

## Attraction of Male Gypsy and Nun Moths to Disparlure and Some of Its Chemical Analogues

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*Summary.* The attractive power of disparlure—the sex attractant of the gypsy moth (*Lymantria/Porthetria dispar*)—vs. four synthetic analogous epoxides was tested in 1972 in a pine forest near Heidelberg. With two levels of concentration in the traps (2 and 20  $\mu\text{g}$ ), a total of 1112 nun moths (*Lymantria/Porthetria monacha*) and 257 gypsy moths were caught in 9 experiments. Approximately equal percentages of the two species were caught with a given compound. Disparlure was by far the most effective attractant. The other substances were between three and twenty times less effective. These experiments support the assumption that disparlure is also at least part of the sexual attractant of the nun moth. In two additional experiments, moth captures by a series of increasing disparlure concentrations (2–100  $\mu\text{g}$ /trap) were determined. The catches of both species increased nonlinearly with the bait concentration. The experiments are discussed with respect to new (unpublished) electrophysiological recordings from disparlure receptor cells in both species. Special attention is given to the supposed masking effect of the disparlure precursor (an olefin). This substance is ineffective as an attractant, but has been reported to reduce the attraction of gypsy moth males to disparlure or to live females. However, the olefin elicits excitatory reactions in the same type of receptor cell that responds to disparlure and the related epoxides. Furthermore, no masking of the electrophysiological response was observed with the receptor cells when the olefin was added to disparlure.

The gypsy moth *Lymantria (Porthetria) dispar* L. is a serious pest of deciduous trees in the Northeastern part of the United States and in some parts of Europe, from whence it was introduced to North America in 1868/69. The nun moth *Lymantria (Porthetria) monacha* L. is still an important pest of conifer forests in large parts of Europe.

Attempts to control gypsy moths in New England by trapping the males with the sexual attractant of the females date back to 1893 (see Schedl, 1936), but critical evaluation of the sex pheromone as a potential means of survey and control was not possible before the attractant was chemically identified (Beroza *et al.*, 1971 a, b; Beroza and Knipling, 1972). The structure of the gypsy moth sex attractant (disparlure) was recently

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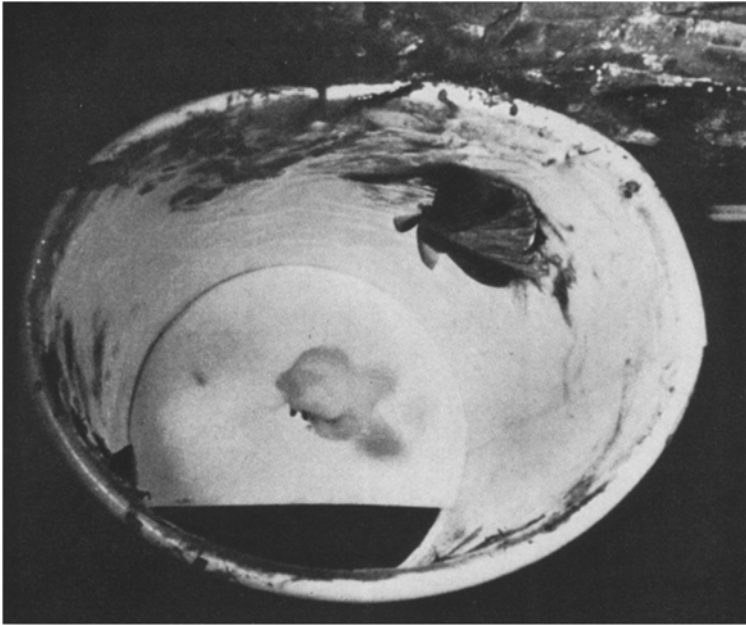
determined (see Table 1) and also verified by synthesis and biological tests (Bierl *et al.*, 1970, 1972). These results have been corroborated by a separate disparlure synthesis with a different technique (Eiter, 1972). The former authors also tested isomers and compounds related to disparlure and found them to be less attractive than disparlure. Additional information on the attractiveness and olfactory potency of disparlure and its chemical analogues came from comparative field and electrophysical tests (Adler *et al.*, 1972; Sarmiento *et al.*, 1972). Disparlure consistently appeared to be the most effective attractant for *L. dispar*; the next most potent compound had to be presented at a concentration more than 10 times higher to elicit the same response or attractiveness.

The female nun and gypsy moth's respective luring capacities were compared in earlier field and behavior experiments with live females and extracts from female pheromone glands (Görnitz, 1949; Schwinck, 1955). According to these reports, females of *L. dispar* are fully attractive to males of both species while females of *L. monacha* exhibit a weaker or doubtful luring effect upon males of *L. dispar*. So far, it remains an open question whether these differences are real or are the result of variations of the relative population densities of the two species. Recent electrophysiological cross tests with these two species, however, gave the full electroantennogram response between them: *L. dispar* ♀ gland stimulated the *L. monacha* ♂ antenna and vice versa (Priesner, unpubl.). Lack of species specificity of some sex attractants has been suggested by many anecdotal and systematic field (as well as laboratory) experiments (for Ref. see Schneider, 1962; Priesner, 1968, 1973; Jacobson, 1972). Recently, field tests proved pure synthetic disparlure to be an effective sexual attractant for male nun moths (Schönherr, 1972).

We have investigated the attraction of disparlure and some of its analogues for the two Lymantriid species in a pine forest in the Rhine valley near Heidelberg (Germany). The main goal of these studies was to determine whether the relative luring power of disparlure and some related epoxides differs with *L. dispar* and *L. monacha*. In two additional experiments, we checked on the relative effectiveness of traps containing a series of increasing amounts of disparlure. Finally, we conducted three preliminary experiments to determine whether the supposed disparlure precursor (Bierl *et al.*, 1970, 1971, 1972) has any luring effect upon the two Lymantriids.

### Material and Methods

The trapping experiments were conducted from July 23 to August 23, 1972. The forests are under observation by entomologists and known to maintain—at least for the last 10 years—a permanent population of *Lymantria dispar* and *L. monacha*.



a



b

Fig. 1. a USDA cup trap (diameter 7 cm). The bait is mounted on a wire in the centre of the cup. A captured moth sticks to the inner cup surface. b Lure cup in the centre of a sticky board on a tree

Table 1. Compounds (samples) used in the trapping experiments. The numbers are identical with Adler *et al.* (1972) and Sarmiento *et al.* (1972)

No.	Name
1	<i>cis</i> -7,8-epoxy-2-methyloctadecane (disparlure)
1 A	87% disparlure + 13% <i>trans</i> -7,8-epoxy-2-methyloctadecane
13	<i>cis</i> -7,8-epoxy-3-methyloctadecane
4	<i>cis</i> -7,8-epoxy-2-methylnonadecane
3	<i>cis</i> -7,8-epoxy-2-methylheptadecane
10	<i>cis</i> -6,7-epoxy-2-methyloctadecane
<i>p</i>	2-methyl- <i>cis</i> -octadecene (olefin)

The traps (see Stevens and Beroza, 1972) were the customary conical USDA-Johnson-type cardboard cups (diameters 5 and 7 cm, height 9 cm; see also Holbrook *et al.*, 1960). The wider side of the cup was fully open, the smaller side partly open (Fig. 1a). The cotton wick which contained the attractant was mounted on a wire centrally in the cup. In some experiments (designated "cups only"), we put the cups in horizontal position directly in the trees, 2.5 m above ground.

In other experiments the cups were placed horizontally through the centre of 35 × 35 cm plastic boards, which were fastened vertically on the trees, 2.5 m above ground (Fig. 1b). The inner wall of the cups and both sides of the plastic boards were covered with a water-insoluble glue (Tacky-Sticker). Care was taken not to put any glue on the wicks.

Table 1 shows the compounds used. In all tables and diagrams, compounds are listed in a sequence based upon electrophysiologically determined effectiveness ratings: substances no. 1:13:4:3:10:p = 1000:50:20:20:10: less than 1. These ratings relate to the respective stimuli eliciting equal responses in the electro-antennogram and the single disparlure receptor cell (Schneider, Kafka, Beroza, unpubl.). This sequence was not identical with the arbitrarily chosen sequence of the samples in the "carousel" shifts (see below).

The given amounts of attractants were applied to the cotton wicks in a keeper substance (trioctanoin) to reduce evaporation. Traps with disparlure dissolved in this keeper have been found to maintain their attractiveness for several months (Beroza *et al.*, 1971b; Beroza and Knippling, 1972).

The experiments were conducted in two neighboring forest areas, Reilingen and Schwetzingen. About 90% of the trees were European pines (*Pinus silvestris* L.), age 40–50 years, approximately evenly spaced, distance 5–10 m (Fig. 2). The rest were broad-leaf trees, *Tilia* and *Fagus*, of approx. the same age.

The traps were arranged in a nearly straight line. Distances from trap to trap were 20–25 m. To equalize the trapping chances for the different compounds, the cups were shifted in a carousel style daily; for example: day *x*, positions 1, 2, 3, 4 with compounds a, b, c, d, respectively; day *x* + 1, positions 1, 2, 3, 4 compounds b, c, d, a, etc. Since individual experiments lasted 8–12 days, the series of 5 or 6 traps rotated about twice by the positions. The sticky boards were stationary and only the cups with the attractants (and also one control cup with trioctanoin but no attractant) were shifted daily. In the experiments with the cups only, one control cup was placed at each position, 30 cm below the lure cup; these control cups were not shifted. The wicks with the lure compounds remained in the cups during each



Fig. 2. Experimental pine forest area. Arrows point at two sticky board. Distance between boards 25 m. Height of board above ground 2.5 m

experiment and were transferred to a new cup only in a few cases (when the adhesive surface was largely covered with moths and/or their scales). Captured moths were counted and removed daily from the sticky surfaces.

Temperatures during the experimental period ranged from 10–20° C in the early morning to 20–30° C in the early afternoon. Relative humidities often reached 90% after midnight and dropped to 50–60% in the early afternoon. The weather was typical for mid-summer in the Rhine valley with mostly sunshine, some overcast and occasional rain. Wind speeds were variable and from different directions.

## Results

### A. Relative Luring Effects of Different Epoxides

As judged from our captures, *L. monacha* was on the wing from the beginning of our trapping period (July 23), but *L. dispar* did not appear until August 1. The numbers of both species declined towards the end of August. We caught *L. monacha* only during the night, while *L. dispar* responded during the day and night. In New England, disparlure trapping of the gypsy moth has its maximum around midday, but is again increased during the first hour of darkness (Cardé *et al.*, 1973a). Since our experiments were not designed to analyze the daily rhythm of the moth, we are unable to judge whether the *L. dispar* population in the New World has a response rhythm different from that in Europe.

Cups + sticky boards gave catches nearly three times greater than cups only. The larger trapping surfaces of the board traps are therefore useful in tests with low-population densities.

Table 2. 20  $\mu\text{g}$  catches of *Lymantria monacha* (L.m.), July 23–30, 1973. Only one single *L. dispar* ♂ was caught during this period

Samples (shifted)	Exp. 1+2, cups + sticky boards		Exp. 3+4, cups only		Controls (stationary)
	L.m.	%	L.m.	%	
13	142	41	41	31	8
4	3	1	0		0
3	not tested		not tested		
10	12	3	1	1	1
Control (shifted)	2	1			
$\Sigma$	346		133		11

Table 3. 20  $\mu\text{g}$  catches of *Lymantria monacha* (L.m.) and *L. dispar* (L.d.); July 30–August 8, 1972

## a) Cups only (exp. 5)

Samples (shifted)	L.m.	%	L.d.	%	Controls (stationary)
1	76	84	34	81	1 <i>L. dispar</i>
13	1	1	6	14	1 <i>L. dispar</i>
4	0		0		0
3	14	15	2	5	0
10	0		0		0
$\Sigma$	91		42		

## b) Cups+sticky boards (exp. 6)

Samples (shifted)	L.m.	%	L.d.	%
1	221	68	72	86
13	31	10	8	9
4	0		0	
3	69	21	3	4
10	2	1	0	
Control (shifted)	0		1	1
$\Sigma$	323		84	

Table 4. 2  $\mu$ g catches of *Lymantria monacha* (L.m.) and *L. dispar* (L.d.)

a) Cups only; July 30–August 10, 1972 (exp. 7)					
Samples (shifted)	L.m.	%	L.d.	%	Controls (stationary)
1	22	64	21	80	1 <i>L. dispar</i>
13	4	12	3	12	0
4	2	6	1	4	0
3	5	15	1	4	1 <i>L. dispar</i>
10	1	3	0		0
$\Sigma$	34		26		

b) Cups+sticky boards; July 30–August 22, 1972 (exp. 8 and 9)					
Samples (shifted)	L.m.	%	L.d.	%	
1	111	60	74	70	
13	35	19	11	10	
4	19	10	6	6	
3	16	9	9	9	
10	3	2	5	5	
Control (shifted)	1	0	0	0	
$\Sigma$	185		105		

Tables 2–4 give an overview of all 9 experiments in which disparlure and some analogous epoxides were exposed. It should be noted that: 1. During the first week (exp. no. 1–4, Table 2) we caught 479 *L. monacha* ♂♂, but only one *L. dispar* ♂; the disparlure used was substance 1A (cf. Table 1); and no samples of substance 3 were exposed. 2. In the experiments no. 5–9 (Tables 3 and 4), we exposed disparlure (subst. 1) in parallel with all the other epoxides. 3. Because the control cups were stationary in the “cups-only” experiments (no. 3, 4, 5, 7), control catches are shown in the corresponding tables in a separate column. 4. In those experiments (no. 1, 2, 6, 8, 9) where the cups were exposed in the centre of the sticky boards and the control cups were members of the shifting (rotating) lure-cup series, data on control cups are listed in the corresponding tables below the lines giving data on the samples.

The obvious overall impression from these experiments is that disparlure is the most effective attractant, not only for *L. dispar* but also

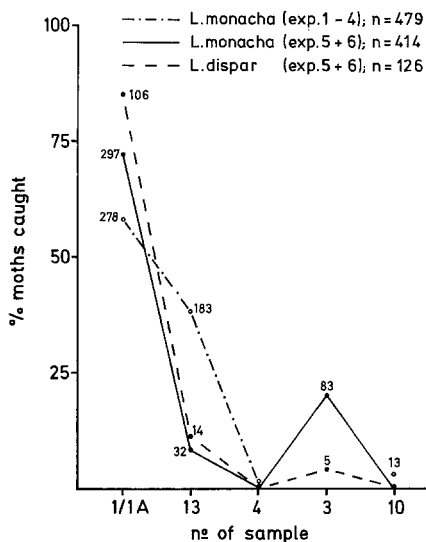


Fig. 3

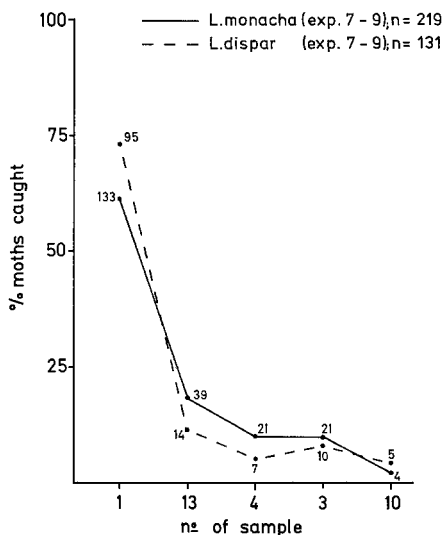


Fig. 4

Fig. 3. *Lymantria* captures with 20  $\mu$ g samples (exp. 1-6).  $n$  = total number of moths caught per curve. Note: with experiments 1-4, no sample of substance 3 was exposed and only one *L. dispar* male was caught

Fig. 4. *Lymantria* captures with 2  $\mu$ g samples (exp. 7-9)

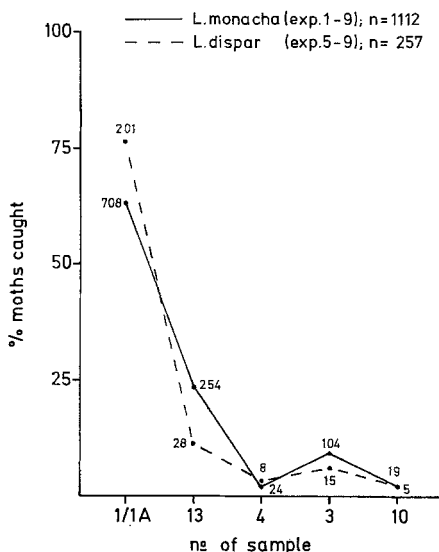
for *L. monacha*. Only in experiment no. 4 (included in Table 2) did substance 13 catch as many *L. monacha* as disparlure.

Since conditions differed in the various experiments, the results had to be separately summarized and plotted (Figs. 3-5). While the Figs. 3 and 4 permit a direct comparison of the species-specific relative effectiveness of a given compound, this is not so with Fig. 5. Here all the catches are summarized, including the experiments 1-4, where *L. dispar* was not present. This is particularly important with respect to substance 13 with its relatively high effect upon *L. monacha* (cf. exp. 1-4 with 5 and 6 in Fig. 3). It is therefore doubtful whether a judgement of the species-related effect of substance 13 may be based upon the summed data of Fig. 5.

The general trend of the 2  $\mu$ g experiments (Fig. 4) corresponds fairly well with the electrophysiologically determined effectiveness sequence, while the totalled data (Fig. 5) still show the somewhat controversial results of the 20  $\mu$ g catches.

In order to ensure the statistical significance of the *relative* catches, we have checked some of our data with the Wilcoxon test (cf. Documenta Geigy, 1968/9).



Fig. 5. Sum of all *Lymantria* captures (exp. 1-9)Table 5. The relative effectiveness of compound 3 vs. disparlure with *L. monacha* and *L. dispar* (exp. 5-9). Absolute numbers of catches

a) <i>Lymantria monacha</i>			b) <i>Lymantria dispar</i>		
	Compound 1	Compound 3		Compound 1	Compound 3
20 $\mu$ g	297	83	20 $\mu$ g	106	5
2 $\mu$ g	133	21	2 $\mu$ g	95	10

Table 6. Statistical treatment of moth captures

Exp. no.	Samples compared	Species	Wilcoxon figure
1-9	1/1 A vs. 13	L. m.	$2\alpha < 1\%$
5-9	1 vs. 13	L. m.	$2\alpha = 1\%$
1-4	1A vs. 13	L. m.	$5\% < 2\alpha < 10\%$
1-9	13 vs. 4	L. m.	$2\alpha < 1\%$
5-9	1 vs. 13	L. d.	$2\alpha < 1\%$
1, 2, 6, 8, 9	13 vs. contr.	L. m.	$2\alpha < 1\%$
1, 2, 6, 8, 9	4 vs. contr.	L. m.	$2\alpha = 10\%$
1, 2, 6, 8, 9	10 vs. contr.	L. m.	$2\alpha < 2\%$
3, 4, 5, 7	13 vs. contr.	L. m.	$2\alpha = 5\%$

These checks (Table 6) are based on the respective percentages of moths caught (Tables 2–4). In one case, differences in the absolute catches (Table 5) were evaluated by the  $\chi^2$ -test.

With *L. monacha*, the sum of all disparlure (subst. 1/1A) catches is different from all catches with substance 13 ( $2\alpha < 1\%$ ). The same is the case with experiments 5–9 alone. (Fig. 3), where disparlure (subst. 1) is likewise better than substance 13 to nearly the same degree ( $2\alpha = 1\%$ ). However, the significance of the difference between disparlure (subst. 1A) and substance 13 is much less (Fig. 3) in the experiments 1–4 alone ( $5\% < 2\alpha < 10\%$ ). Since with *L. monacha* substance 13 is the compound next most effective to disparlure, the other epoxides used as baits in the experiments are also significantly less effective than disparlure.

We now ask—still with respect to *L. monacha*—whether there are significant differences in effectiveness between any of the disparlure analogues used here. Lumping of all experiments (1–9) shows that substance 13 is a better bait than substance 4 ( $2\alpha < 1\%$ ). Aside from the *L. monacha* results, no other differences in effectiveness could be found with reasonable significance at or below the 10% level.

The *L. dispar* results are very clear (Figs. 3–5). Disparlure (exp. 5–9) is a better bait than substance 13 at the 1% level ( $2\alpha = 1\%$ ). Since the numbers of moths caught by the other disparlure analogues barely differ from those caught by substance 13, the other analogues also are significantly less effective than disparlure.

Are there significant differences between the *L. dispar* and *L. monacha* catches? We have compared the respective catches by the disparlure traps in experiments 5–9. It appears that the luring effect of disparlure—in relation to the other compounds used—is *not* significantly different for the two species:  $2\alpha > 10\%$ . Furthermore, none of the other epoxides is a significantly better attractant with either *L. monacha* or *L. dispar*, as long as the 2 and 20  $\mu\text{g}$  catches are lumped. But with substance 3 an interesting difference was observed between the relative effectiveness of the 20 and 2  $\mu\text{g}$  samples with *L. monacha* (cf. Fig. 3 with Fig. 4). In relation to disparlure, the 20  $\mu\text{g}$  sample of substance 3 is significantly more effective than the 2  $\mu\text{g}$  sample ( $P_{\chi^2} < 0.05$ , Table 5a). With *L. dispar*, such an effect is not apparent ( $P_{\chi^2} > 0.05$ , Table 5b).

Are the control catches significantly different from the catches with the chemicals? This is obviously true for disparlure vs. control with both species. But with the other epoxides a statistical treatment is necessary. Here we need to distinguish between the two types of control. It appears from experiments 10 and 11 (Table 7) that the “equal trapping chance” requirement for the control cups is only fulfilled in those cases where the controls shifted with the samples (exp. 3, 4, 6, 8, 9). In the other experiments the stationary control catches increase with the effectiveness of the sample mounted 30 cm above the control (see exp. 11, Table 7). The results of the significance tests for *L. monacha* are listed in Table 6.

Assessment of the control vs. sample catches in the experiments with stationary controls is necessarily different. However, even here it appears that with *L. monacha* substance 3 is different from the control at the 5% level. Since the remaining “cups-only” catches are very low, no difference between them and the control is apparent. Neither substance 3 nor the *L. dispar* experiments could be included in these statistical overall checks because the minimum number of experiments for the Wilcoxon test is four.

## B. Luring Effects of Increasing Disparlure Concentrations

In two experiments (Table 7) we checked the effectiveness of a series of traps with increasing amounts of disparlure (subst. 1A). Because the

Table 7. Concentration test: August 11–22, 1972

Subst. 1 A	Exp. 10, cups + stickyboards				Exp. 11, cups-only				Controls stationary
	L.m.	%	L.d.	%	L.m.	%	L.d.	%	
2 $\mu$ g	17	7	13	9	7	4	13	14	0
5 $\mu$ g	31	12	22	15	22	14	19	20	0
10 $\mu$ g	32	12	24	16	23	15	16	17	0
20 $\mu$ g	45	17	32	21	32	20	18	19	1 L.m.
100 $\mu$ g	133	50	60	40	74	47	28	30	4 L.m.
Control	0	0	0	0					
$\Sigma$	258		151		158		94		5 L.m.

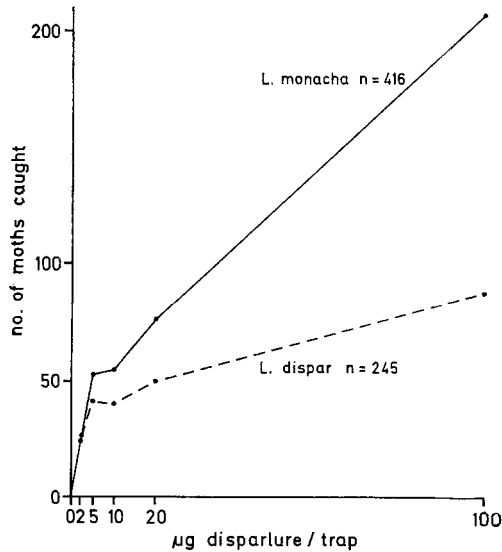


Fig. 6. *Lymantria* captures with different amounts of disparlure per trap. Experiments 10 and 11

solution of disparlure in the keeper was prepared at a fixed concentration in the laboratory and the odor sources had to be prepared in the field, it was necessary to use more than one cotton wick in the 5- and 10- $\mu$ g cups to provide the required disparlure concentration per trap. Therefore, the surface areas of these two odor sources were larger than the others and this might have resulted in higher evaporation rates. The two experiments are summarized in Fig. 6.

### C. Preliminary Comparison of Disparlure and Its Precursor as a Bait

In two experiments we compared the luring effect of the olefinic disparlure precursor (Bierl *et al.*, 1970, 1971, 1972)—substance p of Table 1—with disparlure (subst. 1A) and substance 13. In a third experiment, disparlure (subst. 1) was compared with two different concentrations of substance p.

The first two experiments were conducted in the Schwetzingen and Reilingen areas, respectively (August 11–23, 1973) and the final experiment at Possenhofen (Upper Bavaria) from September 2–9, 1972. In the first two experiments, with cups only, the 2 and 20  $\mu\text{g}$  sources of the epoxides were exposed as usual with the keeper on the cotton wicks. The olefin (2 and 20  $\mu\text{g}$ ), however, could only be exposed without the keeper for technical reasons. This means its evaporation must have been relatively high during the first days and then decreased faster than the epoxides. Because of this expectation, the olefin sources were renewed on the third day. In the Possenhofen experiment, disparlure (100  $\mu\text{g}$ ) and two rather high concentrations of the olefin (10 and 100 mg) were exposed with paraffin oil as keeper in cup traps with sticky boards.

In the first two experiments a total of 40 *L. monacha* and 9 *L. dispar* ♂♂ were trapped by the epoxides and none by the olefin. In the final experiment (in very late season), 8 *L. monacha* ♂♂ were caught with the disparlure traps only.

## Discussion

### A. Is Disparlure the Sexual Attractant of the Nun Moth?

In spite of earlier indications that disparlure may also be the sexual attractant of *L. monacha* (Schönherr, 1972), this pheromone has not yet been isolated from *L. monacha*. Our experiments were designed to determine whether disparlure or any of four related epoxides, which were shown to be relatively potent attractants for the gypsy moth, are possible candidates for the unknown sexual attractant of the nun moth.

Our results indicate that of the 5 epoxides tested (Table 1), disparlure is the most potent attractant for *L. dispar* as well as for *L. monacha*. Disparlure therefore continues to be the best candidate for the sexual attractant of the female nun moth. In addition, no significant differences in the relative luring power of the compounds for the two species were found.

The assessment of the relative effectiveness rating of the disparlure analogues (see also p. 22) is difficult because of the small number of experiments and the comparatively low capture rates. But compound 13 is clearly a better bait for *L. monacha* than compound 4. In addition, the low absolute numbers of moths caught with compounds 4, 3 and 10 make it—even with *L. monacha*—difficult to distinguish their effects from those of the controls. Undoubtedly tests with higher population densities of the moths and/or higher bait concentrations with the resulting higher catches would provide a more accurate evaluation of the disparlure analogues.

Since technical reasons forced us to limit the number of compounds in our field experiments to five, the possibility exists that another analogue of disparlure, not yet field-tested, might be a very effective bait, and therefore perhaps identical or related to the female attractant of *L. monacha*. However, extensive electrophysiological tests of the responses of the nun moth's antennal receptors to disparlure and to 50 related *cis*- and *trans*-epoxides (Kafka, Schneider, Beroza, unpubl.) support the assumption that disparlure is also the sexual attractant of *L. monacha*. An even larger electrophysiological test series with the same compounds on *L. dispar* (Schneider, Kafka, Beroza, unpubl.) gave results identical to the results with *L. monacha*. In this context it should also be remembered, that Priesner's electroantennogram cross-tests (see p. 20) showed that the lure glands of both species elicited equally large olfactory responses in the male antenna of either species.

While these recent observations favor the assumption that the attractants of *L. dispar* and of *L. monacha* are identical, two earlier papers of Görnitz (1949) and Schwinck (1955) appear to contradict this view. In field-trapping experiments, these authors found that the nun moth responded readily to the female gypsy moth (or its crude lure gland extracts), but found the male gypsy moth to be either unresponsive or less responsive to the nun moth or its glandular extract. These results led Görnitz (1949) to conclude that the sexual attractant of the gypsy moth is probably genus specific, while the attractant of the nun moth is species specific. But this explanation is not consistent with our electrophysiological observations that the receptor cells of homologous sensilla in both species respond identically to disparlure and all the other epoxides. The receptor cells in question belong to the long olfactory hairs and are known in the males of several moth species to respond to the respective female sexual attractant (Schneider *et al.*, 1964; Schneider and Steinbrecht, 1965; Kaissling and Priesner, 1970; Priesner, 1973). Here one needs to consider that the "one-attractant" concept, which actually stems from the observations with the silkmoth *Bombyx* (see Kaissling and Priesner, 1970; Kaissling, 1971; Schneider, 1971), might be too simple an interpretation of the situation with other moths (for further references see Priesner, 1973). Additional glandular products, which might have been overlooked in the past and which would stimulate other types of receptor cells on the male antenna, could play an as yet unknown role (cf. the olefin consideration, p. 23). Such compounds might either potentiate (e. g. with *L. dispar*) or inhibit (e. g. with *L. monacha*) the behavioral effect of the disparlure. Such an interplay of several chemical messengers does not need to occur on the same acceptor (receptor site) of the odor cell, or at a different acceptor of the same cell, but may arise by way of neuronal effects in the brain. Since with *Lymantria* nothing is known about such effects, this aspect of olfaction will require special attention in future studies.

Because the moth populations were expected to be low, we decided that in our trapping experiments we would rely not only on the customary cups, but also on the cups placed in the centre of a sticky board, because this type of trap is known to be more effective.

The placement of the control cups on the same tree trunk with the test cups (in the cups only experiments) was a mistake. These control cups caught more moths as the attraction of the nearby test cup increased (see data on "cups only" in Tables 2 and 7). Presumably, the male moths do not approach the traps directly but meet the control cup during their search for the attractive odor source. However, these questionable control catches do not influence our results.

With field experiments, one is always forced to accept the ecological conditions in a given biosystem. While the Rhinevalley sites were selected because they are relatively uniform and also well known to forest entomologists, critical information was lacking. It would certainly have facilitated the assessment of our results, had we known the population density of the moths, their exact daily rhythm, and the details of their orientation and mating behavior. Evidently, not only olfactory, but also visual stimuli play a role, at least with *L. dispar* (Doane, 1968; Doane *et al.*, 1973). So far, we may deduce from our catches that both species are mainly active at night (particularly the nun moth), that the population of the nun moth might have been up to 4 times greater than the gypsy moth population, and that the latter emerged later than the nun moths. But we are not certain that such deductions are admissible.

#### B. Moth Catches as a Function of Disparlure Concentration

Our catches (Table 7 and Fig. 6) compare well with larger scale trapping experiments with *L. dispar* (Beroza *et al.*, 1971 a). The concentration range of 2–100  $\mu\text{g}$  of disparlure per trap corresponds to the middle part of the 0.01–1000  $\mu\text{g}$  range in the earlier study (*l.c.*). The non-linear course of the curves is obvious.

While it would be interesting to compare the behavioral intensity-response curves with the corresponding electrophysiological responses of the olfactory organ, such a comparison needs to be done cautiously: identical, or at least comparable, stimulus conditions are the necessary requirement for this. Only in a few cases was this requirement rigidly fulfilled (see e.g. Kaissling and Priesner, 1970). With our field catches, any such comparison is particularly difficult because we neither know anything of the stimulus spreading nor do we have more than vague ideas about the olfactory orientation of the moths. The rather trivial statement that stronger stimuli probably extend the range of the supra threshold odor distribution farther (see Wilson and Bossert, 1963)

and necessarily lure more moths into the traps, does not help our understanding of the underlying mechanisms. Similar interpretatory difficulties have been observed with *L. dispar* when the luring effects of disparlure and some related compounds were compared with electroantennogram responses (Adler *et al.*, 1972).

The effectiveness of the disparlure analogues in relation to disparlure can be estimated from Fig. 6. The analogues caught between 0 and 30 percent of the number of moths caught with equal amounts of disparlure (Figs. 3-5). From Fig. 6 it can be seen that the analogues are comparable in effectiveness to 1/10 or a lesser amount of disparlure. Thus 20  $\mu\text{g}$  of substance 13 would—with *L. monacha*—be about as effective as 2  $\mu\text{g}$  of disparlure. The relative attraction of compound 13 and of the other analogues would be even less with *L. dispar*.

It is instructive in this connection to consider our recently determined electrophysiological data on disparlure versus the other epoxides. With *L. dispar* electroantennograms (EAG), based on the reference response to 1  $\mu\text{g}$  of disparlure per odor source, the amounts of the analogues needed to produce an equal EAG amplitude were as follows: substance 13, 15  $\mu\text{g}$ ; substance 4, 50  $\mu\text{g}$ ; substance 3, 50  $\mu\text{g}$ ; and substance 10, 80  $\mu\text{g}$ . For the single cell response, the corresponding amounts needed to produce an impulse rate equal to that with 1  $\mu\text{g}$  disparlure per odor source were 24  $\mu\text{g}$ , 50  $\mu\text{g}$ , 50  $\mu\text{g}$ , and 200  $\mu\text{g}$ . Similar ratings have been found with *L. monacha* (Schneider *et al.*, Kafka *et al.*, unpubl.). In general it can be said that our field data compare well with the laboratory data of the olfactory responses.

It should be added that none of the 45 other related epoxides was electrophysiologically more effective than any of the compounds used in our field experiments. The *trans* epoxides were particularly weak stimulants or were even ineffective in the range of reasonable odor concentrations. *Trans*-Disparlure as well as the olefin (subst. p, Table 1) was at least 1000 times less effective than disparlure.

### C. Is the Disparlure-Olefin Interaction in the Trapping Experiments a Natural or Artificial Phenomenon?

The presumed precursor of the sexual attractant of the gypsy moth differs from the final glandular product only at C-atoms 7 and 8: disparlure is a *cis*-7, 8-epoxide and the precursor is the *cis*-7-olefin (Bierl *et al.*, 1970, 1972). Three years before the identification of disparlure, Beroza (1967) observed masking effects when he compared different fractions of the lure gland extracts in field bioassays. Recently, this effect has been found to be due to the disparlure precursor (Cardé *et al.*, 1973 b). These authors also found that the olefin alone has a very low attractiveness for *L. dispar*.

With this background, our very preliminary experiments allow us to conclude tentatively that the olefin attracted neither *L. dispar* nor *L. monacha*. As to the masking effect, we have no judgement because our experiments were not designed to test this assumption.

In their short note, Cardé *et al.* (1973b) have not only considered the olefin to be a useful compound for interference with the chemical communication of the gypsy moth, but they also discuss the receptorial mechanisms of this behavioral inhibitor. They think it likely that the olefin competes with disparlure at the acceptor (receptor site) in the membrane of the receptor cell. While this interpretation would be reasonable under biochemical conditions *in vitro*, it is hardly compatible with the field situation in which only a few molecules will be "competing" with one another on any of the sensory hairs. Furthermore, our single cell recordings do not favor such an interpretation (Kafka *et al.*, Schneider *et al.*, unpubl.). When we tested the relative stimulatory power of disparlure, of 50 related epoxides, and of the olefin, we only found quantitatively different excitatory responses, but no inhibitory ones with the one cell type under observation. The olefin rated low (approx. 1000 times less effective than disparlure) but still higher than some of the other epoxides, particularly the weakly effective or ineffective *trans* compounds. Most important, disparlure-olefin mixtures (100:1 to 1:100) elicited normal EAGs and single-cell responses with no indication of an olfactory inhibition.

Since site competition in the sense of receptor blocking is so far not supported by our electrophysiological data, we propose another mechanism to explain the moth's behavior. Obviously the animal is able to discriminate disparlure from its precursor. Since the one cell type which we studied does not allow this discrimination, we might have either overlooked another cell type that responds only to disparlure (or only to the olefin), or we did not see cell types that have a disparlure/olefin response rate different from the one observed to date. The EAGs are simultaneous responses of many cells and would be in agreement with either interpretation. Whether either proposed mechanism suffices to allow the postulated discrimination remains to be seen. The behavioral situation definitely is complex because the olefin not only inhibits the attractant, but also shows a weak attractive effect when exposed alone (Cardé *et al.*, 1973b).

The interesting thought that the female moth might use the precursor as a signal to escape from disturbing males after her first and only mating has found no experimental support (Cardé *et al.*, 1973b).

Evidence presently available does not support the belief that the olefin naturally plays a role as a communicative signal in the orientation of the gypsy and nun moth.

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