Inhibitors of Sexual Attraction in the Moth *Agrotis exclamationis*

E. Priesner

Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen

Z. Naturforsch. **40c**, 943–945 (1985); received August 26, 1985

Pheromones, Attraction-Inhibitors, Olfactory Receptors, *Agrotis exclamationis*, Noctuidae

The sex-attractant system of the dart moth *Agrotis exclamationis* (L.) (Noctuidae) was re-investigated with electrophysiological and field trapping tests. The identified pheromone components (*Z*)-5-tetradecenyl acetate and (*Z*)-9-tetradecenyl acetate elicited maximum trap captures when combined in a *Z*5-/*Z*9-mixture ratio of between 100/10 and 100/20, in contrast to an earlier reported mixture optimum of 100/5. Each compound activated a particular type of receptor cell located in the male antennal hair sensilla. Three further cell types discovered in these sensilla responded specifically to the non-pheromonal compounds (*Z*)-7-dodecenyl acetate and (*Z*)-7- and (*Z*)-11-tetradecenyl acetate. These latter compounds did not show attractive or synergistic properties in field trapping tests but rather reduced captures when added to the binary pheromone blend as a third component. The biological functions of these three "attraction-inhibitors" remain unidentified.

The female sex pheromone of the dart moth *Agrotis (Scotia) exclamationis* (L.) (Noctuidae: Agrotinae) was identified by Bestmann *et al.* [1] as a binary combination of (*Z*)-5-tetradecenyl acetate and (*Z*)-9-tetradecenyl acetate in a female-produced ratio of *Z*5-14:Ac / *Z*9-14:Ac of 93/7. Their field trapping data showed that the single chemicals were ineffective in capturing male *A. exclamationis* moths, which were maximally attracted to traps containing a 95/5 blend of the two chemicals [1]. Vrkoč *et al.*'s and Löfstedt *et al.*'s independent studies [2, 3] confirmed the chemical identity of the two primary pheromone components and indicated an average *Z*5-/*Z*9-14:Ac ratio of approx. 80/20 for the gland extracts investigated. Vrkoč *et al.* also found evidence for a more complex pheromone blend based on the observation that dodecenyl acetate and hexadecenyl acetate fractions of the gland extracts when added to the two synthetic tetradecenyl acetates increased flight activity of *A. exclamationis* males in a wind tunnel [2].

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen 0341–0382/85/1100–0943 $ 01.30/0

This study reports effects of three other alkenyl acetates, *viz.* (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac) and (*Z*)-7- and (*Z*)-11-tetradecenyl acetate (*Z*7-14:Ac and *Z*11-14:Ac), on the sex-attractant response of this moth species. Each compound was found to activate its own, specialized type of sensory receptor cell located in the long hair sensilla (S. trichodea) on the male antenna. In field trapping tests each compound greatly reduced captures when added to the synthetic binary pheromone as a third component.

Nerve impulse responses of single receptor cells were monitored from the cut ends of individual hair sensilla using technical procedures and sets of test chemicals as in previous work on other noctuid species (see [4] and references therein). A total of five different cell types were discovered. Response spectra to synthetic test chemicals [4–8] showed that these were specialist receptor cells for *Z*5-14:Ac and *Z*9-14:Ac (the two reported sex pheromone components), and *Z*7-12:Ac, *Z*7-14:Ac and *Z*11-14:Ac. Particular efforts were made to detect further cell types (specific to other, mono- or di-unsaturated acetates, or to an olefinic alcohol or aldehyde). No other type of sensory cell was found. The combination of olfactory receptor cells specialized for *Z*7-12:Ac, *Z*5-14:Ac, *Z*7-14:Ac, *Z*9-14:Ac and *Z*11-14:Ac has recently been reported [9] in the pheromone receptor systems of some other noctuid moths, including six species of Agrotinae.

Field trapping tests on the five compounds and related chemicals were conducted in southern Germany near Starnberg and Seewiesen, south-west of Munich. Trap types, odour dispensers and trap placement procedures were similar to those used in studies on other noctuid moth in the same experimental area [4, 10]. Each test series was conducted in 4 to 6 concurrent replications and the data submitted to analysis of variance followed by statistical treatment (Duncan's multiple range test).

Trapping results from the 1978 and 1979 field seasons showed that the local *A. exclamationis* population was maximally responsive to binary *Z*5-/*Z*9-14:Ac combinations in ratios of 100/10 and 100/20. Captures did not differ between these two lures but were significantly lower ($P = 0.05$) for the 100/5 and 100/30 mixtures of the two chemicals. The 100/3 and 100/100 mixtures were even less attractive, and ratio mixtures such as 100/1 and 30/100 did not catch any *A. exclamationis* male (Table I). This pat-

Table I. Trap captures of male *Agrotis exclamationis* moths in response to different combinations of five "receptor key compounds". Summary of results obtained at Starnberg/Seewiesen, 1978–1984.

Amount of chemical [µg/trap]					Catch rate*
*Z*5-14:Ac	*Z*9-14:Ac	*Z*7-12:Ac	*Z*7-14:Ac	*Z*11-14:Ac	
10	100	–	–	–	0
30	100	–	–	–	0
100	100	–	–	–	+
100	50	–	–	–	++
100	30	–	–	–	++
100	20	–	–	–	+++
100	10	–	–	–	+++
100	5	–	–	–	++
100	3	–	–	–	+
100	1	–	–	–	0
100	0.3	–	–	–	0
100	15	0.1	–	–	+++
100	15	0.3	–	–	+++
100	15	1	–	–	+
100	15	3	–	–	0
100	15	10	–	–	0
100	15	–	0.1	–	+++
100	15	–	0.3	–	+++
100	15	–	1	–	++
100	15	–	3	–	+
100	15	–	10	–	0
100	15	–	30	–	0
100	15	–	–	1	+++
100	15	–	–	3	+++
100	15	–	–	10	+
100	15	–	–	30	0
100	15	–	–	100	0

* Capture rates indicated by different symbols are significantly different at P = 0.05 according to Duncan's multiple range test.
+++, ++, +, maximum to low catch; 0, no catch.

tern of response specificity appears to differ from that reported by Bestmann *et al.* [1] who obtained the maximum trap catch with a *Z*5-/*Z*9-14:Ac mixture ratio of 95/5.

Traps containing binary compound combinations other than *Z*5-/*Z*9-14:Ac never caught any *A. exclamationis* male. The effects of the *Z*7-12:Ac, *Z*7-14:Ac and *Z*11-14:Ac ("key compounds" for further cell types disclosed in the receptor recordings) were accordingly studied as additions to the binary synthetic pheromone. The *Z*5-14:Ac + *Z*9-14:Ac mixture of 100 + 15 µg (intermediate between the two maximally effective test ratios of 100/10 and 100/20) was used in all third-component tests considered here. Each of the three chemicals was added to this basic lure in eight different amounts, ranging from 0.03 µg to 100 µg. Traps baited with the 100/15 binary lure alone were included as the reference in all test series.

Although strong modifying effects were evident from the first test series for all three chemicals, accumulation of trap capture data over five successive years was required to fully quantify these effects for the 24 different three-component mixtures. The results are summarized in Table I.

Addition of the third chemical never increased captures over those obtained with the basic lure alone. Additions of *Z*7-12:Ac or *Z*7-14:Ac lower than 1 µg, or *Z*11-14:Ac lower than 10 µg, had no significant effect (P = 0.05). Larger amounts of these chemicals reduced captures, although the amounts required to abolish any catch differed approx. 10-fold between the three compounds, from 3 µg for *Z*7-12:Ac to 30 µg for *Z*11-14:Ac (Table I). These data classified all three chemicals as "inhibitors" of the sex-attractant response of male *A. exclamationis*.

Various other, mono- and di-unsaturated acetates, and also some alcohol and aldehyde analogues, were

tested in an analogous manner. Additions of up to 100 µg of these did not significantly increase or strongly decrease captures. This held for further (*Z*)-monoenic acetates such as *Z*5-10:Ac, *Z*5- and *Z*9-12:Ac, and *Z*5-, *Z*7-, *Z*9- and *Z*11-16:Ac; various (*E*)-monoenic acetates; and the alcohol and aldehyde analogues corresponding to the five "key compounds".

The absence of an increase in captures on addition of a third chemical compound to the two primary pheromone constituents is interesting with respect to recent claims of minor (trace) components involved in the *A. exclamationis* sex pheromone blend [2]. Alcohols and aldehydes are unlikely candidates as minor blend constituents in this species considering that no receptor cell types responsive to these classes of compounds were found and these chemicals showed no modifying effects in field tests. The same holds for 10- or 16-carbon acetates. In the dodecenyl acetate series only the (*Z*)-7 isomer had a corresponding specialist receptor cell. Additions of this compound greater than 0.3% of the major pheromone constituent (*Z*5-14:Ac) were "inhibitory" to trapping responses, as shown above. The present result cannot, however, exclude possible secondary pheromonal functions (not necessarily reflected by trap capture rates) of the *Z*7-12:Ac at even lower amounts. A search for the two other "inhibitors", *Z*7-14:Ac and *Z*11-14:Ac, in gland extracts showed that these were absent even at trace amounts [11].

During the course of the present study, traps baited with binary combinations of *Z*5-14:Ac / *Z*9-14:Ac in ratios of between 100/5 and 100/30 were operated for six consecutive years in the test area throughout the *A. exclamationis* flight period, early June to mid July. Other noctuid moths comprised less than 2% of captures during this time. Another Agrotinae species, *Xestia rhomboidea* (Esp.), was captured by these traps in large numbers later in the year. Its flight period in the test area usually lasted from early to late August, separated from the *A. exclamationis* flight period by 1–2 weeks. Thus, the functional interpretation of the three "inhibitors" in the *A. exclamationis* sex-attractant system as maintaining reproductive isolation from other, pheromonally-related species occurring in the same habitats during the same time cannot, as yet, be substantiated.

A full account of the electrophysiological part of this study will be given elsewhere.

Acknowledgements

I thank Drs. H. Arn (Wädenswil), C. Descoins (Brouessy) and S. Voerman (Wageningen) for supply of test chemicals; P. Witzgall (Seewiesen) for his help in statistical analysis of trapping data; C. Löfstedt (Lund) for communicating prepublication results [3, 11]; and D. Schneider and L. Gardiner (Seewiesen) for their critical comments on the manuscript.

Note added in proof: While this paper was in press, C. Löfstedt acquainted me with work conducted on the pheromone receptor system of male *A. exclamationis* at the Department of Ecology of the University of Lund, Sweden. Their results [12] are in full agreement with those presented here.

[1] H. J. Bestmann, T. Brosche, K. H. Koschatzky, K. Michaelis, H. Platz, O. Vostrowsky, and W. Knauf, Tetrahedron Lett. **21,** 747 (1980).
[2] J. Vrkoč, V. P. Konyukhov, and B. G. Kovalev, Acta entomol. bohemoslov. **80,** 184 (1983).
[3] C. Löfstedt *et al.*, in preparation.
[4] E. Priesner, Z. Naturforsch. **39c,** 845 (1984).
[5] E. Priesner, in: Chemical Ecology: Odour Communication in Animals (F. J. Ritter, ed.), p. 57, Elsevier, Amsterdam 1979.
[6] E. Priesner, in: Insect Neurobiology and Insecticide Action (Neurotox 79), p. 359, Soc. Chem. Ind., London 1980.
[7] E. Priesner, Z. Naturforsch. **38c,** 874 (1983).
[8] E. Priesner, in: Mechanisms in Insect Olfaction (T. L. Payne, M. C. Birch, and C. E. J. Kennedy, eds.), p. 225, Oxford University Press, Oxford 1985.
[9] E. Priesner, Z. Naturforsch. **40c,** 939 (1985).
[10] E. Priesner, Z. Naturforsch. **35c,** 990 (1980).
[11] C. Löfstedt, personal communication.
[12] B. S. Hansson, E. Hallberg, C. Löfstedt, and J. Löfqvist, Naturwissenschaften, submitted.

Verantwortlich für den Inhalt: A. KLEMM
Satz und Druck: Allgäuer Zeitungsverlag GmbH, Kempten