the present main effect is thus completely attributable to the different ages of the males at first exposure (Control A1), especially given the nonsignificant interaction (Experimental R * T) described below. There was a significant reduction with increasing age of the males, as indicated by the main effect of the "criterion-scaled" variable (Experimental CSA1), upon the successive levels of response to pheromone when repeated exposures to pheromone were given to individuals. This term represented the expected linear response decrement with the increasing ages of the males and is therefore consistent with the results of the controls described above (Control A1, Control LNA|A1, and Control A|A1 LNA).

Trials. Repeated exposures to pheromone significantly affected the successive levels of response to pheromone of individual males, as indicated by the rectilinear effect (T1) of the successive trials. This supports the major experimental hypothesis that long-term habituation occurs with repeated pheromonal exposure. There was no significant logarithmic deviation from linearity (Experimental LNT|T1), after the rectilinear term (Experimental T1) was estimated, in the main effect of repeated exposures to pheromone upon successive levels of response to pheromone. Due to the logarithmic interaction reported below (Experimental K * LNT|K * T1), however, the logarithmic main effect had to be both specified and estimated in the optimal regression model. This logarithmic regression weight was negative, indicating decreasing response to pheromone but indicating that the inhibiting effect of repeated stimulation has curvilinearly "diminishing returns." There was also no significant deviation from logarithmic curvilinearity (T|T1 LNT) after both the linear and the logarithmic terms (Experimental T1 and Experimental LNT) were estimated, in the main effect of the repeated exposures to pheromone upon successive levels of response to pheromone.

Doses × Trials Interactions. The reduction in response to pheromone with the repeated trials was significantly greater when the higher dosage was used, as indicated by the significant interaction (Experimental K * T1) between the pheromone dosage and the rectilinear effect of repeated exposures to pheromone upon successive levels of response to pheromone. The curvilinear decrease in successive levels of response to pheromone with repeated exposures to pheromone was significantly different for the different pheromonal dosages, as indicated by the significant logarithmic deviation from linearity (Experimental K * LNT|K * T1), after the rectilinear effect (Experimental K * T1) was estimated, in the interaction between pheromone dosage and repeated exposures to pheromone upon successive levels of response to pheromone. The regression weight for this logarithmic interaction was negative, indicating an even greater (than Experimental LNT) decrease in the level of response to pheromone with the

higher dosage (30 μg) of sex pheromone. Thus, the greater the magnitude of semiochemical stimulation, the higher the curvilinear rate of habituation. Furthermore, there was no significant deviation from logarithmic curvilinearity (Experimental K * T|K * T1 K * LNT), after both the linear and the logarithmic terms (Experimental K * T1 and Experimental K * LNT) were estimated, in the interaction between the pheromone dosage and the effect of repeated exposures to pheromone upon successive levels of response to pheromone.

Replications × *Trials Interaction.* The rate of systematic decrease in successive levels of response to pheromone with repeated exposures to pheromone was equivalent between the different initial age groups of males, as indicated by the lack of significant interaction (Experimental R * T) between the constructive replication and the repeated exposures upon successive levels of response to pheromone. Thus, the age at which habituation trials were started did not influence the slopes of the habituation functions.

Spontaneous Recovery Effects. Surprisingly, successive levels of response to pheromone with repeated exposure to pheromone showed no residual effect of continuous elapsed time in hours (Experimental H|BEST MODEL), indicating that variable intertrial intervals had no significant proportional influence upon subsequent response to pheromone. There were thus no linear "regression discontinuities" due to elapsed time between successive trials, regardless of exact hour of testing. Successive levels of response to pheromone with repeated exposure to pheromone also showed no residual effect of the discrete days of testing (Experimental D|BEST MODEL), indicating that even major intertrial intervals measured as days, disregarding minor ones measured as hours, did not significantly influence subsequent response to pheromone, proportionally or otherwise. There were therefore no discrete or nonlinear "regression discontinuities" between successive trials, regardless of the different days of testing.

Summary of Results

Given repeated exposures to sex pheromone, the successive levels of response to sex pheromone in male oriental fruit moths significantly decreased in (a) a rectilinear function with increased ages of the males, (b) a logarithmic function of successive trials, and (c) a steeper logarithmic function of successive trials with increased dosage of sex pheromone. The baseline levels of responding were not affected by either (a) the higher dosage of sex pheromone, (b) the increased posteclosion age of the males for initial exposures, once the main effect of age itself was estimated, (c) the elapsed time as hours between trials, or (d) the discrete days of testing as integral intervals, disregarding hours within days. The lack of either (a) rectilinear or (b) curvilinear or nonlinear "regression discontinuities" in the habituation functions provide substantial evidence against any "spontaneous recovery" in this insect. Although one can never prove the

null hypothesis, this negative conclusion is supported by the unusually high statistical power of the regression discontinuity analysis applied (Petrinovich and Widaman, 1984).

DISCUSSION

Our results indicate that repeated exposure to pheromone over a period of days causes the male oriental fruit moth response to be significantly decreased. Such long-term habituation to pheromone has not heretofore been demonstrated in insects. It is surprising that even a few exposures plus upwind flight to the source can result in subsequent reduced response even several days later in the same individual. Although this reduction was found statistically significant, it was not exceedingly great, and so it is not clear that there would be any evolutionary significance to this response under field conditions. The septum dosages used emit pheromone at approximately the same rates at which calling females do. For example, a calling female emits pheromone at a rate of 3.2 ng/h, a 10-μg septum at 1.2 ng/h, and a 100-μg septum at 12 ng/h (Baker *et al.*, 1980). Therefore, the repeated stimulations were not performed with unnaturally high emission rates.

Virtually nothing is known about the numbers of males that are attracted to calling females in the field or how many unsuccessful upwind flights might be experienced under the selective pressures of the real world. Habituation under such conditions might be advantageous in that it might prevent males from responding to too many females from too great a distance and thus failing to reach a female before a closer male does. By restricting their attempts to females that are closer, and thus more likely not to be in copula by the time the male arrives,habituated males may be proportionally more successful at locating females at higher population densities.

There are numerous anecdotal reports in pheromone field trapping tests of male captures diminishing rapidly after the first day of deployment. One logical explanation of this effect has been that following the entrapment of a floating pool of previously uncaptured males on the first day, there are fewer males remaining to be captured on subsequent days. However, in light of our results, it appears that some additional reduction in capture levels might also occur due to habituation of uncaptured males. The habituation might even be enhanced by the repeated exposures from the constantly emitting synthetic lures. Our results indicate that even without such continual exposure, long-term habituation from even a few upwind flights could contribute to reduced capture on subsequent days.

These findings strongly suggest that generalizing from laboratory results, using sexually inexperienced males, to natural conditions, involving unknown

distributions of prior experience among wild males, may be a significant source of imprecision in evaluating the probable impact of any proposed field intervention. A greater awareness of the variables controlling the modulation of response to pheromones can enhance laboratory replicability of results, both between and within research institutions, and also provide a basis for comparability of results under naturally varying conditions in the field. Results such as these might thus prove useful in helping to improve either (1) the interpretation of monitoring trap capture results for predicting population densities and thus the optimal timing of insecticidal sprays or (2) the existing and perhaps novel strategies for mating disruption, such as habituating males to unnaturally high dosages of pheromone or conditioning them to unnatural component blends (e.g., Flint and Merkle, 1984).

G. molesta has long been known to be quite susceptible to inadvertent preexposure to sex pheromone, resulting in reduced responses on the same day of exposure to synthetic sources (C. E. Linn, Jr., personal communication). It is not clear whether other species would exhibit a similar degree of long-term habituation. We need to determine whether *G. molesta* males can be made to dishabituate when they are allowed to respond to slightly suboptimal but behaviorally active blends of the three pheromone components. Because of the surprising absence of spontaneous recovery in this species, the possibility also needs to be investigated that positively reinforced associative learning (by successful copulation) might prevent subsequent reduction of response following exposure to pheromone. Thus, much work remains to be done in the service of these various objectives, but the present results should at least serve to establish the need for such research.

ACKNOWLEDGMENTS

This research was supported in part by a Biomedical Research Support Grant to the University of Arizona from the National Institutes of Health (NIH Grant SO7RRO7OO224). Special thanks go to Lewis Petrinovich and Lee Sechrest for reading the manuscript and reviewing the validity of the more involved statistical inferences. We also wish to thank Rick Vetter for the rearing, sexing, and shipping of the insects used in this study and for the preparation of the figures. Our research assistants at the University of Arizona were Heather Asad, Barbara Burtnett, Paul Gatto, Karen Jordan, Rebecca MacDonald, Adam Mann, Kentaro Ogawa, and Michelle Walter. Research instrumentation technician Tony Mascaro also helped in both the design and the construction of the wind tunnel.

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