Gypsy Moth[1] Responses to Pheromone Enantiomers as Evaluated in a Sustained-Flight Tunnel[2]

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ABSTRACT

(−)-Disparlure reduced: (1) the durations of anemotactic flights of male gypsy moths, *Lymantria dispar* (L.), in plumes of (+)-disparlure; (2) the rate of moth flight with respect to a moving floor pattern; and (3) the percentage of moths initiating anemotactic flights. The effects of (−)-disparlure were much more apparent on in-flight than pre-flight behavior.

Field tests (Vité et al. 1976, Miller et al. 1977, Cardé et al. 1977) with 2 different synthetic preparations of disparlure (*cis*-7,8-epoxy-2-methyloctadecane) enantiomers (Iwaki et al. 1974, Mori et al. 1976) proved that: highly optically pure (+)-disparlure is very attractive to male gypsy moths, *Lymantria dispar* (L.); and when (−)- is combined with (+)-disparlure, trap catches are greatly reduced, i.e., racemic (±)-disparlure caught ca. 10 times fewer males than did (+)-disparlure.

The step(s) in the behavioral sequence of attraction to a trap affected by (−)-disparlure remained unknown. Activation bioassays measuring wing-fanning (Yamada et al. 1976) revealed little difference in the activities elicited by (+)- and (±)-disparlure, suggesting that the effect on sexual behavior exerted by the (−) enantiomer must be more apparent at some point after initial stimulation of resting moths.

As a means of documenting further the effects of (−)-disparlure on gypsy moth sexual behavior we have used a sustained-flight tunnel to analyze both the pre-flight and in-flight sexual behavior elicited by (+)- and (−)-disparlure and their mixtures.

Materials and Methods

All experiments were conducted with laboratory-reared male gypsy moths supplied by the USDA Gypsy Moth Methods Development Laboratory, Otis AFB, Mass. Shipped pupae were placed in 40×40×47-cm screened cages and held at 23°C and ca. 70% RH under a 16:8 L/D photoperiod. Emerged males were held at the above conditions and used 2–5 days post-eclosion.

Experiments were conducted 3–9 h prior to scotophase in the sustained-flight tunnel previously described by Miller and Roelofs (1978). A wind velocity of 65 cm sec^{-1} was maintained throughout the experiments and temperatures were held between 22°–26°C.

The enantiomers of disparlure, synthesized and supplied by Mori et al. (1976), were $>$ 98% optically pure. Chemicals were introduced into the flight tunnel on 1×4-cm strips of Whatman No. 1 filter paper placed edgewise to the wind and positioned 18 cm above the canvas belt floor.

In trapping tests, baited Pherocon™ 1C traps opened to 11 cm and having both a sticky top and bottom (Miller et al. 1977) were hung one at a time at the upwind end of the flight tunnel at ca. 40 cm above the tunnel floor. In a test of their relative attractancy, traps baited with (+)- and (±)-disparlure were alternated. Release cages containing 5 male gypsy moths were inserted into the chemical plumes and opened allowing responding males to fly to the traps.

In choice tests, 2 chemical treatments positioned 10 cm apart were introduced into the flight tunnel at once. Smoke generated from sources soaked with titanium tetrachloride demonstrated that resultant plumes were discrete over the upwind half of the tunnel and contiguous near the downwind end. Moths were released into the plumes individually or in groups and a record was kept of the relative time spent in each plume and the number of approaches to within 1 cm from the source. Upon leaving an attractant source, moths usually flew downwind and then began reorienting. When a reorientation had begun, moths were driven to the downwind end of the tunnel by moving the floor pattern (Kennedy and Marsh 1974, Miller and Roelofs 1978). Successive approaches, therefore, spanned the length of the tunnel and data were recorded until flight ceased spontaneously. Between and sometimes during approaches, the sources were rotated.

The durations of anemotactic flights of moths were measured while they were being flown in place by the moving floor pattern. In one type of experiment, paired treatments were tested on individual males, i.e., a moth was allowed to fly to (+)- or (±)-disparlure until it left the plume spontaneously; then, after a 5-min rest, it was induced to fly to the other treatment. The sources were first selected randomly and then alternated until a moth would no longer fly to either one.

A similar test was designed to document male activation and flight behavior elicited by single treatments. Moths that had no previous exposure to disparlure were introduced individually into the flight tunnel and given 5 min to settle. The release cage was opened and the chemical source inserted. Vari-

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ous pre-flight and in-flight responses (Table 1) were measured. Six ratios of (+)- and (−)-disparlure were tested using a randomized complete block design; one block was completed on one day of testing. Great care was taken so that moths received no inadvertant exposure to any disparlure other than the designated treatment. Between each test, release cages and the screening at the downwind tunnel end were rinsed with acetone. Data from this test were transformed to $(X+0.5)^{1/2}$ and submitted to an analysis of variance. Differences were established by a Baysean significant difference test at $P = 0.05$.

Results and Discussion

In 12 replicates, traps baited with 1 μg of (+)- or with 2 μg of (±)-disparlure caught means of 3.3±1.1 (SD) and 2.6±1.4 males, respectively, released 5 at a time. These means were significantly different as determined by a chi-square 2×2 contingency table test ($P < 0.05$); however, this difference was far less than the 10-fold difference manifested in field tests (Miller et al. 1977).

A similar test (12 replicates, 5 moths/test), not using traps but allowing moths to orient to and land on fully exposed filter paper sources of 100 ng (+)- or of 200 ng (±)-disparlure yielded significantly different ($P < 0.001$) means of 3.5±0.8 and 1.8±0.9 for landing and 4.2±0.9 and 2.6±1.7 for preflight wing fanning, respectively. Again, these differences were smaller than those determined in the field.

Likewise, choice tests revealed no great differences between (+)- and (±)-disparlure. When 19 moths were tested individually, 1 μg (+)-disparlure was selected for close approach (<1cm) or landing 33 times and 2 μg (±)-disparlure was chosen 40 times (differences not significant by chi-square test with Yates correction, $P > 0.1$). In 50 orientations, no significant preference was shown with respect to time spent in either plume. During 22 orientations moths spent more time in the (+)- than (±)-disparlure plume and during 28 orientations, the reverse was true. Moths often moved freely from one plume to the other. Similarly, a test on 25 individual moths comparing a 1-μg (+)-disparlure source with a source bearing 1 μg (+)-combined with 3 μg (−)-disparlure yielded, respectively, counts of 23 vs. 29 for approaches and 21 vs. 31 for plume preference during orientations (no significant differences). There was no evidence of the development of a plume preference with successive orientations by individual moths.

Choice tests were conducted on groups of 10 moths released simultaneously into the tunnel. In 4 replicates counts of 104 vs. 70 close approaches were recorded for 1 μg (+)- and 2 μg (±)-disparlure, respectively. This difference was significant ($P < 0.01$) but not dramatic. Choice tests comparing plumes from 1 μg (+)-disparlure vs. 1 μg (−)-disparlure revealed that moths spent only a few seconds in the latter plume and always selected the former for orientation and close approach.

Table 1.—Responses of male gypsy moths, *Lymantria dispar* (L.), to mixtures of disparlure enantiomers in a sustained-flight tunnel.[a]

Treatment	% (+)-disparlure	$\bar{x}$ time wing-fanning begins[b] (sec)	$\bar{x}$ time reaching cage opening (sec)	$\bar{x}$ time leaving cage (sec)	$\bar{x}$ time initiating anemotactic flight (min)	$\bar{x}$ time flying in plume (min)	$\bar{x}$ distance flown with respect to floor (m)	$\bar{x}$ rate of flight, m/min
1 μg(+) and 10 ng(−)	99[c]	7.9 a	43.9 a	70.8 a	2.9 a	38.5 a	208.3 a	6.3 a
1 μg(+) and 250 ng(−)	80	13.3 a	37.8 a	40.3 a	2.3 a	10.8 b	64.6 b	4.8 a
1 μg(+) and 1 μg(−)	50	17.2 a	53.7 a	68.9 a	3.0 a	3.3 b	8.8 c	2.1 b
1 μg(+) and 3 μg(−)	25	33.9 a	92.6 a	112.0 a	3.1 (33%)	1.8 (21%)	3.7 (30%)	2.5 (30%)
1 μg(+) and 9 μg(−)	10	25.0 (11%)[d]	70.8 (11%)	89.0 (11%)	1.1 (56%)	1.4 (43%)	4.9 (60%)	1.2 (60%)
10 ng(+) and 1 μg(−)	1	— (100%)	— (100%)	— (100%)	— (100%)	— (100%)	— (100%)	— (100%)

[a] The floor pattern was moved to prolong anemotactic flights; each observation was replicated 9–14 times.

[b] Means followed by the same letter in each column are not significantly different ($P > 0.05$).

[c] The purest enantiomers of Mori et al. (1976) were taken to be 99%.

[d] Numbers in parentheses represent the percentage of non-responders. The means were derived from responders only and statistical tests were performed only on those groups with non-responders.

The successive flights of 28 moths were sustained by a moving floor pattern and timed as sources of (+)- and (±)-disparlure were alternated for each successive flight by an individual. Mean flight durations for 45 paired flights were 4.0±11.4 and 2.5± 6.4 min, respectively. This difference was significant in a sign test for paired data ($P < 0.01$).

Thus far, all laboratory tests had failed to demonstrate a major difference between the activities elicited from gypsy moths by (+)- and (±)-disparlure. However, in the paired flight endurance tests, a trend became apparent. The longest flights (> 30 min) occurred when a fresh male was flown to (+)-disparlure. Subsequent flights to (+)- after an exposure to (±)-disparlure rarely lasted more than 5 min. This suggested that (−)-disparlure might have a lasting antagonistic effect on sexual behavior and emphasized that each disparlure treatment should be tested on a separate individual.

The results of such a test are presented in Table 1. Males responded to the disparlure treatments by initiating wing-fanning, walking upwind to the opening of the release cage, and in many cases walking onto the screening enclosing the downwind tunnel end. Flight was initiated from either this screening or from the outside of the release cage.

As the quantity of (−)-disparlure increased (Table 1), there was a trend toward a longer elapsed time from source introduction until initiation of wing-fanning; however, due to the extensive individual variability, this difference was not significant in an analysis of variance test. The exception was (−)-disparlure, to which no males responded sexually.

For responders, there was no difference among treatments in the time moths exited from the release cages or initiated anemotactic flights. However, for the treatments with highest amounts of (−)-disparlure, the percentage of non-responders increased with major steps in response complexity, i.e., wing-fanning vs. anemotactic flight initiation.

This test revealed major differences among disparlure treatments with respect to in-flight responses. With great care that moths were not exposed to any extraneous (−)-disparlure, flights to (+)-disparlure averaged over 30 min. As greater quantities of (−)-disparlure were added, the durations of anemotactic flights and concommitant distance flown with respect to the moving floor decreased dramatically. Interestingly, the decreases in flight duration closely paralleled the decreases in trap catch found in the field (Miller et al. 1977), i.e., there was ca. a 10-fold decrease between values for (+)- and (±)-disparlure in both tests.

(−)-Disparlure also significantly reduced the forward rate of moth flight. With increases in the (−)-enantiomer, greater numbers of moths hovered in flight without making much forward progress.

In summary, these data indicate that the strongest orientation responses are elicited by highly pure (+)-disparlure and that simultaneous or previous exposures to (−)-disparlure dramatically reduce the duration and intensity of in-flight responses. Also reduced is the percentage of responders.

The mechanism whereby (−)-disparlure disrupts gypsy moth behavioral responses to (+)-disparlure remains unknown. By EAG studies, Miller et al. (1977) documented 2 different receptor types on the male gypsy moth antenna; one had greater affinity for (+)- and the other had greater affinity for (−)-disparlure. Single neuron recordings should help differentiate whether the disruptive effect of (−)-disparlure is a peripheral or CNS phenomenon.

The present experiments also illustrate the utility of a sustained-flight tunnel in evaluating the response of insects to airborne sex pheromones. The feature of a moving floor pattern for sustaining anemotactic flights was of utmost importance in these experiments for it allowed the measurement of the more discriminating in-flight responses. Based on the excellent agreement of these tests with previous gypsy moth field tests (Miller et al. 1977, Cardé et al. 1977), we predict that sustained-flight tunnel tests will have greater relevance to field results than will most other laboratory sex pheromone bioassays heretofore reported.

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