Analysis of Pheromone-Mediated Behaviors in Male *Grapholitha molesta*, the Oriental Fruit Moth (Lepidoptera : Tortricidae)[1]

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Reprinted from the
ENVIRONMENTAL ENTOMOLOGY

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ABSTRACT

Environ Entomol 8: 956–968 (1979)

In *Grapholitha molesta* (Busck), the Oriental fruit moth, the behavioral effects of 3 female sex pheromone components, (*Z*)-8-dodecenyl acetate (Z8-12:Ac), (*E*)-8-dodecenyl acetate (E8-12:Ac), and (*Z*)-8-dodecenyl alcohol (Z8-12:OH), are described most precisely only when each component is considered in combination with the other two, rather than individually or in binary blends. In a blend ratio approximating that emitted by *G. molesta* females, these 3 components elicited increases in both early (long-range) and late (close-range) behaviors in the male response sequence. Hence, these components act as a unit. An added fourth component, dodecanol (12:OH), had significant, but subtle, effects upon the hairpencil display when Z8-12:OH was at suboptimal levels, in contrast to the stronger behavioral effects previously ascribed to 12:OH. Z8-12:OH appears important to the reproductive isolation between *G. molesta* and *G. prunivora* (Walsh), because this component strongly reduced trap capture of *G. prunivora* males when present at a dispenser dosage of only 1% of the acetates, and virtually eliminated capture at 10%. There was a strong correlation between pheromone plume behaviors of pre-flight wing fanning while walking and upwind flight, suggesting that these behaviors may be closely associated, functioning to locate the pheromone source by ground or air, respectively.

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 female glands by Roelofs et al. (1969). In field trapping experiments a small amount of (*E*)-8-dodecenyl acetate (E8-12:Ac), the opposite geometric isomer, was required in addition to the (*Z*) isomer for capture of *G. molesta* males; from 5 to 9% (*E*) isomer yielded optimal male captures (Beroza et al. 1973a,b, Roelofs and Cardé 1974, 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 50–100% capture increase (Roelofs et al. 1973, Beroza et al. 1973a, Roelofs and Cardé 1974, Gentry et al. 1974, 1975, Rothschild and Minks 1977). (*Z*)-8-dodecenyl alcohol (Z8-12:OH) produced similar increases in capture at very low ratios to the acetates (Cardé et al. 1975a, Rothschild and Minks 1977). All 4 compounds are emitted by *G. molesta* females (Cardé et al. 1979) and all except 12:OH are suggested by GLC and MS to be in the female abdominal tips (Biwer et al. 1979), although the position and configuration of the double bonds were not verified beyond GLC retention times.

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. 1975a,b). 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, 2 components whose roles were not satisfactorily known, in 1976 we initiated new behavioral observations and trapping studies in western Michigan. Our findings differed from previous reports. We 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 report we 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

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 (Cardé et al. 1979). Other volatile 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 volatile 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 volatile 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 volatile impurities.

Mixtures of these chemicals were formulated as de-

[1] Published as Journal Article No. 8888 of the Michigan State University Agric. Exp. Stn. Received for publication Feb. 8, 1979.

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scribed below for each experiment in either hexane or ether solutions and pipetted in 10 μl solution onto rubber septum dispensers (Arthur H. Thomas Co.). For most of the experiments component ratios in solution 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.

Release Rates from Rubber Septa

Baker et al. (unpubl.) measured the release rates of various dosages of pure Z8-12:Ac and pure Z8-12:OH on rubber septa at 25°C. 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., in the emitted blend 1% Z8-12:OH on the septum is about 3% of the acetates. A similar relationship likely exists for 12:OH as a percent of the acetates. Rates of Z8-12:Ac release for 10-, 100-, and 1000-μg septum dosages were 1.2 (± 3.75 SD), 11.8 (± 3.7), and 220 (± 69) ng/hour. Rates of Z8-12:OH release for 10-, 100-, and 1000-μg septum dosages were 2.8 (± 1.4), 33.0 (± 10.4), and 665 (± 201) ng/hour. Mean rate of Z8-12:Ac released by a calling female was 3.5 (± 1.6) ng/hour.

Trapping Experiments

Usually behavioral activity of different mixtures was 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 6×13-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 control treatment of a septum impregnated with 10 μl of solvent. 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 $\sqrt{(X + 0.5)}$ 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 $\alpha = 0.05$.

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 one m above the ground and placed in the middle of aisles between rows of trees. A single observer watched for downwind males judged to be starting upwind flight toward the arena. Individuals in an aisle sometimes could be observed in upwind flight as far away as 10 m. To be scored, males flying near a tree had to break away clearly from the foliage and start upwind. 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); (3) landing (including touching arena); (4) wing fanning while walking on arena (for one or more sec); (5) hairpencil display 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 usually could 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. The numbers of males observed were standardized per observation-hour.

Because 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 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 held 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 were used for analysis.

Other behavioral observations were made in a 2.8×1.4×0.8-m laboratory wind tunnel (Cardé and Ha-

gaman 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 h 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, 15×15-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 release platform. A 2- to 5-day-old male was taken from its holding cage in the wind tunnel room and placed in an 11×7 cm diam copper screen cylinder open at both ends. Ten seconds were allowed for the male to acclimatize. If after 10 seconds 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 seconds while in the tunnel. If after 30 seconds a male in the cylinder was not sitting, observations continued until he either remained sitting for 5 seconds or flew from the cylinder. In the latter case, we continued to observe his behavior until normal termination, described below.

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 one second, exhibiting regular lateral oscillations and without significant downwind or upwind progress or touching the wind tunnel surface (possibly the reversing 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 number of displays per male. Wing fanning while stationary, another possible category, may be distinctly different from wing fanning while walking. This behavior was not scored because it occurred infrequently among some males that left the plume and touched the tunnel surface. Observation was terminated when a male touched the tunnel surface.

Some behaviors were timed, including first response latency, 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).

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. Flight speeds were calculated using the time taken to traverse the fixed 80-cm interval on the tunnel floor. 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 10 μg Z8-12:Ac + 0.7 μg E8-12:Ac + 0.1 μg Z8-12:OH + 30 μg 12:OH.

Results

Z8-12:Ac and E8-12:Ac Mixtures

More *G. molesta* males were captured at Z8-12:Ac containing 3–7% (*E*) than at other (*Z*) - (*E*) mixtures (Fig. 1). For a related species, *G. prunivora* (Walsh), 5.1% (*E*) was also the optimum 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 1974).

Based upon observations of males approaching the arenas, 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 and land on the arenas (Table 1). Proportions of (*E*) exceeding 5.1% lowered the proportion responding at each behavioral state that we monitored except for wing fanning while walking, for which the trend implies reduced response to 12.1% (*E*) (Table 1). No hairpencil displays occurred. No males initiated upwind flight to pure Z8-12:Ac, although a few males seemed to be in stationary flight 10 m or more from the arena.

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 apparently the appropriate low (*E*) percentage between 0 and 3.2% was not tested to note such an effect. No males were observed to exhibit upwind flight to 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 1).

Possible responses in the sequence occurring prior to upwind flight (such as flight initiation) could not feasibly be observed in the field, and it is not clear whether frequencies of these early 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 (Fig. 3). 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. The (*Z*)-(*E*) mixture also elicited a greater percentage of stationary flight and upwind flight compared to the pure (*Z*) group, paralleling field observations. However, we observed no landing or wing fanning, perhaps because the wind tunnel dosage was 10 times lower than that employed in the

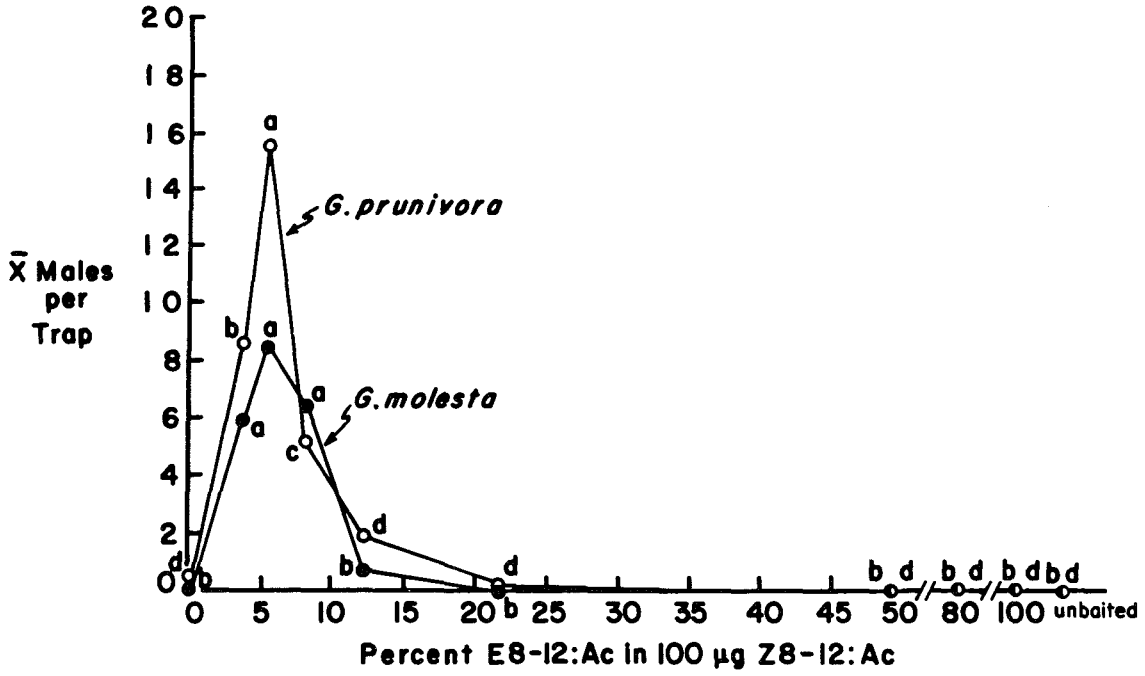

FIG. 1.—Effect of different binary mixtures of Z8- and E8-12:Ac upon capture of male *G. molesta* and *G. prunivora*. Amount of Z8-12:Ac was held at 100µg. Percentages of E8-12:Ac were, as checked by GLC: 0% (no detectable) (*E*); 3.2%; 5.1%; 7.1%; 11.1%; 22.2%; 48.4%; 77.5%; and 100 µg pure (*E*). Experiment 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 ($P < 0.05$).

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.

Addition of Z8-12:OH or 12:OH

The addition of 1% of Z8-12:OH to the optimal acetate mixture elevated male capture by about 10-fold compared to the acetate mixture alone (Fig. 2). Moreover, the Z8-12:OH-containing mixture appeared to accomplish this increase by competition. When tested alongside treatments containing Z8-12:OH, the acetate mixture alone was statistically indistinguishable from a solvent blank-baited trap (Fig. 2), whereas in earlier tests this treatment caught significant numbers of males (Fig. 1).

Table 1.—Behavior of *G. molesta* males on or near 50-cm radius observation arenas in response to mixtures of 100 µg Z8-12:Ac plus various percentages of E8-12:Ac on rubber septa dispensers. To avoid compounding behavioral effects occurring in a sequence, percentages were calculated using as N, the number of individuals exhibiting the immediately preceding behavior.

Treatment (on rubber septum)	No. ♂/h observed beginning upwind flight[a] (no.)	% ♂ flying to 10 cm of arena's edge[b] (no.)	Of those flying to edge, % ♂ landing[b] (no.)	Of those landing, % ♂ wing fanning while walking[b] (no.)	Of those fanning while walking, % ♂ displaying hairpencils (no.)	Of those flying to edge x̄ closest approach to dispenser[c,d] (cm) (± SD)	Of all ♂ observed, x̄ orientation time (sec) (± SD)
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 a (± 9.83)	8.9 b (± 5.50)
22.2% (*E*)	41 c (29)	3.4 c (1)	—	—	—	50.0	5.3 c (± 1.37)

[a] Numbers having no letters in common are significantly different according to χ^2 ($P<0.05$) using a null hypothesis of equal numbers of observations per hour.
[b] Percentages in same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence with Yates' correction ($P<0.05$).
[c] Means in same column having no letters in common are significantly different according to a LSD test ($P<0.05$). Data were first submitted to a one-way analysis of variance for unequal replication.
[d] 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 arena but not landing were scored as 50 cm approaches.

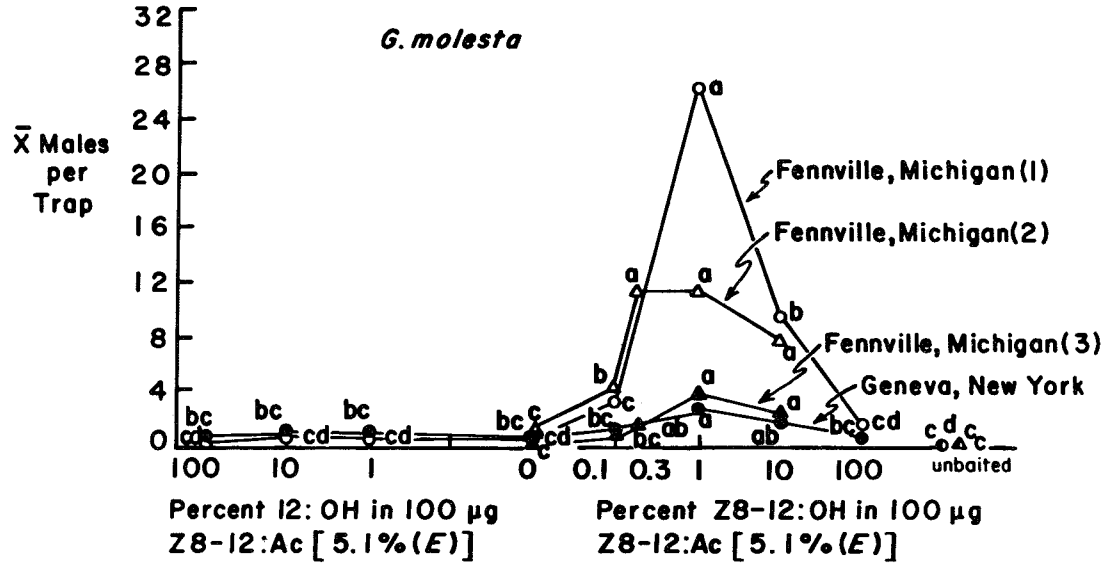

FIG. 2.—Effect of adding various percentages of either Z8-12:OH or 12:OH to 100 µg Z8-12:Ac {5.1% (*E*)} upon capture of *G. molesta* males. Fennville (1) experiment was conducted May 13–16, 1977; Fennville (2), September 4–8, 1976; Fennville (3), September 20 to October 5, 1976; Geneva experiment, May 19–22, 1977. Within each experiment means having no letters in common are significantly different according to Student-Newman-Keuls' multiple range test ($P<0.05$).

The capture patterns between Geneva, New York and Fennville (Fig. 2) were similar, confirming that of the percentages tested, 1% Z8-12:OH in the acetates produced the highest capture, eliminating population differences as an explanation of the disparity between our results and previous reports. Only a limited number of Z8-12:OH dosages was tested and it is possible that some intermediate percentage (e.g., 3%) of Z8-12:OH might produce a higher capture. In both locations, 12:OH at several dosages added to the acetates did no better than either the acetates alone or a solvent-blank septum.

Increased trap capture to Z8-12:OH-containing treatments was caused by an increase in both early and late behaviors. In field observations, 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 2). 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 this alcohol. When Z8-12:OH was present, more

Table 2.—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 100-µg 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. ♂/h observed beginning upwind flight[a] (no.)	% ♂ flying to 10 cm of arena's edge[b] (no.)	Of those flying to edge, % ♂ landing[b] (no.)	Of those landing, % ♂ wing fanning while walking[b] (no.)	Of those fanning while walking, % ♂ displaying hairpencils[b] (no.)	Of those flying to edge, x closest approach to dispenser[c,d] (cm) (± SD)	Of all ♂ observed, x orientation time (sec) (± SD)
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 (92)	93.5 a (86)	86.0 a (74)	63.5 a (47)	23.4a (11)	25.6 c (± 19.28)	27.6 a (± 21.5)
300µg 12:OH	76 a (94)	72.3 b (68)	27.9 c (19)	10.5 c (2)	0 a	46.6a (± 6.76)	10.3 d (± 8.44)

[a] Numbers having no letters in common are significantly different according to χ^2 ($P<0.05$) using a null hypothesis of equal numbers of observations per hour.
[b] Percentages in same column having no letters in common are significantly different according to a χ^2 2×2 test of independence with Yates' correction ($P<0.05$).
[c] Means in same column having no letters in common are significantly different according to a LSD test ($P<0.05$). Data were first submitted to a one-way analysis of variance for unequal replication.
[d] Males flying to within 10 cm of edge were scored as approaching to 50cm; 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.

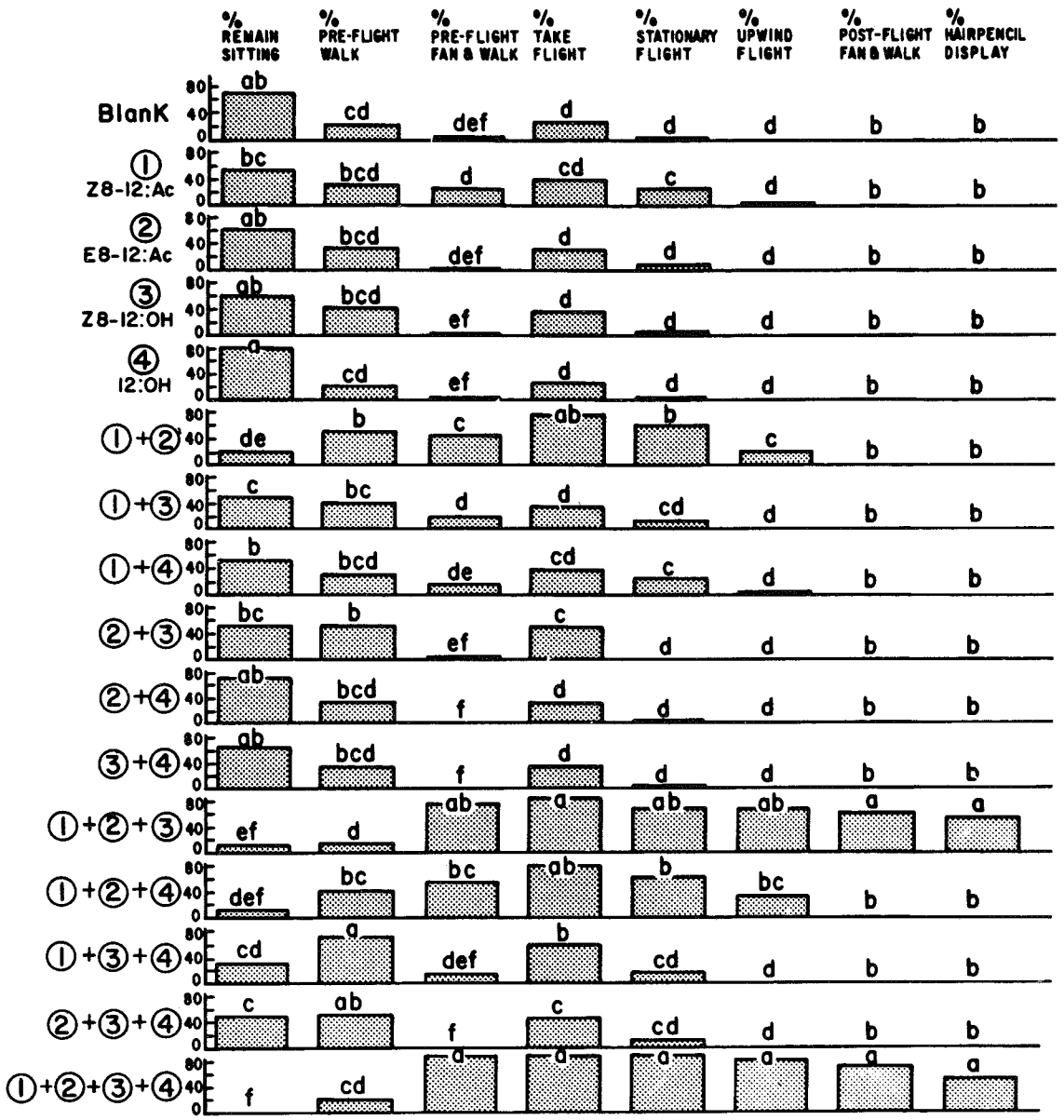

FIG. 3.—Percentage response of male *G. molesta* observed individually in a laboratory wind tunnel to all possible combinations of four *G. molesta* pheromone components emitted at rates and ratios similar to those emitted by *G. molesta* females. Septa contained, either singly or in combination as indicated, 10 μg Z8-12:Ac, 0.7 μg E8-12:Ac, 1 μg Z8-12:OH, and 1 μg 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 a χ^2 2×2 test of independence ($P<0.05$). N = 40 for each treatment.

males walked while fanning on the table and their approach to the dispenser was significantly closer than with treatments lacking Z8-12:OH (Table 2). Mean orientation 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 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 appear to increase the frequency with which males were observed flying upwind, but this conclusion may be incorrect due to an observational bias. There were shorter duration flights to the treatment lacking Z8-12:OH and the observer was able to note approaches of other males, thereby increasing observation frequency; during both

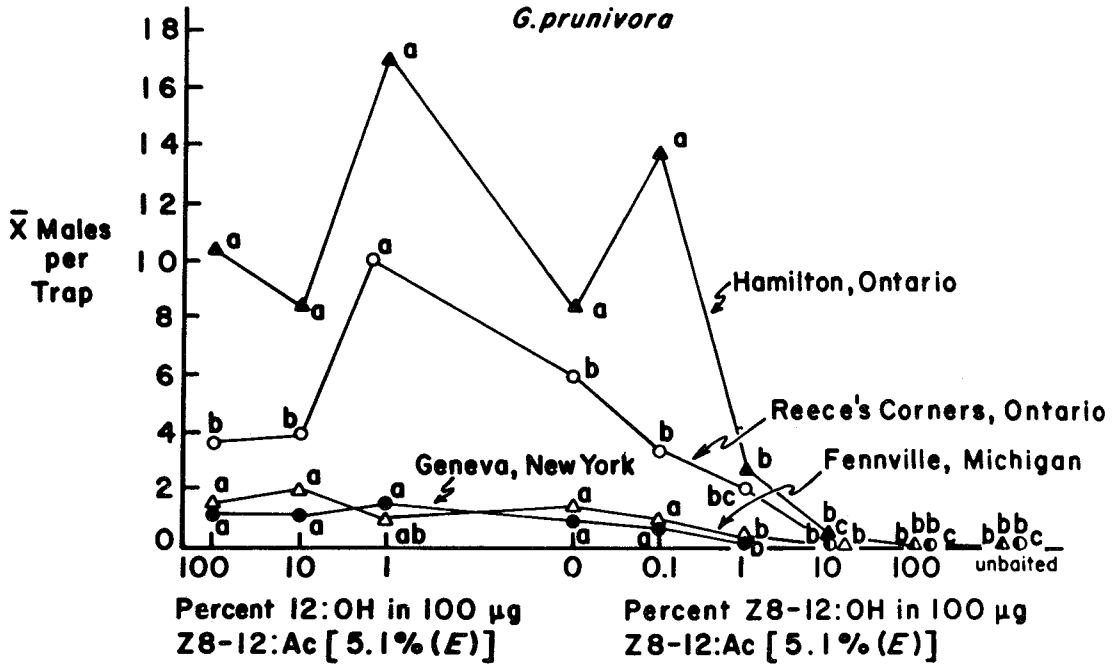

FIG. 4.—Effect of adding various percentages of either Z8-12:OH or 12:OH to 100 µg Z8-12:Ac {5.1% (*E*)} upon capture of *G. prunivora* males. Hamilton, and Reece's Corners, Ontario experiments were conducted May 18–23, 1977; Geneva, May 19–22, 1977; and Fennville, May 13–16, 1977. Within each experiment, means having no letters in common are significantly different according to Student-Newman-Keuls' multiple range test ($P<0.05$).

longer duration flights and wing fanning of Z8-12:OH - responding males many simultaneous approaches of other males went unrecorded. In the wind tunnel upwind flight frequency was increased when Z8-12:OH was added to the two acetates (Fig. 3), as was a pre-flight behavior, wing fanning while walking. Later behaviors such as post-flight wing fanning while walking and hairpencil display also were increased by addition of Z8-12:OH, in agreement with field observations.

The pattern of response to the component mixtures in the wind tunnel indicates that the 3-component blend of the acetates plus Z8-12:OH acts as a unit to increase both early and late stages of behavior. At this approximation to the natural emission rate, no component emitted alone had a significant effect upon behavior, except Z8-12:Ac which slightly increased the amount of stationary flight (Fig. 3). The only binary mixture having a major behavioral effect was the Z8- and E8-12:Ac mixture which increased the early behaviors of pre-flight wing fanning while walking, flight initiation, stationary flight, and upwind flight. Moreover, when Z8-12:OH was also present the acetate mixture caused increases in the later behaviors as well, including hairpencil display. We conclude that Z8-12:OH added to the acetate mixture significantly increases both the earliest and later behaviors in the sequence.

In addition to increasing the trap capture of *G. molesta* males, as little as 1% of Z8-12:OH added to the acetates reduced capture of *G. prunivora* in two locations in Canada and in New York and Michigan (Fig. 4). When 10% Z8-12:OH was present, *G. prunivora* capture was further reduced to no greater than a solvent blank-baited trap. In contrast, addition of 12:OH to the acetates did not alter *G. prunivora* capture; in 3 of the 4 locations there were no increases or decreases in trap catch and the one significant increase was at the lowest 12:OH dosage. We attribute the large variation in the two Canadian orchards to no trap re-randomization, and position effects.

Addition of E8-12:OH to Z8-12:OH

Addition of various percentages of E8-12:OH into the mixture containing 100 µg Z8-12:Ac [5.1% (*E*)] plus one µg pure Z8-12:OH, did not affect capture of males (Table 3). 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.

Simultaneous Addition of Z8-12:OH and 12:OH

Addition of various quantities of 12:OH to the optimal Z8-12:OH - Δ8-12:Ac 3-component mixture had no significant effect upon male capture (Table 4), but the trend toward increase at higher dosages of 12:OH implied there might be some behavioral effect. However, 300 µg 12:OH added to 100 µg Z8-12:Ac [4.9% (*E*)] plus one µg Z8-12:OH [5.7% (*E*)] elicited no significant increases in behavior, except for a closer mean closest approach to the dispenser (Table 5, 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 5, Test 2).

However, in the wind tunnel, at a 10-fold lower dosage more closely approximating the emission rate of the female (Baker et al., unpubl.), the 12:OH-added treat-

Table 3.—Effect on *G. molesta* male capture of various percentages of E8-12:OH added to 1 μg Z8-12:OH (pure) in 100 μg 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-Keuls' multiple range test ($P<0.05$).

Treatment (on rubber septum)	$\bar{x}$ ♂/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 4.—Effect of addition of various quantities of 12:OH to 1 μg Z8-12:OH (pure) plus 100 μg 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)	$\bar{x}$ ♂/trap
100 μg Z8-12:Ac {4.9% (*E*)}	6.4 b
100 μg Z8-12:Ac {4.9% (*E*)} + 1μg Z8-12:OH (pure)	16.7 a
100 μg Z8-12:Ac {4.9% (*E*)} + 1μg Z8-12:OH (pure) + 1 μg 12:OH	15.6 a
100 μg Z8-12:Ac {4.9% (*E*)} + 1μg Z8-12:OH (pure) + 10 μg 12:OH	18.8 a
100 μg Z8-12:Ac {4.9% (*E*)} + 1μg Z8-12:OH (pure) + 100 μg 12:OH	18.6 a
100 μg Z8-12:Ac {4.9% (*E*)} + 1μg Z8-12:OH (pure) + 300 μg 12:OH	21.3 a
100 μg Z8-12:Ac {4.9% (*E*)} + 1μg Z8-12:OH (pure) + 1000 μg 12:OH	15.6 a
100 μg Z8-12:Ac {4.9% (*E*)} + 300μg 12:OH	6.8 b
Solvent-impregnated septum	0.0 c

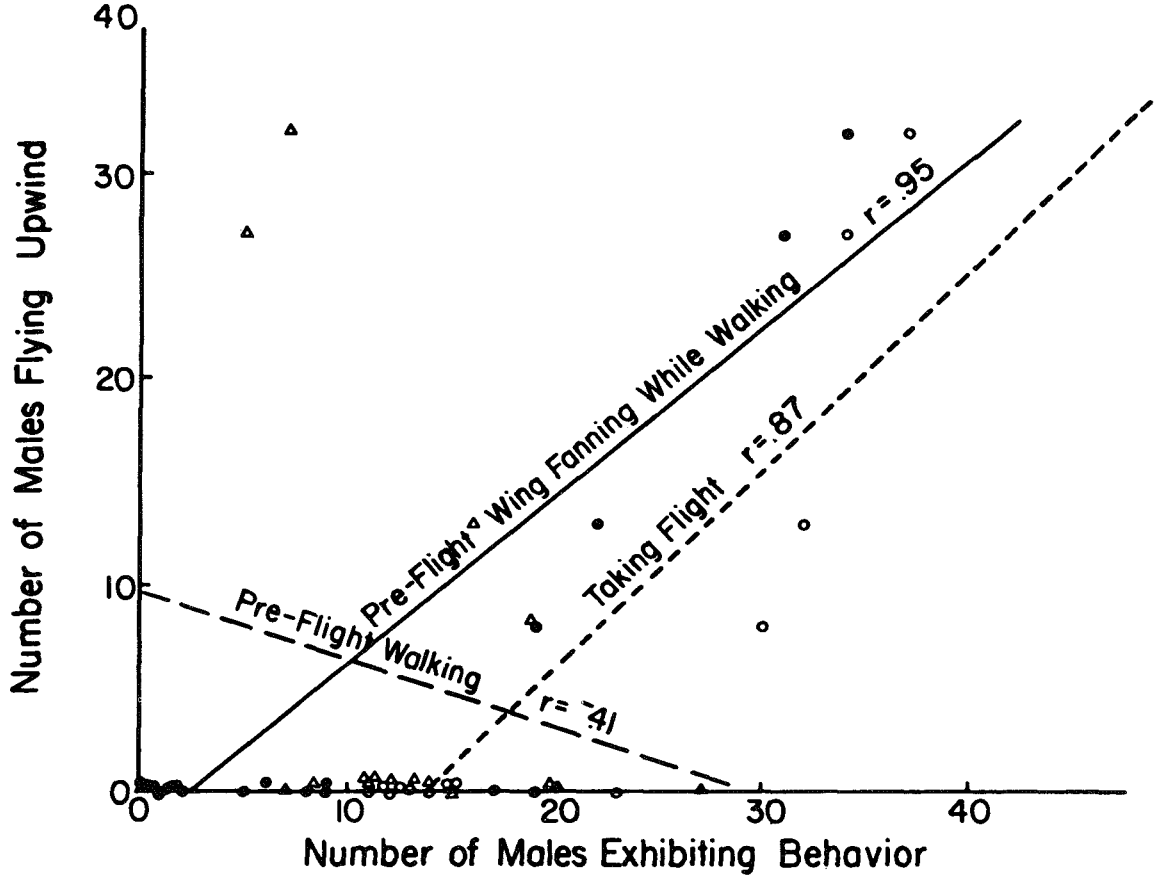

FIG. 5.—Correlations between number of males exhibiting either pre-flight wing fanning while walking (solid circles), taking flight (open circles), or pre-flight walking (triangles), and the number of males flying upwind in response to each of the 16 wind tunnel treatments listed in Fig. 4 and Table 7.

Table 5.—Behavior of *G. molesta* males on or near 50-cm radius observation arenas 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 septum 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, 1977 at Fennville, Michigan.

Treatment (on rubber septum)	No. ♂/h observed beginning upwind flight[a] (no.)	% ♂ flying to 10 cm of arena's edge[b] (no.)	Of those flying to edge, % ♂ landing[b] (no.)	Of those landing, % ♂ wing fanning while walking[b] (no.)	Of those fanning while walking, % ♂ displaying hairpencils[b] (no.)	Of those hairpencilling $\bar{x}$ no. displays/♂[c] (± SD)	Of those flying to edge, x closest approach to dispenser[c,d] (cm) (± SD)	Of all ♂ observed, $\bar{x}$ orientation time (sec) (± SD)
Test 1								
100 μg Z8-12:Ac {4.9% (*E*)}	51	96.1	75.5	94.6	2.9	1.0	30.2	36.0
+ 1 μg Z8-12:OH {5.7% (*E*)}	(51)	(49)	(37)	(35)	(1)	—	(± 16.5)	(± 26.71)
100 μg Z8-12:Ac {4.9% (*E*)}	38 NS	97.9 NS	89.4 NS	90.5 NS	15.8 NS	4.0	20.8 **	49.0 NS
+ 1 μg Z8-12:OH {5.7% (*E*)}	(48)	(47)	(42)	(38)	(6)	(± 3.69)	(± 18.2)	(± 37.5)
+ 300 μg 12:OH								
Test 2								
100 μg Z8-12:Ac {4.9% (*E*)}	47	100.0	81.7	86.6	32.8	2.74	20.2	35.4
+ 1 μg Z8-12:OH (pure)	(82)	(82)	(67)	(58)	(19)	(± 1.74)	(± 19.6)	(± 24.5)
100 μg Z8-12:Ac {4.9% (*E*)}	55 NS	100.0 NS	91.7 NS	83.0 NS	17.8 NS	2.7 NS	22.1 NS	37.0 NS
+ 1 μg Z8-12:OH (pure)	(96)	(96)	(88)	(73)	(13)	(± 1.7)	(± 18.5)	(± 24.4)
+ 300 μg 12:OH								

[a] NS; number not significantly different from one above it according to χ^2 using a null hypothesis of equal numbers of observations per hour ($P>0.05$).
[b] NS; percentage not significantly different from one above it according to a χ^2 2×2 test of independence with Yates' correction ($P>0.05$).
[c] **; mean significantly different from one above it according to the *t*-test ($P<0.05$). NS; mean not significantly different from one above it according to the *t*-test ($P>0.05$).
[d] 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 6.—Hairpencil display behavior of *G. molesta* males in laboratory wind tunnel to 30 μg 12:OH added to 10 μg Z8-12:Ac {4.9% (*E*)} plus 0.1 μg Z8-12:OH (pure). There were no differences in other behavior such as upwind flight, landing, or post-flight wind fanning while walking according to a χ^2 2×2 test of independence with Yates' correction ($P > 0.05$).

Treatment (on rubber septum)	% ♂ displaying hairpencils[a]	Of males displaying hairpencils,[b] $\bar{x}$ no. displays/♂ (± SD)
10 μg Z8-12:Ac {4.9% (*E*} + 0.1 μg Z8-12:OH (pure)	38.8 (N = 116)	2.9 (± 1.75) (N = 45)
10 μg Z8-12:Ac {4.9% (*E*)} + 0.1 μg Z8-12:OH (pure) + 30 μg 12:OH	54.3 * (N = 116)	3.9 (± 2.20) * (N = 63)

[a] *; Percentages significantly different according to a χ^2 2×2 test of independence with Yates' correction ($P<0.05$)
[b] *; means significantly different according to the *t*-test ($P<0.05$)

ment increased the percentage of males giving hairpencil displays, and also the mean number of displays per male (Table 6). Earlier behaviors were not affected. Thus, when Z8-12:OH was present at 1% of the acetates, addition of 12:OH produced some subtle but significant increases in a close-range behavior. Because the hairpencil display occurs only after close approach to the source, it is unlikely 12:OH added to this 3-component mixture could significantly increase trap catch by increasing levels of this behavior alone; sticky traps presently in use routinely ensnare males and terminate "normal" orientation 5–15 cm from the septum.

That 12:OH discernibly affects behavior when Z8-12:OH is 1% of the acetates is difficult to interpret in terms of a precise definition of a pheromone component. Z8-12:OH is emitted by females at a considerably higher, and 12:OH at a lower, ratio to the acetates than the formulation we used which showed 12:OH-mediated hairpencil display increases (Baker et al., unpubl., Cardé et al. 1979). However, when one μg 12:OH was added either to the 10 μg Z8-12:Ac [6.7% (*E*)] plus one μg Z8-12:OH mixture (approximating the female emission rates of Z8-12:OH and 12:OH) or to the binary acetate mixture, 12:OH had no discernable behavioral effect (Fig. 3). There were also no differences in mean number of hairpencil displays (3.33±1.78 SD, n = 25 and 2.76±1.51, n = 20, respectively) average flight speed (14.9 cm/sec ± 5.3 and 15.4 cm/sec ± 5.0) or response latency (3.7 sec ± 3.24 and 3.8 sec ± 2.95) between the Z8-12:Ac [6.7% (*E*)] plus Z8-12:OH mixtures with or without 12:OH.

Table 7.—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 Fig. 3. 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 (± SD)	No. ♂ flying upwind
Blank	0.7±0.28 (N=2)	0
1 Z8-12:Ac 10 μg	1.5±1.60 (N=9)	1
2 E8-12:Ac 0.7 μg	0.6±0.07 (N=2)	0
3 Z8-12:OH 1 μg	—	—
4 12:OH 1 μg	—	—
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

Certain patterns of behaviors across treatments were evident. Pre-flight wing fanning while walking and upwind flight were highly correlated (r = 0.95) (Fig. 5). 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 (Fig. 5).

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 7). The strong correlation between the amount and duration of wing fanning while walking and upwind flight implies that in other species wing fanning while walking may be the best "key response" to score in traditional "stimulation" olfactometers where upwind flight cannot be measured directly.

Optomotor Response

Males took significantly longer (17.6 sec ± 8.2 vs. 3.8 sec ± 1.1, n = 13) (*t*-test $P<0.01$) to fly upwind over an 80-cm distance in response to the same chemical stimulus when the striped floor pattern was moved backward at 26 cm/sec than 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 1979), and undoubtedly represents a mechanism widespread in the insects to gauge upwind progress while in an aerial odor plume.

Discussion

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 preflight wing fanning and upwind flight was the 2 acetates, although we tested only a limited number of all possible dosages and component ratios. Percentages of E8- in Z8-12:Ac [ca. 5–7% (*E*)] close to the natural percentage elicited an increase in male behaviors both at the early (pre-flight wing fanning while walking, flight initiation, upwind flight initiation) and late 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 late (post-flight wing fanning while walking, hairpencil display), and early behaviors. We conclude that the blend of these 3 components acts as a unit to affect all stages of male response. This unitary mode of action is in contrast to components in the sex pheromone blend of *Argyrotaenia velutinana* (Walker), the redbanded leafroller moth. At the single dosage and ratio tested, dodecyl acetate added to the optimal (*E*)- and (*Z*)-11-tetradecenyl acetate ratio increased only the "close-range" behaviors such as landing and wing-fanning while walking near the source (Baker et al. 1976) while apparently not affecting the frequency of upwind flight. Thus the behavioral effects of blends containing newly-added components may vary according to species. Detectable increases in behavior may be seen only in late behaviors close to the source, or may extend to the earliest behaviors observable at long-range.

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 is the 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 hairpencil displays, a late behavior, only when in blends containing reduced amounts of Z8-12:OH plus the acetates. There was no discernible effect either when Z8-12:OH was 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. 1975a,b), and to the consistent substantial trap capture increases with the addition of 12:OH reported by Beroza et al. (1973a), Roelofs and Cardé (1974), Gentry et al. (1975), and Rothschild and Minks (1977). The differences between previous reports and the current findings are likely related to contamination of the optimal (*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 increased trap catch only 2-fold (Cardé et al., 1975a). 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 hairpencil behavior only when Z8-12:OH was already present at low ratios (ca. 1%), not higher (10%), or absent entirely. Although the contribution of 12:OH to the behavior-modifying properties of the 4-component blend appears minimal, it may affect behavior in ways we have not yet measured.

Classifying Identified Sex Pheromone-Mediated Behaviors

We first described the behavioral effects of *G. molesta* sex pheromone components in terms of the forms of the behaviors evoked. Then the position of each behavior in the response "sequence" was estimated, thereby categorizing the behavioral effect as being relatively "early" or "late." Although such a classification avoids possible ambiguities of "close-range" and "long-range" labels on behavioral acts (Kennedy 1978), it rests upon precise definitions of early and late behaviors in a sequence. An ordered series of behaviors may arise in a particular environment because spatial constraints impose a particular pattern on the behaviors (Slater and Olasson 1973). For instance, males were required to fly to the pheromone source. Had they been released directly onto the upwind platform in the plume, 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, unpubl.). In the field there are a number of spatial problems (of which our arena was but one) for males to solve while remaining in the pheromone plume to locate the source. Hence the sequence of response may be highly dependent on the experimental environment. Such dependencies may make correlations between behaviors more difficult to interpret, but if the dependencies can be removed, then positive correlations between behaviors may mean they are closely related according to the function they perform. For example, the high positive correlation of wing fanning while walking with upwind flight suggests that both may be functionally similar solutions to the same problem: location of the chemical source either by locomotion via air or ground. When viewed in this way measuring upwind progress by leg movement against a fixed substrate or by a moving ground pattern over ommatidia does not seem very different, even though the

wing fanning while walking and upwind flight behaviors have a dissimilar form. Consolidation of sex pheromone-mediated behaviors according to function rather than position in a sequence or distance from the source at which they occur may prove illuminating.

Assigning Functions to Chemical Stimuli

The sequential, orderly addition of chemical components, one at a time, would appear to allow for an accounting of the various behaviors they evoke. However, for *G. molesta* components, the added chemical alone was not responsible for the new behaviors observed. Rather, the new *blend* elicited the behavior and to consider Z8-12:OH as simply a "courtship component" would be misleading. Only in combination with the optimal (*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 of Roelofs and Cardé (1977) these 3 chemicals could be designated as primary sex pheromone components. Considering the dependency of each upon the other two and their largely unitary mode of action, the mixture itself could be considered as a primary component. When there are so many primary components this classification system may lose 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 upwind flight thresholds appeared lowest to the (*E*)-(*Z*) acetate mixture compared to all other binary blends and single components, although a dosage-response experiment is needed to prove this. These thresholds appeared lower still to the acetates-plus-Z8-12:OH mixture, although again, a dosage series is needed. In the sex pheromone communication system of *G. molesta* there are probably few behaviors that are uniquely affected by a change in blend. More likely there are a graded series of quantitative changes in behaviors, of which only the larger can be measured by our crude methods. At some point the behavioral changes become so small that they fall below *our* detection threshold.

Relation of Wing Fanning While Walking to Attraction

Both the percentage and duration of pre-flight fanning while walking were highly correlated with upwind flight, a behavior normally associated with attraction. Thus, where upwind flight cannot be directly measured, as in stimulation olfactometers, duration of fanning while walking may 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 (Baker et al. 1976).

Wing Fanning While Walking and Trap Competition

The acetates-alone blends did moderately well at capturing males (Fig. 1) unless Z8-12:OH was added at various dosages (Fig. 2), whereupon the acetates alone were indistinguishable from unbaited traps. Behavioral observations showed that to traps containing Z8-12:OH plus the acetates, males were at least as likely to fly upwind as to the acetates alone. However, they were more likely to land and wing fan while walking near the source to the 3-component blend (Table 2) and thus be captured on the sticky surface of traps. Flying, as opposed to sitting males are more likely to encounter pheromone plumes from neighboring traps; thus a treatment evoking flight but little landing will lose more potential males to neighboring traps eliciting flight and *much* landing than predicted from a comparison of capture levels of the traps in isolation. Our evidence suggests that competition from other traps or calling females is directly related to capture efficiency, i.e., the percentage of upwind-flying males that land, wing fan while walking near the source and are caught. Thus in some cases the "competition effect" reported at high population densities, where greater numbers of females are presumed to somehow compete more strongly with pheromone traps for males (Minks 1977), may be accentuated by traps with pheromone blend-produced sub-optimal capture efficiency.

Conclusions

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 (long-range) and late (close-range) stages of male sexual behavior. The behavioral effects of these components were described most precisely only when each was considered in combination with the others, rather than individually or in binary blends. Addition of the pheromone component 12:OH elicited an increase only in a late stage, the hairpencil display, but only when 12:OH was emitted at higher, and Z8-12:OH at lower, than natural emission rates.

The behavioral effects of blends containing a newly-added component may vary according to the component's chemical structure, its dosage, and the species studied. Detectable increases in behavior may be seen only in later behaviors close to the source, or may extend to the earliest observable behaviors at long-range.

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 many pheromone investigations are inadequate.

The strong positive 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 of some moth species where upwind flight cannot be measured directly, wing fanning while walking may be the best "key response" to measure.

The bioassay environment itself may contribute to the order and frequency of behaviors in a sequence. More meaningful functional relationships between behaviors may be revealed only when such bioassay design effects are understood and analyzed.

Acknowledgment

We thank P. Castrovillo for helpful discussion and assistance, and M. Benson, B. Miclay, and D. Eldridge for excellent technical assistance.

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