J. Chem. Ecol., 1975, Vol. 1, No. 4, pp. 475–491

ETHOLOGICAL FUNCTION OF COMPONENTS OF A SEX ATTRACTANT SYSTEM FOR ORIENTAL FRUIT MOTH MALES, *Grapholitha molesta* (LEPIDOPTERA: TORTRICIDAE)

R.T. CARDÉ,[1] T.C. BAKER, and W.L. ROELOFS

New York State Agricultural Experiment Station
Geneva, New York 14456

(*Received January 30, 1975; revised March 10, 1975*)

Abstract—Field studies of male Oriental fruit moth, *Grapholitha molesta* (Busck), behavior indicated that a mixture of *cis*-8-dodecenyl acetate (c8–12:Ac) and ca. 7% *trans*-8-dodecenyl acetate (t8–12:Ac) was requisite for upwind anemotaxis. The simultaneous emission of dodecyl alcohol (12:OH) and the attractant blend of c8–12:Ac containing ca. 7% t8–12:Ac elicited a behavioral repertoire including close-range orientation, landing near the chemical source, wing fanning, and extrusion of the males' abdominal hairpencils in precopulatory display. The effect of 12:OH in increasing trap catches was, therefore, not due to its effect on upwind anemotaxis, but rather to its importance in eliciting landing and other close-range precopulatory behavior. Interestingly, *cis*-8-dodecen-1-ol in extremely low ratios to the attractants (1:333) appeared to duplicate the activity of 12:OH used in ratios of 3:1 to the attractants. Laboratory observations of mating sequences revealed that male hairpencil eversion always preceded copulation. The evidence supports a male response sequence based on specific component combinations and concentrations eliciting successive behavioral steps rather than a response hierarchy dependent on increases in concentration of a single chemical or blend. Additionally, the closeness of the males' approach to c8–12:Ac containing ca. 7% t8–12:Ac was optimal at a discrete emission rate, and male responses were diminished within 30–60 sec after the males' arrival at the attractant source.

Key Words—Tortricidae, *Grapholitha molesta*, Oriental fruit moth, sex pheromone, attractant, synergist, hairpencil, aphrodisiac, *cis*-8-dodecenyl acetate, *trans*-8-dodecenyl acetate, dodecyl alcohol, *cis*-8-dodecen-1-ol.

[1] Present address: Department of Entomology, Michigan State University, East Lansing, Michigan 48824.

INTRODUCTION

Although the majority of Lepidoptera appear to employ female sex pheromone blends (Roelofs and Cardé, 1974*b*), behavioral investigations have been limited to the description of blend attractiveness in nature and excitation in the laboratory. The exact communicative role of the individual pheromone components has remained undefined. The sex pheromone of the Oriental fruit moth, *Grapholitha molesta* (Busck) (Tortricidae: Oleuthrutinae), was identified as *cis*-8-dodecenyl acetate (Roelofs et al., 1969). Recently the requirement for attractancy of ca. 7% *trans*-8-dodecenyl acetate was demonstrated (Beroza et al., 1973*a,b*; Roelofs and Cardé, 1974*a*) although this component has not yet been characterized from the female. Male trap catches also were increased approximately twofold by the addition of two intrinsically nonattractive chemicals: dodecyl alcohol (Roelofs et al., 1973; Roelofs and Cardé, 1974*a*) and 8-propoxyoctan-1-ol (Roelofs et al., 1973) in ratios of 4:1 or 3:1 and 10:1, respectively, to 200 μg of attractant pheromone. Neither dodecyl alcohol or 8-propoxyoctan-1-ol are known to be part of the natural pheromone bouquet of *G. molesta*. The present paper details the communicative role of *cis*- and *trans*-8-dodecenyl acetates and two chemicals which increase trap catches. Some preliminary aspects of this study were reported elsewhere (Cardé et al., 1975*a*).

METHODS AND MATERIALS

Mixtures of *cis*-8-dodecenyl (c8–12:Ac) and *trans*-8-dodecenyl acetates (t8–12:Ac) and of *cis*-8-dodecen-1-ol (c8–12:OH) and *trans*-8-dodecen-1-ol (t8–12:OH) were analyzed quantitatively with a 3% phenyldiethanolamine succinate (HI-EFF 10BP, Applied Science Laboratories, State College, Pennsylvania) on 100–120 mesh Chromosorb-W-AW-DMCS gas chromatographic column. Pure c8–12:Ac was obtained by TLC; pure c8–12:Ac was saponified to the alcohol. All test chemicals were placed on 5 × 7-mm rubber septa (Arthur H. Thomas Co., Philadelphia, Pennsylvania) dispensed in 10-μl Skellysolve B solvent. White sticky traps employed were either Sectar® I with a restricted entrance (Roelofs et al., 1973) or Pherotrap® IC (both supplied by Zoecon Corp., Palo Alto, California).

All experiments were conducted in non-insecticide-sprayed apple orchards in Geneva, New York. Relative attractiveness of various chemical blends in sticky traps was evaluated in a randomized complete-block design with 10 replicates positioned 1 trap/tree at a height of 1.5 m on a 10-m tree spacing. Traps were cleaned of males and rerandomized at least every second day. Data were transformed to $\sqrt{x+0.5}$ and submitted to an analysis of variance, with differences among means being determined according to Duncan's new multiple-range test.

Wild male behavior in the vicinity of a pheromone-baited sticky trap was quantified by 1 or 2 observers situated 2 or 3 m from the attractant source who described individual male behavior onto a tape recorder. Male responses over a 4-min interval near various attractant sources were recorded by allowing individual wild males to enter clear plastic cages 20 × 14 × 10 cm, open to air currents at each of the 4 sides with 2.5 × 5-cm wire screens. The attractant dispenser was positioned on the center of the floor of these cages. Male behavioral patterns were described for 4 min on tape. These behavioral observations were conducted in May, 1974, between 14:00 and 19:00 (Eastern Standard Time), in accordance with the males' diel response interval as shown by an automated trap (Comeau, 1971) indicating time of attraction to synthetic pheromone. Data from the tape recordings were analyzed subsequently with the aid of a stop watch.

Additional studies of male behavior close to an attractant dispenser were conducted using a flat, circular, table-top arena of galvanized sheet metal with a 60-cm radius. The chemical dispenser was located on the table center. Concentric circles marked on the table surface delineated 10-cm intervals from the center. These arenas were set out at a height of ca. 0.7 m approximately 2–3 m from apple trees.

Recordings of visual observations and subsequent analyses of male behavior were undertaken as described previously over observational periods of 10–15 min. Two arenas were employed simultaneously by single observers. To minimize the possibilities of contamination the lowest dispenser dosages of the components were presented first: subsequent treatments utilized increased charge levels. The arenas were moved to new trees 24 m or more crosswind prior to the start of the next observational period. Both experiments were conducted on several days in September, 1974, between 17:00 and 19:30 (EST) with all treatments being accorded equal observational time on each day. Dispenser septa were stored between tests in individual stoppered vials, and the arenas were rinsed with acetone prior to each test series.

Our brief reports of laboratory mating sequences were undertaken with laboratory reared moths in about the 12th hr of photophase under a 16:8 light/dark regime at 22°C.

RESULTS

Attractancy of Males to c8–12:Ac and to c8–12:Ac with ca. 7% t8–12:Ac

The attractancy (trap catch) of a multicompound blend such as 180 μg c8–12:Ac + 20 μg t8–12:Ac or these compounds plus either 600 μg 12:OH or 0.6 μg c8–12:OH, could result from individual components acting in a medley in which each component affects all phases of behavior or by some combinations of components eliciting positive anemotaxis and others mediating close-range searching behavior. We investigated the comparative attractiveness of 200 μg of c8–12:Ac (containing <0.5% t8–12:Ac, undetectable by GLC) and 200 μg c8–12:Ac with 6.8% t8–12:Ac. In several tests, traps baited with the pure c8–12:Ac caught no males, whereas traps baited with the appropriate *cis–trans* mixture captured several hundred males (>200) in 10 hr of observations. By situating 2 observers downwind of the pheromone source, it was possible to follow individual males flying upwind to the traps baited with c8–12:Ac containing 6.8% t8–12:Ac. Males routinely were followed 10 m or more to the trap. No instances of *G. molesta* male orientation or positive upwind anemotaxis toward the pure c8–12:Ac were noted, although equal observational time was accorded both dispensers. A related apple-feeding oleuthrutine, *G. prunivora* (Walsh), would be lured to pure c8–12:Ac (Roelofs and Cardé, 1974*a*), and in flight could be confused with *G. molesta*. Although the temporal distributions of these 2 species overlap broadly, adult *G. prunivora* were not present during the period of these observations, as determined by separate monitoring traps. These findings indicate that male attraction or upwind anemotaxis under natural conditions is elicited by the combination c8–12:Ac and t8–12:Ac and that c8–12:Ac alone is intrinsically unattractive to *G. molesta*.

Effect of c8–12:OH on Male Trap Catches

The roughly twofold increase in male trap catch effected by the addition of dodecyl alcohol (12:OH) (Roelofs et al., 1973; Roelofs and Cardé, 1974*a*) and 8-propoxyoctan-1-ol (PrO-8:OH) (Roelofs et al., 1973) to c8–12:Ac (3–7% t8–12:Ac) suggested the potential of c8–12:OH and 12:OH for affecting male behavior, since these 4 compounds likely could interact with the same hydroxyl and terminal C_{12} active sites on a male antennal pheromone acceptor (Roelofs et al., 1973). Preliminary studies in Australia (G. Rothschild, private communication) indicated that low percentages of c8–12:OH did increase *G. molesta* attractancy.

Numerous field trials were conducted in 1973 and 1974 using 200 μg of c8–12:Ac with 4–8% t8–12:Ac (attractant) and various added quantities of c8–12:OH. One trial (Table 1, Test 1) showed that c8–12:OH reduced male trap catch at attractant/secondary compound ratios of 1:3 to 33:1, with the use of 7.8% t8–12:OH in the c8–12:OH giving a lower catch than the use of 3.5% t8–12:Ac. Tests (Table 1, Tests 2 and 3) of c8–12:OH at ratios greater than 33:1 showed some increases in trap catches, with a dispenser load of ca. 333:1 giving roughly a twofold increase in male catch.

TABLE 1. COMPARISONS OF ATTRACTIVENESS OF c8–12:Ac WITH 6.6% t8–12:Ac (ATTR.) WITH VARIOUS ADDED AMOUNTS OF c8–12:OH USING SECTAR I TRAPS

Treatment	Males/trap ($\bar{X}$)[a]
Test 1 (August 13–20, 1973)	
200 μg Attr.	40.6 a
200 μg Attr. + 6 μg c8–12:OH (3.5% t8–12:OH)	43.0 a
200 μg Attr. + 60 μg c8–12:OH (3.5% t8–12:OH)	17.4 c
200 μg Attr. + 600 μg c8–12:OH (3.5% t8–12:OH)	8.2 d
200 μg Attr. + 6 μg c8–12:OH (7.8% t8–12:OH)	29.2 b
200 μg Attr. + 60 μg c8–12:OH (7.8% t8–12:OH)	18.2 c
200 μg Attr. + 600 μg c8–12:OH (7.8% t8–12:OH)	4.6 d
Unbaited	0.0 e
Test 2 (August 27–31, 1973)	
200 μg Attr.	20.6 cd
200 μg Attr. + 0.6 μg c8–12:OH (< 0.1% t8–12:OH)	33.3 a
200 μg Attr. + 2 μg c8–12:OH (< 0.1% t8–12:OH)	23.8 bc
200 μg Attr. + 6 μg c8–12:OH (< 0.1% t8–12:OH)	16.3 d
200 μg Attr. + 20 μg c8–12:OH (< 0.1% t8–12:OH)	9.9 e
Unbaited	0.0 f
Test 3 (May 17–22, 1974)	
200 μg Attr.	8.0 b
200 μg Attr. + 0.06 μg c8–12:OH (6.6% t8–12:OH)	11.2 ab
200 μg Attr. + 0.2 μg c8–12:OH (6.6% t8–12:OH)	11.3 ab
200 μg Attr. + 0.6 μg c8–12:OH (6.6% t8–12:OH)	17.4 a
200 μg Attr. + 2 μg c8–12:OH (6.6% t8–12:OH)	14.5 ab
Unbaited	0.0 c

[a] Within each test, treatment means followed by the same letter are not significantly different at the 5% level.

Together these data show that at certain low ratios relative to the attractant, c8–12:OH will elicit an increase in male trap catch, similar to the effects of 12:OH and PrO-8:OH at much higher ratios.

The Role of 12:OH in Close-Range Searching Behavior

The increase in male trap catch effected by the addition of discrete quantities of PrO–8:OH, 12:OH, and c8–12:OH to c8–12:Ac (6.8% t8–12:Ac) could be related to an increase in the frequency of elicitation of male anemotaxis downwind of the attractant source or in a modification of close-range searching behavior. Comparative observations of male behavior within 1 m of Pherotraps IC baited with either 200 μg c8–12:Ac (6.8% t8–12:Ac) or 200 μg c8–12:Ac (6.8% t8–12:Ac) and 600 μg 12:OH revealed distinctly disparate patterns of close-range searching behavior. Individual males were followed continuously from the time upwind orientations brought them within approximately 3 m of the trap until they were ensnared on the trap sticky surface or until they ceased apparent searching behavior by leaving the trap vicinity. The direction of departure was almost always directly downwind. Males terminating close-range searching behavior were observed until they flew at least 5 m from the trap, although searching behavior was scored as terminated once males flew more than 3 m from the trap. None of our observations indicated that males which had terminated searching behavior later reoriented to the attractant source. Nonetheless, the possibility of multiple observations of a single moth was minimized by repositioning traps 20 m crosswind within the orchard at 15 min intervals. No more than 13 males were observed prior to trap repositioning.

Male flight within 1 m of a trap was characterized by a decreased precision of orientation and an undulating flight, during which the distance of the males to the attractant source remained relatively constant, while males generally flew up, down, and from side to side in "casting" movements of roughly 5–20 cm and 1–2 sec in duration. Such close-range searching behavior (before capture or flight away from the trap) occurred for a mean of 19.8 sec (range of 5–69 sec, $n = 39$) with a trap baited with 200 μg c8–12:Ac (6.8% t8–12:Ac), whereas the mean time of searching flight with a trap baited with c8–12:Ac (6.8% t8–12:Ac) plus 600 μg 12:OH was 12.5 sec (range 3–50, $n = 40$). These means are significantly different at $P<0.05$ according to the t test.

The frequency of males landing on the trap's lower sticky surface (and thereby approaching within at least 10 cm of the attractant) differed markedly with the addition of 12:OH to the attractant dispenser. The attractant alone produced a trap capture rate of 56.4%, where the confidence interval (c.i.) at $t = 0.95$ extends from 40.8 to 72.0%, whereas the attractant plus 12:OH resulted in 92.5% of the males being captured, where the c.i. at $t = 0.95$ extends from 80.1 to 98.4%. The communicative function of 12:OH added to the c8–12:Ac (6.8% t8–12:Ac) attractant did not seem to be effecting an increase in the number of males attracted to within 3 m of the attractant source. On the contrary, in this behavioral context, 12:OH appears either to modify the males' close-range searching behavior, or to cause the males to land, or both. Since the ensnarement of males on the sticky trap surface terminates the behavioral response, analyses were conducted utilizing assays which allowed continued observations of male responses.

Behavioral Effects of 4 Minutes' Exposure to Multicomponent Systems

Males were allowed to orient toward and enter a baited 20 × 14 × 10-cm clear plastic cage hand held at a height of 1.5 m. Immediately after a male had flown into the assay device, the top of the box was replaced and recording of behavior commenced. Four distinct categories of behavior were evident: (1) flying and hovering; (2) wing fanning on the cage surface (concurrent with rapid walking or while stationary); (3) rapid walking; and (4) quiescence.

Analyses of these behavioral modalities for the 4-min observational period revealed distinct changes in their relative frequencies dependent on the nature of the stimulus and the time elapsed (Figure 1). During the first

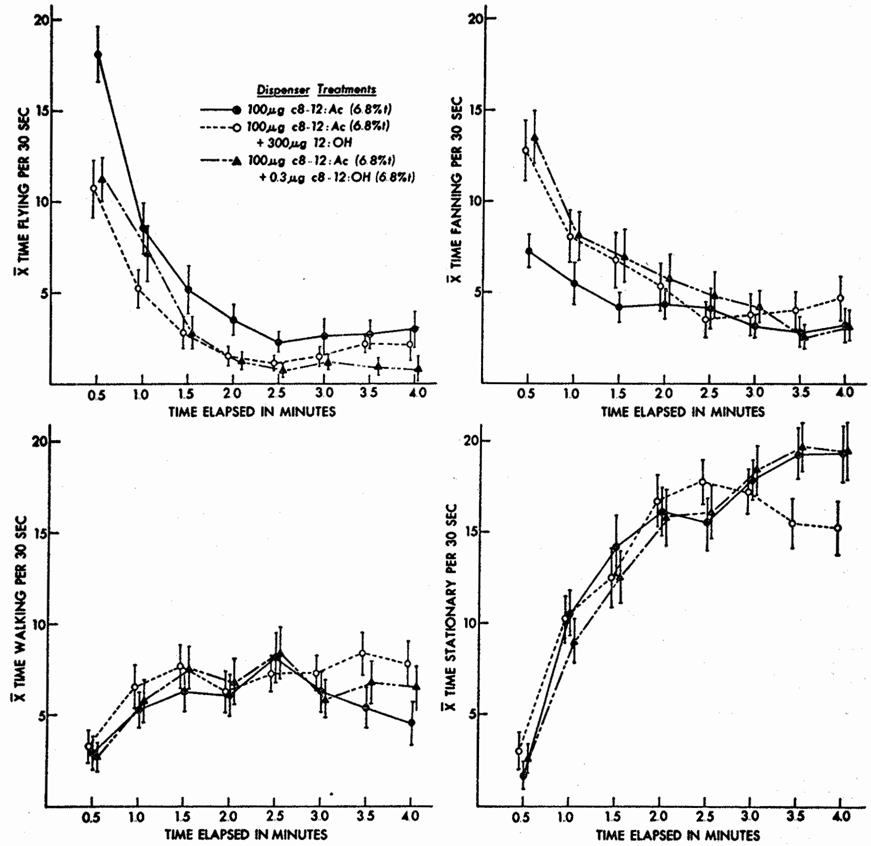

FIG. 1. Comparisons of the behavioral responses of males to blends during the first 4 min confinement after upwind attraction. Bars enclose the standard errors of the means for 30-sec intervals.

TABLE 2. TEMPORAL ANALYSES OF BEHAVIOR DURING THE FIRST 30 SEC ELAPSED AFTER UPWIND ORIENTATION

Stimulus	During first 30 sec		Initial behavior	
	Time flying ($\bar{X}$)	Time fanning/ walking ($\bar{X}$)	Time 1st flying ($\bar{X}$)	Time 1st fanning/ walking ($\bar{X}$)
100 μg c8–12:Ac (6.8% t8–12:Ac)	18.10	7.32	15.00	3.71
100 μg c8–12:Ac (6.8% t8–12:Ac) + 300 μg 12:OH	10.79***[a]	12.86**[a]	5.07**[a]	6.93
100 μg c8–12:Ac (6.8% t8–12:Ac) + 0.3 μg c8–12:OH (6.8% t8–12:Ac)	11.32***[a]	13.48***[a]	10.67	8.90**[a]

[a] **, *** differ from $\bar{X}$ of c8–12:Ac (6.8% t8–12:Ac) at $P < 0.01$ and $P < 0.001$, respectively, according to the t test.

30 sec of observations (Table 2) c8–12:Ac (6.8% t8–12:Ac) alone elicited flying or hovering behavior for a significantly longer time than these compounds plus 12:OH or c8–12:OH (6.8% t:–12:OH). During the first 30 sec the mean time of males fanning or fanning concurrent with walking was enhanced by the presence of 12:OH or c8–12:OH (6.8% t8–12:OH). These same trends are evident if one contrasts the mean times of the first occurrence (initial behavior, columns 3 and 4 of Table 2) of these behavioral responses during the initial 30 sec of observations.

For the three chemical mixtures presented, several trends are evident during the remaining 3.5 min of observations. The relative time males engaged in flying, fanning, or fanning while walking decreases concurrent with increases in time spent walking or not moving. There are no significant ($P < 0.05$) differences among any of the treatment means for the last 3.5-min observation period when behavioral modalities are compared at the same elapsed times. Such time-dependent changes may indicate a degree of habituation (alteration of the pheromone response threshold) within 30–60 sec from the time of attraction.

Effect of Dispenser Charges of 1 to 3000 μg c8–12:Ac (6.8% t8–12:Ac)

Observations of male behavior within 1 m of the 60-cm-radius table top baited with various charges of c8–12:Ac (6.8% t8–12:Ac) showed varying responses in several behavioral catagories (Table 3). Intermediate dosages effected quantitatively significant increases in frequencies of male landing and

TABLE 3. MALE BEHAVIOR NEAR 60-CM-RADIUS METAL ARENAS BAITED WITH 1 TO 3,000 μG OF *cis*-8-DODECENYL ACETATE (c8–12:Ac) WITH 6.8% t8–12:Ac ISOMER

μg c8–12:Ac (6.8% *trans*)	No. males observed	% Landing on table top[a]	% Fanning on table top[a]	% Displaying on table top	Orientation time, sec ($\bar{X}\pm$SE)[b]	Fanning time, sec ($\bar{X}\pm$SE)	Closest approach, cm($\bar{X}\pm$SE)[b]
1	14	36 cde	0 d	0	3.2 d±0.5	0	55.4 a±1.9
3	25	24 de	0 d	0	6.6 c±0.9	0	57.5 a±1.0
10	36	53 bc	22 b	8	14.9 a±3.2	8.0±1.0	43.2 b±3.9
30	43	65 ab	16 bc	0	17.6 a±2.3	8.7±0.5	44.7 b±2.9
100	54	69 ab	17 bc	0	14.1 a±1.7	2.9±0.2	45.1 b±2.5
300	67	37 cd	1 d	0	9.9 b±1.1	0	55.9 a±0.9
1,000	55	40 cd	5 cd	0	7.5 b±0.6	3.7±0.3	56.0 a±1.0
3,000	53	17 e	4 cd	0	5.1 c±0.4	1.5±0.1	58.7 a±0.6
100+300 μg 12:OH	46	80 a	48 a	2	16.5 a±2.4	6.5±1.1	36.9 c±3.2

[a] Percentages in the same column followed by the same letter do not differ at the 5% level when compared according to a 2×2 test of independence using the *G* statistic with Yates' correction.

[b] Means in the same column followed by the same letter do not differ at the 5% level according to the *t* test. Orientation time includes all categories within 0.5 m of the table top. In tabulating closest approach to the dispenser, males flying within 0.5 m of the table top but not landing were scored as approaching within 60 cm. The remaining approaches were by males walking on the table top.

TABLE 4. MALE BEHAVIOR NEAR 60-CM ARENAS BAITED WITH 10 μG *cis*-8-DODECENYL ACETATE (6.8% t8–12:Ac) PLUS DODECYL ALCOHOL (12:OH)

μg 12:OH	No. males observed	% Landing on table top[a]	% Fanning on table top[a]	% Displaying on table top[a]	Orientation time, sec ($\bar{X}\pm$SE)[b]	Fanning time, sec ($\bar{X}\pm$SE)[b]	Closest approach, cm ($\bar{X}\pm$SE)[b]
—	37	68	27	3	15.0 ± 1.8	7.3 ± 0.7	47.5 ± 2.5
1	40	88*	73**	8	20.7 ± 3.1	9.3 ± 1.6	33.6*** ± 3.2
3	39	77	36	23*	23.7** ± 2.6	16.5** ± 2.0	36.7* ± 3.9
10	33	94**	83**	42**	23.6* ± 3.1	15.9* ± 1.9	19.9*** ± 3.9
30	40	80	45	15	18.6 ± 2.7	11.4 ± 1.4	35.3** ± 3.6
100	30	83	43	30*	25.4 ± 5.1	18.3** ± 2.5	28.5*** ± 4.3

[a] Percentages in the same column compared to the treatment lacking 12:OH according to a 2×2 test of independence using the *G* statistic with Yates' correction: * indicates $P < 0.05$, ** $P < 0.01$.

[b] Means in the same column compared to the treatment lacking 12:OH according to a *t* test: * indicates $P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Orientation time includes all categories of behavior within 0.5 m of the table top. In tabulating closest approach to dispenser, males flying within 0.5 m of the table top but not landing were scored as approaching within 60 cm. The remaining approaches were by males walking on the table top.

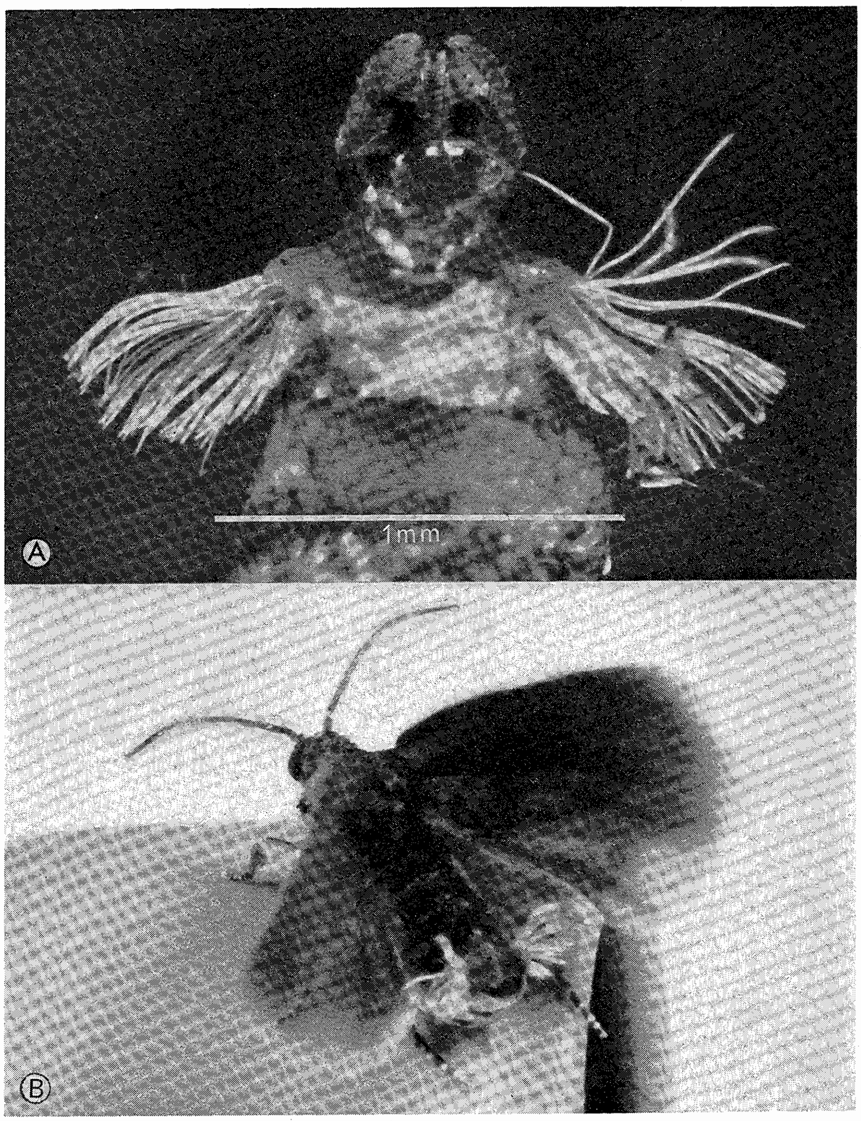

FIG. 2. (A) Male abdominal tip with hairpencils forcibly extruded. (B) Laboratory male everting his hairpencils on a rubber septum charged with 10 μg *cis*-8-dodecenyl acetate (6.8% *trans*) and 10 μg dodecyl alcohol. Electronic flash duration was 1/1000 sec.

(*Facing p.* 485)

walking concurrent with wing fanning, in mean times of orientation (flight or walking on the table top) and wing fanning, and in the closest approach to the chemical dispenser while walking on the table top. Since the lowest dispenser dosages (1 μg) would emit relatively reduced amounts of attractant, the lowered mean orientation time and close approach to this dispenser is probably related to the visual cues presented by the experimental observation table. Otherwise, continued upwind anemotaxis toward the chemical dispenser would be expected.

Effect of 12:OH with 10 µg c8–12:Ac (6.8% t8–12:Ac)

Addition of 300 μg of 12:OH to 100 μg of c8–12:Ac (6.8% t8–12:Ac) produced responses generally similar to those evoked by 100 μg c8–12:Ac (6.8% t8–12:Ac) alone, although the simultaneous emission of 12:OH elevated the frequency of landing and produced a closer approach to the chemical dispenser. Since 10 μg of attractant on the dispenser was the lowest charge level that elicited increased responses in the tabulated behavioral parameters, male responses to a combination of this charge plus 1–100 μg of 12:OH were examined in another experiment (Table 4). The response values for 10 μg of attractant alone in this test correlate very closely with those found in the previous test (Table 3). Concurrent release of 12:OH produced significant increases in the frequencies of landing on the observation table and wing fanning while walking, in mean times of orientation and fanning, and in closest approach to the chemical dispenser. Hairpencil display behavior (Figure 2)—found in a low percent of males in the previous observations—was noted in 42% of the males (51% of those landing) observed in the 10 μg attractant:10 μg 12:OH treatment.

Hairpencil display in general involved wing fanning while walking to within 1 to 2 cm of the rubber septum dispenser. Continuing to fan their wings males then turned away from the septum and everted their hairpencils 1–8 times, followed by a turning to face the septum, repeating this sequence until the male would abruptly fly away. Males would often crawl onto the septum, curl their abdominal tips toward the septum, or push the septum with their heads before departing. The temporal patterns of hairpencil eversions and turns are listed in Table 5. All dispenser dosages of 12:OH tested appeared to evoke similar patterns of precopulatory display.

Mating Behavior: The Role of Hairpencilling

Mating behavior was observed in the laboratory in a 0.15 × 1.5-m clear plastic wind tunnel with a wind speed of ca. 12 m/min. Males were released 1.2 m downwind of a calling female. Responsive males walked upwind while

TABLE 5. BEHAVIOR OF MALES ENGAGED IN HAIRPENCIL DISPLAY (CONCURRENT WITH WING FANNING)[a]

10 μg c8–12:Ac (6.8% t)+μg 12:OH	No. males displaying	Time display, sec ($\bar{X} \pm$ SE)	No. pencil eversions ($\bar{X} \pm$ SE)	No. eversions prior to turn ($\bar{X} \pm$ SE)	No. turns ($\bar{X} \pm$ SE)
0	1	2.0	4.0	2	2
1	3	7.7	8.0	3.2	2.5
3	9	14.9±1.4	11.4±1.8	3.7±0.7	3.0±0.6
10	14	12.6±1.8	9.6±1.2	3.1±0.8	4.1±0.6
30	6	15.5±1.1	15.0±0.7	3.8±0.8	5.2±0.6
100	9	10.9±1.9	7.9±1.2	3.2±0.8	3.3±0.5

[a] These are the males of Table 4, column 5.

fanning their wings. In all (n = 18) successful copulations, male hairpencil eversion preceded the males' attempts to couple. Males walked to within 1–2 cm of the female, turned 180° away, and everted the hairpencils toward the females' anterior or sides while continuing to fan their wings. During 14 of 18 sequences of hairpencil extrusion displays, the female either turned toward the males' posterior, or walked rapidly toward the male, or both. In some instances the females' run toward the male brought their antennae into apparent contact with the males' abdominal tip, whereas in others no tactile stimulus preceded the males' 180° whirl and subsequent curling of the posterior of his abdomen preceding a copulatory attempt. Initial experiments indicate that an olfactory cue is involved in eliciting female orientation, since methylene chloride extracts of a single male's hairpencils when presented on filter paper within 1–2 cm of a calling female generally elicit walking toward the stimulus. It is probable that the hairpencils also act as an aphrodisiac, producing female acquiescence to copulatory attempts (Birch, 1974).

DISCUSSION

In the Lepidoptera the ubiquitous nature of communication systems involving two or more chemicals has been elucidated only recently, and so it is hardly surprising that little is known of the behavioral function of individual chemical components. Apparent attractancy of isolated natural or synthetic compounds has been considered by most investigators as the prime behavioral response evoked by so-called attractant pheromones, when indeed pheromone-mediated upwind anemotaxis has been demonstrated (Kennedy and

Marsh, 1974) in but a few species. Single compounds or combinations thereof which produce a trap catch have been regarded as being intrinsically attractive when, as Kennedy (1972) has pointed out, the behavioral effect could be of quite a different nature, such as eliciting landing when in the vicinity of a trap. Similarly, nonattractive chemicals that increase trap catch (so-called synergists) and those that decrease trap catch (so-called inhibitors or anti-attractants) when released simultaneously with attractants could mediate close-range behavior rather than upwind anemotaxis.

In *G. molesta* the present data indicate that the combination of c8–12:Ac and t8–12:Ac elicits upwind orientation. In concert these two compounds are attractants in the sense of evoking apparent upwind anemotaxis. Three related chemicals, PrO–8:OH, 12:OH and c8–12:OH, increase male trap catches although they do not appear to possess intrinsic attractiveness. In the case of 12:OH the effects appear unrelated to long-range searching behavior. In the presence of c8–12:Ac and t8–12:Ac, 12:OH apparently evokes a repertoire of precopulatory behavior: close approach to the chemical emitter, landing, and wing vibration, either while stationary or concurrent with walking, and hairpencil extrusion in precopulatory display. Similar responses in landing and wing vibration and increases in trap catches were evoked by c8–12:OH at certain low ratios, whereas at high ratios trap catch was decreased. Such dual effects of c8–12:OH on trap catch are quite similar to those reported by Baker et al. (1975) for the alcohols corresponding to the *Playtnota stultana* (Walsingham) attractant pheromone, *cis*- and *trans*-11-tetradecenyl acetates (17:83). When the alcohols were present as 0.2–2% of the attractant pheromone mixture, the trap catch was elevated; at >20% the catch was suppressed. In *P. stultana* it is not known if the mechanism responsible involves modification of close-range behavior. In *G. molesta* the modification of close-range orientation is apparently related to increases in the catch on sticky traps baited with c8–12:Ac, t8–12:Ac plus 12:OH. Hence, in *G. molesta* the term attractant "synergist" is clearly inappropriate to the behavior evoked by 12:OH.

When c8–12:Ac with ca. 7% t8–12:Ac was presented at 1–3000 μg per rubber septum dispenser, charges of 10, 30, and 100 μg elicited the closest mean approaches to the dispensers and the longest mean searching times (Table 3). Such a concentration-dependent response pattern appears analogous to the behavior of two noctuid moth species in which the male catches were reported to be optimal at certain discrete emission rates of *cis*-7-dodecenyl acetate (Kaae et al., 1973).

Males approaching the arena's edge frequently engaged in "casting" flight for 10 or more seconds within 1–20 cm of the arena's edge. The flight pattern undulated both laterally and vertically as much as 30 cm, and the precision of orientation would appear to be altered from the more direct

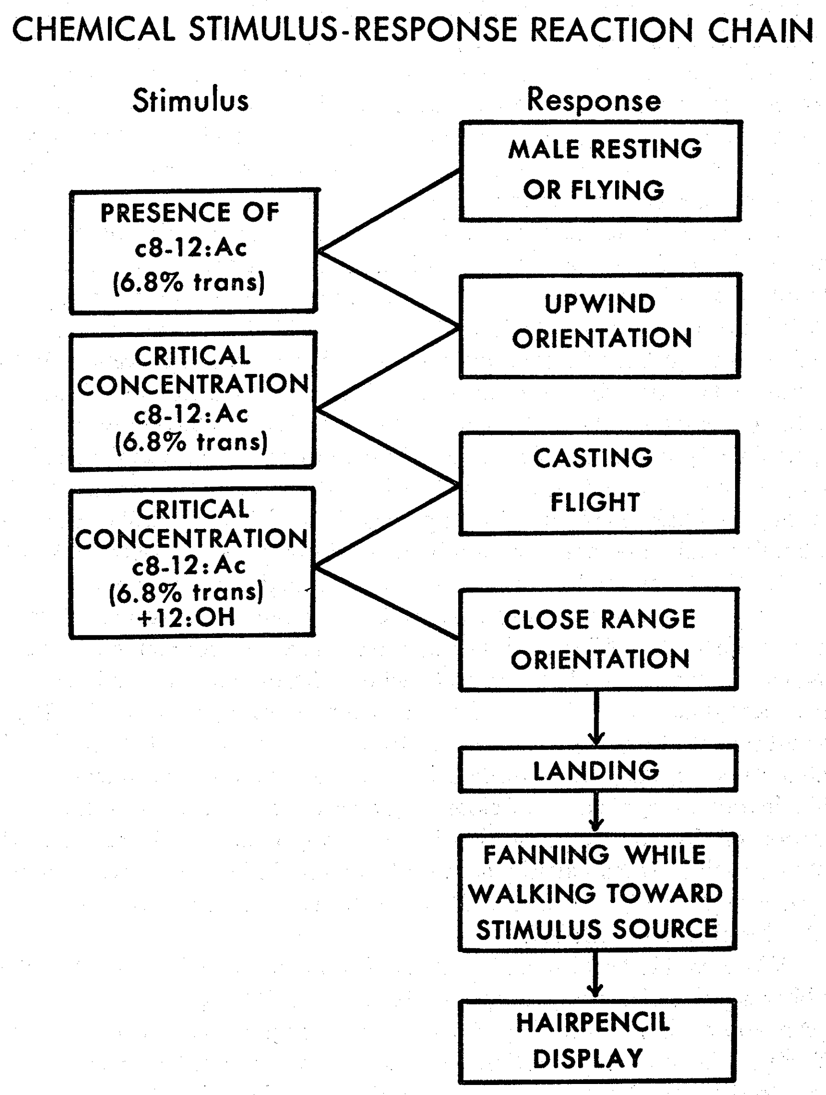

FIG. 3. Stimulus–response reaction chain. The behavior is indicated on the right and the chemical stimulus mediating the response is presented on the left.

flight path taken in upwind orientation. While the role of vision was not assessed directly, the presence of the arena surface is probably related to the mean closest approach to the chemical dispensers: males often flew for extended periods within several centimeters of the arena's edge.

The mean time spent within 20 cm of the arena, frequency of landing on the arena's surface, the mean time of wing fluttering concurrent with walking, and mean closest approach to the chemical dispenser were all elevated in response to the simultaneous emission of 12:OH in the presence of critical concentration of c8–12:Ac and t8–12:Ac over the emission of c8–12:Ac and t8–12:Ac alone. Extrusion of male hairpencils in precopulatory display ritual was evoked by 12:OH. Interestingly, such mediation of hairpencil display by a particular chemical component has not been reported previously, although George (1965) observed hairpencil extrusion in natural pheromone-stimulated *G. molesta* males. Indeed, the very existence of male brush organs within the Tortricoidea is not well documented.

The accumulated observations suggest that in *G. molesta* upwind anemotaxis is governed by a two-chemical blend, whereas close-range orientation and precopulatory display is effected by the addition of a third component. This mechanism contrasts with the hierarchy of behavior concept in which successive steps of the pheromone response sequence are elicited by increasing pheromone concentrations (Schwinck, 1955). This latter sort of pheromone-regulated behavior would be most likely in those species utilizing a communication system based on one chemical. The sequence of events in *G. molesta* as presently envisaged is summarized in the chemical stimulus–response reaction chain presented in Figure 3.

As yet only c8–12:Ac is known to be present in the pheromone-producing female abdominal tip of *G. molesta* (Roelofs et al., 1969), although it is reasonable to suppose on these results that additional components are involved in the natural mating communication system of this species. Notwithstanding, the finding that a specific chemical component can modify close-range orientation and precopulatory behavior has importance to the mating disruption technique, in which the pheromone or pheromone-related chemicals are dispersed throughout the air to prevent mating. Obviation of long-distance mate finding may not prevent mating (Cardé et al., 1975*b*) since attraction and mating behavior each may be modulated by a precise blend and concentration of components.

The complexity of manipulating pheromonal communication has been pointed out by Rothschild (1974) in disruption trials with *G. molesta* in Australia. Dodecyl acetate (12:Ac) emitted simultaneously with an attractant blend of c8–12:Ac containing ca. 3–7% t8–12:Ac from a single dispenser locus depressed male trap catch relative to the attractant blend alone (Roelofs et al., 1973; Roelofs and Cardé, 1974*a*; Rothschild, 1974). However, atmospheric permeation with 12:Ac released from spaced evaporator stations elevated the catches of nearby traps baited with the attractant blend alone (Rothschild, 1974). When permeating the atmosphere, 12:Ac may habituate or modify the close-range behavioral repertoire normally elicited by additional components such as 12:OH. This habituating effect on close-range behavior could result in increased trap catches with traps baited only with the attractant blend.

Acknowledgments—This research was supported in part by the Rockefeller Foundation and by NSF Grant GB-38020. We thank G. Catlin and R. McMillen for preparation of the figures, Dr. A. Hill for analyses of the chemical mixtures, and Dr. M. Birch for his comments on this paper.

REFERENCES

BAKER, J.L., HILL, A.S., CARDÉ, R.T., KUROKAWA, A., and ROELOFS, W.L. 1975. Sex pheromone trapping of the omnivorous leafroller, *Platynota stultana*. *Environ. Entomol.* 4:90–92.

BEROZA, M., GENTRY, C.R., BLYTHE, J.L., and MUSCHIK, G.M. 1973*a*. Isomer content and other factors influencing captures of Oriental fruit moth by synthetic pheromone traps. *J. Econ. Entomol.* 66:1307–1311.

BEROZA, M., MUSCHIK, G.M., and GENTRY, C.R. 1973*b*. Small proportion of opposite geometrical isomer increases potency of synthetic pheromone of Oriental fruit moth. *Nature* 244:149–150.

BIRCH, M.C. 1974. Aphrodisiac pheromones in insects, pp. 115–134, *in* M.C. Birch (ed.), *Pheromones*. North Holland Publ. Co., Amsterdam.

CARDÉ, R.T., BAKER, T.C., and ROELOFS, W.L. 1975*a*. Behavioural role of individual components of a multichemical attractant system in the Oriental fruit moth. *Nature* 253:348–349.

CARDÉ, R.T., TRAMMEL, K., and ROELOFS, W.L. 1975*b*. Disruption of sex attraction of the redbanded leafroller (*Argyrotaenia velutinana*) with microencapsulated pheromone components. *Environ. Entomol.* 4:448–450.

COMEAU, A. 1971. Physiology of sex pheromone attraction in Tortricidae and other Lepidoptera (Heterocera). Ph.D. Thesis, Cornell University, Ithaca, New York.

GEORGE, J.A. 1965. Sex pheromone of the Oriental fruit moth, *Grapholitha molesta* (Busck) (Lepidoptera: Tortricidae). *Can. Entomol.* 97:1002–1007.

KAAE, R.S., SHOREY, H.H., and GASTON, L.K. 1973. Pheromone concentration as a mechanism for reproductive isolation between two lepidopterous species. *Science* 179:487–488.

KENNEDY, J.S. 1972. The emergence of behaviour. *J. Aust. Entomol. Soc.* 11:168–176.

KENNEDY, J.S., and MARSH, D. 1974. Pheromone-regulated anemotaxis in flying moths. *Science* 184:999–1001.

ROELOFS, W.L., and CARDÉ, R.T. 1974*a*. Oriental fruit moth and lesser appleworm attractant mixtures refined. *Environ. Entomol.* 3:586–588.

ROELOFS, W.L., and CARDÉ, R.T. 1974*b*. Sex pheromones in lepidopterous species, pp. 96–114, *in* M.C. Birch (ed.), *Pheromones*. North Holland Publ. Co., Amsterdam.

ROELOFS, W.L., COMEAU, A., and SELLE, R. 1969. Sex pheromone of the Oriental fruit moth. *Nature* 224:723.

Roelofs, W.L., Cardé, R.T., and Tette, J. 1973. Oriental fruit moth attractant synergists. *Environ. Entomol.* 2:252–254.

Rothschild, G.H.L. 1974. Problems in defining synergists and inhibitors of the Oriental fruit moth pheromone by field experimentation. *Entomol. Exp. Appl.* 17:294–302.

Schwinck, I. 1955. Weitere Untersuchungen zur Frage Geruchsorientierung der Nachtschmetterlinge: Partielle Fühleramputation bei Spinnermännchen, insbesondere am Seidenspinner *Bombyx mori* L. *Z. Vergl. Physiol.* 37:439–458.