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Attractancy of Optically Active Pheromone for Male Gypsy Moths[1,2]

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ABSTRACT

Synthetic attractant lures enriched in the (+) enantiomer of *cis*-7,8-epoxy-2-methyloctadecane were many times more effective in eliciting trap catch of male gypsy moth, *Lymantria dispar* (L.), than racemic *cis*-7,8-epoxy-2-methyloctadecane. The lowered trap catch at the racemate was due to the antagonistic effects of the (−) enantiomer on the male behaviors of long-range upwind flight, the likelihood of landing on the baited trap and duration of the persistent "searching" behavior of wing fanning concurrent with walking on the trap. The admixture of 2-methyl-(*Z*)-7-octadecene with the (+) enantiomer also effected a reduction in trap catch.

The gypsy moth, *Lymantria dispar* (L.), attractant pheromone was characterized from combined data on a behaviorally active component in the natural attractant and on an olefin in the natural female extract that had been epoxidized with *m*-chloroperbenzoic acid to produce a behaviorally active component (Bierl et al. 1970). The identified compound, *cis*-7,8-epoxy-2-methyloctadecane (named "disparlure") was synthesized as the racemate and, in field tests with released laboratory-reared males, was indicated to be superior to the attractancy of laboratory-reared females (Beroza et al. 1971). However, Richerson (1976) found laboratory-reared females less effective than wild females in eliciting orientation responses. Additionally, Cardé et al. (1974) pointed out that (±)-disparlure was a less efficacious lure than feral females when the male trap catch was contrasted at the maximum of the females' diel rhythm of attractiveness.

Because the epoxide is chiral, disparlure exists as (+) and (−) enantiomers. Iwaki et al. (1974) synthesized optically active disparlures in reported purities of 94% (+) and 94% (−). In simulated field attraction tests with Japanese *L. dispar,* Yamada et al. (1976) showed the 94% (+) to lure males whereas the 94% (−) was unattractive. Mori et al. (1976), via a different route, produced disparlure estimated to be >98% (+). In field tests with wild males in West Germany, the (+) enantiomer of Mori et al. was found to elevate trap catch ca. 3-fold over the reconstituted racemate (Vité et al. 1977). The configurational purity of the natural communication system of *L. dispar* remains unresolved.

This paper reports trapping and behavioral tests on the attractiveness of disparlure racemates and the antipodes of Iwaki et al. (1974) and Mori et al. (1976). These tests were conducted concurrently with those of Miller et al. (1977).

Materials and Methods

Field Tests

Trapping and behavioral studies were conducted in Natchaug State Forest in Eastford, Conn. in July, 1976. Test compounds were dispensed in 100 μl petroleum ether onto cotton wicks 1 cm in diam × 1 cm long. The disparlure and olefin combinations (Table 5) were placed on separate dispensers. The charged lures were placed inside traps modified (Cardé et al. 1977) from the design of Granett (1973). Insects were killed by a 2×2-cm plastic strip containing 18% 2,2-dichlorovinyl dimethyl phosphate. Traps were hung at a height of 1.8 m on sapling trees 3–8 cm in diam that had been cleared of branches and leaves within 50 cm of the trap. Trap trees were situated 10 m apart and at least 2 m away from trees of >8-cm diam. This procedure isolated the traps from large vertical silhouettes (such as large trees) which, in the presence of phermone, elicit investigative behavior (Richerson et al. 1976, Cardé et al. 1975b) and can result in postive catch even in unbaited, uncontaminated traps (Cardé et al. 1977). Treatments were set out in a randomized complete block design with 5 replicates. Trap data were transformed to $\sqrt{(x + 0.5)}$ and submitted to analyses of variance.

Behavioral Observations

Male attraction to synthetic-baited traps was monitored at traps baited with 10 μg Iwaki et al. (1974) 94% (+) enantiomer and 20 μg of (±)-disparlure reconstituted from equal parts of 94% (+) and 94% (−) synthetics. Traps were placed on sapling trees as in the previous section in a crosswind line with 30 m between traps. The 2 entrance ports at each end of the trap were aligned with the prevailing wind. Traps were observed for 10-min inter-

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Table 1.—Attractancy to *Lymantria dispar* males of disparlure enantiomer combinations. Test conducted from July 12–18, 1976, Eastford, Conn.

Treatment 50 µg (+):(−)		x̄ males trap/sample[a]
Iwaki et al.	94:6	21.3 a
Iwaki et al.	75:25	8.0 b
Iwaki et al.	50:50	2.9 c
Iwaki et al.	25:75	0.9 d
Iwaki et al.	6:94	0.1 d
Farchan	50:50	3.0 c
unbaited		0.5 d

[a] Means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls multiple range test. Treatments rerandomized 6 times.

vals, after which traps were positioned to new sites and the observers rotated to a different treatment. The observers at each trap were situated 10 m crosswind where the movements of males up to ca. 20 m downwind could be seen readily. Behavioral sequences were transcribed onto portable tape recorders for subsequent analyses by stopwatch.

Chemicals

The Iwaki et al. (1974) and Mori et al. (1976) enantiomers were as described in the literature. The 2-methyl-(*Z*)-7-octadecene and one of the (±)-disparlure samples was obtained from Farchan Chemicals. Another (±)-disparlure sample was obtained from ChemSampCo. The plastic laminate dispenser was a 1976 sample from Hercon Corp. and contained 10 mg (±)-disparlure.

Results

Field Trapping Experiments

Attractancy trials (Table 1) with combinations of Iwaki et al. (1974) enantiomers and mixtures with nominate (+) to (−) ratios of 94:6, 75:25, 50:50, 25:75 and 6:94 suggested that the 94% (+) was ca. 7-fold as attractive as reconstituted racemic. The (±)-disparlure from Farchan produced a trap catch statistically indistinguishable from the reconstituted racemate. The (−) enantiomers appeared to possess a negative effect on the trap catch elicited by the 94% (+)-disparlure, acting as a behavioral antagonist. Because the level of (+) enantiomer was not held constant in this test, the trap catch evoked by various dispenser levels of 94% (+) and (±)-disparlure at a range of dispenser dosages was contrasted (Tables 2 and 3). The catches at the racemates, while above those of the unbaited experimental controls, did not exhibit a dose-response relationship. Although the higher dispenser doses would yield an increased rate of attractant emission (and therefore increase in the active space), the concomitant increase in (−) enantiomer emission could have an increased antagonistic effect on male behavior, by affecting the long or close range orientation behaviors.

The trap catch at various dispenser dosages of

Table 2.—Attractancy to *Lymantria dispar* males to a range of dispenser dosages of Iwaki et al. 94% (+) and Farchan (±). Test conducted July 21–26, 1976, Eastford, Conn.

Treatment	x̄ males trap/sample[a]
500 µg Iwaki et al. 94% (+)	32.2 a
50 µg Iwaki et al. 94% (+)	27.7 b
5 µg Iwaki et al. 94% (+)	13.7 c
500 ng Iwaki et al. 94% (+)	4.5 d
50 ng Iwaki et al. 94% (+)	2.2 e
1000 µg Farchan (±)	3.5 de
100 µg Farchan (±)	4.1 d
10 µg Farchan (±)	4.7 d
1 µg Farchan (±)	3.0 de
100 ng Farchan (±)	2.0 e
unbaited	4.0 f

[a] Means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls multiple range test. Treatments rerandomized 6 times.

Iwaki et al. (1974) 94% (+) enantiomers revealed that male catch increased at rates of up to 500 µg. Since the area and particularly the downwind projections of the active space would be increased with the higher rates of emission found with greater dispenser loads, trap catch should be elevated accordingly. In opposition, high emission rates near the attractant source may act antagonistically (Cardé et al. 1975a) or the nominate 6% (−) enantiomer may decrease long or short range orientation success. At both 500 and 50 ng of 94% (+) enantiomer/dispenser, the trap catch did not differ significantly from the catches elicited by the Farchan racemate.

A comparison (Table 4) of the trap catch effected by the >98% (+) enantiomer of Mori et al. (1976) with the 94% (+) enantiomer of Iwaki et al. (1974) indicated that these products were of comparable attractiveness. However, admixture of 2.5 µg Mori et al. (1976) >98% (−) enantiomer to 50 µg of the (+) enantiomer resulted in reduced trap catch as compared to the 94% (+) Iwaki et al. (1974)

Table 3.—Attractancy to *Lymantria dispar* males of Iwaki et al. 94% (+) enantiomer and disparlure racemates. Test conducted July 25–27, 1976, Eastford, Conn.

Treatment	x̄ males trap/sample[a]
200 µg Iwaki et al. 94% (+)	22.4 a
20 µg Iwaki et al. 94% (+)	10.3 b
1000 µg ChemSampCo (±)	1.5 c
100 µg ChemSampCo (±)	1.4 c
10 µg ChemSampCo (±)	3.6 c
1000 µg Farchan (±)	1.9 c
100 µg Farchan (±)	3.6 c
10 µg Farchan (±)	4.0 c
Hercon 1970 dispenser (±)	1.5 c
unbaited	0.0 d

[a] Means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls multiple range test. Treatments rerandomized twice.

Table 4.—Attractancy to *Lymantria dispar* males of the enantiomeric combinations of Mori et al. and Iwaki et al. 94% (+). Test conducted July 21–28, 1976, Eastford, Conn.

Treatment	$\bar{x}$ males trap/sample[a]
50 μg (+) Mori et al. 98% (+)	29.6 a
50 μg (+) Mori et al. 98% (+) + 1.0 μg 98% (−)	13.3 b
50 μg (+) Mori et al. 98% (+) + 2.5 μg 98% (−)	6.2 c
50 μg (+) Mori et al. 98% (+) + 5.0 μg 98% (−)	4.0 cd
50 μg (+) Mori et al. 98% (+) + 7.5 μg 98% (−)	2.8 d
50 μg (+) Iwaki et al. 94% (+)	34.2 a
unbaited	0.1 e

[a] Means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls multiple range test. Treatments rerandomized twice.

product, even though the nominate (+) to (−) ratios should have been close.

Emission of the olefin analogue of disparlure (Table 5) with >98% Mori et al. (1976) (+) enantiomer reduced male catch, a reduction similar to the diminution reported with olefin emitted with either the racemate or virgin calling female (Cardé et al. 1973). The olefin appeared to act as a behavioral antagonist, similar to the effect of the (−) enantiomer.

Behavioral Observations

The criterion of trap catch for evaluation of the efficacy of an attractant may serve only as an approximate measure of the complex sequences of behavioral events involved in pheromone response (Roelofs and Cardé 1977). In instances where *L. dispar* males were captured in traps baited with 94% Iwaki et al. (1974) (+)-disparlure, males, after initial flight into the attractant plume up to 10 or 20 m downwind of the trap, flew upwind in a zig-zag pattern to the vicinity of the trap. The long range anemotactic flight generally was followed by stationary casting flight or hovering within ca. 1 m or less of the trap at the trap height, and then landing on the trap surface. Once landed, males engaged in wing fanning and walked (occasional bouts of stationary wing fanning occurred in some males) over the trap until entering the port (the males not captured at this point of the sequence terminated "searching" by a rapid flight away from the trap).

Table 5.—Attractancy to *Lymantria dispar* males of Mori et al. enantiomer and combinations of enantiomer and 2-methyl-(Z)-7-octadecane (olefin). Test conducted July 17–21, 1976, Eastford, Conn.

Treatment	$\bar{x}$ males trap/sample[a]
50 μg Mori et al. 98% (+)	50.1 a
50 μg Mori et al. 98% (+) + 500 ng olefin	43.6 a
50 μg Mori et al. 98% (+) + 5 μg olefin	28.8 a
50 μg Mori et al. 98% (+) + 50 μg olefin	2.8 b
unbaited	0.5 b

[a] Means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls multiple range test. Treatments rerandomized once

Table 6.—Behavioral responses of *Lymantria dispar* male to traps baited with either 10 μg Iwaki et al. 94% (+)-disparlure or 20 μg Iwaki et al. reconstituted (±)-disparlure. Test conducted July 18, 1976, Eastford, Conn.

Behavior	Treatment: 10 μg 94% (+)	Treatment: 20 μg (±)
Number males observed	34	27
Males/observation min[a]	0.57	0.23
Capture efficiency (orienting-males captured)[b]	47%	4%
$\bar{x}$ sec (± SD) within 2–2.5 m of trap[c]	52.2±51.7 (n = 34)	11.6±8.8 (n = 27)
$\bar{x}$ sec (± SD) within 2–2.5 m of trap until capture	66.9±62.9 (n = 16)	9 (n = 1)
$\bar{x}$ sec (± SD) wing fanning on trap entrance funnel[d]	24.8±18.0 (n = 24)	5.5±5.7 (n = 4)
Proportion wing fanning on trap entrance funnel[b]	70% (n = 34)	15% (n = 27)

[a] The traps baited with 10 μg 94% (+) and 20 μg (±) disparlure were observed for 60 and 120 min, respectively.
[b] Percentages differ by χ^2 in 2×2 test of contingency at $P<0.01$.
[c] Means differ at $P<0.01$ by Student's *t* test. Mean sum of seconds within 2–2.5 m of the trap until capture or termination of searching.
[d] Sum of seconds spent wing fanning (generally while walking but occasionally while stationary) on the funnel entrance to the trap.

Comparative analyses of the behaviors (Table 6) indicated that long-range anemotaxis is more apt to be elicited by 94% (+)-disparlure. The numbers of males exhibiting flight orientation per minute of observation toward the trap baited with enriched (+)-disparlure was elevated ca. 3-fold over the racemate. Many males (not reported in Table 6) traversed through the presumed plume area of the (±) lure in rapid flight without apparent orientation behavior. A number of additional behaviors (Table 6) are suppressed by the addition of the (−) enantiomer to the stimulus: (1) the mean cumulative times spent either in in-flight orientation within 2–2.5 m of the trap and wing fanning on the trap until capture; (2) the time of the same behavior until termination of "searching" by flight away from the trap or capture; (3) likelihood of an individual male wing fanning on the trap; and (4) the duration of wing fanning. Of especial importance from a trapping standpoint is the disparity of capture after observed positive orientation (the trapping efficiency).

Discussion

The attraction of male *L. dispar* to various enantiomeric combinations indicated that disparlure enriched in the (+) enantiomer is much more effective than the racemate in eliciting trap catch. The catch at (±)-disparlure, although statistically above that of unbaited traps, remained at the same level at a range of dispenser dosages from 100 ng–1000 μg. The Iwaki et al. (1974) 94% (+)-disparlure, on the other hand, exhibited a positive dose-response relation.

The (−)-disparlure enantiomers of both Iwaki et al. (1974) and Mori et al. (1976) possessed a negative effect on the catch elicited by the enriched (+)-disparlure, apparently acting as an antagonist toward male behavior. It will be of interest to determine if the 2 enantiomers interact with distinct antennal acceptor sites or if the behavioral effect is effected via interaction with the same acceptor site. The olefin, previously determined to act as an effective behavioral antagonist to the attractancy of (±)-disparlure and feral virgin females (Cardé et al. 1974), also proved effective as an antagonist to enriched (+)-disparlure. The olefin with (±)-disparlure combination emitted an attractive component and 2 behavioral antagonists, the (−)-disparlure and the olefin.

Observations of male behavior in the vicinity of traps baited with 10 μg Iwaki et al. 94% (+) and 20 μg of reconstituted Iwaki et al. (±) indicated that the parameter of trap catch is comprised of a sequence of events. Long-range anemotaxis to the vicinity of the trap is followed by close-range in-flight orientation in which the velocity of upwind flight is apparently decreased. In all instances where the male was observed to be captured, the male usually landed on the outside surface of the trap first, although occasionally landing first occurred on the sapling tree supporting the trap or on nearby foliage. The male then engaged in "searching" behavior consisting of wing fanning while walking with occasional bouts of stationary wing fanning. This behavior was a prerequisite to eventual orientation to the funnel entrance of the trap and capture.

The marked increase in trap catch at the enriched (+) enantiomer over the racemate was due to several factors. The enriched (+) enantiomer elicited both a several-fold increase in males exhibiting positive anemotaxis 10 m or more downwind and a more persistent "searching" at the trap due to a prolongation of wing fanning while walking. Because the amount of (+) enantiomer emitted was equivalent in the 2 treatments, the long and close range differences in behavior seem explicable in terms of antagonistic effects of the (−) enantiomer.

Because only a portion of the feral males responded positively to the racemate, there would appear to be different classes of responders. The basis of variation in the males' behavioral responses is of both evolutionary and practical importance. However, trap catch, even if supplemented by direct behavioral observations, cannot distinguish the proportion of behavioral types unless the classes are mutually exclusive (Cardé et al. 1976). Because individual males have not been retested, preferential response among several treatments cannot be established, although such variation is possible. The positive anemotactic response and capture of a minority of wild males with (±)-disparlure may be due to prior physiological conditioning or effects dependent on varying peak atmospheric concentration of stimulus rather than to some males being particularly responsive to the racemate.

The disparity in the trap catch effected by the 2 (+)-disparlure samples was inconsistent with their stated purities. The >98% (+) synthetic of Mori et al. (1976) when admixed with >98% (−) to produce a ratio nominally equivalent to the 94% (+) synthetic of Iwaki et al. (1974) did not yield a lure of equivalent potency. It is important to note that the enantiomeric purities of either these synthetics or the natural pheromone have not been established. The optical purities of the available (+) and (−) enriched enantiomers were assumed to be identical with those of the precursors (Iwaki et al. 1974, Mori et al. 1976). However, some racemization in the conversion to the epoxide is mechanistically possible. Definition of the optimally attractive system for *L. dispar* awaits further chemical and behavioral studies.

The results reported herein suggest that the past utilizations of (±)-disparlure in population monitoring and mating disruption (Beroza 1976) deserve reappraisement. The enriched (+)-disparlure clearly would be more sensitive than the racemate for monitoring male distribution and abundance, a factor particularly important at low population densities. Similarly, although previous field tests indicated a degree of disruption with (±)-disparlure, this combination may not be the most efficacious disruptant. Because the (+) enriched disparlure elicited an increased level of male response, it is possible that the disruptive effect of the (+) enriched antipode may be significantly greater than that of the racemate, especially if the elicitation of male behavioral response of itself contributes to habituation at the central nervous system. The antagonistic effects of both the (−) enriched enantiomer and the olefin on male orientation also suggest that these compounds (perhaps in combination) may have potential as mating disruptants.

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