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THE SEXUAL COMMUNICATION SYSTEM OF THE ORIENTAL FRUIT MOTH, *GRAPHOLITHA MOLESTA* (BUSCK)

by

Thomas Charles Baker

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

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1979

ABSTRACT

THE SEXUAL COMMUNICATION SYSTEM
OF THE
ORIENTAL FRUIT MOTH, GRAPHOLITHA MOLESTA (BUSCK)

by
Thomas Charles Baker

The sexual communication system of Grapholitha molesta (Busck), the Oriental fruit moth, involves a female-emitted pheromone consisting of (Z)-8-dodecenyl acetate, (E)-8-dodecenyl acetate, (Z)-8-dodecenyl alcohol, and dodecanol. Periodicities of both female calling and male response to sex pheromone were determined in part by circadian rhythms. The lights-on photoperiodic cue was partly responsible for setting the phase of the calling rhythm. Absolute temperature levels and not necessarily a decrease in temperature modified the timing of calling; there were both high (34°C) and low (15°C) thresholds of temperature and, at particular photoperiod times, a temperature range optimal for calling. Previous performance of calling may establish a refractory period. The ability to use both an endogenous clock and exogenous temperature cues to synchronize sexual activity appears adaptive for a temperate-zone insect exposed to both long periods of favorable climatic conditions in summer and harsh, unpredictable conditions in spring or fall.

Three G. molesta sex pheromone components (Z)-8-dodecenyl acetate, (E)-8-dodecenyl acetate, and (Z)-8-dodecenyl alcohol acted as a unit to increase male behaviors both early and late in the orientation sequence. A fourth component, dodecanol, added to the other 3 only increased a later behavior, the hairpencil display. The pheromone

components' behavioral effects are described most precisely only when each component is considered in combination with the others, not individually or in binary or 3-component blends. There was a strong correlation between pre-flight wing fanning while walking and upwind flight in the pheromone plume, suggesting that these behaviors should be considered similar in function. Fitting pheromone-induced behavioral responses into slots in a sequence will not prove illuminating unless the sequence is independent of spatially-induced and bioassay design-related artifacts.

Courtship behavior, as analyzed by conditional probability matrix techniques, consists of a relatively stereotyped sequence of behaviors culminating in an elaborate hairpencil display directed toward and performed 1 or 2 cm from the female. The display is comprised of multiple rhythmic extrusions and retractions of the white hairpencil organs and claspers at the end of the abdomen, accompanied by corresponding 45 and 90 cm/sec "puffs" of wind generated by the vibrating wings and directed toward the female. The stimuli contained in the display, primarily chemical and anemo-tactile (wind movement), attract the female, who walks to the hairpencils, where contact with the end of the abdomen causes the female to cease walking. The tactile stimulus from the female causes the male to turn quickly and attempt copulation. The male hairpencils and courtship behavior of this species are hypothesized to have evolved primarily by means of sexual selection involving "female choice", in which a female preference and preferred male trait become linked as a result of the mating advantage conferred upon male offspring of discriminating females. Male

courtship pheromones of other Lepidoptera and "coyness" in females also may have evolved by this mechanism. The hairpencil display is viewed as a form of clasper extension behavior that has become "ritualized" through the process of sexual selection.

To My Mother and Father,

Ygerne and Tom

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INTRODUCTION

The Oriental fruit moth, Grapholitha molesta (Busck) is a world-wide pest of peaches and apples, and was introduced into North America from Japan in about 1912. It is a problem on these crops not only because it inflicts direct damage to fruit making them unmarketable, but because the larvae often feed on growing shoots. This feeding kills the shoots and causes severe damage to young trees. The adults accomplish mating by means of a sex attractant pheromone, first demonstrated by George (1965). A major component of this perhomone was identified by Roelofs et al. (1969), and the addition of two other components produced a commercially available synthetic sex attractant which has been used throughout the 1970's to monitor the adult males of this pest.

For economically important insects like G. molesta, sex attractant pheromones provide a sensitive, species-specific means of population sampling and are presently an integral part of many pest management systems. The rate of isolation and identification of new pheromone structures in Lepidoptera is increasing rapidly, but minimal attention has been paid to the precise roles of pheromone components in communication. The efficacy of attractants has usually been expressed in terms of relative numbers of males captured in traps, somewhat understandably, since their primary use has been as lures in monitoring traps. But if the utility of pheromones is ever to be

expanded, for instance using them to estimate absolute population density, then knowledge of species' sexual communication systems must be expanded accordingly.

With the advent of effective controlled-release devices to permeate the atmosphere with pheromone, the use of pheromones as communication disruptants to directly control pest populations is a reality. Unfortunately, in most cases it is not known what is being communicated nor how the disruption is effected. Intelligent, efficient formulation of pheromone components as disruptants may only be possible after studying the behavioral effects of these components in the context of the natural communication system. Understanding the communication systems of several species may allow general principles to be used in a predictive way for newly identified pheromone systems.

In this dissertation I examine in three chapters three phases of sexual communication in G. molesta which can be roughly described as: 1) initiation of communication, in which males and females maintain synchrony through both circadian rhythms and responses to environmental cues, and the female begins releasing pheromone; 2) attraction phase, in which the male's behavioral responses to female-emitted pheromone components result in location of the female; and 3) courtship, where there is a rapid exchange of information between male and female resulting in copulation.

CHAPTER 1

Endogenous and Exogenous Factors Affecting Periodicities of Female Calling and Male Sex Pheromone Response in *Grapholitha molesta*

INTRODUCTION

Knowledge of diel sexual periodicity in moths is important from at least two standpoints. From an applied aspect, the duration of diel male sexual responsiveness to pheromone traps can determine the trap capture magnitude, which in turn influences estimates of population density. Pest management decisions in the future may be made using trap capture frequency to infer population size. Secondly, knowledge of the diel temporal activity pattern of a population is important to understanding intraspecific communication and its role in the temporal organization of a community.

Several reports have indicated that adult Oriental fruit moth, Grapholitha molesta (Busck), activity in the field occurs in the 2-3 hours preceeding sunset (Dustan 1961, Rothschild and Minks 1974, Gentry et al. 1975). Laboratory observations also showed mating behavior to occur in the few hours before lights-off (Dustan 1964, George 1965). Rothschild and Minks determined in the field that cool spring temperatures seemed responsible for an advancement of male attraction to pheromone to earlier, warmer hours of the day, implying that these exogenous temperature factors exerted some control over response time. However, there was some evidence of a circadian rhythm, because time of male attraction remained advanced even on those spring days when the temperature remained above the flight threshold until late in the day. Also, laboratory mating periodicity persisted in continual

light (George 1965), further evidence for a circadian rhythm. There has been no direct experimental work to determine the factors influencing the observed periodicities of G. molesta female calling behavior and male pheromone response. We report here laboratory experiments indicating that these periodicities are both endogenously and exogenously controlled.

MATERIALS AND METHODS

A. Rearing.--G. molesta adults were from a laboratory colony originating from Michigan apples and maintained at Michigan State University since 1975. Larvae were reared on small green apples at 25-26°C, 70% relative humidity, and a 16:8, light:dark photoperiod regime. Photophase (daylight) light intensity was ca. 2100 lux and scotophase (night) intensity was ca. 0.3 lux. When available, feral adults were added to the mating stock. Adults were segregated by sex as pupae (George 1965), and the subsequent adult males and females held in separate cages according to emergence date.

B. Female Calling Observations.

1. Female age, circadian rhythm, photoperiod cues, and temperature effects.--Females greater than 1 day old, except in those experiments measuring female age effects, were placed in individual clear plastic cups, 4 x 4 cm top diam., having plastic-lined cardboard lids. In the female age experiment, groups of females were 0 - 9 hrs, and 1, 2, 3, 4, 5, and greater than 6 days old. Each cup contained a 1 cm long dental wick soaked with distilled water to maintain ca. 100% relative

humidity. Unless a special temperature effect was tested, calling observations were performed at the rearing temperature, 25 - 26°C. A female was scored as calling if at least the ovipositor's anal papillae were extruded or, when the abdomen was turned away from the observer, if the female assumed the typical calling posture. In this posture, the female's wings are elevated, the legs extended, and the abdomen raised above the substrate (Figure 1). The mean time of calling was calculated as the time at which 50% of the calling hours had occurred on a given day.

Photophase light intensity was 2100 lux and scotophase intensity 0.3 lux. During scotophase viewing was accomplished with a light having a Kodac Wratten filter eliminating light below 6800 nm. Temperature changes were accomplished using two environmental cabinets and two large walk-in chambers. When an experimental group was transferred to a different chamber, the control group was sham-transferred back into the original chamber to control for temporary exposure to different temperatures and lower light intensity.

2. Relative humidity.--The effects of relative humidity were investigated using 10 x 23 x 31 cm plastic boxes. Fifteen clear plastic cups described above, with bottoms removed, were glued over screen-covered holes in the box lids to create a continuous air space between the box and cup interiors. The boxes containing varying-concentration KOH solutions resulting in relative humidities of 25, 50, 75, and 100% (pure H_2O) in airspaces over the solutions (Solomon 1951). Each cup contained two 3-6-day-old females which were used once.

C. Male wing fanning while walking response to pheromone.--Diel fluctuations of male sexual response were measured using an

Figure 1. G. molesta female in calling position. Note the extended legs, the elevated body and wings, and the extruded ovipositor containing the pheromone gland.

olfactometer similar to that described by Sower et al. (1973). It was comprised of a series of glass orientation tubes 100 x 2 cm diam, each connected to a 105° 3-way connecting tube at the upwind end. Filtered laboratory air was blown into a glass manifold which distributed the air flow equally to all connecting tubes and thence to the orientation tubes at a velocity of 80 cm/sec.

Five males 2 days or more old, were placed into each tube at least 1 hr before testing. Males were 0-9 hrs, and 1, 2, 3, and 4 days old in experiments determining effects of age upon response to pheromone.

The synthetic pheromone mixture was 4 identified G. molesta components in the amounts 1 µg (Z)-8-dodecenyl acetate, 0.07µg (E)-8-dodecenyl acetate, 0.01 µg (Z)-8-dodecenyl alcohol, and 3 µg dodecanol on a rubber septum dispenser (A. Thomas Comp.). Before introduction of pheromone, background levels of wing fanning while walking were assessed. These background levels were subtracted from post-pheromone-introduction levels to result in percent response to pheromone. Wing fanning while walking was chosen as the key response to observe because it was highly correlated with attraction (upwind flight) in a wind tunnel study of behavioral responses (Baker and Cardé, 1979). In these tubes unrestricted upwind flight could not occur. Wing fanning while walking was recorded during the first 15 seconds and then at 25-35 seconds. The maximum number of males simultaneously displaying this behavior during these periods were the number scored. Males were usually used only once, but if used more than once, then only once per 24 hour period. Both assay and connecting tubes were rinsed thoroughly with acetone after each use. The

septum was stored at -10° in a glass vial. Photophase light intensity for all the assays was 2100 lux. Scotophase intensity was 0.7 lux, the diffuse, low light level provided by an incandescent light-box immediately beneath the tubes.

RESULTS

A. Female calling.

1. Effect of age.--Newly emerged females, 1-9 hrs. old, did not call (Figure 2). Hour of calling onset and mean hour of calling were similar for all groups 1 day old or greater, with only some slight differences in percentage calling between groups at particular times. Calling periodicity appeared to coincide closely with the adult male attraction pattern, observed in the field to occur in the few hours prior to sunset in the summer (Dustan 1961, Gentry 1975, Rothschild and Minks 1974); at 25° in the laboratory calling commenced about 3.5 hours before, and terminated by about 0.5 hours after, lights-off. The calling rhythm also closely matched the oviposition pattern of females observed concurrently in the rearing box.

2. Effect of photophase onset.--Advancing or retarding lights-on by 4 hrs slightly accelerated and delayed, respectively, the subsequent mean calling time (Figure 3). However, the magnitude of the shift in mean calling time was less than the shift in lights-on. This implies that either photophase onset is not the only phase-setting cue, or there was resistance to a shift by an underlying rhythm whose phase was not completely reset by the cue.

3. Demonstration of circadian rhythm.--Calling persisted with

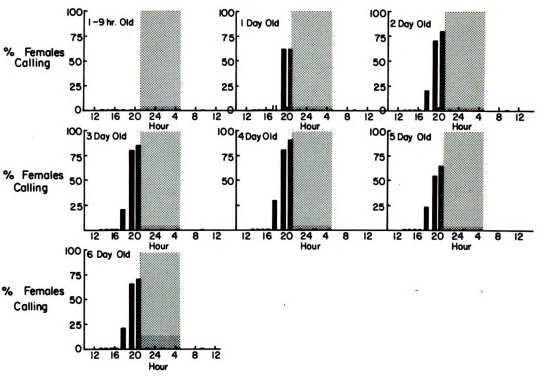

Figure 2. Effect of age upon female calling. The different age groups were observed simultaneously on the same day. N = 50 for 0-9-hr-old group; N = 86 for 5-day-old group; N = 100 for all other age groups. Temperature was 25°. Shaded areas represent scotophase.

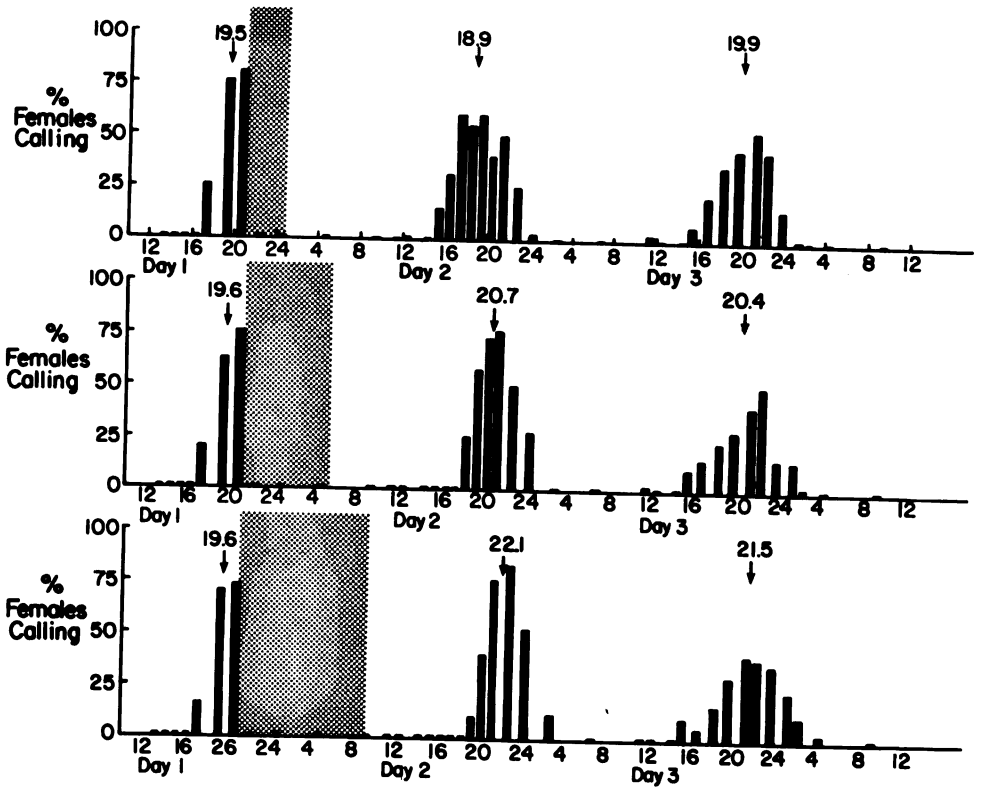

Figure 3. Effect upon female calling of varying the photophase onset cue by 4 hours. Scotophase (shaded area) in the middle group was the normal, 8 hour duration. Numbers above arrows denote mean hours of calling (decimal hours). On day 3, calling periodicity persisted in continual light, indicating the presence of a circadian rhythm. N = 200 for each group. Temperature was 25°.

approximately the same 24 hour periodicity in continual light at 25° (Figure 3). Thus calling periodicity is at least partially determined by a circadian rhythm.

4. Effect of relative humidity.--Calling proportion between 6 and 0.5 hours prior to scotophase was not obviously affected by relative humidities of 25, 50, 75, and 100% (Table 1). Neither onset of calling nor percentage calling during peak hours differed significantly between humidities except for a slight distinction 3 hours before lights-off.

5. Effect of temperature.--Calling was eliminated by temperatures above or below a threshold range falling between ca. 15° to 32°. However, the behavioral bases for this suppression were different at high versus low temperatures. At cold temperatures of ca. 15° or below, reduced calling was characterized by the absence of leg extension, body and wing elevation, and locomotor activity. At high temperatures of ca. 32° or greater, however, lack of calling often resulted from females walking and flying, although there were also immobile, non-calling females.

Temperature changes within the 15° to 32° range appeared to alter the time at which calling occurred. Calling onset was advanced by as much as 4 hours when the temperature was decreased from 25° to 20° at various times during photophase (Table 2). This decrease did not advance the termination of calling, as evidenced by no reduction in calling in the few hours immediately preceeding scotophase.

Longer exposure to altered temperatures also changed the calling times. Mean hour of calling was advanced by a temperature decrease to 20° and delayed by an increase to 31° during the 12 hours before

Table 1.--Effect of relative humidity upon female calling. N = 60 for all groups. Percentages in the same row having no letters in common are significantly different according to a χ^2 2 x 2 test of independence with Yates' correction ($P < 0.05$).

Hours Before Scotophase	Percent Females Calling (25°)			
	25% R.H.	50% R.H.	75% R.H.	100% R.H.
- 6	0 a	0 a	0 a	0 a
- 5	0 a	0 a	0 a	3.3 a
- 4	6.7 a	5.0 a	5.0 a	10.0 a
- 3	23.2 a	8.3 b	11.7 ab	26.7 a
- 2	66.7 a	63.3 a	56.7 a	65.0 a
- 1	85.0 a	76.7 a	80.0 a	81.7 a
- 0.5	76.7 a	78.3 a	68.3 a	73.3 a

Table 2.--Effect of a 5° temperature decrease upon percentage of females calling at various hours before lights-off. N = 50 for all groups. Females were used for one temperature decrease and discarded. **; percentage significantly different from that of the constant 25° group at the same hour according to a χ^2 2 x 2 test of independence with Yates' correction ($P < 0.01$). NS; percentage not significantly different to that in the constant 25° group at the same hour according to a χ^2 2 x 2 test of independence ($P \geq 0.05$).

Hours Before Scotophase	Percent Females Calling		
	Constant 25°	Min. at 20°	
		20	60
- 9	0	2 NS	2 NS
- 8	0	0 NS	0 NS
- 7	0	22 **	36**
- 6	0	40 **	58**
- 5	0	44 **	78**
- 4	0	72 **	76**
- 3	25	76 **	83**
- 2	72	86 NS	84 NS
- 1	70	66 NS	--

lights-off (Figure 4). Temperatures of 15° and 10° appeared to suppress calling. Returning the temperature for the group held at 31° to 25° the next day resulted in a mean calling time "normal" for 25°. However, the 10°-, 15°-, and 20°-exposed females returned to 25° exhibited advanced mean calling times. Some females in the 10° group called as soon as the temperature was raised (Figure 4).

When the temperature was oscillated between 34° and 10°, maximal calling occurred only within a certain temperature range, approximately 18°-25° (Figure 5). Moreover, the calling increases occurred during both rising and falling temperatures. Thus female calling was influenced by the absolute temperature. At a particular photophase hour, calling occurred if the temperature was within an absolute range; very low or high temperatures eliminated calling. The low and high thresholds appeared to vary with photoperiod hour. For instance, in the hours immediately before and after lights-off, high temperatures did not appear to reduce calling as much as during earlier photophase hours (Figure 5).

Complicating the temperature modulation of calling time is the possibility that calling behavior itself exerts some control over the timing of the next day's calling. This was first evidenced in the experiment where the calling times of previous day cold-exposed females were advanced even after females were returned to warmer temperatures (Figure 4). The fact that calling had not occurred or had occurred earlier on the cold-exposure day seemed to allow earlier calling on the following, warmer, day. A similar effect was observed in another experiment involving temperature oscillations (Figure 6).

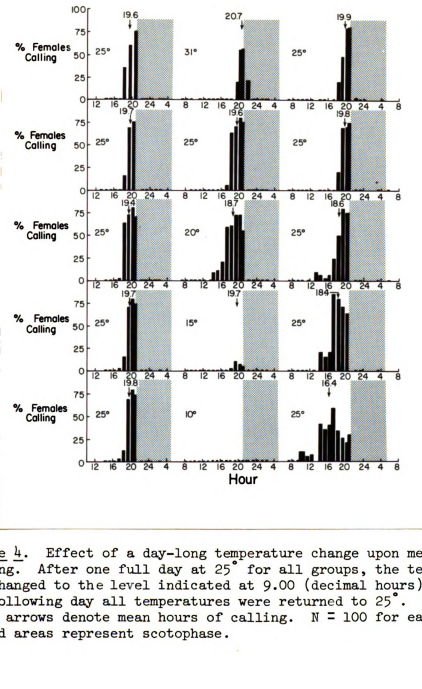

Figure 4. Effect of a day-long temperature change upon mean hour of calling. After one full day at 25° for all groups, the temperature was changed to the level indicated at 9.00 (decimal hours). At 9.00 the following day all temperatures were returned to 25°. Numbers above arrows denote mean hours of calling. N = 100 for each group. Shaded areas represent scotophase.

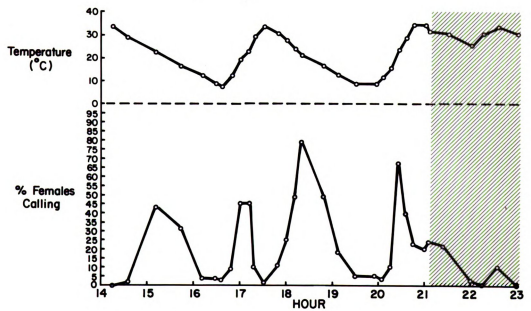

Figure 5. Effect of an oscillating temperature upon percentage of females calling at various photoperiod times. Intermediate temperature levels appeared favorable for calling whether they were arrived at by an increase or decrease. Extreme high or low temperatures suppressed calling. N = 125. Shaded area represents scotophase.

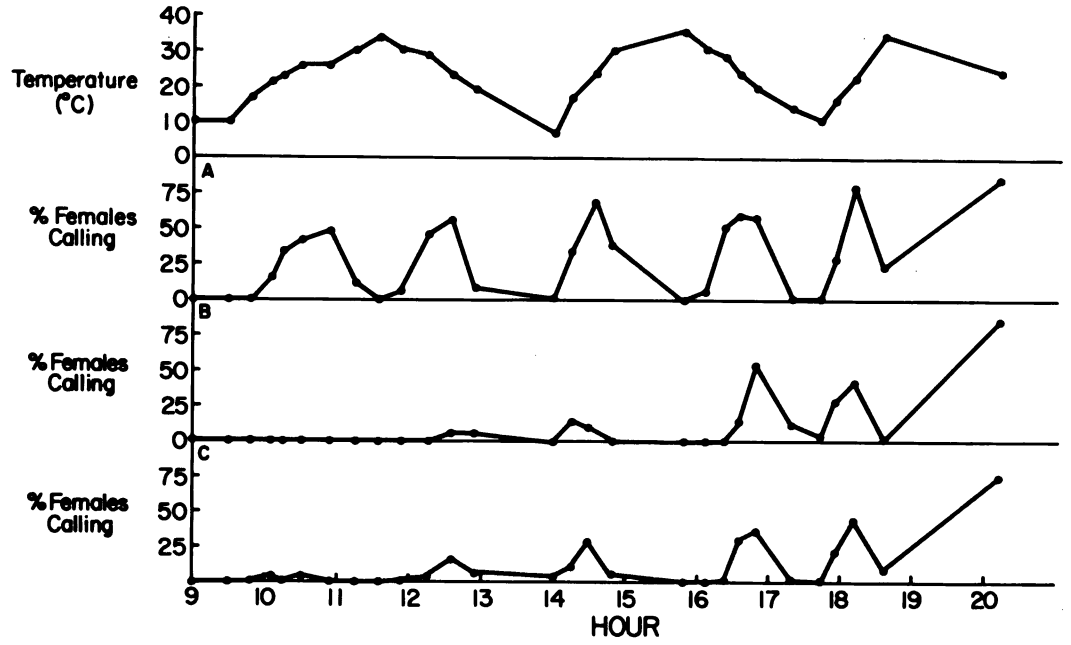

Figure 6. Effect of previous day's calling upon subsequent response to oscillating temperature. A; females not calling during the previous day due to a temperature decrease to 10° just before onset of calling; temperature was maintained at 10° until 9.30 (decimal hours) on the day of testing. B; control group experiencing normal calling on previous day and held at continual 25° until 10.30 on day of testing, whereupon the temperature oscillation was identical to the 2 other groups'. C; control group placed in 10° immediately after (21.30) experiencing normal calling on previous day and maintained at 10° until 9.30 on the day of testing. N = 50 for each group.

Females experiencing a decrease in temperature from 25° to 10° at 3.5 hours prior to scotophase did not call until the first increase in temperature on the following morning (Figure 6A). Females placed at 10° immediately after the previous day's calling had ended (0.5 hrs after lights-off), did not call with the first favorable temperatures (Figure 6C). Their calling in favorable temperatures was quite similar to that of females held at 25° (Figure 6B). The effect of the previous day's calling may involve a refractory period during which calling cannot begin again. Such a refractory period would likely be related to the endogenous oscillator determining the calling rhythm. Alternatively, the previous day's temperature decreases themselves may have acted as exogenous cues that reset the rhythm's phase. This is less likely since one decrease to 10° occurred at 9.00 (decimal hours) (Figure 4) and another at 17.5, yet calling commenced in both cases at about 10.0 the next morning soon after the temperature was increased (Figure 4, Figure 6A).

B. Male wing fanning while walking response to pheromone.

1. Age of males.--Percentage of males wing fanning while walking in response to the synthetic pheromone blend increased with age (Table 3). Males 1 day old or less had comparatively lower response levels, 2 day old intermediate response, and 3 to 4 day old the highest responses to pheromone.

2. Demonstration of circadian rhythm.--In continual light at 25° the periodicity of response persisted, indicating that male pheromone responsiveness is at least partially determined by an underlying rhythm (Figure 7). In continual light, mean hour of response was

Table 3.--Effect of age upon percentage of males wing fanning while walking during the first 15 sec of exposure to synthetic pheromone. Males were exposed to pheromone once and then discarded. Percentages having no letters in common are significantly different according to a χ^2 2 x 2 test of independence with Yates' correction ($P < 0.05$).

Age of Males	Percent Males Wing Fanning While Walking
0-9 hrs old	9 c (N = 58)
1 day old	15 c (N = 60)
2 days old	65 b (N = 60)
3 days old	80 ab (N = 58)
4 days old	84 a (N = 58)

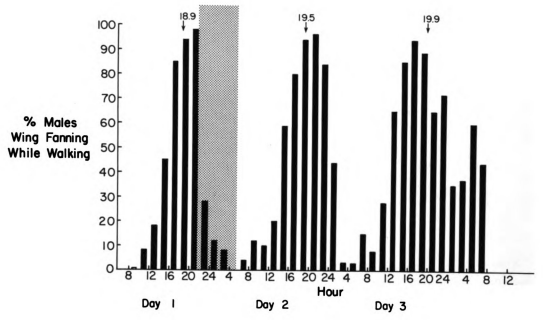

Figure 7. Effect of continual light upon periodicity of male wing fanning while walking response to sex pheromone. Since response periodicity persisted, it was at least partly determined by a rhythm which appeared circadian. Numbers above arrows denote mean hours of response. Males were used for no more than one exposure to pheromone. N = 60 for most individual testing hours. Shaded area represents scotophase.

slightly delayed compared to males experiencing scotophase, apparently due to a slower rate of decline of response rather than a delayed response onset. However, the rhythm appeared circadian.

3. Effect of a decrease in temperature.--Diminishing the temperature from 25° to 20° at various periods before lights-off did not significantly increase wing fanning while walking response compared to males maintained at continual 25° (Table 4). Thus, unlike females, the male sexual activity period could not be shifted by this 5° decrease. In fact, at some hours of testing there were slight but significant decreases in response in the temperature-decreased group.

DISCUSSION

A. Endogenous factors influencing sexual behavior periodicity.--In G. molesta, periodicities of female calling and male pheromone response are determined in part by a circadian rhythm. The female rhythm's phase was directly influenced by the lights-on photoperiod cue. The magnitude of the phase shift did not correspond, however, to the cue-shift magnitude, and so the phase-setting mechanism may involve more than the simple lights-on signal. Alternatively, the phase-setting effect of lights-on may have been partially obscured by the self-sustaining effect of the rhythm itself.

The circadian rhythm may persist by a type of interval-timer mechanism using the previous performance of calling (or pheromone response) to time the interval until the behavior can next occur. My results indicate that when the interval expires, calling is induced and may somewhat override exogenous temperature cues (Figure 4).

Table 4.--Effect of a 5° temperature decrease upon percentage of males wing fanning while walking during the first 15 seconds of exposure to pheromone at various hours before light-off. *; percentage in same row significantly different according to a χ^2 2 x 2 test of independence with Yates' correction ($P < 0.05$). N = 40 for all groups except those at 3, 2, and 1 hr before scotophase where N = 34, 25, and 35, respectively.

	Percent Males Wing Fanning While Walking	
Hours Before Scotophase	20°	25°
- 8	15	0 NS
- 7	43	32 NS
- 6	18	18 NS
- 5	55	63 NS
- 4	53	76 NS
- 3	74	97 *
- 2	96	96 NS
- 1	71	92 *

Rothschild and Minks (1974) observed what perhaps may be the rhythm's similar influence upon G. molesta male attraction. In the spring males fly earlier in the day and are "not influenced by the occasional warm day". A possible similar "residual" effect of previous calling hour upon the following day's calling onset can be seen in the data of Cardé et al. (1975; Figure A) for the redbanded leafroller moth, Argyrotaenia velutinana (Walker). Perhaps the circadian rhythms of calling in these two insects are driven by a similar mechanism.

B. Exogenous factors.--In addition to the endogenous influence of a circadian rhythm upon female calling periodicity, an exogenous factor, temperature, modified the rhythm's expression. Constant high temperature (31°) delayed mean calling time by delaying calling onset and extending offset further into scotophase. Continual low temperature (20°) advanced the mean calling hour mainly by advancing calling onset. At 15° or lower, expression of the calling rhythm was almost completely repressed, a low temperature threshold consistent with the 15° adult flight activity threshold in the field resported by other authors (Rothschild and Minks 1974, Armstrong 1929, Reichart and Bodor 1972). Rapid temperature decrease to 20° resulted in an onset of calling advancement of up to 4 hrs. However, as discussed below, the decrease itself may not actually induce calling but rather calling may result from an interaction of the current absolute temperature with both the hour of the photoperiod and the interval established by the previous performance of calling.

Male response to pheromone was not advanced by a temperature decrease from 25° to 20°. It is not clear why no advancement was

observed even in this limited experiment. A field study of G. molesta male attraction periodicity to synthetic pheromone indicated that the quite discrete attraction period of only a few hours was advanced during cooler spring periods compared to summer (Rothschild and Minks 1974). In New York the hour of 50% male trap capture in the field also appeared to be earlier at lower temperatures (Baker and Cardé unpubl.), although it is unclear whether this advancement was caused by an immediate or long-term exposure to cooler temperatures.

It seems inconsistent that females should exhibit a temperature-sensitive response for calling behavior while males, for pheromone response, do not. Possibly my method of measuring male periodicity of responsiveness was insufficient, and a more discriminating assay would reveal male temperature-sensitivity. Using an identical olfactometer, male Laspeyresia pomonella (L.), also an olethreutine, also failed to exhibit a temperature-modulated hour of response shift whereas females clearly advanced their calling time at lower temperatures (Castrovillo and Cardé 1979). However, males of Argyrotaenia velutinana, a tortricine, to colder temperatures did exhibit an advanced response period of the same magnitude as female calling advancement. It is possible that these differences in male response to changes in temperature are related to differen t bioassay methods. A. velutinana males were assayed in still air. Clearly additional experimentation with G. molesta needs to be done before it can be concluded that temperature cues affect only female calling and not male responsiveness.

C. Optimal temperature range of calling.--Advancement of calling time

has now been reported for the cabbage looper, Trichoplusia ni (Hubner) (Sower et al. 1971), the spruce budworm, Choristoneura fumiferana (Clemens) (Sanders and Lucuik 1972), A. velutinana (Cardé et al. 1975), L. pomonella (Castrovillo and Cardé 1979), and G. molesta. Many of the authors have rightly noted the adaptive value of early activity on cold spring days, especially for relatively small moths such as tortricids whose large surface area to volume ratio facilitates heat loss (Cardé et al. 1975). Although a decrease in temperature alone evokes an advancement in calling time, evidence for G. molesta indicates that the temperature level has a major effect regardless whether this level is reached by a temperature decrease or increase. There appears to be a temperature range during which calling can be expressed optimally for a particular photoperiod time and state of the underlying rhythm. The optimal range may increase under the influence of the rhythm; during late photophase and early scotophase, temperatures of 30°-33° did not reduce calling proportion to the levels seen earlier in photophase at the same temperatures. In G. molesta, and perhaps other species of moths which exhibit temperature-modulated calling advancement, the temperature decrease may act to increase early calling by disinhibition rather than inducement. The lower temperature level may remove calling suppression caused by higher temperatures, allowing expression of the calling rhythm earlier in the photoperiod.

D. The function of the rhythm.--In G. molesta the periodicity of female calling results from an interaction between endogenous and exogenous factors. Male periodicity of pheromone response also results from an endogenous factor (a circadian rhythm) and probably also from its interaction with exogenous factors although I was not able to

define this interaction. Corbet (1966) hypothesized that field periodicities observed at the lowest latitudes are most likely the expression of a rhythm alone, and at the highest latitudes responses to only exogenous factors. Behavioral periodicities of temperate insects should be determined by both endogenous and exogenous factors (Corbet 1966). For G. molesta calling behavior, this combination is indeed the case. During its many generations a year G. molesta may be exposed to both the harsh, unpredictable conditions of early spring (or fall) placing energetic and physiological constraints upon diel activity periods, in contrast to the relatively stable summer conditions. The combination of rapid response to exogenous cues modifying an underlying stability-lending rhythm appears to allow the moth to function under differing seasonal conditions. Temperate region insect species packing is less dense than in the tropics (Price 1975). For the adult stage only, this is especially true in early spring. Thus, in the spring there is greater temporal flexibility to respond to often fleeting favorable conditions without incurring detrimental levels of interspecific competition. In summer G. molesta may achieve optimal temporal synchrony with its potential mates and the rest of the more tightly packed community of insect adults mainly by its circadian rhythm. For a number of moth species, such diel temporal isolation has probably been important as a reproductive isolating mechanism (Roelofs and Cardé 1974). A rhythm-dominated periodicity would appear to be able to achieve greater intraspecific temporal synchrony in the rather unvarying condition of midsummer.

CHAPTER 2

Analysis of Pheromone-Mediated Behaviors in Male *Grapholitha molesta*

INTRODUCTION

The sex pheromone of Grapholitha molesta (Busck), the Oriental fruit moth, is comprised of a blend of at least 4 chemicals. A major component, (Z)-8-dodecenyl acetate (Z8-12:Ac), was identified from excised females' glands by Roelofs et al. (1969). In field experiments using sticky traps a small amount of the opposite geometric isomer (E)-8-dodecenyl acetate (E8-12:Ac) was required in addition to the (Z) isomer for capture of G. molesta males; from 5 to 9% (E) isomer yielded optimale male captures (Beroza et al. 1973a, b, Roelofs and Cardé 1974b, Gentry et al. 1974, 1975, Rothschild and Minks 1977). Also, two 12 carbon alcohols increased male captures. First, dodecanol (12:OH) at about a 3:1 ratio to the acetates gave a 50 to 100% capture increase (Roelofs et al. 1973, Beroza et al. 1973, Roelofs and Cardé 1974b, Gentry et al. 1974, 1975, Rothschild and Minks 1977). (Z)-8-dodecenyl alcohol (Z8-12:OH) produced similar capture increases at very low ratios to the acetates (Cardé et al. 1975b, Rothschild and Minks 1977). All 4 compounds are now known to be emitted by G. molesta females (Cardé et al. 1979) and these along with Z8-12:Ac can be considered sex pheromone components.

Based upon observations of feral males, 12:OH added to Z8-12:Ac [6.8%(E)] caused an increase in landing, wing fanning while walking, and hairpencil display close to the chemical source. The 12:OH-containing blend elicited no discernable increase in "long-range"

behavior such as upwind flight (Cardé et al. 1975b, c). Increased trap catch at 12:OH-containing treatments thus could be explained by increased landing and walking while wing fanning rather than initiation of upwind flight.

To define the behavioral effects of E8-12:Ac and Z8-12:OH, two components whose roles were not satisfactorily known, I initiated new behavioral observations in 1976 and trapping studies in western Michigan. My findings differed from previous reports. I now report that Z8-12:OH has major behavioral effects when emitted with the Z8-, E8-12:Ac mixture; addition of 12:OH only subtly affects behavior and then only when Z8-12:OH is present at suboptimal levels. Moreover, Z8-12:OH is apparently a strong behavioral antagonist to the closely-related G. prunivora (Walsh), causing reduced male captures when present at only 1% of the acetates. In this chapter I also discuss some of the problems in defining behavioral effects of individual components without regard to the total blend, and in classifying effects in terms of their positions in a sequence.

MATERIALS AND METHODS

A. Chemical solutions.--Z8-12:Ac was obtained from Farchan Corporation and purified by liquid chromatography on a 10% silver nitrate column. After purification it contained no detectable quantities of either E8-12:Ac or any 12-carbon alcohols as checked on 10% XF-1150 and 3 OV-1 GLC columns. Other impurities were less than 0.1%. The Z8-12:OH was made by saponifying the above-purified Z8-12:Ac. After clean-up it contained no detectable amounts of the (E) isomer as checked on

XF-1150 and no detectable quantities of any 12 carbon acetates or other impurities on OV-1. The E8-12:Ac was obtained from Farchan Corporation and used without further purification. It contained no detectable quantities of the (Z) isomer, less than 0.03% of any 12 carbon alcohols and no detectable quantities of other impurities. Dodecanol was purchased from Eastman Kodak and used without further purification. It contained no detectable amounts of any 12-carbon acetates or other 12-carbon alcohols and was greater than 98% free of other impurities.

Mixtures of these chemicals were formulated as described below for each experiment in either hexane or ether solutions and pipetted onto rubber septum dispensers (Arthur H. Thomas Co.). Serial dilutions usually were made so that the desired dosage could be extracted in 10µl of solution. Component ratios from solutions for most of the experiments were checked using either the XF-1150 (for isomer ratios) or OV-1 (alcohol-acetate ratios) GLC columns. Solutions were stored at -10°C. Before use, all solutions were warmed to room temperature and agitated to dissolve crystals sometimes observed in the stronger concentrations of alcohol-containing solutions.

B. Release rates from rubber septa.--Baker et al. (1979) measured the release rates of various dosages of pure Z8-12:Ac and pure Z8-12:OH on rubber septa at 25°C. They found that at a given septum dosage Z8-12:OH was emitted at a 2-3 times higher rate than Z8-12:Ac. Therefore, actual Z8-12:OH emitted as a percentage of (E) and (Z) acetates is probably about 2-3 times its percentage dosage on the septum; i.e., 1% Z8-12:OH on the septum is about 3% of the acetates in the emitted blend. A similar relationship likely exists for 12:OH as a percent of the acetates. Rates of Z8-12:Ac release for 10, 100, and 1000µg septum

dosages were 1.2 (± 3.75 S.D.), 11.8 (± 3.7 S.D.), and 220 (± 69 S.D.) ng per hour. Rates of Z8-12:OH release for 10, 100, and 1000µg septum dosages were 2.8 (± 1.4 S.D.), 33.0 (± 10.4 S.D.), and 665 (± 201 S.D.) ng per hour.

C. Trapping experiments.--Usually trends in behavioral activity of different mixtures were assessed initially by captures of males in sticky traps. All experiments were conducted using Pherocon-2, fold-out style sticky traps, 15 cm long with 6 x 13 cm restricted entrances. They were deployed in apple orchards at a height of ca. 2 m on outer tree branches. At Fennville, Michigan, where most experiments were conducted, the trees were semi-dwarfs and traps were spaced ca. 7 m apart. This block was on an insecticide-free, fungicides-only spray program each year and was adjacent to a block of peach trees. The only other orchards used were in Hamilton and Reece's Corners, Ontario, and in Geneva, New York for one experiment. The trees in Ontario were full-sized and trap spacing was ca. 10 m, whereas in Geneva the trees were semi-dwarf and spacing was ca. 7 m. Treatments always included a septum impregnated with 10 µl of solvent as a control. Traps were deployed in a randomized, complete-block design and were re-randomized within blocks whenever they were checked (usually every other day) at which time males were counted and removed. Since experiments were never deployed for more than 3 weeks, septa were not replaced. Traps were replaced whenever they were excessively soiled with scales or had lost appreciable glue. Trap data were transformed to $\sqrt{X + .5}$ and analyzed with a 2-way analysis of variance for randomized, complete-block design. Differences between means were tested for significance using the Student-Newman-Keuls' multiple range test at $\alpha = 0.05$.

D. Behavioral observations.--Male G. molesta responses to component mixtures were observed both in the field and in a laboratory wind tunnel. All field observations were conducted at Fennville, Michigan in the same semi-dwarf block used for trapping experiments. An individual septum was placed in the center of a 50 cm radius circular sheet-metal arena, similar to those used by Cardé et al. (1975a, b) with 10 cm intervals inscribed on the surface. The arenas were supported 1 m above the ground and placed in the middle of aisles between rows of trees. A single observer watched for males downwind judged to be starting upwind flight toward the arena. Individuals could sometimes be observed in upwind flight as far away as 10 m, but only when in an aisle. When flying near a tree, a male had to clearly break away from the foliage and start upwind before being scored. Behavior was described verbally onto a portable cassette tape-recorder and later transcribed. The behaviors scored were: 1) male observed flying upwind; 2) flying at the arena's edge (within 10 cm of arena); 3) landing (at least touching arena); 4) wing fanning while walking on arena (for 1 sec or more); 5) giving a hairpencil display when on the arena (usually at the dispenser); 6) mean duration of wing fanning while walking on arena; 7) mean orientation duration (from beginning of upwind flight to departure); and 8) mean closest approach to the dispenser (only males flying at the edge, landing, or wing fanning while walking were scored). For some observations the mean number of hairpencil displays, usually 3 or 4 separate hairpencil extrusions (Baker and Cardé 1979), were calculated. The percentages of males exhibiting a particular behavior were calculated using the number of males having exhibited the previous behavior. Thus true differences in the effects of chemical treatments could be

determined at each behavioral step, with the differences not merely being compounded at each stage whenever there were inter-stage dependencies.

Often more than one male would respond simultaneously. Two males' behaviors could usually be kept separate and described accurately for analysis, but when 3 or more responded simultaneously, or the observer confused 2 males, the observations were disregarded. Thus, despite attempts to keep observation times of all treatments identical, they were not, and the numbers of males observed were standardized per observation-hour.

Since response to pheromone could possibly differ with time within the male response period (usually beginning 2-3 hours before, and ending with sunset), treatments were observed for 5 minutes in a randomized complete-block fashion to minimize possible time effects. The response period was judged to have begun when 5-10 males were seen orienting toward an arena containing one of the more "active" treatments in the series. Then the first treatment was deployed on a different arena and observations commenced. The arenas were moved to different areas of the orchard each time a treatment was changed to minimize multiple observations of the same males and other unknown effects upon males exposed to different treatments. Immediately after use, each arena was washed with liberal amounts of acetone. Septa were capped in teflon-lidded vials while other treatments were observed. If darkness or rain prevented completion of the final block of observations, none of those in that block was used for analysis.

Other behavioral observations were made in a 2.8 x 1.4 x 0.8 m laboratory wind tunnel (Cardé and Hagaman 1979) housed in a controlled

environment chamber. Wind velocity was 70 cm/sec, light intensity 150 lux, temperature 22-25°C, and relative humidity 50-70%. An exhaust system removed pheromone from the tunnel.

Males were from a laboratory colony maintained at Michigan State University since November 1975 on green apples at 25-26°c on a 16:8, light:dark, photoperiod regime. Observations usually began 3 hours before, and ended with, lights-off of the laboratory photoperiod regime.

A treatment-impregnated septum was placed in the center of a 15 cm-high, 15 x 15 cm galvanized steel platform situated on the wind tunnel floor 34 cm from the upwind end and 150 cm upwind of a second, identical, male released platform. A 2- to 5-day-old male was taken from its holding cage in the wind tunnel room and placed in an 11 x 7 cm diam. copper screen cylinder open at both ends. Ten seconds were allowed for the male to acclimatize. If after 10 sec the male was not in sitting position somewhere on the inside of the cylinder, it was not used. If the male remained sitting, the cylinder was placed on end, male on the upwind side, on the release platform located in the pheromone plume 34 cm from the back of the tunnel. The plume location was checked initially using a titanium tetrachloride-impregnated septum to generate "smoke." Observations ended for males remaining sitting for 30 sec while in the tunnel. If after 30 sec a male in the cylinder was not sitting, observations continued until he either remained sitting for 5 sec or flew from the cylinder. In the latter case, I continued to observe his behavior until normal termination.

Each male was scored for exhibiting the following behaviors, usually occurring in this order: 1) either pre-flight walking, pre-flight wing fanning while walking, or both; 2) taking flight;

3) stationary flight of at least 1 sec without significant downwind or upwind progress or touching the wind tunnel surface [possibly anemomenotactic flight of Kennedy (1978)]; 4) upwind flight (flying to at least 10 cm upwind of the release cylinder while in the plume); 5) post-flight wing fanning while walking on the septum platform; and 6) hairpencil display at the dispenser and mean number of displays per male. Wing fanning while stationary, another possible category, may be distinctly different from wing fanning while walking but this behavior was not scored because it occurred infrequently, except when males had left the plume and touched the tunnel surface. Observation was terminated when a male touched the tunnel surface.

Some behaviors were timed, including latency to first response, duration of pre-flight wing fanning while walking, and mean upwind flight speed (measured as the time taken to traverse an 80 cm distance marked on the wind tunnel floor).

E. Optomotor response.--To test whether males were responding to the striped floor pattern while flying upwind in the plume, males were released individually from the cylinder and the floor pattern was randomly either rotated backward at 26 cm/sec or left stationary. Using the time taken to traverse the fixed 80 cm interval on the floor, the flight speeds were calculated. The same male could be tested repeatedly to both conditions by moving the floor rapidly enough to bring him back downwind to the original starting point. The blend used was 10µg Z8-12:Ac + 0.7 µg E8-12:Ac + 0.1µg Z8-12:OH + 30µg 12:OH.

RESULTS

A. Z8-12:Ac and E8-12:Ac Mixtures.--More G. molesta males were captured at Z8-12:Ac [5.1% (E)] than at other (Z)-(E) mixtures (Figure 8). For a related species, G. prunivora (Walsh), 5.1% (E) was also the optimal blend. The Fennville, Michigan population of G. molesta thus responded similarly to those in Georgia and New York, where males were captured optimally at about 5 to 7% (E) (Beroza et al. 1973a, b, Gentry et al. 1974, 1975, Roelofs and Cardé 1974b).

Based upon behaviors on and near the arena, the optimal (5.1%) percentage of (E) in (Z) increased both the number of males initiating upwind flight and the percentage of males continuing to fly upwind, land and wing fan while walking on the arena (Table 5). Proportions of (E) exceeding 5.1% lowered the proportion responding at each behavioral state that we monitored except for wing fanning while walking. In this latter behavioral state the trend implies reduced response to 12.1% (E) (Table 5). No hairpencil displays occurred. No males initiated upwind flight to pure Z8-12:Ac although a few males seemed to be in stationary flight without making upwind progress.

Thus, increased male captures to the 5.1% (E) blend result from increased responses at all behavioral stages. Reduced captures to higher percentages of (E) are a result of the compounding of reduced responses both early (e.g., initiation of upwind flight) and late in the sequence (e.g., landing near the source). A reduced trap catch at mixtures containing little (E) also is probably due to a compounding effect at each stage, but the appropriate (E) percentage between 0 and 3.2% may not have been tested to note such an effect. No males were

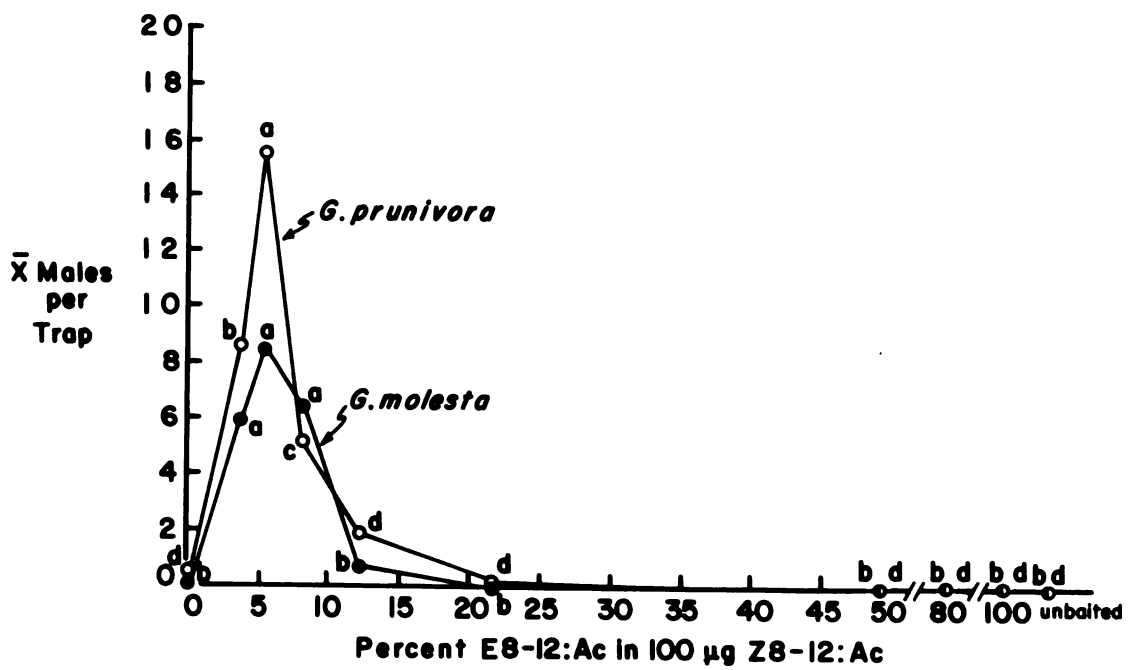

Figure 8. Effect of different binary mixtures of Z8- and E8-12:Ac upon capture of male G. molesta and G. prunivora. Amount of Z8-12:Ac was held at 100µg. Percentages of E8-12:Ac were, as checked by GLC: 0% (no detectable) (E); 3.2%; 5.1%; 7.1%; 11.1%; 22.2%; 48.4%; 77.5%; and 100µg pure (E). Experiment was conducted at Fennville, Michigan, August 20 to September 7, 1976. For each species, means having no letters in common are significantly different according to Student-Newman-Keuls' multiple range test ($P < 0.05$).

Table 5.--Behavior of G. molesta males on or near 50 cm radius observation arenas in response to mixtures of 100µg Z8-12:Ac plus various percentages of E8-12:Ac on rubber septa dispensers. To avoid the compounding behavioral effects occurring in a sequence, percentages were calculated using N as the number exhibiting the immediately preceding behavior.

Treatment (on rubber septum)	No. Males/Hr. Observed Beginning Upwind Flight[1] (No.)	% Males Flying to 10 cm of Arena's Edge[2] (No.)	Of Those Flying to Edge, % Males Landing[2] (No.)	Of Those Landing, % Males Wing Fanning While Walking[2] (No.)	Of Those Fanning While Walking, % Males Displaying Hairpencils (No.)	Of Those Flying to Edge, $\bar{X}$ Closest Approach to Dispenser[3,4] (cm)(± S.D.)	Of All Males Observed, $\bar{X}$ Orientation Time (sec) (± S.D.)
0% (E)	0 d	--	--	--	--	--	--
3.2% (E)	90 ab (69)	78.3 a (54)	66.7 ab (36)	47.2 a (17)	0	41.7 a (± 11.59)	14.0 a (± 8.92)
5.1%(E)	102 a (106)	84.0 a (89)	68.5 a (61)	41.0 a (25)	0	39.1 a (± 15.81)	14.4 a (± 8.69)
11.1%(E)	67 b (67)	49.3 b (33)	39.4 b (13)	15.4 a (2)	0	46.2 (± 9.83) a	8.9 b (± 5.50)
22.2%(E)	41 c (29)	3.4 c (1)	--	--	--	50.0	5.3 c (± 1.37)

[1]Numbers having no letters in common are significantly different according to χ^2 ($P < .05$) using a null hypothesis of equal numbers of observations per hour.

Table 5 (cont'd.).

[2]Percentages in same column having no letters in common are significantly different according to a χ^2 2 x 2 test of independence with Yates' correction ($P < .05$).

[3]Means in same column having no letters in common are significantly different according to a LSD test ($P < .05$). Data were first submitted to one-way analysis of variance for unequal replication.

[4]Males flying to within 10 cm of edge were scored as approaching to 50 cm; males landing on arena surface were scored for their closest approach while contacting surface. Those flying over table but not landing were scored as 50 cm approaches.

observed to exhibit upwind flight toward pure Z8-12:Ac and 3.8% (E) was sufficiently high in (E) so that responses were similar to those elicited by 5.1% (E) (Table 5).

Possible responses in the sequence occurring prior to upwind flight (such as initiation of flight) could not feasibly be observed in the field, and it is not clear whether frequencies of these earlier behaviors also were increased by 5.1% (E). However, the initiation of the orientation sequence was studied in the wind tunnel. In this situation these earlier behaviors were indeed increased by a small amount (6.7%) of (E) added to pure Z8-12:Ac (Figure 10). A greater percentage of moths wing fanned while walking before flight and initiated flight to 6.7% (E) compared to those exposed to pure (Z). In the latter group, a greater percentage remained sitting (Figure 10). The (Z)-(E) mixture also elicited a greater percentage of anemomenotactic ("stationary") flight and upwind flight compared to the pure (Z) group, paralleling field observations. However, I observed no landing or wing fanning, perhaps because the wind tunnel dosage was 10 times lower than that employed in the field, and thus possibly below threshold for these behaviors.

The combined field and wind tunnel data support the hypothesis that optimal E8-Z8-12:Ac mixtures increase male response during the earliest as well as later behavioral stages compared to other (E)-(Z) mixtures at the same dosage. These behaviors may have lower thresholds for Z8-12:Ac containing 5-7% (E) compared to other (Z)-(E) mixtures.

B. Addition of Z8-12:OH or 12:OH.--The addition of Z8-12:OH to the optimal acetate mixture elevated male capture by about 10-fold compared

to the acetate mixture alone. Moreover, Z8-12:OH accomplished this increase evidently by competition with the other treatments (Figure 9, Fennville 2, 3). The acetate mixture alone here was statistically indistinguishable from a solvent blank-baited trap whereas earlier it had caught significant numbers of males (Figure 8). A treatment containing the acetates plus 300% 12:OH (not shown in Figure 9, Fennville 2, 3) also elicited a trap catch indistinguishable from the blend. This is the lure blend used widely for monitoring of G. molesta populations.

The capture pattern between Geneva, New York and Fennville (Figure 9, Fennville 1, Geneva) was similar, confirming that 1% Z8-12:OH in the acetates produced the highest capture, eliminating population differences as an explanation of the disparity between my results and previous reports. In both locations, 12:OH added to the acetates at several dosages did no better than either the acetates alone or a solvent-blank septum.

In behavioral observations in the field, Z8-12:Ac [5.1% (E)] containing as little as 0.1% Z8-12:OH caused a greater percentage of the males starting upwind flight to continue all the way to the arena's edge (Table 6). Percentages of males both landing and wing fanning while walking likewise were elevated by increasing the Z8-12:OH levels. The only hairpencil displays were to treatments containing the highest amounts of Z8-12:OH, although with this level of replication these percentages were not significantly higher than those treatments lacking the alcohol. When Z8-12:OH was present, more males walked while fanning on the table and their approach to the dispenser was significantly closer than with treatments lacking Z8-12:OH (Table 6). Mean orientation

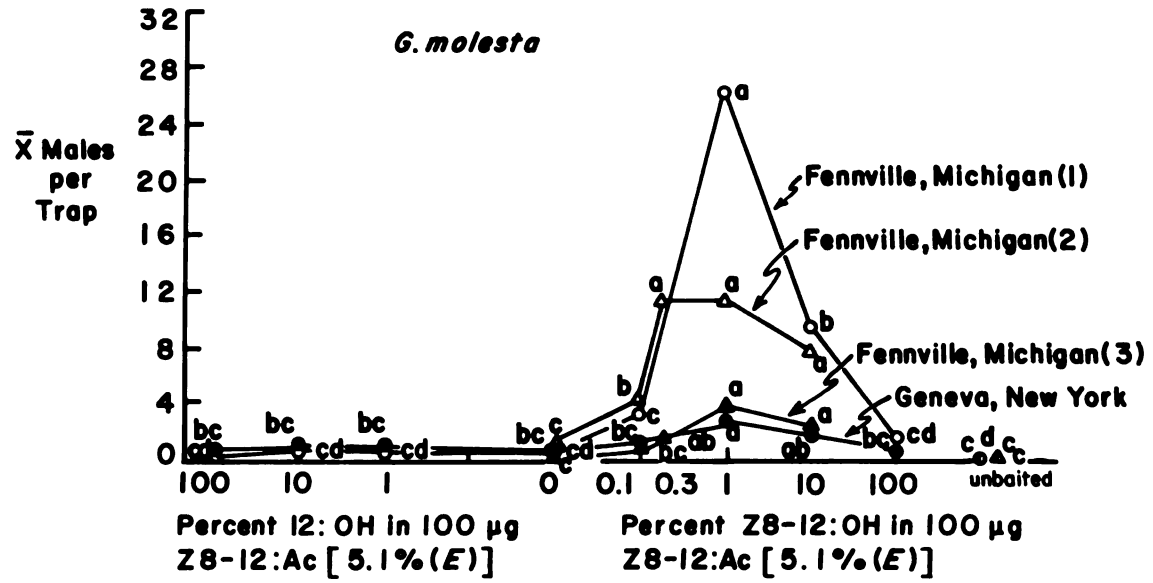

Figure 9. Effect of adding various percentages of either Z8-12:OH or 12:OH to 100µg Z8-12:Ac [5.1% (E)] upon capture of G. molesta males. Fennville (1) experiment was conducted May 13-16, 1977; Fennville (2), September 4-8, 1976; Fennville (3), September 20 to October 5, 1976; Geneva experiment, May 19-22, 1977. Within each experiment means having no letters in common are significantly different according to Student-Newman-Kuels' multiple range test ($P < .05$).

Table 6.--Behavior of G. molesta males on or near 50 cm radius observation arenas in response to various percentages of Z8-12:OH [12.4% (E)] added to 100µg Z8-12:Ac [5.1% (E)] on rubber septa dispensers. To avoid compounding behavioral effects occurring in a sequence, percentages were calculated using N as the number exhibiting the immediately preceding behavior. Experiment was conducted September 8-12, 1978 at Fennville, Michigan.

Treatment (On Rubber Septum)	No. Males/Hr. Observed Beginning Upwind Flight[1] (No.)	% Males Flying to 10 cm of Arena's Edge[2] (No.)	Of Those Flying to Edge, % Males Landing[2] (No.)	Of Those Landing, % Males Wing Fanning While Walking[2] (No.)	Of Those Fanning While Walking, % Males Displaying Hairpencils (No.)	Of Those Flying to Edge, $\bar{X}$ Closest Approach to Dispenser[3,4] (cm)(± S.D.)	Of All Males Observed, $\bar{X}$ Orientation Time (sec) (± S.D.)
0µg Z8-12:OH	99 a (99)	69.7 b (69)	27.5 c (19)	26.3 bc (5)	0 a	46.1 a (± 8.38)	11.1 cd (± 7.12)
0.1µg Z8-12:OH	88 a (97)	86.6 a (84)	57.1 b (48)	31.3 bc (15)	0 a	38.1 b (± 17.50)	14.8 bc (± 13.94)
0.3µg Z8-12:OH	79 a (79)	91.1 a (72)	62.5 b (45)	40.0 b (18)	22.2 a (4)	37.6 b (± 16.90)	17.7 b (± 13.57)
1.0µg Z8-12:OH	74 a (86)	95.4 a (82)	70.7 b (58)	67.5 a (39)	18.0 a (7)	27.2 c (± 19.91)	26.7 a (± 18.32)
10.0µg Z8-12:OH	81 a (82)	93.5 a (86)	86.0 a (74)	63.5 a (47)	23.4 a (11)	25.6 c (± 19.28)	27.6 a (± 21.5)
300µg 12:OH	76 a (84)	72.3 b (68)	27.9 c (19)	10.5 c (2)	0 a	46.6 a (± 6.76)	10.3 d (± 8.44)

Table 6 (cont'd.).

[1]Numbers having no letters in common are significantly different according to χ^2 ($P < .05$) using a null hypothesis of equal numbers of observations per hour.

[2]Percentages in same column having no letters in common are significantly different according to a χ^2 2 x 2 test of independence with Yates' correction ($P < .05$).

[3]Means in same column having no letters in common are significantly different according to a LSD test ($P < .05$). Data were first submitted to a one-way analysis of variance for unequal replication.

[4]Males flying to within 10 cm of edge were scored as approaching to 50 cm; males landing on arena surface were scored for their closest approach while contacting surface. Those flying over table but not landing were scored as 50 cm approaches.

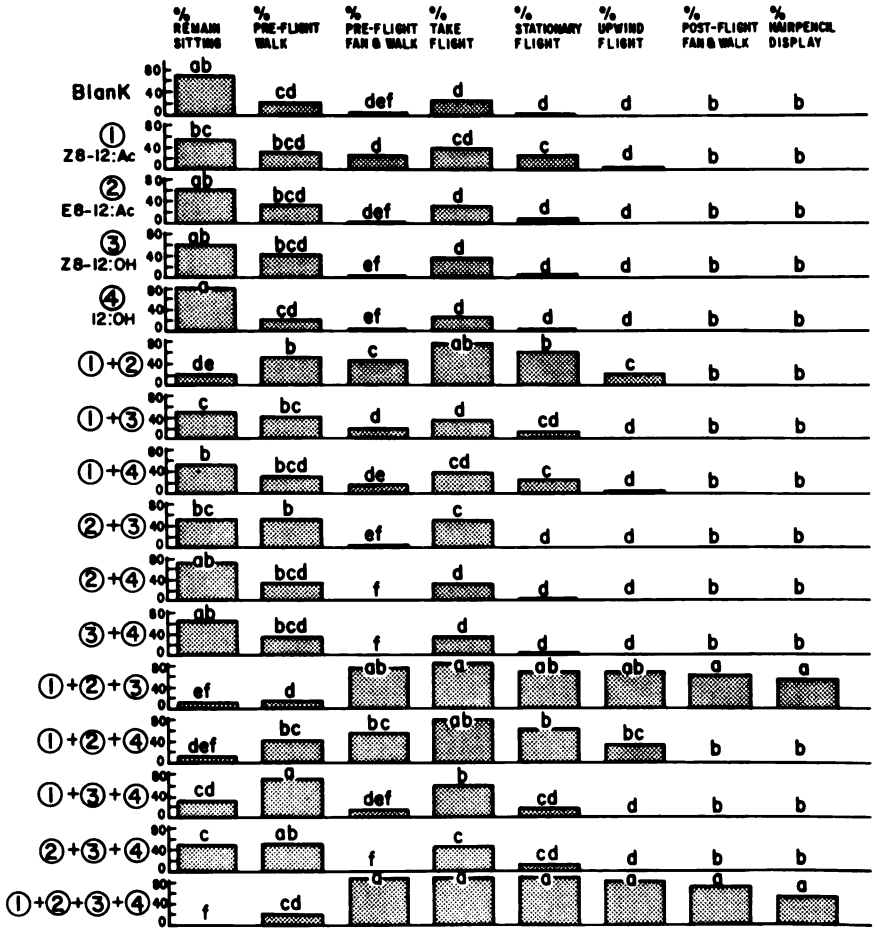

Figure 10. Percentage response to male G. molesta observed individually in a laboratory wind tunnel to all possible combinations of four G. molesta pheromone components emitted at rates and ratios similar to those emitted by G. molesta females. Septa contained, either singly or in combination as indicated, 10µg Z8-12:Ac, 0.7µg E8-12:Ac, 1µg Z8-12:OH, and 1µg 12:OH. Behavioral categories are as described in text, but in general the sequence of behavior proceeds from left to right. Percentages in same column having no letters in common are significantly different according to χ^2 2 x 2 test of independence ($P < .05$). N = 40 for each treatment.

times were also longer, reflecting not only an increased duration of wing fanning while walking on the arena to Z8-12:OH-containing treatments, but the brevity of upwind flight to treatments lacking Z8-12:OH. Once males ceased making upwind progress they hovered in stationary (anemomemotactic) flight only momentarily before returning rapidly downwind, usually by simultaneously flying slightly skyward. In none of the behaviors measured did Z8-12:Ac [5.1% (E)] plus 300 µg 12:OH differ significantly from the acetates alone, a finding that was not surprising considering their similarity as measured by male capture in traps.

In the field, addition of Z8-12:OH did not influence the frequency with which males were observed flying upwind, therefore, it would appear that this compound did not affect initiation of upwind flight but rather its duration. This conclusion may be incorrect for two reasons. First, there may have been a bias in monitoring the frequency of upwind flights which favored treatments eliciting shorter duration flights especially when several males responded within a short period. With the shorter flights to treatments lacking Z8-12:OH the observer was free to note approaches of other males thereby increasing observation frequency; during the longer duration observations of Z8-12:OH-responding males many simultaneous approaches of other males went unrecorded. Secondly, males in the wind tunnel did exhibit an increased frequency of upwind flight when Z8-12:OH was added to the two acetates (Figure 10). Flight initiation (taking flight) was not increased, but a pre-flight behavior, wing fanning while walking, was. Later behaviors in the sequence such as post-flight wing fanning while walking and hair-pencil display were increased by addition of Z8-12:OH, in agreement

with the field observations. The pattern of response to the component mixtures in the wind tunnel leads to the conclusion that the 3-component blend of the acetates plus Z8-12:OH acts as a unit to increase all stages of behavior. At this approximation to the natural emission rate, no component emitted alone had even a slight effect upon behavior, except Z8-12:Ac which slightly increased the amount of stationary flight (Figure 10). The only binary mixture having a major behavioral effect was the Z8- and E8-12:Ac mixture which increased pre-flight wing fanning while walking, flight initiation, stationary flight, and upwind flight. However, when Z8-12:OH was present the acetate mixture caused increases at all stages of behavior, including hairpencil display, compared to the single acetates. Finally, Z8-12:OH had a major behavioral effect only when added to the (E)-(Z) mixture, not alone or when admixed to either acetate. I conclude that Z8-12:OH added to the acetates significantly increases both the earliest and later behaviors in the sequence.

In addition to increasing the trap capture of G. molesta males, mixtures containing Z8-12:OH strongly reduced captures of G. prunivora. In two locations in Canada, and in New York and Michigan, G. prunivora capture was significantly reduced with as little as 1% of Z8-12:OH added to the acetates, and was further reduced to be statistically indistinguishable from a solvent blank-baited trap when only 10% Z8-12:OH was present (Figure 11).

The effect of 12:OH on G. prunivora capture was negligible (Figure 11); in 3 of the 4 locations there were no increases or decreases in trap catch at the dosages tested and the one significant increase was at the lowest dosage. We attribute the large variation in the two

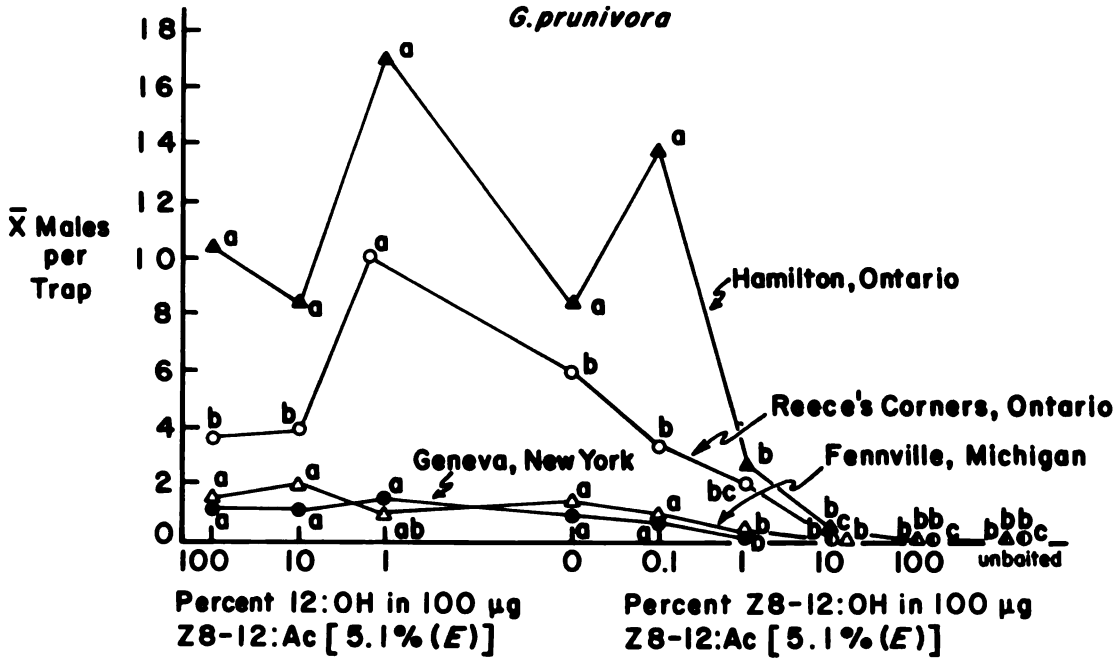

Figure 11. Effect of adding various percentages of either Z8-12:OH or 12:OH to 100µg Z8-12:Ac [5.1% (E)] upon capture of G. prunivora males. Hamilton, and Reece's Corners, Ontario experiments were conducted May 18-23, 1977; Geneva, May 19-22, 1977; and Fennville, May 13-16, 1977. Within each experiment, means having no letters in common are significantly different according to Student-Newman-Keuls' multiple range test ($P < .05$).

Canadian orchards to no trap re-randomization, and position effects.

C. Addition of E8-12:OH to Z8-12:OH.--Addition of various percentages of E8-12:OH into the mixture containing 100µg Z8-12:Ac [5.1% (E)] plus 1µg pure Z8-12:OH, did not affect capture of males (Table 7). No further experiments utilizing E8-12:OH were conducted, although behavioral effects not measured by trap capture could occur in the presence of this compound.

D. Simultaneous addition of Z8-12:OH and 12:OH.--Addition of various quantities of 12:OH to the optimum Z8-12:OH - Δ8-12:Ac 3-component mixture had no significant effect upon male capture (Table 8), but the trend toward increase at higher dosages of 12:OH (also in other small experiments not reported here) implied there might be some behavioral effect. However, 300µg 12:OH added to 100µg Z8-12:Ac [4.9% (E)] plus 1µg Z8-12:OH [5.7% (E)] elicited no significant increases in behavior, except for a closer mean closest approach to the dispenser (Table 9, Test 1). A similar experiment conducted a few days later using the same components and dosages (except pure Z8-12:OH) resulted in no significant effects of the 12:OH-added mixture (Table 9, Test 2).

In the wind tunnel, at a 10-fold lower dosage more closely approximating the emission rate of the female (Baker at al. 1979), the 12:OH-added treatment increased the percentage of males giving hairpencil displays, and also the mean number of displays per male (Table 10). Virtually identical results were obtained when the experiment was repeated later. Thus, when Z8-12:OH was present at 1% of the acetates, addition of 12:OH produced some subtle but significant increases in response. Because the affected behavior (hairpencil display) occurs

Table 7.--Effect on G. molesta male capture of various percentages of E8-12:OH added to 1µg Z8-12:OH (pure) in 100µg Z8-12:Ac [5.1% (E)]. Experiment was conducted April 30 to May 12, 1977 at Fennville, Michigan. Means having no letters in common are significantly different according to Student-Newman-Keul's multiple range test ($P < 0.05$).

Treatment (On Rubber Septum)	$\bar{X}$ Males Per Trap
100µg Z8-12:Ac [5.1% (E)]	2.8 b
100µg Z8-12:Ac [5.1% (E)] + 1µg Z8-12:OH [0.0% (E)]	29.0 a
100µg Z8-12:Ac [5.1% (E)] + 1µg Z8-12:OH [5.7% (E)]	32.6 a
100µg Z8-12:Ac [5.1% (E)] + 1µg Z8-12:OH [8.9% (E)]	31.9 a
100µg Z8-12:Ac [5.1% (E)] + 1µg Z8-12:OH [47.2% (E)]	30.6 a
Solvent-impregnated Septum	0.0 b

Table 8.--Effect of addition of various quantities of 12:OH to 1µg Z8-12:OH (pure) plus 100µg Z8-12:Ac [4.9% (E)]. Experiment was conducted August 3-12, 1977 at Fennville, Michigan. Means having no letters in common are significantly different according to Student-Newman-Keuls' multiple range test ($P < 0.05$).

Treatment (On Rubber Septum)	$\bar{X}$ Males Per Trap
100µg Z8-12:Ac [4.9% (E)]	6.4 b
100µg Z8-12:Ac [4.9% (E)] + 1µg Z8-12:OH (pure)	16.7 a
100µg Z8-12:Ac [4.9% (E)] + 1µg Z8-12:OH (pure) + 1µg 12:OH	15.6 a
" "	
+ 10µg 12:OH	18.8 a
" "	
+ 100µg 12:OH	18.6 a
" "	
+ 300µg 12:OH	21.3 a
" "	
+ 1000µg 12:OH	15.6 a
100µg Z8-12:Ac [4.9% (E)] + 300µg 12:OH	6.8 b
Solvent-impregnated Septum	0.0 c

Table 9.--Behavior of G. molesta males on or near 50 cm radius observation arena in response to 300 µg 12:OH added to 100 µg Z8-12:Ac [4.9% (E)] plus 1 µg Z8-12:OH [5.7% (E)] on a rubber septa dispenser. To avoid compounding behavioral effects occurring in a sequence percentages were calculated using N as the number exhibiting the immediately preceding behavior. Test 1 was conducted August 23-25, 1977 and Test 2, August 25-27 at Fennville, Michigan.

Treatment (On Rubber Septum)	No. Males/Hr. Observed Beginning Upwind Flight[1] (No.)	% Males Flying to 10 cm of Arena's Edge[2] (No.)	Of Those Flying to Edge, % Males Landing[2] (No.)	Of Those Landing % Males Wing Fanning While Walking[2] (No.)
Test 1				
100 µg Z8-12:Ac [4.9% (E)] + 1 µg Z8-12:OH [5.7% (E)]	51 (51)	96.1 (49)	75.5 (37)	94.6 (35)
100 µg Z8-12:Ac [4.9% (E)] + 1 µg Z8-12:OH [5.7% (E)] + 300 µg 12:OH	38 NS (48)	97.9 NS (47)	89.4 NS (42)	90.5 NS (38)
Test 2				
100 µg Z8-12:Ac [4.9% (E)] + 1 µg Z8-12:OH (pure)	47 (82)	100.0 (82)	81.7 (67)	86.6 (58)
100 µg Z8-12:Ac [4.9% (E)] + 1 µg Z8-12:OH (pure) + 300 µg 12:OH	55 NS (96)	100.0 NS (96)	91.7 NS (88)	83.0 NS (73)

Table 9 (cont'd).

Treatment (On Rubber Septum)	Of Those Fanning While Walking, % Males Displaying Hairpencils[2] (No.)	Of Those Hairpencilling, $\bar{X}$ No. Displays Per Male[3] (±S.D.)	Of Those Flying to Arena's Edge, $\bar{X}$ Closest Approach to Dispenser[3,4] (±S.D.)	Of All Males Observed, $\bar{X}$ Orientation Time (±S.D.)
Test 1				
100 µg Z8-12:AC [4.9% (E)]	2.9	1.0	30.2 cm	36.0 sec
+ 1 µg Z8-12:OH [5.7% (E)]	(1)	--	(±16.5)	(±26.71)
100 µg Z8-12:Ac [4.9% (E)]	15.8 NS	4.0	20.8 cm **	49.0 sec NS
+ 1 µg Z8-12:OH [5.7% (E)]	(6)	(±3.69)	(±18.2)	(±37.5)
+ 300 µg 12:OH				
Test 2				
100 µg Z8-12:Ac [4.9% (E)]	32.8	2.7	20.2 cm	35.4 sec
+ 1 µg Z8-12:OH (pure)	(19)	(±1.74)	(±19.6)	(±24.5)
100 µg Z8-12:Ac [4.9% (E)]	17.8 NS	2.7 NS	22.1 cm NS	37.0 sec NS
+ 1 µg Z8-12:OH (pure)	(13)	(±1.7)	(±18.5)	(±24.4)
+ 300 µg 12:OH				

[1]NS; number not significantly different from one above it according to χ^2 using a null hypothesis of equal numbers of observations per hour ($P > .05$).

Table 9 (cont'd.).

[2]NS; percentage not significantly different from one above it according to a χ^2 2 x 2 test of independence with Yates' correction ($P > .05$).

[3]**; mean significantly different from one above it according to the t-test ($P < .05$). NS; mean not significantly different from one above it according to the t-test ($P > .05$).

[4]Males flying to within 50 cm of the edge were scored as approaching to 50 cm; males landing on arena surface were scored for their closest approach while contacting surface. Those flying over arena but not landing were scored as 50 cm approaches.

Table 10.--Hairpencil display behavior of G. molesta males in laboratory wind tunnel to 30µg 12:OH added to 10µg Z8-12:Ac [4.9% (E)] plus 0.1µg Z8-12:OH (pure). There were no differences in other behavior such as upwind flight, landing, or post-flight wing fanning while walking according to a χ^2 2 x 2 test of independence with Yates' correction ($P < .05$).

Treatment (On Rubber Septum)	% Males Displaying Hairpencils (No.)[1]	Of Males Displaying Hairpencils[2], $\bar{X}$ No. Displays Male (±S.D.)
10µg Z8-12:Ac [4.9% (E)]	38.8	2.9 (±1.75)
+ 0.1µg Z8-12:OH (pure)	(n = 116)	(n = 45)
10µg Z8-12:Ac [4.9% (E)]	54.3*	3.9 (±2.20 S.D.)*
+ 0.1µg Z8-12:OH (pure)	(n = 116)	(n = 63)
+ 30µg 12:OH		

[1]*; Percentages significantly different according to a χ^2 2 x 2 test of independence with Yates' correction ($P < .05$).

[2]*; Means significantly different according to the t-test ($P < .05$).

only after close approach to the source, it is unlikely 12:OH could ever significantly increase trap catch by increasing levels of this behavior alone; traps presently in commercial use routinely terminate "normal" orientation 5-15 cm from the septum.

The 12:OH discernably affects behavior when Z8-12:OH is 1% of the acetates is perhaps a moot point. Z8-12:OH is emitted by females at considerably higher, and 12:OH at lower, ratios to the acetates (Baker et al. 1979, Cardé et al. 1979). When 1 µg 12:OH was added either to the 10µg Z8-12:Ac [6.7% (E)] plus 1µg Z8-12:OH mixture (approximating the female emission rates of Z8-12:OH and 12:OH) or to the acetate mixture, 12:OH had no discernable behavioral effect (Figure 10). There were also no differences in mean number of hair-pencil displays (3.33 ± 1.78 S.D., N = 25 and 2.76 ± 1.51 S.D., N = 20, respectively) average flight speed (14.9 cm/sec ± 5.3 and 15.4 cm/sec ± 5.0 S.D.) or response latency (3.7 sec ± 3.24 and 3.8 sec ± 2.95 S.D.) between the Z8-12:Ac [6.7% (E)] plus Z8-12:OH mixtures with or without 12:OH.

Certain patterns of behaviors across treatments were evident. Pre-flight wing fanning while walking and upwind flight were highly correlated ($r = 0.95$) (Figure 12). The correlation was greater than that between taking flight and upwind flight, behaviors which would appear *a priori* more inter-dependent than the former pair. Pre-flight walking and upwind flight were poorly correlated (Figure 12).

The duration of pre-flight wing fanning while walking also appeared correlated with the occurrence of later behaviors such as hairpencil display, post-flight wing fanning while walking, and upwind flight (Table 11). Some of these relationships may result from the spatial

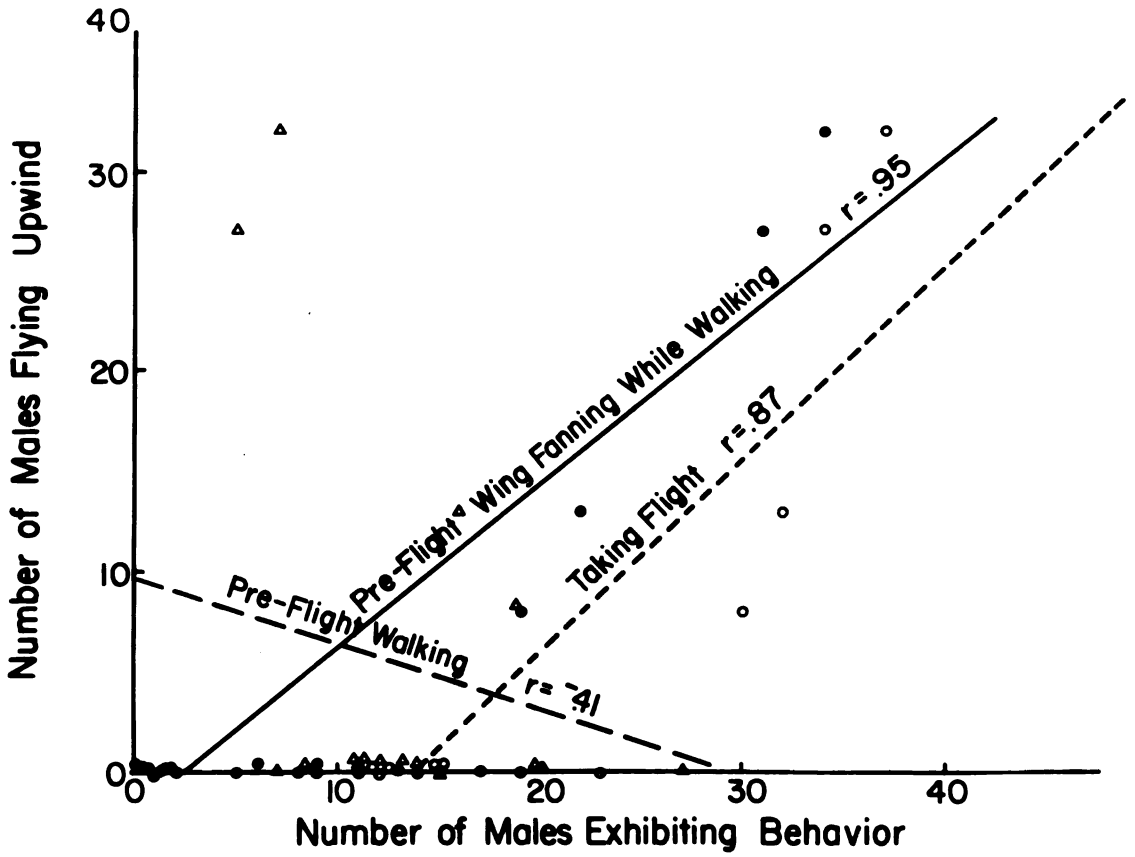

Figure 12. Correlations between number of males exhibiting either pre-flight wing fanning while walking (solid circles), taking flight (open circles), or pre-flight walking (triangles), and the number of males flying upwind in response to each of the 16 wind tunnel treatments listed in Figure 10 and Table 11.

Table 11.--Correlation (r = 0.98) between mean duration of pre-flight wing fanning while walking (discontinuous occurrences included) and the number of males flying upwind in the laboratory wind tunnel to the 16 treatments in Figure 10. Only treatments having 2 or more males fanning while walking were used so that means could be calculated.

Treatment	$\bar{X}$ Duration (Sec) of Pre-Flight Wing Fanning While Walking (± S.D.)	No. of Males Flying Upwind
Blank	0.7 ± 0.28 (N = 2)	0
1 Z8-12:Ac 10µg	1.5 ± 1.60 (N = 9)	1
2 E8-12:Ac 0.7µg	0.6 ± 0.07 (N = 2)	0
3 Z8-12:OH 1µg	--	
4 12:OH 1µg	--	
1 + 2	8.0 ± 13.93 (N = 19)	8
1 + 3	5.4 ± 5.02 (N = 8)	0
1 + 4	1.5 ± 1.58 (N = 6)	1
2 + 3	--	
2 + 4	--	
3 + 4	--	
1 + 2 + 3	30.4 ± 42.27 (N = 31)	27
1 + 2 + 4	8.6 ± 13.81 (N = 22)	13
1 + 3 + 4	1.9 ± 2.25 (N = 5)	0
2 + 3 + 4	--	
1 + 2 + 3 + 4	32.8 ± 50.13 (N = 34)	32

requirements: males first had to move up inside the screen cylinder by either flying, walking, or wing fanning while walking before they could fly upwind. The strong correlations among the amount and duration of wing fanning while walking and attraction (upwind flight) and courtship implies that wing fanning while walking would be the best "key response" to score in traditional "stimulation" olfactometer where attraction cannot be measured directly.

E. Optomotor response.--Males took significantly longer (17.6 sec ± 8.2 vs. 3.8 sec ± 1.1 S.D., N = 13) (t-test, $P < 0.01$) to fly upwind over 80 cm distance in response to the same chemical stimulus when the striped floor pattern was moved backward at 26 cm/sec compared to when the pattern was stationary. This optomotor response itself was sufficiently strong as to use it to re-test individual males, guiding them back downwind by moving the floor more rapidly than during the test situation. This behavior is similar to that described for several pyralids (Kennedy and Marsh 1974), Argyrotaenia velutinana (Walker) (Miller and Roelofs 1978a) Lymantria dispar (L.) (Miller and Roelofs 1978b, Cardé and Hagaman 1979), and Bradysia impatiens (Johannsen) (Diptera) (Alberts 1978), and undoubtedly represents a mechanism widespread in the insects to gauge upwind progress while in an aerial odor plume.

F. Blend quality vs. quantity.--A trapping experiment using the optimum and two "off" ratios of E8- and Z8-12:Ac indicated that males are sensitive not only to blend quality, but also quantity (Figure 13). An "off" ratio, 1.9% (E) at ca. a 4 times higher release rate captured as many males as the optimum ratio, but only when the latter was emitted at a suboptimal rate (for trap capture). Both these

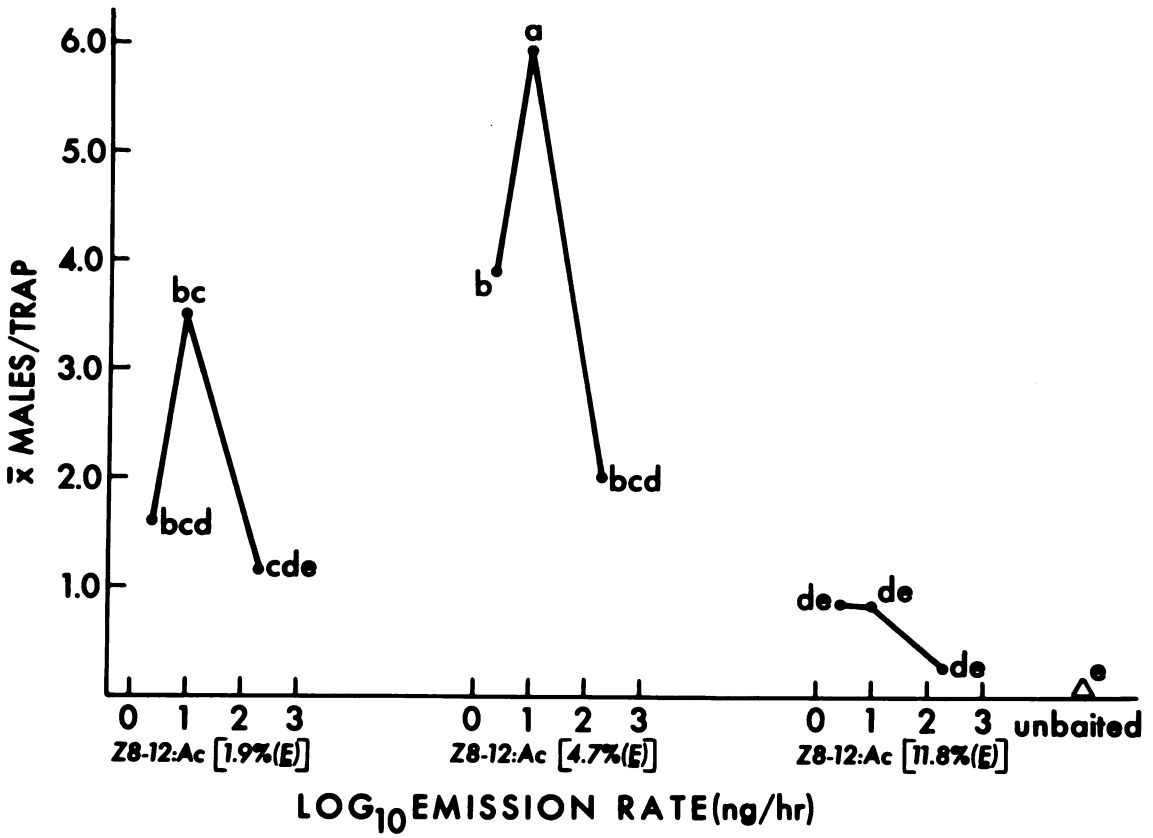

Figure 13. Effect of varying the emission rate and the (Z)-(E) ratio upon capture of G. molesta males. Rates were measured in still air at 25.5°C (Baker et al. 1979). All treatments contained a septum dosage of 1% Z8-12:OH with the acetates. Treatments having no letters in common are significantly different according to Student-Newman-Keuls' multiple range test ($P < 0.05$).

ratios elicited optimal male capture at the same release rates, although only a small sample of rates was tested. Thus, over some range of release rates a blend of inferior quality can apparently be compensated for by increasing its release rate. However, the release rate can only be increased to an apparent absolute amount regardless of the blend indicating male behaviors are affected by both stimulus quality and quantity. Cardé et al. (1975a) observed that both male approaches to and landings on arenas decreased after the dosage of the acetates on the septum surpassed 100µg.

DISCUSSION

A. Behavioral effects of the 4 components.--Only 3 of the 4 identified G. molesta sex pheromone components, Z8-12:Ac, E8-12:Ac, and Z8-12:OH significantly affect male behavior when emitted at rates and ratios approximating those of G. molesta females. None affected behavior in a significant way when emitted singly, and the only binary mixture eliciting pre-flight wing fanning and upwind flight was the 2 acetates. Percentages of E8- in Z8-12:Ac closest to the "natural" percentage [ca. 5-7% (E)] elicited an increase in male behaviors both at the earliest (pre-flight wing fanning while walking, flight initiation, upwind flight initiation) and later stages of the orientation sequence (upwind flight near the source, landing, wing fanning while walking). The acetate mixture's effect on the later stages was more obvious when Z8-12:OH was present. The 3-component blend of the 2 acetates plus Z8-12:OH elicited further increases in both the latest (post-flight wing fanning while walking, hairpencil display), and earlier behaviors.

I conclude that the blend of these 3 components acts as a unit to affect all stages of male response.

Blends containing Z8-12:OH also caused a sharp reduction of G. prunivora capture at the very ratios increasing G. molesta capture. This is the first chemical found to have such opposite effects on these two species, which seem to share at least the acetate portion of their communication system. Z8-12:OH may be the single-most important component yet discovered causing reproductive isolation between these sympatric, and largely synchronic species.

The fourth pheromone component, 12:OH, evoked a small but significant increase in one of the later behaviors, hairpencil display, and only when occurring in blends containing low amounts of Z8-12:OH plus the acetates; there was no discernable effect when Z8-12:OH was either completely absent or emitted at its "natural" higher ratio to the acetates, approximated by a dispenser dosage of 10% Z8-12:OH in the acetates. This much-diminished effect of 12:OH-containing blends is in contrast to the comparatively major behavioral role previously ascribed to this combination (Cardé et al. 1975 b, c), and to the consistent substantial trap capture increases with the addition of 12:OH reported by several investigators (Beroza et al. 1973, Roelofs et al. 1974, Gentry et al. 1975, Rothschild and Minks 1977). The differences between previous reports and the current findings are likely related to contamination of the optimum (E)-(Z) acetate mixture with a small (0.1-0.3%) percentage of Z8-12:OH. Such contamination might be a synthesis by-product or it could result from saponification of the Z8-12:Ac with water contacting the septum dispenser (our treatments lacking Z8-12:OH often improved after a soaking of the septa in

rainstorms). In this report as little as 0.1% to 0.3% Z8-12:OH elicited significant behavioral effects. The sample of Z8-12:Ac [6.8% (E)] used at Geneva for both trapping and behavioral observations had a heretofore undetected 0.4% of Z8-12:OH as a contaminant (A. Hill and W. Roelofs pers. comm.). This level of contamination would explain why in the past addition of 0.3% Z8-12:OH seemingly increased trap catch only 2-fold (Cardé et al. 1975b). The portion of the Z8-12:OH present as a contaminant would already have accounted for an appreciable increase in trap catch over the acetates alone, and addition of more Z8-12:OH could cause only a further small increase in capture. Thus, it now appears that the past behavioral effects of 12:OH-containing blends were significantly influenced by Z8-12:OH. In this report 12:OH-containing blends increased various behavior only when Z8-12:OH was already present at low ratios (ca. 1%), not higher (10%), or absent entirely. Had Z8-12:OH been at less than 1%, perhaps addition of 12:OH would have produced the level of behavioral effects similar to those previously reported, not just increases in hairpencil behavior. Although the contribution of 12:OH to the behavior-modifying properties of the 4-component blend appears minimal, it may affect behavior in as yet unknown ways. For instance, it may influence attraction or courtship in ways I have not yet measured, or possibly its affect upon behaviors I measured will be more apparent under certain environmental conditions.

B. Classifying identified sex pheromone-mediated behaviors.--I have described the behavioral effects of the G. molesta sex pheromone components first in terms of specific behaviors evoked which were named. Then the temporal position of each behavior in the orientation

"sequence," was determined, thereby categorizing the behavioral effect as being relatively "early" or "late." Such a classification avoided the ambiguities of "close-range" and "long-range" labels on behavioral acts (Kennedy, 1978), but my categorization rests upon precise definition of "early" and "late" behaviors in a sequence. An ordered series of behaviors may arise in a particular environment merely because spatial constraints impose a particular pattern on the behaviors (Slater and Olasson, 1973). For instance, in the wind tunnel prior to stationary or upwind flight, from the release cage males had to walk, wing fan while walking, or take flight. Also, males were required to remain in the pheromone plume in flight to reach the source. Had they been released directly onto the platform with the septum, the truncated "sequence" would have lacked flight. During male courtship of calling females where males were released ca. 10 cm from the female, no males flew, and they initiated hairpencil display within seconds of wing fanning while walking (Baker and Cardé, 1979). Here fanning while walking could be considered both an "early" and "late" behavior. In the field there are an infinite number of spatial considerations (of which our arena was but one) for responding males in the pheromone plume. Hence a "normal" sequence or "hierarchy" of response (Schwinck, 1958) may be partially artifacts of the experimental environment. Indeed, unless we can remove artificial environmental constraints, the ordering of behaviors may be so context-dependent that relationships between behaviors that are functional in all environments will remain obscured. For example, the close correlation between duration of pre-flight wing fanning while walking and likelihood of post-flight wing fanning while walking suggests that fanning while walking is the same irrespective of

its spatial occurrence "early" or "late" in a sequence. Furthermore, the high correlation of wing fanning while walking with upwind flight indicates that these two behaviors may be functionally similar solutions to the same problem--location of the chemical source either by air or ground. Measuring upwind progress by leg movement against a fixed substrate or by a moving ground pattern over ommatidia does not seem very different when viewed in this way, even though the behaviors themselves have a dissimilar form. Hence, it is difficult to classify behavioral effects as "close" or "long-range" or as occurring "late" or "early" in the response "hierarchy," although we attempted the latter classification throughout this study for both orchard and wind tunnel environments. The first system depends upon arbitrary distances and the second upon sequences which are organized not necessarily by function but largely by the environment's spatial pattern. Perhaps, after initial quantification of the various behaviors of a response, behaviors should be consolidated according to their function independent of environmental design, thereby gaining a greater understanding of the real processes involved and possibly uncovering real, concentration-dependent hierarchies.

C. Assigning functions to chemical stimuli.--Similar difficulties occur in the classification of the chemical stimuli. The sequential, orderly addition of components, one at a time, would appear to allow for an accounting of the various behaviors they evoke--a sort of dissection of stimuli. However, for G. molesta components an added chemical was never singly responsible for the new behaviors observed. Rather, the blend caused the behavior and to consider Z8-12:OH a "courtship component," for instance, would have been misleading unless

it was proved that at its natural dosage it could, emitted singly, elicit hairpencil displays from males. Only in combination with the optimum (Z)-(E) acetate blend did it evoke hairpencilling, and so Z8-12:OH's effect became impossible to separate from that of the total blends', which was an increase in all behaviors including hairpencilling. E8-12:Ac added to Z8-12:Ac and Z8-12:OH also increased all behaviors, as did Z8-12:Ac added to the other two. Moreover, irrespective of the order in which the 3 were added, a new one entering a partial blend changed the "roles" previously ascribed to the others. According to the classification method suggested by Roelofs and Cardé (1977) these 3 chemicals should be designated as primary sex pheromone components. Considering the dependency of each upon the other two and their unitary mode of action, perhaps this mixture itself should be named the primary component; 12:OH, on the other hand, in some blends can act as a secondary component apparently affecting only hairpencil behavior. It would appear that when there are so many primary components this classification system loses much of its usefulness.

It does appear that behavioral thresholds were lower for some "partial" blends than others. For instance, wing fanning while walking and upwing flight thresholds appeared lowest to the (E)-(Z) acetate mixture compared to all other binary and single component blends, although a dosage-response experiment is needed to prove this. These thresholds appeared lower still to the 3-component Z8-12:OH-containing mixture. Careful experimentation is needed to demonstrate whether one set of behaviors is uniquely affected by chemical blend; if this could be demonstrated, then a particular blend may indeed have a qualitatively

different effect. In reality, however, there are probably few such well-defined qualitative effects, but a graded series of quantitative effects only the larger of which can be measured by our crude methods. At some point the behavioral differences become so small that they fall below our detection threshold.

D. Relation of wing fanning while walking to attraction.--Different blends appear to evoke quantitative behavioral differences on the individual level, such as increased durations of orientation within the plume, and wing fanning while walking. Both the percentage and duration of pre-flight fanning while walking were highly correlated with later behaviors such as upwind flight, suggesting that where attraction cannot be directly measured, as in "stimulation" olfactometers, duration of fanning while walking would be the best "key response" to measure. For Argyrotaenia velutinana percentage and duration of wing fanning while walking in orientation tube and box olfactometers were accurate indicators of attraction of males to various blends as measured by trap capture and field observation. Similar to Z8-12:OH-containing blends in G. molesta, a blend of all identified components including dodecyl acetate increased duration of wing fanning while walking in laboratory olfactometers and the percentage of males landing and wing fanning while walking near the source, accounting for higher A. velutinana trap capture (Baker et al. 1976).

E. Wing fanning while walking and trap competition.--The behavioral differences observed between the E8-, Z8-12:Ac blend either lacking or containing Z8-12:OH were reflected in trap catch. The acetates-only blends did moderately well at capturing males (Figure 8) until Z8-12:OH was added at various dosages (Figure 9), whereupon the acetates alone

were indistinguishable from unbaited traps. The partial blend of acetates evoked a "partial" behavioral response, getting the males "up and flying" upwind. However, near the source males were not likely to wing fan while walking and be captured (Table 5). To Z8-12:OH-containing traps, males were at least as likely to be "up and flying" upwind, but they were more likely to wing fan while walking and thus be captured on the sticky surface near the source (Table 6). If males already in flight are more likely to encounter from neighboring traps another pheromone plume that elicits more complete behavior, then it is clear that a treatment evoking flight but not landing will lose more potential males over predicted by mere comparison of capture levels of the isolated treatments. The intensity of the effect appears greater at higher population levels (Figure 9). This may be the behavioral basis for the "competition effects" sometimes observed in high population densities where greater numbers of females are presumed to somehow "compete" more strongly with pheromone traps for males (Minks 1977). Our evidence suggests that manifestation of the effect depends directly upon pheromone-related capture efficiency and the behavioral response for that efficiency.

F. Internal control of behavior.--Although fanning while walking and upwind flight may be closely related in function, the balance between the pheromone-evoked states of remaining fanning while walking or taking flight upwind probably depends upon both external (pheromone quality and quantity, wind velocity and direction, visual cues) and internal factors such as antagonistic induction (Kennedy, 1974). The latter is when the duration of performance of one behavior makes the occurrence of another behavior more likely. For instance, in the wind

tunnel wing fanning of longer duration occurred before flight in response to Z8-12:OH plus the acetates than to 12:OH plus acetates. Latency of upwind flight may have been increased because the quality of the stimulus required greater amounts of fanning while walking to induce upwind flight. Although I did not investigate them, the contributions of possible internal factors controlling transitions between behavior should not be ignored. Careful experimentation will be needed to observe their contribution to pheromone-mediated behavior.

SUMMARY

1) The female G. molesta sex pheromone chemicals Z8-12:Ac, E8-12:Ac, and Z8-12:OH acted as a unit to elicit increases in both early and late stages of male sexual behavior. Addition of the chemical 12:OH elicited an increase only in a later stage, the hairpencil display, and only when 12:OH was emitted at higher, and Z8-12:OH at lower, than "natural" emission rates.

2) A pheromone component's effect in G. molesta was described most precisely only when the component was considered in combination with the others, rather than individually, or in binary or 3-component blends.

3) Significant behavioral increases were elicited by the addition of as little as 0.1% Z8-12:OH to the two acetates. This suggests that standards of purity employed in most pheromone investigations are inadequate.

4) The strong correlation in G. molesta between pre-flight wing fanning while walking and upwind flight in the pheromone plume suggests that these behaviors should be considered very similar in function. In bioassays where upwind flight cannot be measured directly, for some moth species wing fanning while walking may be the best "key response" to measure.

5) Spatially-induced artifacts of the bioassay environment may contribute to the order and frequency of behaviors in a "sequence." More meaningful functional relationships between behaviors may be revealed only when a sequence is free of such context-dependent effects.

CHAPTER 3

Courtship Behavior of *Grapholitha molesta*

Experimental Analysis and Consideration

of the Role of Sexual Selection

in the Evolution of Courtship Pheromones in the Lepidoptera

INTRODUCTION

Many males of the Lepidoptera possess elaborate brush organs or hairpencils, which are kept in pockets or pouches on various areas of the body until courtship, when they are everted in the presence of a female. While most of the courtship pheromones in Lepidoptera described to date elicit in females such responses as flight cessation, quiescence and/or abdominal extension (see Birch 1974 for review), orientation responses by females to courtship pheromones have been rarely described. I report here that males of a tortricid pest of peaches and apples, the Oriental fruit moth, Grapholitha molesta (Busck), give a stereotyped elaborate hairpencil display whose chemical and wind stimuli cause locomotion and attraction of a female to the hairpencils from up to 2 cm away. I propose that the display represents a form of ritualized clasper extension which evolved via "female choice" sexual selection (Fisher 1958).

George (1965) first described the eversion but not the function of "scent-pencils" of male G. molesta exposed to pheromone from a neighboring container of females. Roelofs et al. (1969) identified the major component of the G. molesta female sex pheromone and it was later found that additional components were necessary for optimal catch of males in sticky traps (Roelofs and Cardé 1974b, Beroza et al. 1973a, b). Some of these components at close range caused increases in the amount of landing, wing fanning while walking, and hairpencil display behavior,

and the hairpencil extrusions appeared to attract calling females from short distances (Cardé et al. 1975[b, c]).

I present here a detailed analysis of the sequence of male courtship behavior, the female stimuli which release the male behaviors, and the stimuli contained in the male hairpencil display which affect female behavior.

MATERIALS AND METHODS

A. Rearing.--Grapholitha molesta adults came from a laboratory colony maintained at Michigan State since October 1975 on apples on a 16:8 light:dark photoperiod regime at 25°C. Feral adults were added to the mating stock when available each summer.

B. Description and sequence analysis of courtship behavior.--I recorded courtship behavior in the laboratory by placing a calling female (emitting pheromone) onto an open horizontal observation platform of black paper or cloth and then releasing, from a glass vial downwind, a male. The ensuing behavior was recorded on a Sony AVC-3540 videorecorder through a Sun III time base generator to number the frames each 1/30 sec. I played back the tapes on a Concord VTR 820 deck through a time base decoder onto a television monitor and transcribed the behavior frame by frame onto paper for analysis of sequence and temporal patterns. Durations of hairpencil extrusion and retraction were measured from the transcribed data using the first and last visible appearances of white hairpencils as markers for the start and finish of an extrusion and the interval between (no white hairpencils visible) as the duration of retraction.

I used the sequence analysis technique that others have used for similar studies (e.g., Baerends et al. 1955, Halliday 1975). Frequencies of transitions from one behavior to another were first tabulated from the transcribed recordings and then consolidated into a transition probability matrix using only first-order transitions. Self-transitions (i.e., when, after a predetermined time interval, the animal remains in the same behavioral state) were not included in these matrices. In sequence analysis, the importance of transitions between behaviors can be obscured if self-transitions are included, as the latter usually occur within long "bouts" of one behavior and are therefore more numerous (Slater and Ollason 1973). Hence, the final matrices and resulting flow diagrams (Figure 17) are of conditional probabilities, i.e., the probability of a particular transition occurring given a change in behavioral state will occur.

C. Effect on male behavior of visual stimulus at pheromone source.--I constructed a special platform (Figure 14) and placed it in a laboratory wind tunnel (described in Cardé and Hagaman 1979) having a photophase (daytime) lighting intensity of 150 lux, to test males' visual response to calling females. The platform was a thin sheet of white polystyrene plastic 23.5 x 24.5 x 0.05 cm covered by a black cloth, with a 1 mm diameter hole through the center of only the plastic into which the tip of a Pasteur pipette was placed. A rubber septum was impregnated with 4 components of the female-produced sex pheromone (Cardé et al. 1979): 9.5 µg(Z)-8-dodecenyl acetate, 0.5 µg (E)-8-dodecenyl acetate, 0.1 µg (Z)-8-dodecen-1-ol, and 10 µg n-dodecanol, all pipetted in 10 µl from a hexane solution. From gas chromatography, I determined that all compounds were greater than 99% pure; the

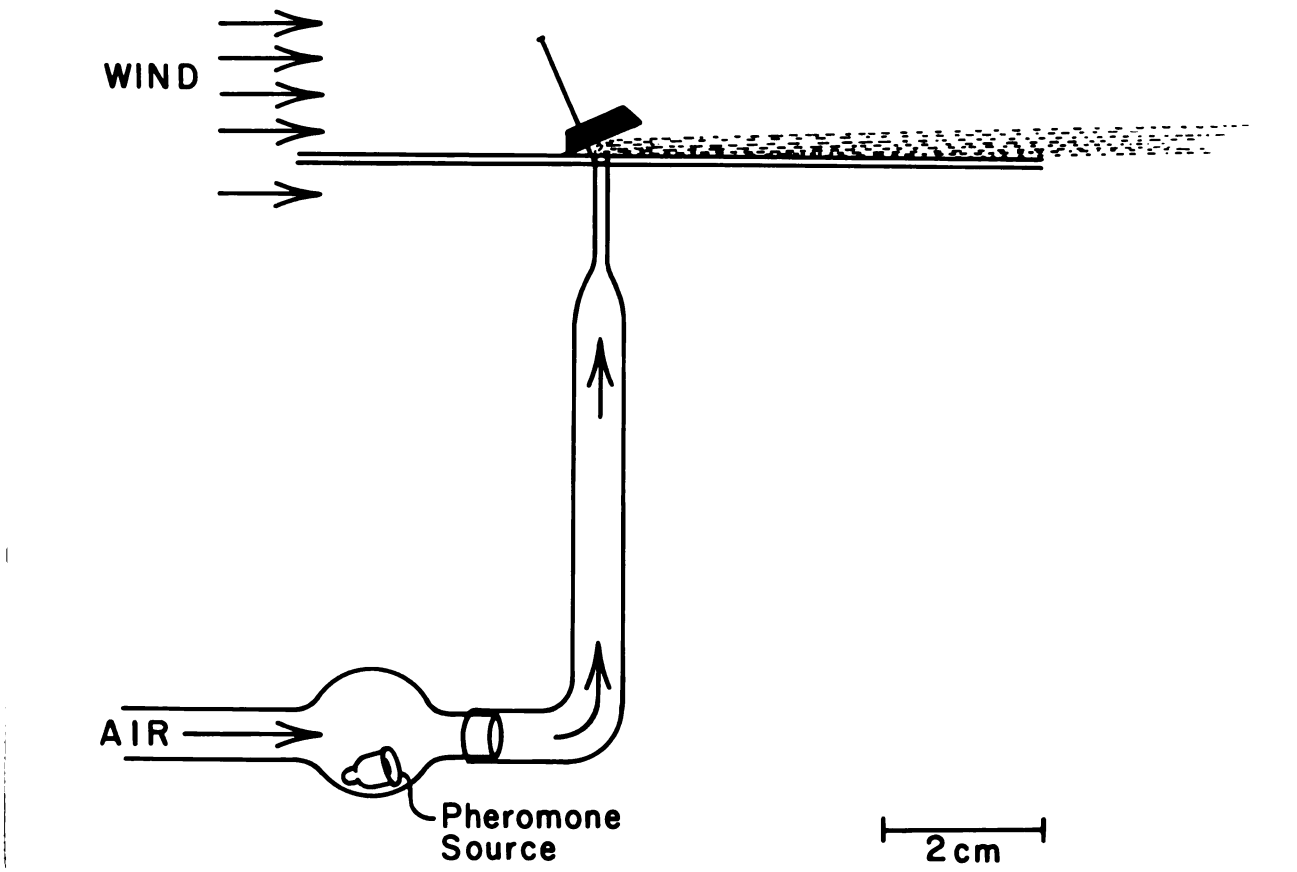

Figure 14. Diagram of the lateral view of the observation platform used for presenting G. molesta sex pheromone both with and without a visual stimulus to males flying upwind from the right. Pheromone-laden air was blown through the tip of the pipette through a layer of black cloth covering the plastic platform surface and travelled downwind in the wind tunnel at a rate of 0.7 m/sec.

acetates (Farchan Corp.) contained less than 0.03% 12-carbon alcohols and the (Z)-8-dodecen-1-ol no detectable amounts of the (E) isomer. I placed the septum in a glass vessel through which filtered air was blown at the rate of 0.4 ml/sec, and the pheromone-laden air passed up the pipette and through the cloth on top of the platform; thus no visual cues were present on the black cloth surface.

Wind in the tunnel at a velocity of 0.7 m/sec blew the pheromone to the downwind end (where it was exhausted from the building), and I released males individually within the pheromone plume from an 11 x 7 cm diameter screen cylinder from which males flew to the platform, landed, and approached the chemical source by fanning their wings while walking.

I used 3 different 3-dimensional visual models to test male courtship response: a dead male washed with acetone and pinned to the platform over the hole, a gray piece of construction paper (7 mm long, 3 mm high, 3 mm wide at the posterior end) (Figure 23) folded in half and pinned over the hole, and a rubber septum (Figure 20) (Arthur Thomas Co.) 15 x 9 mm diameter at the widest end. Models were tested against the control situation of no visual stimulus present, and the number of males in which hairpencil display was elicited was recorded.

D. Effect of spatial separation of visual and chemical stimuli.--I modified the platform described above to test the effect of moving a visual model away from the chemical source. I removed the black cloth and drew in pencil on the plastic to divide the surface into quadrants: upwind, downwind, left, and right. A small piece of white tape was placed loosely on the center of the platform to conceal the hole and the tip of the Pasteur pipette, as preliminary trials had indicated

that these visual cues could increase the frequency of hairpencil display behavior. The gray paper model described above was pinned 1 cm from the pheromone source in one of the quadrants or on top of the source or removed completely, using a completely randomized design. Males were released at the downwind end of the tunnel and their behavior upon landing on the platform was recorded on videotape. For each male, the number of hairpencil displays and percentage of time spent in each of the quadrants both away from (>0.5 cm) and at (≤ 0.5 cm) the chemical source were calculated after a frame-by-frame analysis of the tapes. All displays, regardless of their direction, were scored for each model location. The percentage times spent in quadrants for each individual were transformed to arcsin $\sqrt{x}$ and the data were submitted to a 2-way analysis of variance for unequal replication. Means were compared using the LSD test. Mean numbers of hairpencil displays per male were compared by the LSD test after a one-way analysis of variance for unequal replication.

E. Effect of female movement on the form of male hairpencil displays.--Videotape recordings revealed a positive correlation between the duration of the first hairpencil extrusion of the first display and the success of males in attracting females. To test whether female movement toward the male was causing longer extrusion durations, a rubber septum was impregnated with the pheromone mixture described earlier and affixed from beneath by an insect pin to the observation platform. When a male began his display I either rotated the model rapidly 180° back and forth by twirling the pin from beneath, or else left it stationary. The behavior was video-recorded and the extrusion durations measured later frame-by-frame.

F. Effect of tactile stimulus from female after attraction to male abdomen.--Using the platform and gray paper model, the hairpencil display was evoked in males landing on the platform after flying upwind in the tunnel. I tried to touch males' abdominal tips with a camel's-hair brush dusted with Day-Glo rocket red powder during their first display at the model, and observed the subsequent behavior. The males were recaptured and the hairpencils checked using ultraviolet light for evidence of transfer to the powder. Transfer of powder was considered proof that contact with the abdomen had actually been made by the brush. I ran controls at random, holding the brush near the abdomen, but trying not to touch it.

G. Stimuli from male during hairpencil display affecting female courtship behavior.--The male hairpencil display contains many types of stimuli of potential importance in attracting the female to the male. Three stimuli thought to be important were: 1) chemicals emanating from the hairpencil surfaces; 2) visual stimuli presented by a male's white hairpencils; and 3) the mechanical stimulation from wind generated by the male's vibrating wings. I investigated the relative importance of these 3 stimuli in the following way.

I used 2 groups of calling females, 1 group sham-operated, and the other "blinded" by the following procedure. After being chilled for a few minutes in a freezer, females were picked up by their wings with a pair of forceps and a small amount of white water-soluble glue was applied to both compound eyes with an insect pin until no ommatidia were visible. I sham-operated females by placing a drop of glue between, but not covering, the compound eyes. During the normal calling period, I tested individual females from both groups in a randomized complete

block design for their response to a hairpencil extract chemical stimulus, wind stimulus, chemical and wind stimuli combined, and respective solvent blank controls. The response of blinded versus sham-operated females to the extract- or solvent-impregnated white filter paper presented against the black background was considered a measure of the importance of vision in female orientation to hairpencils.

A female, either blinded or sham-operated, was placed on the horizontal cloth-covered platform and allowed to adjust to the new conditions for about 1 minute. Then 1 of the 4 treatments described below was directed toward the female from 1 cm directly upwind and the resulting behavioral response recorded.

I made hairpencil extract by taking frozen males, excising the terminal 3 abdominal segments including hairpencils, and extracting these in re-distilled methylene chloride. Approximately 5 male equivalents (50 µl) were pipetted onto a piece of folded filter paper attached to and protruding 1 cm from the tip of a disposable pipette (1 mm i.d.). The solvent was allowed to evaporate for 20 sec. and then the tip of the paper was placed 1 cm upwind of a calling female (ambient wind velocity of about 33 cm/sec). For another treatment, additional wind was added by blowing filtered air at a velocity of about 76 cm/sec (measured 1 cm from the tip) through the pipette, over the filter paper, and thence onto the female 1 cm downwind of the filter paper. Solvent blank controls were made using 50 µl of methylene chloride.

H. Measurement of wind velocity generated by male wing fanning during hairpencil display.--To measure the velocity of wind generated posterior to a male during hairpencilling, I constructed an anemometer consisting

of a 6 cm long section of a single branch of a peacock feather suspended vertically from a horizontal bend on a 6 cm high piece of wire. The wire was affixed to the observation platform so that the feather hung just beside and behind the gray cardboard model pinned directly over the pheromone source (Figure 24A). Males hairpencilling at the model caused the feather to be deflected backwards, and during playback analysis of videotapes made of these males, the deflection magnitudes were measured on the television screen for the periods when the hairpencils were fully extruded and retracted. Immediately after a session of taping male behavior, I also made recordings of feather deflections from a known velocity of air emanating from a tube (0.6 cm i.d.) placed 2 cm directly upwind of the feather. Wind velocities of 25, 51, 102, 152, and 254 cm/sec were used to deflect the feather, as first measured on a Hastings-Raydist model AB-27 anemometer, and the deflections measured on the television monitor. I then converted the magnitude of deflection caused by hairpencilling males to wind velocities in cm/sec by interpolation.

I. Measurement of female preference for males with intact hairpencils.--I first chilled males to immobility in a freezer about 6 hr before lights-off, and while the male was gently grasped by the wings and middle of the abdomen with forceps, the end of the abdomen was exposed and dabbed with a drop of clear glue from an insect pin. Glued males were incapable of extruding their hairpencils. I handled sham-operated males identically except that I rubbed a clean insect pin 4 or 5 times across the abdominal tip; a second sham-operated group consisted of males having glue applied to the pronotum.

To test female response to males from different groups, at 0.5-3

hours before lights-off I placed an individual "glued" male onto the observation platform located in a laboratory exhaust hood. A calling female was then placed upwind of the male, and the ensuing hairpencil displays and female response were noted. After the male's courtship attempt was terminated (usually after the female walked or flew away), I removed the male, returned the same female to the middle of the platform, and introduced a sham-operated male (from the first sham group) downwind of the female. Hairpencil displays and female response were recorded. To test the possibility that dried glue on the male's body was influencing female response, males from the 2nd sham-operated group, containing glue on their thorax, were introduced downwind from naïve calling females; the resulting behavior was observed and recorded.

RESULTS

A. Description of the courtship sequence.--Analysis of 49 courtship sequences revealed that a fairly fixed pattern of male and female behavior occurs. After the male walks towards the female from downwind, fanning his wings (Figure 15A), the usual sequence is this: 1) pauses briefly less than 1 cm from the calling female, facing her (Figure 15B); 2) moves past her into a new quadrant, which usually brings him upwind of her; 3) turns and points his abdomen's tip at her, thus facing away from her (Figure 15C); 4) extrudes his hairpencils (Figure 15D) which are located between the 7th and 8th abdominal segments and associated with the claspers. After the first extrusion, the female ceases calling and begins walking towards the male's abdomen tip (Figure 15E, F). While the female approaches, the male extrudes and retracts his

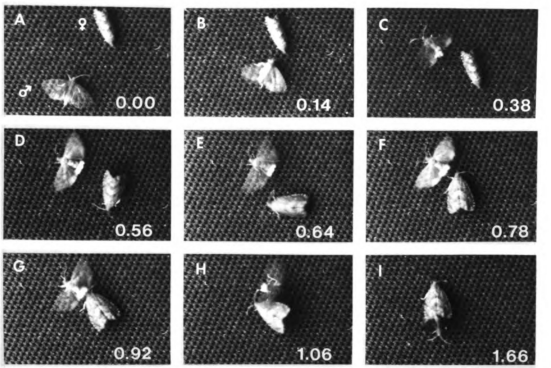

Figure 15. The sequence of events occurring during courtship and copulatory behavior: A) male approaching calling female from downwind by wing fanning while walking; B) male faces female; C) male has moved past female and faces away, female still stationary; D) male extrudes hairpencils, female begins walking towards male; E), F) male hairpencils remain extruded, female continues walking towards male; G) female about to make contact with abdomen; H) contact with abdomen made, male begins to attempt copulation; I) copulation, with male's wings about to settle on top of female's. Numbers in lower right hand corner indicate time elapsed in seconds. Wind direction is from top of picture to bottom.

hairpencils 1 or 2 more times until the female touches his abdomen with her head and antennae (Figure 15G), pausing momentarily. The contact causes the male to attempt copulation, first arching the tip of his abdomen even higher (Figure 23B) and then abruptly whirling in a type of horizontally performed cartwheel while touching the female (Figures 15H, 23C). During the male's attempted copulation, the female continues walking, but with the wing on the side of the male raised, possibly due to the force of his attempt (Figure 15H); her abdomen is grasped by the male's claspers and copulation occurs. The male's wings rest on top of the female's (Figure 15I).

Males not inducing females to make contact with their abdomen repeated the courtship sequence many times (Figure 16) before finally attempting to copulate, usually unsuccessfully. As a result of the movement into new quadrants between hairpencil displays, males directed the displays towards the female from many different angles (Figure 16); sometimes males only attracted females after 2 or 3 displays from different positions. Other unsuccessful males were those touching the female while moving past her either before or between displays, causing her to fly or walk rapidly away; these males remained wing fanning while walking (N = 25). Although these encounters were not included in further analyses of courtship behavior, they are important in showing that an "improper" performance of even the earliest stages of the courtship behavior can reduce the probability of mating.

B. Sequence analysis of male courtship behavior.--As evidenced by the high conditional probabilities of proceeding only to the next step in the courtship sequence, male courtship behavior is fairly "stereotyped" (Figure 17). For males both successful (Figure 17A) and unsuccessful

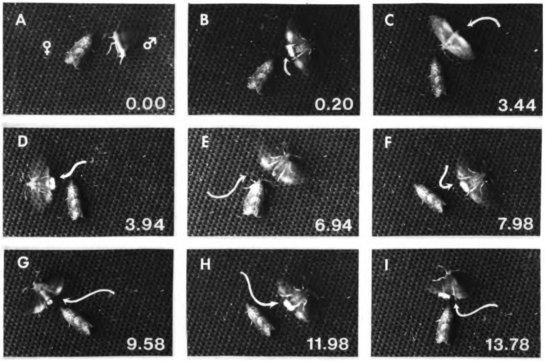

Figure 16. Male courtship behavior, consisting of 7 different hairpencil displays when the female does not make contact with the male's hairpencils. Arrows indicate the path taken by the male. In Figures B, E, and H, the female is about to move towards the male but not touch his abdomen, and then ends up sitting in a new position in C, F, and I. A) male moves past female after approaching from downwind; B) male faces away and gives the hairpencil display consisting of 4 extrusions; C) male turns and faces female after finishing the display; D) male moves past female, faces away and gives hairpencil display; E) as in C and D; F) as in C and D; G) as in C and D, hairpencils beginning extrusion phase; H) as in C and D; I) as in C and D. Numbers in lower right hand corners indicate time elapsed in seconds.

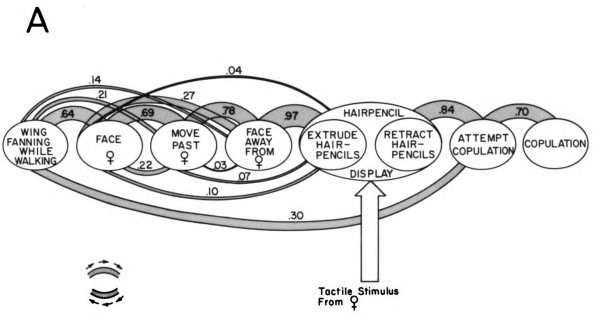

Figure 17. Sequence of courtship behavior observed in G. molesta males both successful (A) (N=27) and unsuccessful (B) (N=22) in attracting females to their abdomens during hairpencil display. Decimal numbers and corresponding thicknesses of shaded bands are the conditional probabilities of a particular transition occurring between 2 behaviors. Behavior flows from left to right in the upper bands and right to left in lower bands.

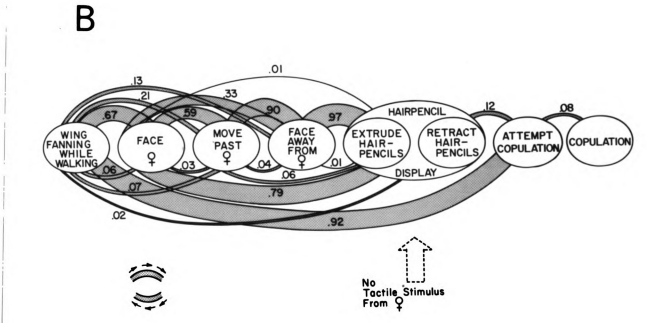

Figure 17. (Cont.)

(Figure 17B), in attracting females to their abdomens (N = 27 and 22, respectively), there is a high probability that once the first step in the sequence is taken the rest will follow. The probability of hairpencil retraction immediately following extrusion is so high that, for sequence analysis, little information is gained by keeping these as separate behaviors; they are presented separately in the diagram, however, to make it clear that the hairpencils are repeatedly extruded and retracted.

The major difference between the behavior of males successful and unsuccessful in attracting females is seen following the hairpencil display. Successful males, i.e., those receiving the apparent "touch" on the abdomen from the female, have a high probability (0.84) of making a copulatory attempt following hairpencilling (Figure 17A); for unsuccessful males the probability is low (0.12) (Figure 17B). For successful males the attempt results in copulation 70% of the time. Rather than attempting copulation after hairpencilling, unsuccessful males have a high probability (0.79) of returning to an earlier step, facing the female, and then running through the sequence again. The high probability of "moving past the female" each time through the loop indicates that these males often move to a different side of the female during each successive display, possibly to help improve the "angle" or directionality to evoke a female response. With each successive display the number of hairpencil extrusions decreases from an initial mean of 3.8 during the first to 2.1 during the 5th display (Figure 18), until a copulatory attempt is finally made. Such attempts are usually unsuccessful and the female either flies or walks away. The male then has a high probability of returning all the way to wing fanning (0.92)

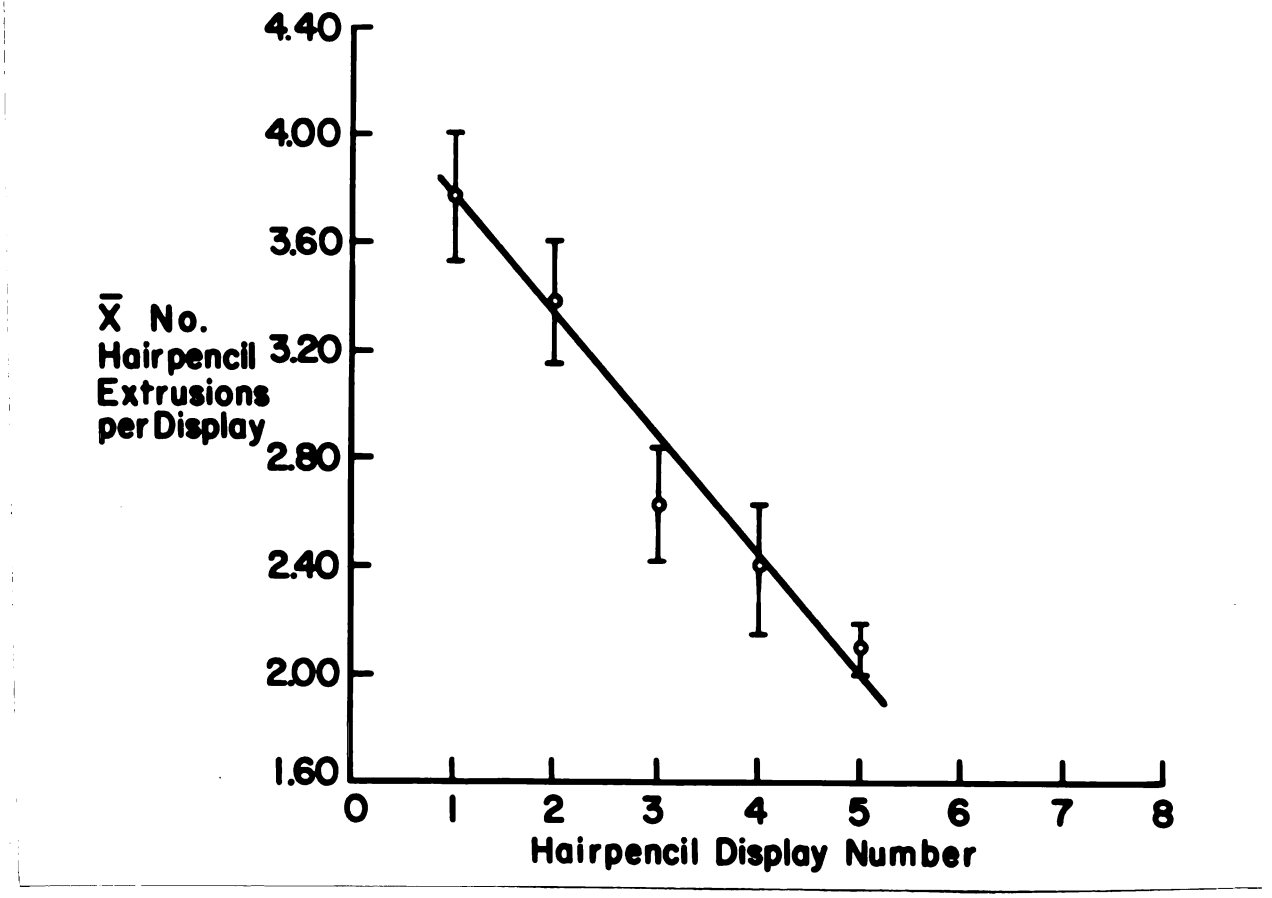

Figure 18. The decline of mean number of hairpencil extrusions during later displays. Brackets around means denote standard errors; solid line is weighted linear regression line, N = 22 for display no. 1, 21 for display no. 2, 17 for display no. 3, 14 for display no. 4, and 8 for display no. 5.

to either follow the disturbed female or "search" the area she has vacated. No behavioral steps preceding wing fanning are included in this analysis, but when the female departs, the male eventually stops wing fanning and returns to earlier behaviors such as flying downwind or sitting.

C. Temporal analysis.--The entire G. molesta courtship sequence occurs in a short time span, generally less than 1.5 sec from momentarily pausing and facing the female to successful copulation. For males whose displays attracted females, the mean time from facing the female to the beginning of hairpencil display was 0.31 sec (±0.16 S.D.; N = 18). The mean duration of the interval beginning hairpencil display to copulatory attempt was 1.04 sec (±0.36 S.D.; N = 27). The copulatory attempt (the quick whirl of a touched male) is difficult to observe on videotape and appears to have a duration no longer than 1/30 sec. Females took mean times of 0.36 sec (±0.21 S.D.) and 0.66 sec (±0.36 S.D.; N = 27) to walk to males' abdomens from the beginning of the first extrusion and from calling position, respectively. The mean duration of the male's first extrusion was longer when females were moving than when they were stationary--a significant temporal difference (Table 12). Within a particular display the first extrusion lasts longer than the last (Figure 19), but this trend is exaggerated for the first display of males successfully attracting females (solid circles, Figure 19). That both the number of extrusions per display (Figure 18) and the duration of extrusions (Figure 19) within a display decrease through time may be a result of a number of factors such as sensory adaptation, habituation, fatigue of the efferent system or a decrease in internal drive. I do not know

Table 12.--Effect of female locomotion toward male upon mean hairpencil extrusion durations. Only males which had females move toward them were used in this comparison. **, entry in same column significantly different according to the t-test ($P \leq 0.01$); NS, entry in same column not significantly different according to the t-test ($P > 0.05$).

	Mean Duration of Hairpencil Extrusions (± S.D.)		
	1st Extrusion	2nd Extrusion	3rd Extrusion
While female is moving toward male	0.54 ± 0.31 sec** N = 22	0.36 ± 0.61 sec NS N = 20	0.30 ± 0.12 sec NS N = 23
While female is stationary	0.33 ± 0.09 sec N = 20	0.29 ± 0.12 sec N = 19	0.27 ± 0.05 sec N = 22

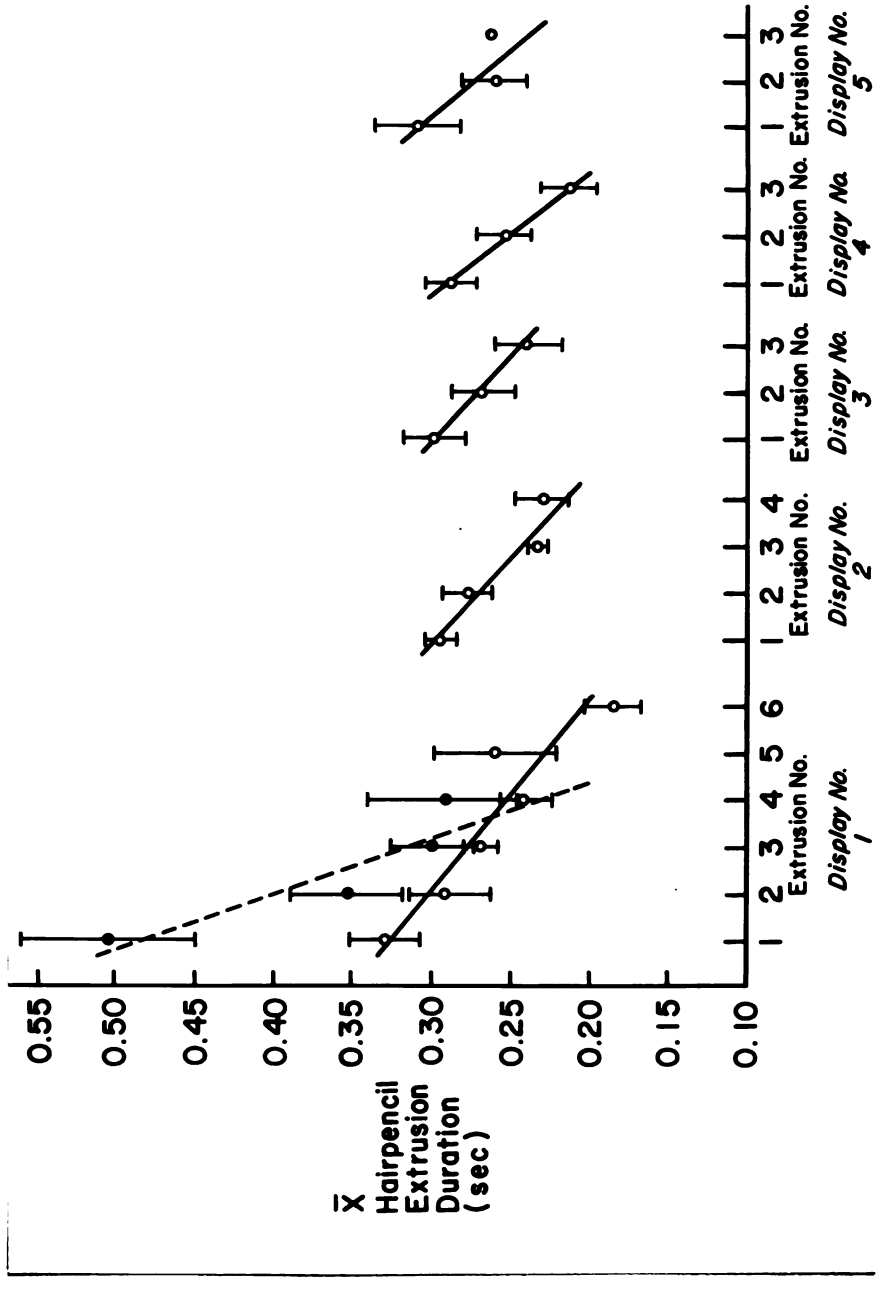

Figure 19. Pattern of mean durations of hairpencil extrusions within successive hairpencil displays toward: females which did not move toward and touch male's abdomen (open circles); and females which moved toward and touched male's abdomen (solid circles). Solid and broken lines are linear regression lines for the stationary and moving female groups respectively. Brackets around means denote the standard errors. Total number of observations, 231 for open circles, 62 for solid circles.

the factors responsible for these changes in displays.

The correlation between success and extrusion duration could be caused by a number of factors: an innate difference in male hairpencil behavior conferring an advantage on those who keep their organs extruded longer; a difference in female stimuli releasing hairpencil display whereby females more likely to respond to the display also emit stimuli more likely to initiate a better display; or, males watch the female and adjust their display according to whether she approaches them or not. Results of a later experiment show that the last hypothesis is at least 1 of the causes of this display difference.

D. Effect of visual stimuli at pheromone source.--The percentage of males giving the hairpencil display increased significantly when female models were at the origin of the pheromone source (Table 13). Of males reaching the source by wing fanning while walking, 64% displayed at dead acetoned males, 47% at the gray paper model, and 52% at the rubber septum (Figure 20). The controls elicited 13, 0 and 5% displays, respectively. Males in both groups not hairpencilling remained wing fanning while walking on top of either the chemical source or the models before sitting or flying back downwind. Displaying males always faced away from, and thus directed their displays toward, the models or the source.

These results indicate that a visual stimulus in conjunction with the pheromone blend (Cardé et al. 1979, Baker and Cardé 1979) releases courtship behavior more frequently than the chemicals alone. Because effectiveness of the 3 models was not contrasted in a single experiment, comparisons between them cannot be made, but it is clear that each one was effective in eliciting the hairpencil display when

Table 13.--The effect of the presence of visual models at the pheromone source upon frequency of hairpencil behavior; **, entry significantly different from the 1 immediately below it according to a χ^2 2 x 2 test of independence with Yates' correction ($P \leq 0.01$).

	No. of Males Wing Fanning at Pheromone Source	No. of Males Giving Hairpencil Display (%)
Dead male present	28	18 (64%)**
Dead male absent	32	4 (13%)
Paper model present	30	14 (47%)**
Paper model absent	30	0 (0%)
Rubber septum present	21	11 (52%)**
Rubber septum absent	21	1 (5%)

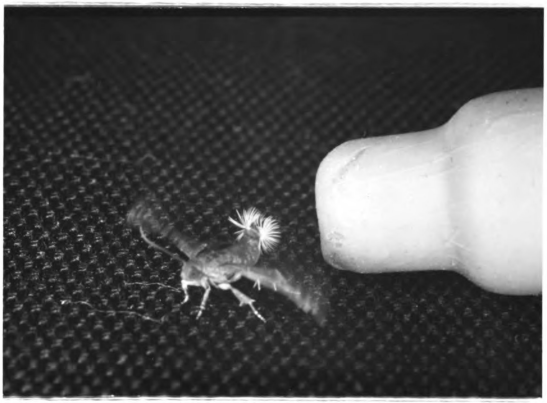

Figure 20. G. molesta male directing hairpencil display toward a rubber septum placed directly upwind of the pheromone source. Male approached on the platform from the right by wing fanning while walking and moved past the model before facing away and displaying his hairpencils.

combined with chemical stimuli. Their effect can be viewed in one way as causing a lowering of the chemical threshold for hairpencil display behavior; or, the visual and chemical stimuli can be viewed as acting together as an algebraic sum which surpasses the minimum value necessary for hairpencilling to occur. The latter explanation has been called the "heterogeneous summation" of stimuli (Lorenz 1950) and used to explain the many ways a behavioral response can sometimes be elicited by varying the relative stimulus intensities.

E. Effect of separation of visual and chemical stimuli.--The gray paper model was most effective in releasing hairpencil behavior when it was placed directly upon the chemical source, less effective downwind or to the left, and no more effective than no model at all when it was upwind or to the right of the source (Figure 21). No matter where the model was placed around the source, the percentages of time spent in a particular quadrant by males did not significantly differ from each other (with 1 exception) (Figure 22A, B). The apparent preference for the left quadrant rather than an equal left-right distribution was explained after the experiment was done when a smoke plume was generated through the apparatus. The piece of white tape concealing the tip of the pipette deflected the pheromone-laden air decidedly to the left and in the control situation males were spending more time in contact with the pheromone plume both downwind but also to the left of the source. The only time this changed was when the model was placed directly over the source, changing the plume's course closer to the "expected" downwind direction and altering the amount of time spent by males close to the model (Figure 22B) in this instance. Regardless of the absence or presence of a

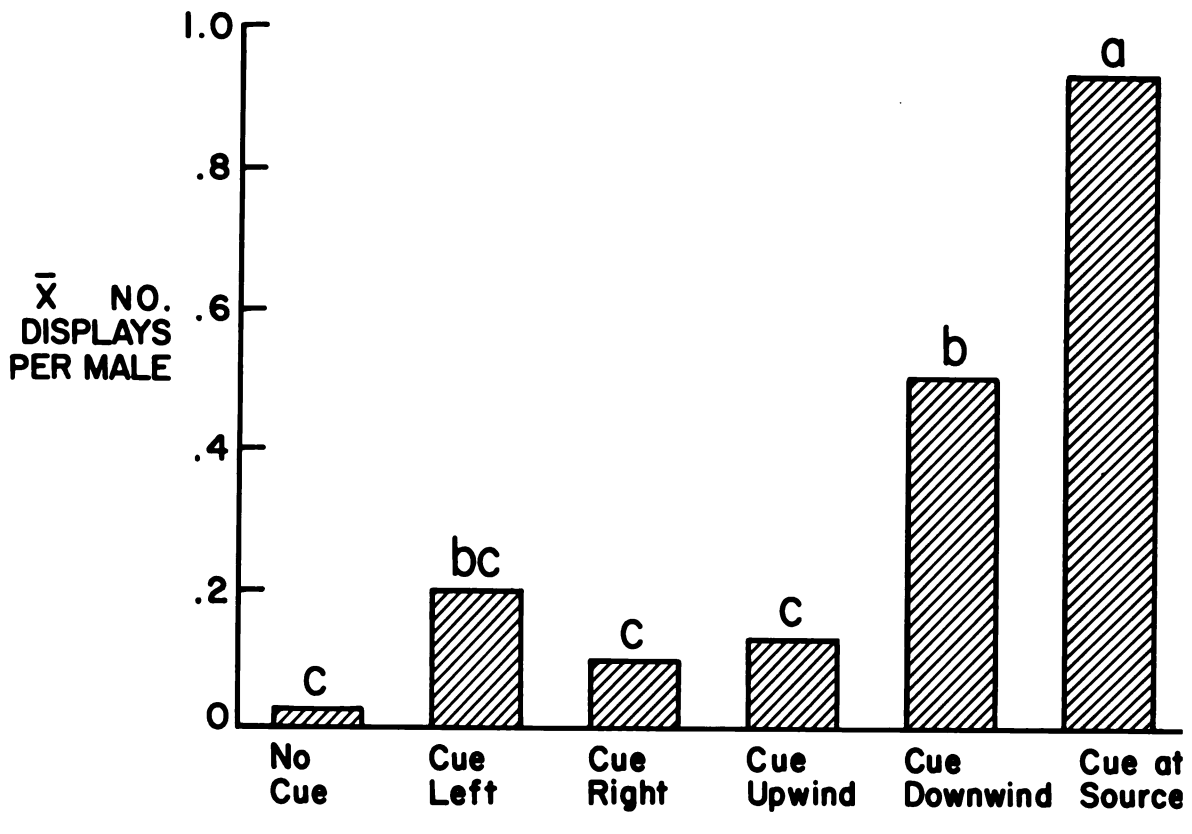

Figure 21. Mean number of hairpencil displays per male elicited when the visual cue (paper model) was in the position indicated. Means having no letters in common are significantly different by the LSD test ($P<0.05$). For the visual cue: absent, N = 22; to the left, N = 24; to the right, N = 26, upwind, N = 26; downwind, n = 27; over the pheromone source, N = 28 males.

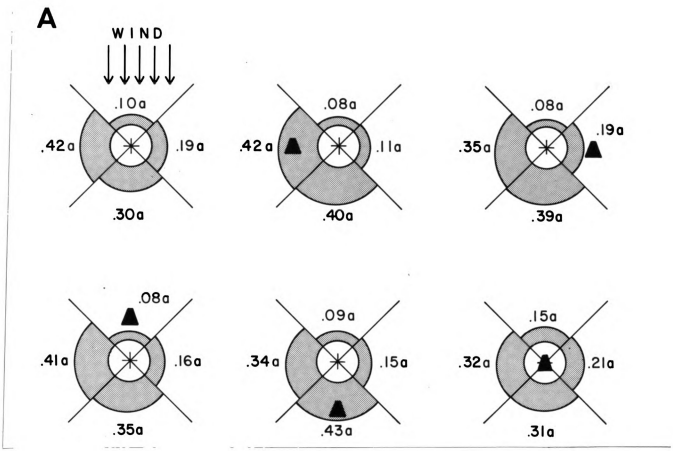

Figure 22. Effect of different placements of the gray paper model 1 cm from the pheromone source upon percentage of time spent by males in each of the quadrants on the observation platform. The "+" in the center of each figure is the location of the pheromone source, and the solid black figure represents the model's position. Decimal numbers and corresponding widths of shaded areas represent the mean percentage time spent by males in each quadrant; A) away from the pheromone source ($0.5 \text{ cm} < x < 2 \text{ cm}$) and B) at the pheromone source ($X < 0.5$ cm). For a particular quadrant, means for each of the 6 different model positions having no letters in common are significantly different by the LSD test ($P < 0.05$). For the model: absent, N = 22; to the left, N = 24; to the right, N = 26; upwind, N = 26; downwind, N = 27; over the pheromone source, N = 28 males.

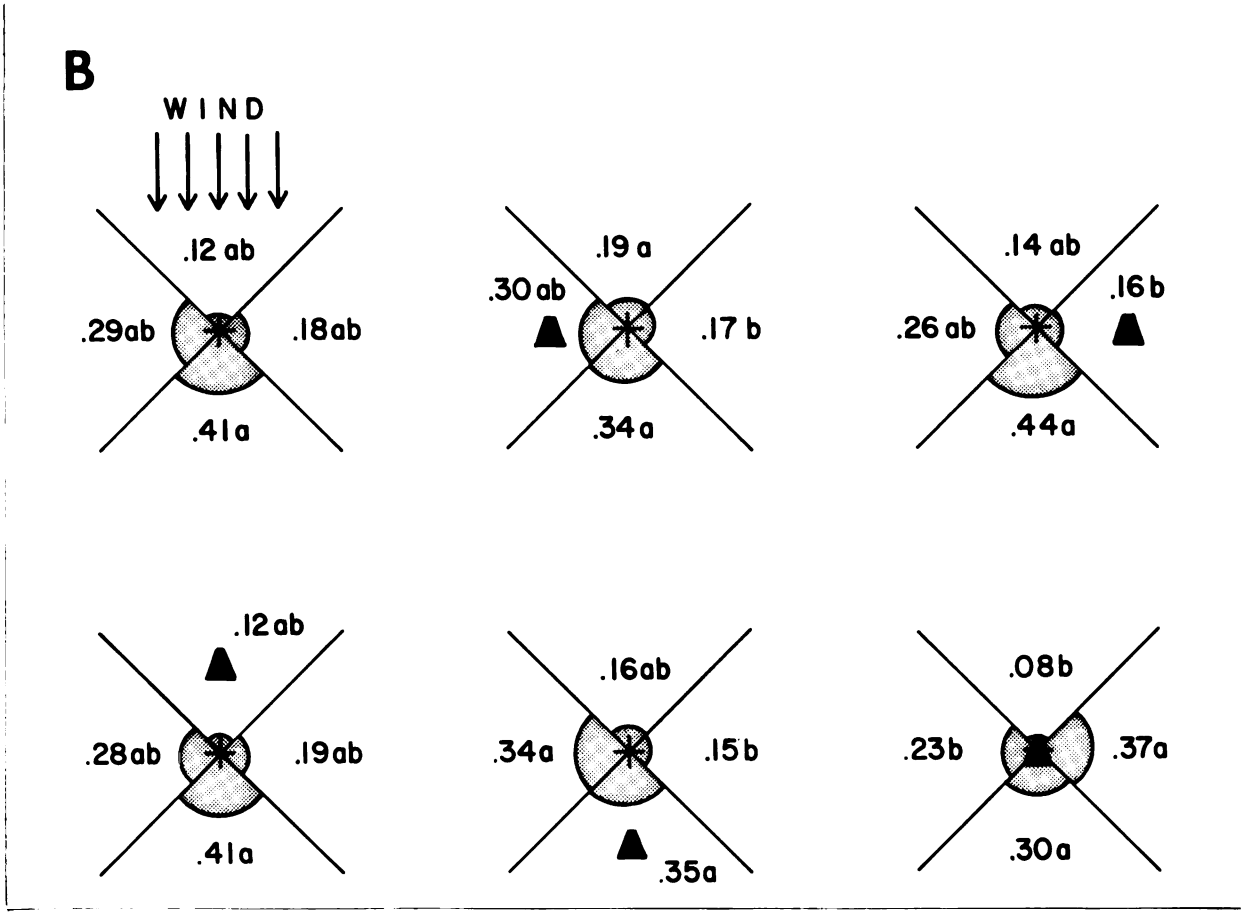

Figure 22 (cont'd.).

visual model in various positions around the source, males oriented primarily to the chemical stimulus and spent most of their time in those quadrants with a greater concentration of pheromone.

The differences in hairpencilling behavior elicited by models in different positions may be explained by whether or not the visual and chemical stimuli are spatially "connected". When they are, there is a greater chance that the display will be released, especially if the chemical and visual cues have a common origin (when the model is directly over the chemical source) (Figure 21).

These results resemble those of Shorey and Gaston (1970) with the cabbage looper moth Trichoplusia ni. (Hbn.). After showing that the major means of orientation in T. ni. was chemical, they demonstrated that the frequency of copulatory attempts increased significantly when a model was placed directly over the pheromone source, and these attempts were directed toward the source more frequently when a model was present. A wide variety of 2- and 3-dimensional models were effective in evoking copulatory attempts. Similarly, Colwell et al. (1978) found that in Pectinophora gossypiella (Saunders) a visual model located downwind within the pheromone plume evoked male copulatory behavior more often than the chemical alone or a model located upwind from, and therefore not connected to, the pheromone stimulus. Thus hairpencil display in G. molesta and copulatory attempts in T. ni. and P. gossypiella are more frequently evoked by, and directed toward, visual models associated with a pheromone stimulus. A major difference is that G. molesta is sexually active in daylight whereas T. ni. and P. gossypiella are nocturnal.

F. Effect of female movement on hairpencil extrusion duration.--The

first hairpencil extrusion is prolonged significantly when the rubber septum model is rotated 180° immediately after initiation of the display (Table 14). When the septum is not rotated, but the pin merely held from beneath, the extrusion duration is significantly shorter. The male apparently watches the female during his display and, if she moves, the hairpencils are held out for a longer period of time. These results explain at least in part the correlation between the number of males successful in attracting females and the duration of the first extrusion.

That visual feedback affects an apparently "fixed" behavior pattern at so late a stage in the sequence, was not expected. Mere rotation of this crude model prolonged only the first extrusion; it is possible that more realistic models and movements toward a hairpencilling male might also prolong the 2nd.

G. Effect of tactile stimulus on hairpencilling males.--All males for which touching with the brush was attempted during hairpencil display immediately made copulatory attempts in the brush's direction (Figure 23B, C), whereas all males in the control group with the brush placed close to but not touching the hairpencils continued their displays and made no immediate attempts at copulation (Figure 23A). That contact had actually occurred and not occurred in the experimental and control groups, respectively, was proven by the transferral of powder to 22 of the 23 males in the former group and lack of powder on the abdominal tips of all 21 males in the latter. The tactile stimulus given to males in other behavioral states does not release copulatory behavior; for instance, males wing fanning while walking touched with the brush either showed no change

Table 14.--Effect of manual movement of the rubber septum model upon mean hairpencil extrusion durations. ***, entry in same column significantly different according to the t-test ($P \leq 0.001$); NS, entry in same column not significantly different according to the t-test ($P > 0.05$).

	Mean Duration of Hairpencil Extrusions (± S.D.)	
	1st Extrusion	2nd Extrusion
Rubber septum moved	1.00 ± 0.33 sec*** N = 19	0.25 ± 0.13 sec NS N = 22
Rubber septum stationary	0.63 ± 0.35 sec N = 25	0.28 ± 0.12 sec N = 26

Figure 23. Effect of tactile stimulus applied to male abdomen during hairpencil display: A) brush dusted with red fluorescent powder and placed near, but not touching male; B) contact made with abdomen (note initial phase of copulatory attempt which includes a hyper-extension of the abdomen; C) beginning of second phase of copulatory attempt, in which male turns quickly around and extends claspers.

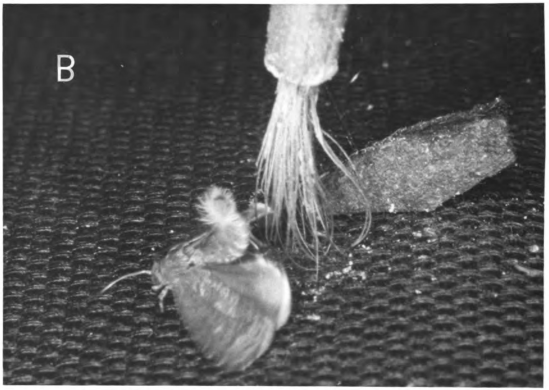

Figure 23 (cont'd.).

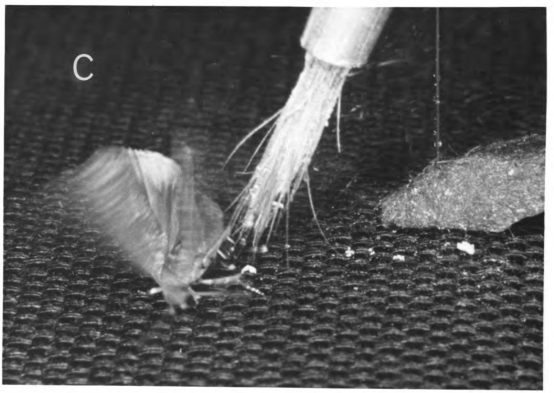

Figure 23 (cont'd.).

(N = 2/19), began walking (N = 5/19), or flew (N = 12/19). These results demonstrate experimentally that the female-delivered "touch" to the male's abdomen released copulatory behavior in males in the hairpencilling phase of courtship.

H. Relative importance of chemical, visual and anemotactic stimuli from hairpencil display.--The most important stimulus in the hairpencil display is chemical, indicated by increased responses in all 3 behavioral categories (locomotion, upwind locomotion, touch the paper) to treatments containing hairpencil extract as opposed to solvent alone (Table 15). The hairpencil pheromone is a short-range attractant to females. Wind added to the solvent blank elicited responses significantly greater than to the solvent blank with ambient wind in all 3 behavioral categories, and wind added to the hairpencil extract evoked higher levels of locomotion compared to all other treatments. Thus wind does appear to have some effect by itself in eliciting locomotion and orientation, but not so much as the hairpencil pheromone.

Blinding the females had no significant effect upon female response in any of the categories (Table 15). so vision contributed little to the orientation response of the female in this experiment. This does not eliminate vision as a cue used by females in orienting to hairpencilling males; however, its role is apparently minor compared to the olfactory and anemo-tactile modalities.

I. Measurement of wind velocities generated during hairpencil display. Males hairpencilling from 1-2 cm directly upwind of the gray paper model (Figure 24) generated wind from their vibrating wings generally at 2 basic velocities. During extrusion the mean maximum velocity was

Table 15.--Responses of blinded and sham-operated calling females to filter paper impregnated with male hairpencil extract or solvent alone and held 1 cm upwind on a horizontal observation platform. Mean ambient wind velocity was 33.3 cm/sec (± 17.29 S.D.) and wind was also added by blowing filtered air at a mean velocity of 76.3 cm/sec (± 12.58 S.D.) over the filter paper and onto the female. Locomotion involved walking in any direction, upwind locomotion was defined as any walking movement which included at least some direct upwind motion, and touching the paper included those females which walked upwind and touched or walked onto the filter paper. Percentages in any column having no letters in common are significantly different according to a χ^2 2 x 2 test of independence with Yate's correction ($P < 0.05$).

			No. ♀'s Tested	Locomotion	Upwind Locomotion	Touch Paper
Solvent blank	No wind added	Not blinded	86	5 (5.8%) d	3 (3.5%) c	2 (2.3%) c
		Blinded	92	4 (4.3%) d	2 (2.2%) c	2 (2.2%) c
	Wind added	Not blinded	86	22 (25.6%) c	17 (19.8%) b	8 (9.3%) bc
		Blinded	92	27 (29.3%) bc	18 (19.6%) b	13 (14.1%) b
Hairpencil extract	No wind added	Not blinded	92	40 (43.5%) b	34 (37.0%) a	29 (31.5%) a
		Blinded	92	37 (40.2%) bc	33 (35.9%) a	27 (29.3%) a
	Wind added	Not blinded	92	59 (64.1%) a	44 (47.8%) a	33 (35.9%) a
		Blinded	92	55 (59.8%) a	44 (47.8%) a	32 (34.8%) a

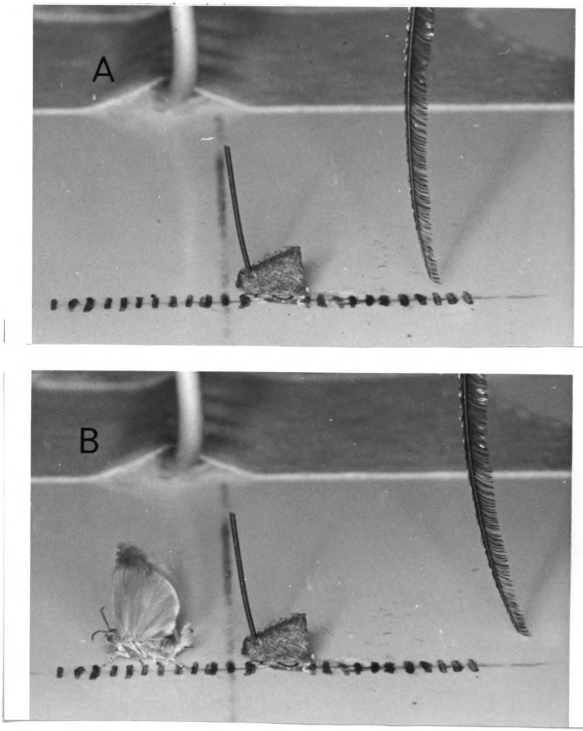

Figure 24. The feather anemometer: A) in resting position with an ambient wind flow from left to right of 0.7 m/sec; B) deflected to the right by wind generated during hairpencil display by the male's vibrating wings.

about 45 cm/sec (~1 mph), and during retraction it increased to 90 cm/sec (~2 mph). Changes in the apparent angle of wing vibration appears to accompany these wind velocity changes. If the female were 1 cm away, the pheromone could be puffed from the hairpencils to the female in approximately 1/45 sec. Also, since it would always originate at the male, the wind would impart a directionality to the signal and allow the female to use pheromone-mediated positive anemotaxis to locate him.

J. Measurement of female preference for males with intact hairpencils.--Females are attracted only to males able to extrude their hairpencils during display. Males with glued abdomens evoked no female movement in 169 displays (Table 16). No aspect of male courtship behavior other than hairpencil extrusion differed from "normal" courtship. The number of attempts at extrusion could be observed during each display by watching changes in the angle of wing vibration which accompany changes in wind velocity. Sham-operated males were attractive to the same females which rejected hairpencil-less males immediately following the latter males' unsuccessful courtship attempts (Table 16). That the dried glue was not the cause of diminished female response to operated males was proven when females responded no differently to sham-operated males bearing dried glue on their thoraxes than to the other sham-operated males (Table 16). Females thus have a clear "preference" for males able to extrude their hairpencils during display and the basis of the female response (or lack of one), as demonstrated earlier, is largely chemical. As observed many times during the course of this study, males having naturally deformed, reduced, or incompletely extrusible hairpencils appeared

Table 16.--Responses of females presented first with males unable to extrude their hairpencils followed immediately by sham-operated males with apparently normal hairpencil extrusions. A second control group was comprised of sham-operated males with glue applied to their thoraxes which did not hinder hairpencil extrusion; these males were exposed to naïve females. Entries in same column having no letters in common are significantly different according to a χ^2 2 x 2 test of independence with Yates' correction ($P \leq 0.0001$).

	No. of Females Tested	No. of Females Attracted to and Touching Male's Abdomen	No. of Females in Which at Least Locomotion was Evoked	Total No. of Displays
Males with glued hairpencils	29	0 (0%) b	0 (0%) b	137
Sham-operated males (same females as used above)	29	30 (69%) a	26 (90%) a	61
Sham-operated males with glue on thorax (naïve females)	25	16 (64%) a	22 (88%) a	129

less able to attract, and less likely to copulate with, calling females.

DISCUSSION

A. Grapholitha molesta courtship behavior.--G. molesta courtship behavior is unique among the Lepidoptera studied thus far in including a display of hairpencil organs by the male whose chemical stimuli attract the female over a range of 1-2 cm after he already has been attracted to the female by her sex pheromone. Males of the lesser wax moth, Achroia grisella (F.), attract females over longer range by a 2-component pheromone emitted from wing glands, and the sound of vibrating wings (Dahm et al. 1971), and thus the A. grisella male pheromone acts as a long-range attractant. In the greater wax moth, Galleria mellonella (L.), the male-emitted wing gland pheromone evokes similar behavior in conspecific females (Roller et al. 1968, Leyrer and Monroe 1973, Finn and Payne 1977). In the Indian meal moth Plodia interpunctella (Hbn.) (Grant and Brady 1975), and Vitula edmandsae (Packard) (Grant 1976a), a combination of male hairpencil pheromone and tactile cues causes the female to turn 180° to face a male approaching from the rear or side. These appear to be the only other occurrences of pheromone-evoked female locomotion to males in the Lepidoptera. Apart from P. interpunctella, V. edmandsae and G. molesta, all other lepidopterous male courtship pheromones elicit in females "acceptance" by quiescence (Tinbergen 1942, Brower et al. 1965, Birch 1970, Pliske and Eisner 1969, Grant and Brady 1975) and/or abdominal extension (Tinbergen 1942, Grant and Brady 1975, Rutowski

1977). In some species, the hairpencils, although actively displayed, have little or no behavioral effect upon females, and when removed have no effect upon male success rate in mating (Gothilf and Shorey 1976, Pliske 1975, Grant and Brady 1975). In the case of the monarch butterfly, Danaus plexipus (L.) (Pliske 1975), whose hairpencil organs are somewhat reduced in size and apparently lack sufficient pheromone to influence female behavior, males rely upon tactile stimuli, aerially grabbing the female to force acquiescence when the pair hit the ground. Male queen butterflies, D. gillipus (Cramer), aerially induce female quiescence by means of hairpencil pheromones alone from their well-developed organs (Brower et al. 1965, Pliske and Eisner 1969); hairpencil removal results in an inability to "seduce" females and a decrease in mating success (Pliske and Eisner 1969).

In G. molesta, correctly performed courtship behavior is necessary for the greatest frequency of mating success. If a male touches a female as he walks past while wing fanning during the earliest stages of courtship, she walks or flies away. Likewise, an unattractive hairpencilling male attempting copulation has a minimal probability of succeeding (Figure 17); again the female either walks or flies away.

G. molesta courtship involves a complex series of behavioral responses and corresponding signals sent in at least 3 sensory modalities over a time span of approximately 1.5 seconds; but basically it is a "chemical dialogue" between a calling female and an answering hairpencilling male. That males use a chemical for rapid communication during the 1 or 2 seconds in which courtship proceeds at first appears unusual. Among the available modalities, chemicals could be considered the most inefficient for rapid communication because their transmission

depends upon diffusion or, at best, ambient wind velocity and direction; the last 2 are subject to high variability. G. molesta males overcome these limitations by supplying their own wind, using wing vibration to propel the chemical; the signal will reach a female 1 cm away in about 1/45 sec or less. In addition, the self-generated wind gives the chemical signal directionality regardless of a male's position with respect to ambient wind direction, and may enable the female to use upwind anemotaxis to locate the male. During hairpencil display, the male is usually upwind or sidewind of the female and always faces away from her, generating wind posteriorly; the series of extrusions and retractions must continue under reduced, or perhaps in the absence of, female pheromone stimulation. Therefore, after its initial release, each display appears to exhibit "momentum" (Lorenz 1950), the male obtaining renewed pheromone stimulation only when he faces the female between displays.

Visual stimulation, however, is present at all times. Although the male courtship sequence is a fairly rigid fixed action pattern, visual feedback in the form of female movement toward the male can alter the form of the hairpencil display. The hairpencilling male apparently watches the effect of his display upon the female; if movement results, the hairpencils are held out longer. If the female is slightly out of alignment when she reaches the male, he will sometimes move his abdomen toward her to make contact with her head, although this may be due to tactile as well as visual stimuli. In addition, the hairpencil display is released by the visual stimulus of a calling female's body combined with a precise blend of female-emitted pheromone chemicals (Baker and Cardé 1979, Cardé et al.1979).

It is not clear why males pulse their chemical and anemo-tactile signals. It may be that males not successful in attracting the female during the first extrusion retract their hairpencils in order to "recharge" them. Hairpencils of successful first extrusion males might have a reduced need to be recharged and so are extruded longer. Possibly a pulsed rather than continuous signal contains more information, but it remains to be demonstrated that such an amplitude-modulated signal would cause a greater female response.

The courtship behaviors of V. edmandsae (Grant 1976a), P. interpunctella and the almond moth, Cadra cautella (Walker), (Grant and Brady 1975) include eversion of male hairpencils from the forewing costal margins plus apparent male-female exchange of visual and tactile stimuli, and are therefore similar in complexity to G. molesta courtship. Unlike those of V. edmandsae and P. interpunctella, C. cautella hairpencils have little or no effect upon mating success. Males do have, associated with the claspers, an auxillary set of brush organs which are everted during an "unusually prolonged" copulatory thrust (Grant and Brady 1975) and may influence female acceptance. These abdominal organs would appear to be similar to those of G. molesta and the other Grapholitha species investigated by Heinrich (1926).

B. The possible role of sexual selection in the evolution of male hairpencils.--An outstanding question in the evolution of courtship behavior in Lepidoptera is a combination of behaviors not easily explained by natural selection: a high degree of female coyness accompanied by correspondingly elaborate male hairpencil behavior. For reproductive isolation, a species-specific chemical signal from the male would appear to be taken care of nicely by a small quantity of a specific chemical

blend, as it has for female-emitted pheromones over a much longer distance. Yet the trend in the Noctuidae is for the structures of chemicals released in copious amounts to be most similar among closely related species and to diverge among more distant relatives (Birch 1974). Similarly, Manning (1966) found a direct correlation between phylogeny and courtship behavior in Drosophila and concluded that the relationship should be an inverse one for species-specific behavior. The chemical specificity of the danaines also does not appear very precise even though there are large quantities of compounds present; however, this could be due to an emphasis upon visual cues typical of butterfly courtship.

Besides reproductive isolation (Grant et al. 1975, Grant 1976b), other evolutionary hypotheses have included hairpencils' possible importance as release surfaces for primer pheromones that stimulate oogenesis, deterrents to other males, and long-range sex attractants presumably before this function was taken over by female pheromone glands (Birch 1974). Each of these ideas has some merit, and yet as Birch (1974) states, "The question remains as to how the male brush-organs, hair-pencils and coremata originally evolved." It may be that to answer this question, one need look no further than the primary role they play in eliciting female acceptance, because in G. molesta courtship and that of other lepidopterous species, it is the female who ultimately accepts or rejects a displaying conspecific male through her behavioral response. We suggest that G. molesta hairpencils and behavior as well as those of some other male lepidoptera may have arisen primarily as a result of "female choice" sexual selection (Fisher 1958).

In his theory of sexual selection, Darwin (1898, p. 335) wrote of

insects: "When we see many males pursuing the same female, we can hardly believe that the pairing is left to blind chance--that the female exerts no choice, and is not influenced by the gorgeous colours or other ornaments with which the male is decorated." Richards (1927, p. 348) hypothesized that female "coyness" or hesitation to mate was the reason insect displays evolved, yet did not appear to favor a sexual selective mechanism to explain how selection for these displays would proceed. Indeed, he concludes, "...Although the displays, considered as a whole, have a survival value, yet it is difficult to see any use in the specific differences in secondary sexual structures of behaviour. It is not at all evident how selection could establish, in a population, a character whose only effect would be to isolate individuals possessing it from those who did not."

Fisher (1958) broadened the distinction made by Darwin (1898) between the type of sexual selection based on male fighting or other direct interactions in which the "winning" male mates with the receptive female, and the type in which a male display directed toward a female causes her to "choose" him over the other males for copulation. In the female choice type, the preferred trait(s) may be important solely for attracting females, thereby increasing mating success, apparently as with the plumage of male birds of paradise (Mayr 1972). In Fisher's model, a female preference character for a male character is initially favored by natural selection and at a low frequency in the population. Female preference behavior rapidly becomes genetically associated with the preferred male character because discriminating females are more likely to choose only males having the preferred character. Such females will pass on more of their genes because their sons will more

likely be accepted for mating by discriminating females in the next generation. Before long both the male character and female preference behavior will develop beyond the level they might reach through natural selection. O'Donald (1962, 1967) formulated mathematical models substantiating Fisher's hypothesis; in his models the "runaway" tendency of sexual selection eventually is opposed and slowed by natural selection.

There is evidence in Drosophila of rapid sexual selection for differences in male courtship behaviors due to competitive mating under laboratory conditions, and these disappear when pairs are coupled in separate bottles, removing the competitive aspects of mating (Ewing 1961). Ehrman (1972) demonstrated frequency-dependent sexual selection by females who "choose" rare males for mating under laboratory conditions. Sexual selection was suggested by Manning (1966) to be linked to the evolution of male courtship behavior in Drosophila, possibly through selection for male behaviors correlated with vigor or fitness; female coyness would allow time to assess the male's stimuli before accepting or rejecting him. Grant (1976b) viewed reproductive isolation as a possible reason for the evolution of female coyness and corresponding male courtship behavior in the moth P. interpunctella due to the species-isolating effects of its courtship behavior and that of another closely related moth, C. cautella (Grant et al. 1975).

Two fundamental conditions must be met for sexual selection to occur. First there must be a sexual preference in at least 1 sex, and second, there must be "bionomic conditions in which such a preference shall confer a reproductive advantage" (Fisher 1958). The latter

conditions, including protandry, male-female sex ratio favoring males, and a broader male than female daily sexual activity period, favor polygyny, and combined with sexual preference, result in some males mating more frequently with more females at the reproductive expense of other males. For G. molesta (this paper), D. gillipus (Pliske and Eisner 1975), a noctuid moth (Birch 1970), P. interpunctella (Grant and Brady 1975) and V. edmandsae (Grant 1976a), sexual preference was demonstrated when females mated preferentially with males possessing intact hairpencils. The major hairpencil stimulus was in all cases apparently chemical. Bionomical situations favorable for sexual selection commonly occur in the Lepidoptera. For example, G. molesta males confined in cages with an excess of virgin females are capable of mating an average of 1.2 times per day during their first week as an adult. Spermatophore counts of wild females indicated that the majority (79% over the 3-year study) had mated only once and most of the rest only twice; of caged females supplied with an equal number of males, 60% mated once, 20% twice and 10% 3 times over a 21-day period (Dustan 1964). Thus males can mate at least once per evening whereas the majority of females will mate only once during their lifetime. In addition, our field observations indicate that a calling female or a synthetic pheromone dispenser may be visited by multitudes of males either simultaneously or within a few seconds of each other.

The evolution of hairpencil behavior in the Oriental fruit moth might have proceeded in the following sequence. First a male character arises for which the corresponding female preference is favored by natural selection. The female preference behavior might entail hesitating slightly longer than normal during courtship until an assessment

of the male character can be made. The initial frequency of female preference alleles in the population could be extremely low. The preferred male trait might indicate: 1) male "vigor" or quality (through behavioral response or quantity of chemical odor on body); 2) gender or species (e.g., male-specific odor or species-specific behavior or odor). Males possessing an odor developed as a deterrent to predators also could be preferred by females. If the species was polygynous, promoting increased mating frequency for preferred males, then linkage of female preference with the male trait could occur; rapid directional selection would predominate until a stable equilibrium was reached. The rate of selection would depend upon the severity of male exclusion, the initial allelic frequencies, whether the trait was dominant or recessive (recessives are favored), and the resistance offered by natural selection (O'Donald 1967). Before the process would slow down, however, the directional selection could result in an exaggerated scent dissemination mechanism or courtship behavior. In addition, the reciprocal directional selection for female coyness behavior could result in a higher degree of hesitancy to mate.

The present G. molesta hairpencil display may represent a type of clasper extension "ritualized" through the process of sexual selection. It may have originated from a genital odor emitted coincidentally during a prolonged copulatory attempt involving clasper extension. Through sexual selection, scent disseminating structures associated with the claspers could have become highly developed and the corresponding clasper extension prolonged or even repeated several times in succession. The concurrent directional selection for female preference behavior may have resulted in the coyness currently exhibited by G.

molesta females: they walk or fly from males who either touch them before hairpencilling or attempt copulation after a non-attractive display. Sometimes a male may display several times before the female moves and touches his abdomen.

For other polygynous Lepidoptera, female preference could conceivably develop for any of a wide variety of male traits. The diverse group of hairpencil structures observed today may be a result of the possible cavalier way a male trait could be favored and then exaggerated by rapid sexual selection. Certain groups having been exposed to a higher incidence of female preference initiators such as incomplete reproductive isolation by female pheromone may be those most likely to have evolved functional hairpencils and female "coyness." Sexual selection involving direct male-male fights and competition near a receptive female (Darwin 1898, Fisher 1958), considered natural selection by many authors (Mayr 1972), may be another mechanism favoring the evolution of hairpencils in other species. In Hawaiian Drosophila, Spieth (1968) found evidence of inter-male aggression near their "leks" and sexual dimorphism appeared to be greatest in "lekking" species. However, observation of G. molesta indicate male displays actually attract, rather than deter, other males, the converse of what might be expected if competition and/or fighting were occuring. Such attraction and homocourtship also occurs in V. edmandsae (Grant 1967a).

Cross-attraction with Grapholitha prunivora (Walsh), the lesser apple-worm, does sometimes occur at a level of less than 5% and male G. prunivora courtship behavior also includes a hairpencil display. However, other steps in its courtship sequence differ significantly from that of G. molesta (Baker and Cardé unpublished). If sexual

selection caused the development of hairpencil behavior in these species, the initially preferred male trait possibly could have been a species-specific male odor or behavior, although perhaps not between these 2 species; they have been sympatric for less than a century. Further studies of male courtship and female sex pheromones may define the relative importance of sexual and natural selection in the evolution of chemical communication in the genus Grapholitha.

LIST OF REFERENCES

LIST OF REFERENCES

Alberts, S. 1978. The laboratory testing of the pheromone communication system of a greenhouse pest, Bradysia impatiens (Johannsen). Master's Thesis, Michigan State University.

Armstrong, T. 1929. Notes on the life history of the Oriental peach moth at Vineland Station. Rep. Entomol. Soc. Ont. 59: 65-72.

Baerends, G.P., R. Brower, and H.T. Waterbolk. 1955. Ethological studies on Lebistes reticulatus (Peters). Behavior 8: 249-334.

Baker, T.C. and R.T. Cardé. 1979. Analysis of pheromone-mediated behaviors in male Grapholitha molesta, the Oriental fruit moth (Lepidoptera: Tortricidae). J. Chem. Ecol. Submitted.

Baker, T.C., and R.T. Cardé. Courtship behavior of the Oriental fruit moth (Grapholitha molesta): experimental analysis and consideration of the role of sexual selection in the evolution of courtship pheromones in the Lepidoptera. Ann. Entomol. Soc. Am. (In press).

Baker, T.C., R.T. Cardé, and W.L. Roelofs. 1976. Behavioral responses of male Argyrotaenia velutinana (Lepidoptera: Tortricidae) to components of its sex pheromone. J. Chem. Ecol. 2: 333-352.

Baker, T.C., J.R. Miller, and R.T. Cardé. 1979. Emission rates of Grapholitha molesta (Busck) sex pheromone components from calling collection devices. J. Chem. Ecol. In preparation.

Beroza, M., G.M. Muschik, and C.R. Gentry. 1973. Isomer content and other factors influencing captures of Oriental fruit moth by synthetic pheromone traps. J. Econ. Entomol. 66: 1307-1311.

Beroza, M., G.M. Muschik, and C.R. Gentry. 1973. Small proportion of opposite geometric isomer increases potency of synthetic pheromone of Oriental fruit moth. Nature 244: 149-150.

Birch, M.C. 1970. Pre-courtship uses of abdominal brushes by the nocturnal moth, Phlogophora meticulosa (L.) (Lepidoptera: Noctuidae). Anim. Behav. 18: 310-316.

Birch, M.C. 1974. Aphrodisiac pheromones in insects. In M.C. Birch (Ed.), "Pheromones." North Holland Publ. Co., pp. 115-134.

Brower, L.P., J. Van Z. Brower, and F.P. Cranston. 1965. Courtship behavior of the queen butterfly, Danaus gillippus berenice (Cramer). Zoologica 50: 1-39.

Cardé, A.M., T.C. Baker, and R.T. Cardé. 1979. Identification of a four component sex pheromone of the Oriental fruit moth, Grapholitha molesta (Lepidoptera: Tortricidae). J. Chem. Ecol. (In press).

Cardé, R.T., and T.E. Hagaman. 1979. Behavioral responses of the gypsy moth in a wind tunnel to air-borne enantimers of disparlure. Environ. Entomol. (In press).

Cardé, R.T., A. Comeau, T.C. Baker, and W.L. Roelofs. 1975a. Moth mating periodicity: temperature regulates the circadian gate. Experientia 31: 46-48.

Cardé, R.T., T.C. Baker, and W.L. Roelofs. 1975b. Ethological function of components of a sex attractant system for Oriental fruit moth males, Grapholitha molesta (Lepidoptera: Tortricidae). J. Chem. Ecol. 1: 475-491.

Cardé, R.T., T.C. Baker, and W.L. Roelofs. 1975c. Behavioral role of individual components of a multichemical sex attractant system in the Oriental fruit moth. Nature 253: 348-349.

Castrovillo, P.J., and R.T. Cardé. 1979. Environmental regulation of female calling and male pheromone response periodicities in the codling moth (Laspeyresia pomonella). J. Insect Physiol. (Submitted).

Colwell, A.E., H.H. Shorey, L.K. Gaston, and S.E. van VorhisKey. 1978. Short-range precopulatory behavior of males of Pectinophora gossypiella (Lepidoptera: Gelechiidae). Behav. Biol. 22: 323-335.

Corbet, P.S. 1966. The role of rhythms in insect behavior. In Haskell, P.T. (Ed.), "Insect Behavior." Symp. R. Ent. Soc. Lond. 3: 13-28.

Dahm, K.H., D. Meyer, W.E. Finn, V. Reinhold, and H. Röller. 1971. The olfactory and auditory mediated sex attraction in Achroia grisella (Fabr.). Naturwiss. 58: 265-266.

Darwin, C. 1898. The Descent of Man and Selection in Relation to Sex. Revised second edition, D. Appleton and Co., New York. 688pp. First published in 1871.

Dustan, G.G. 1961. The Oriental fruit moth Grapholitha molesta (Busck) (Lepidoptera: Olethreutidae) in Ontario. Proc. Ent. Soc. Ont. 91: 215-227.

Dustan, G.G. 1964. Mating behavior of the Oriental fruit moth Grapholitha molesta (Busck) (Lepidoptera: Olethreutidae). Can.Entomol. 96: 1087-1093.

Ehrman, L. 1972. Rare male advantage and sexual selection in Drosophila immigrans. Behav. Gen. 2: 79-84.

Ewing, A.W. 1961. Body size and courtship behavior in Drosophila melanogaster. Anim. Behav. 11: 93-99.

Finn, W.E. and T.L. Payne. 1977. Attraction of greater wax moth females to male-produced pheromones. Southw. Entomol. 2: 62-65.

Fisher, R.A. 1958. The genetical theory of natural selection. Second revised ed., Dover, New York. First published in 1930.

Gentry, C.R., M. Beroza, J.L. Blythe, and B.A. Bierl. 1974. Efficacy trials with the pheromone of the Oriental fruit moth and data on the lesser appleworm. J. Econ. Entomol. 67: 607-609.

Gentry, C.R., M. Beroza, J.L. Blythe, and B.A. Bierl. 1975. Captures of the Oriental fruit moth, the pecan bud moth, and the lesser appleworm in Georgia field trials with isomeric blends of 8-dodecenyl acetate and airpermeation trials with the Oriental fruit moth pheromone. Environ. Entomol. 4: 822-824.

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

Gothilf, S. and H.H. Shorey. 1976. Sex pheromones of Lepidoptera: examination of the role of scent brushes in courtship behavior of Trichoplusia ni. Environ. Entomol. 5: 115-119.

Grant, G.G. 1976a. Courtship behavior of a phycitid moth, Vitula edmandsae. Ann. Entomol. Soc. Am. 69: 445-449.

Grant, G.G. 1976b. Female coyness and receptivity during courtship in Plodia interpunctella (Lepidoptera: Pyralidae). Can. Entomol. 108: 975-980.

Grant, G.G. and U.E. Brady. 1975. Courtship behavior of phycitid moths. I. Comparison of Plodia interpunctella and Cadra cautella and role of male scent glands. Can. J. Zool. 53: 813-826.

Grant, G.G., E.B. Smithwick, and U.E. Brady. 1975. Courtship behavior of phycitid moths. II. Behavioral and pheromonal isolation of Plodia interpunctella and Cadra cautella in the laboratory. Can. J. Zool. 53: 827-832.

Halliday, T.R. 1975. An observational and experimental study of sexual behavior in the smooth newt, Triturus vulgaris (Amphibia: Salamandridae). Anim. Behav. 23: 291-322.

Heinrich, C. 1926. Revision of the North American moths of the subfamilies Laspeyresiinae and Olethreutinae. Smiths. Inst. Bull. 132: 1-216.

Kennedy, J.S. 1974. Changes of responsiveness in the patterning of behavioral sequences. In L. Barton-Browne (Ed.), "Insect Behavior." pp. 1-6.

Kennedy, J.S. 1978. The concepts of olfactory 'arrestment' and 'attraction.' Physiol. Entomol. 3: 91-98.

Kennedy, J.S. and D. Marsh. 1974. Pheromone-regulated anemotaxis in flying moths. Science 184: 999-1001.

Leyrer, R.L. and R.E. Munroe. 1973. Isolation and identification of the scent of the moth, Galleria mellonella and a re-evaluation of its sex pheromone. J. Insect Physiol. 19: 2267-2271.

Lorenz, K. 1950. The comparative method in studying innate behavior patterns. Symp. Soc. Exp. Biol. 4: 221-269.

Manning, A. 1966. Sexual Behavior. In P.T. Haskell (Ed.), "Insect Behaviour." Symp. R. Soc. Lond. No. 3. p. 59-68.

Mayr, E. 1972. Sexual selection and natural selection. In Campbell (Ed.), "Sexual Selection and the Descent of Man 1871-1971." Aldine, Chicago. pp. 87-104.

Miller, J.R. and W.L. Roelofs. 1978a. Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. J. Chem. Ecol. 4: 187-198.

Miller, J.R., and W.L. Roelofs. 1978b. Gypsy moth responses to pheromone enantiomers as evaluated in a sustained-flight tunnel. Environ. Entomol. 7: 42-44.

Minks, A.K. 1977. Trapping with behavior-modifying chemicals: feasibility and limitations. In Shorey, H.H. and John J. McKelvey, Jr. (Eds.), "Chemical Control of Insect Behavior, Theory and Application." John Wiley and Sons. pp. 385-394.

O'Donald, P. 1962. The theory of sexual selection. Heredity 17: 541-552.

O'Donald, P. 1967. A general model of sexual and natural selection. Heredity 22: 499-518.

Pliske, T.E. 1975. Courtship behavior of the monarch butterfly. Ann. Entomol. Soc. Am. 68: 143-151.

Pliske, T.E., and T. Eisner. 1969. Sex pheromones of the queen butterfly: biology. Science 164: 1170-1172.

Reichart, G. and J. Bodor. 1972. Biology of the Oriental fruit moth (Grapholitha molesta Busck) in Hungary. Acta Phytopathol. Acad. Sci. Hung. 7: 279-295.

Richards, O.W. 1927. Sexual selection and allied problems in the insects. Biol. Rev. 2: 298-360.

Roelofs, W.L. and R.T. Cardé. 1974a. Sex pheromones in the reproductive isolation of lepidopterous species. In M.C. Birch (Ed.), "Pheromones." pp. 96-114.

Roelofs, W.L. and R.T. Cardé. 1974b. Oriental fruit moth and lesser appleworm attractant mixtures refined. Environ. Entomol. 3: 586-588.

Roelofs, W.L. and R.T. Cardé. 1977. Responses of Lepidoptera to synthetic sex pheromone chemicals and their analogues. Ann. Rev. Entomol. 22: 377-405.

Roelofs, W.L., A. Comeau, and R. Selle. 1969. Sex pheromone of the Oriental fruit moth. Nature 244: 723.

Roelofs, W.L., R.T. Cardé, and J. Tette. 1973. Oriental fruit moth attractants and 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. Ent. Exp. et Appl. 17: 294-302.

Rothschild, G.H.L., and A.K. Minks. 1974. Time of activity of male Oriental fruit moths at pheromone sources in the field. Environ. Entomol. 3: 1003-1007.

Röller, H., K. Biemann, J. Bjerke, D. Norgard, and W. McShan. 1968. Sex pheromones of the pyralid moths. I. Isolation and identification of the sex attractant of Galleria mellonella L. (Greater wax moth). Acta Entomol. Bohemoslov. 65: 208-211.

Rutowski, R.L. 1977. Chemical communication in the courtship of the small sulphur butterfly. Eurema lisa (Lepidoptera: Pieridae). J. Comp. Physiol. 115: 75-85.

Sanders, C.J. and Lucuik. 1972. Factors affecting calling by eastern spruce budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae). Can. Entomol. 103: 498-502.

Schwinck, I. 1958. A study of olfactory stimuli in the orientation of moths. Proc. Tenth Int. Cong. Entomol. 2: 577-582.

Shorey, H.H. and L.K. Gaston. 1970. Sex pheromones of noctuid moths. XX. Short-range visual orientation by pheromone-stimulated males of Trichoplusia ni. Ann. Entomol. Soc. Am. 63: 829-832.

Slater, J.B. and J.C. Ollason. 1973. The temporal patterning of behaviour in isolated male zebra finches: transition analysis. Behaviour 2: 248-269.

Solomon, M.E. 1951. The control of humidity with potassium hydroxide, sulfuric acid or other solutions. Bull. Entomol. Res. 42: 543-59.

Sower, L.L., H.H. Shorey, and L.K. Gaston. 1971. Sex pheromones of noctuid moths. XXV. Effects of temperature and photoperiod on circadian rhythm of sex pheromone release by females of Trichoplusia ni. Ann. Entomol. Soc. Am. 58: 597-600.

Sower, L.L., K.W. Vick, and J.S. Long. 1973. Isolation and preliminary biological studies of the female-produced sex pheromone of Sitotroga cerealella (Lepidoptera: Gelechiidae). Ann. Entomol. Soc. Amer. 66: 184-187.

Spieth, H.T. 1968. Evolutionary implications of sexual behavior in Drosophila. Evolutionary Biology 2: 157-193.

Tinbergen, N., B.J.D. Meeuse, L.K. Boerema, and W.W. Varossieau. 1942. Die Bals des Samtfalters, Eumenis (=Satyrus) semele (L.). Z. Tierpsychol. 5: 182-226.

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