Specificity of Sexual Attractants in *Xestia triangulum* Hufn. and *X. ditrapezium* Schiff. (Lepidoptera: Noctuidae)

E. Priesner

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

Z. Naturforsch. **40c**, 939–942 (1985); received August 7, 1985

Pheromones, Sex Attractants, Attraction-Inhibitors, Olfactory Receptors, *Xestia,* Noctuidae

Electrophysiological analysis of male antennal Sensilla trichodea in *Xestia triangulum* and *X. ditrapezium* showed that they contain the same five types of receptor cells in both species. These cells responded specifically to the (*Z*)-alkenyl acetates *Z*7-12:Ac, *Z*5-14:Ac, *Z*7-14:Ac, *Z*9-14:Ac and *Z*11-14:Ac. Yet, in field trapping tests, male *X. triangulum* were highly attracted to pure *Z*5-14:Ac as a single compound, whereas male *X. ditrapezium* responded specifically to a ternary combination of *Z*5- / *Z*7- / *Z*9-14:Ac with the *Z*7 isomer as the major constituent. The four other "key compounds" in *X. triangulum,* and the other two in *X. ditrapezium,* inhibited trap captures. The results are considered in view of the close taxonomic relationship of the two species and their potential reproductive isolation from other, pheromonally-related noctuid moths.

This study reports on synthetic sex-attractants and their specific inhibitors for two Noctuidae: Agrotinae species, the double square-spot moth *Xestia (Amathes, Rhyacia) triangulum* (Hufn.) and its taxonomic sibling *X. ditrapezium* (Schiff.). The attractant systems were characterized by combining sensory (electrophysiological) and behavioural (field trapping) data. The electrophysiological results were obtained from a comparative survey of pheromone receptor systems in European species of the subfamily Agrotinae. Field trapping experiments were carried out on the two noctuid species to disclose behavioural functions associated with the "key compounds" of the different cell types found in the receptor recordings. The results show that the two species use pheromone receptor systems composed of similar sets of sensory receptor cells. However, there are basic differences in the composition of their specific sexual attractants, the one being pure (*Z*)-5-tetradecenyl acetate and the other a three-component blend with (*Z*)-7-tetradecenyl acetate as its major constituent.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/1100–0939 $ 01.30/0

The nerve impulse responses of individual receptor cells were recorded from the cut ends of Sensilla trichodea on male antennae of *X. triangulum* and *X. ditrapezium.* Technical procedures were as in other noctuid moth studies [1, 2]. The test chemicals included the usual series of mono- and di-unsaturated acetates, alcohols, and aldehydes. The same set of cell types as characterized by maximally stimulatory compounds were present in both species. Analysis of response spectra to test chemicals [1–5] showed that these were specialist receptor cells for (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac) and (*Z*)-5-, (*Z*)-7-, (*Z*)-9- and (*Z*)-11-tetradecenyl acetate (*Z*5-14:Ac, *Z*7-14:Ac, *Z*9-14:Ac and *Z*11-14:Ac). No additional cell types were found. The type A cell in the receptor system (showing the largest nerve impulse amplitude in the extracellular recordings) responded to *Z*5-14:Ac in *X. triangulum* but to *Z*7-14:Ac in *X. ditrapezium.*

Field trapping experiments were carried out in woodland near Starnberg and Seewiesen, south-west of Munich (southern Germany). The flight period of *X. triangulum* in this area usually lasts from mid June until early August and that of *X. ditrapezium* from early June until late July. Traps, odour dispensers and trap placement procedures were as in field work on other noctuid moths [2, 6]. A test series consisted of between 8 to 18 traps each baited with a different chemical formulation, deployed in 3 or 4 concurrent replications. The experiments took place from early June until early August, 1979 to 1984.

Pure *Z*5-14:Ac on its own was a highly potent attractant for male *X. triangulum.* There was no increase in trap captures for 100 µg sources of *Z*5-14:Ac when small amounts of *Z*7-12:Ac, *Z*7-14:Ac, *Z*9-14:Ac or *Z*11-14:Ac (the "key compounds" of the other four cell types) were added. However, these chemicals at higher doses strongly reduced captures (Table I). *X. triangulum* thus appears to use a single-component sexual attractant counteracted by four different "attraction-inhibitors", each perceived by a specific sensory cell.

In contrast, male *X. ditrapezium* were only captured with ternary combinations of *Z*5-14:Ac / *Z*7-14:Ac / *Z*9-14:Ac, where the *Z*7 isomer was the major constituent. Maximum captures occurred with mixtures of these three positional isomers in ratios of 10/100/10, 10/100/20 and 10/100/30. Captures were not significantly different for these three but were lower ($P = 0.05$) for the mixture ratios 3/100/10, 3/100/30,

Table I. Captures of male *Xestia triangulum* in tetratraps baited with synthetic sex-attractant (*Z*5-tetradecenyl acetate) and four different attraction-inhibitors (*Z*7-dodecenyl acetate and *Z*7-, *Z*9- and *Z*11-tetradecenyl acetate). Seewiesen, 1980–1984.

Amount [μg/trap] of					$\overline{X}$ males/trap in test series*				
*Z*7-12:Ac	*Z*5-14:Ac	*Z*7-14:Ac	*Z*9-14:Ac	*Z*11-14:Ac	I (1980)	II (1981)	III (1982)	IV (1983)	V (1984)
–	100	–	–	–	23.33	17.75	27.5	19.5	12.0
0.1	100	–	–	–	–	–	–	15.75	16.75
0.3	100	–	–	–	–	19.0	–	18.0	10.25
1	100	–	–	–	6.33	1.25	4.5	2.0	2.75
3	100	–	–	–	–	0	0.25	0	0
10	100	–	–	–	0	0	–	–	–
30	100	–	–	–	–	0	–	–	–
100	100	–	–	–	0	–	–	–	–
–	100	0.1	–	–	–	–	–	11.75	10.5
–	100	0.3	–	–	–	4.25	–	3.5	3.5
–	100	1	–	–	1.0	0	0	0	0.75
–	100	3	–	–	–	0	0	0	0
–	100	10	–	–	0	0	–	–	–
–	100	30	–	–	–	0	–	–	–
–	100	100	–	–	0	–	–	–	–
–	100	–	0.1	–	–	–	–	5.25	6.0
–	100	–	0.3	–	–	4.0	–	2.0	0.25
–	100	–	1	–	2.66	0	3.0	0	0.5
–	100	–	3	–	–	0	0	0	0
–	100	–	10	–	0	0	–	–	–
–	100	–	30	–	–	0	–	–	–
–	100	–	100	–	0	–	–	–	–
–	100	–	–	0.3	–	–	30.0	–	11.25
–	100	–	–	1	21.0	–	23.25	20.75	14.0
–	100	–	–	3	–	–	11.75	12.0	5.0
–	100	–	–	10	3.66	–	2.5	4.5	5.75
–	100	–	–	30	–	–	0.25	0	0.25
–	100	–	–	100	0	–	0	0	–

* Each series exposed from mid June to late July in 3 (1980) or 4 (1981–1984) concurrent replications.

30/100/10, 30/100/30, 10/100/3 and 10/100/100 (Table II). No *X. ditrapezium* males were caught with two-component mixtures or one chemical alone.

The effects of *Z*7-12:Ac and *Z*11-14:Ac ("key compounds" for further cell types) on the sex-attractant response of male *X. ditrapezium* were studied by adding them separately to the 10/100/20 combination of *Z*5-14:Ac / *Z*7-14:Ac / *Z*9-14:Ac. They did not improve trap captures in any test combination; an addition of more than 3% actually reduced catches (Table III). Thus both *Z*7-12:Ac and *Z*11-14:Ac are "inhibitors" in the *X. ditrapezium* sex-attractant system.

Various other chemicals were tested in an analogous manner on both *X. triangulum* and *X. ditrapezium*. These included other (*Z*)-alkenyl acetates known as lepidopterous pheromone and sex-attractant components (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), and the (*E*)-isomers and alcohol and aldehyde analogues corresponding to the five acetate "key compounds". Pure *Z*5-14:Ac and the 10/100/20 mixture of *Z*5-14:Ac / *Z*7-14:Ac / *Z*9-14:Ac were again the reference lures. Additions of 1% or 10% of the test chemicals did not markedly modify capture rates.

The female sex pheromones of *X. triangulum* and *X. ditrapezium* are as yet chemically unidentified. The high potency and specificity of the synthetic sex-attractants reported here make them likely candidates for the natural pheromones of these species. All three compounds, *Z*5-14:Ac, *Z*7-14:Ac and *Z*9-14:Ac, are common Agrotinae pheromone and sex-attractant components [7, 8]. Attraction of males to sources of pure *Z*5-14:Ac has already been noted

Table II. Captures of male *Xestia ditrapezium* in tetratraps baited with three sex-attractant components (*Z*5-, *Z*7- and *Z*9-tetradecenyl acetate) in varying combinations. Seewiesen, 1980/81.

Amount [μg/trap] of			$\bar{X}$ males/trap in test series*	
*Z*5-14:Ac	*Z*7-14:Ac	*Z*9-14:Ac	I (1980)	II (1981)
10	100	–	0	0
–	100	10	0	0
1	100	1	–	0
10	100	1	7.0	4.75
1	100	10	0	1.5
10	100	3	12.66	–
3	100	10	–	5.5
10	100	10	24.0	17.25
30	100	10	–	7.75
10	100	30	–	15.0
100	100	10	1.66	2.0
10	100	100	7.66	6.5
100	100	100	0	0

* Each series exposed from early June until late July in 3 (1980) or 4 (1981) replications.

Table III. Captures of male *Xestia ditrapezium* in tetratraps baited with a 10/100/20 μg mixture of *Z*5-/*Z*7-/*Z*9-tetradecenyl acetate as the basic lure and *Z*7-dodecenyl acetate or *Z*11-tetradecenyl acetate as fourth components. Seewiesen, 1982–1984.

Amount [μg] of added		$\bar{X}$ males/trap in test series*		
*Z*7-12:Ac	*Z*11-14:Ac	III (1982)	IV (1983)	V (1984)
–	–	20.0	13.75	11.0
0.1	–	–	15.5	8.25
0.3	–	–	11.25	8.0
1	–	9.5	7.5	2.75
3	–	–	1.0	0
10	–	0	0	0
30	–	–	0	0
100	–	0	0	–
–	0.1	–	16.75	7.5
–	0.3	–	12.0	12.0
–	1	22.5	16.0	10.75
–	3	–	10.5	5.5
–	10	2.0	4.0	1.75
–	30	–	0	0
–	100	0	0	–

* Each series exposed from early June until late July in 4 replications.

for the New World *Agrotis obliqua* (Smith) [7]. The 100/1/10 combination of *Z*5-14:Ac / *Z*7-14:Ac / *Z*9-14:Ac is the reported optimized sex-attractant for male *Euxoa auxiliaris* (Grote) in Canada [9]. However, synergistic combinations of these three chemicals, which have the *Z*7 isomer as their major constituent, were not known as lepidopteran sex-attractants until now. Also, the occurrence in the same moth species of specialist receptor cells for four different positional isomers is reported here for the first time.

Surprisingly few males of other noctuid species were found in the traps baited with pure *Z*5-14:Ac or the 10/100/10 to 10/100/30 combinations of *Z*5-14:Ac / *Z*7-14:Ac / *Z*9-14:Ac during the flight period of the two *Xestia* species. This is particularly noteworthy since a number of the more common noctuid species active in the test area at this time of year use sexual attractants based on either *Z*5-14:Ac or *Z*7-14:Ac as the major constituent. Comparative analyses of sex-attractant systems indicate that reproductive isolation from the two *Xestia* species is maintained mainly by the contrasting effects of minor attractant components, which act as essential attraction-synergists in one species but as attraction-inhibitors in the other. Illustrative examples are provided by the heart-and-dart moth *Agrotis exclamationis* (L.) (Agrotinae) and the marbled white-spot moth *Lithacodia pygarga* (Hufn.) (= *fasciana* auct.) (Jaspidiinae). Both noctuids use pheromone receptor systems composed of specialist cells for *Z*7-12:Ac, *Z*5-14:Ac, *Z*7-14:Ac, *Z*9-14:Ac and *Z*11-14:Ac [10] (the same as found in *X. triangulum* and *X. ditrapezium*). The female sex pheromone of *A. exclamationis* is a 9/1 combination of *Z*5-14:Ac / *Z*9-14:Ac [11, 12] to which males are highly attracted in the field [11-13]. No male *A. exclamationis* were captured in traps baited with a single chemical. Reproductive isolation from *X. triangulum* is thus apparently maintained through the contrasting effects of the *Z*9-14:Ac, which is an essential attractant component for *A. exclamationis* but a strong inhibitor for *X. triangulum*. The female sex pheromone of *L. pygarga* is as yet chemically unknown but field trapping tests have shown [10] that the 10/100 combination of *Z*5-14:Ac / *Z*7-14:Ac is a highly potent and specific attractant for the males. The addition of *Z*9-14:Ac strongly reduced captures of male *L. pygarga* which were thus totally absent from traps baited with the ternary *ditrapezium* lure [10].

This situation changed after the flight periods of the two *Xestia* species when two other Agrotinae moths were caught in large numbers in traps baited with the synthetic *triangulum* and *ditrapezium* lures.

These were the lesser yellow underwing *Noctua comes* (Hbn.) and the barred chestnut moth *Diarsia dahlii* (Hbn.). Male *N. comes* responded strongly to pure *Z*5-14:Ac but were inhibited by the addition of *Z*7-12:Ac, *Z*7-14:Ac or *Z*9-14:Ac [10]. The same overall pattern of attraction specificity was found in *X. triangulum*. Male *A. comes* flights to sex-attractant sources in the Seewiesen test area usually started in mid August, after summer aestivation, and were separated from the *X. triangulum* flight period by at least 1 week, with no overlap. Traps baited with the ternary *ditrapezium* mixture began to catch many *D. dahlii* males in mid August. This noctuid species occurs only locally in Central Europe but is quite common in the moorlands surrounding Seewiesen. Analysis of the *D. dahlii* sex-attractant system [10] showed that this species uses the same five types of receptor cells and is maximally attracted to the same mixture ratio of *Z*5-14:Ac / *Z*7-14:Ac / *Z*9-14:Ac as is *X. ditrapezium*. This indicates a remarkable convergence in sex-attractant responses between species of different noctuid genera. The results provide additional evidence for the hypothesis that noctuid species with separate seasonal flight periods may share a common sex-attractant signal (see [13]).

The combination of specialist receptor cells for *Z*7-12:Ac, *Z*5-14:Ac, *Z*7-14:Ac, *Z*9-14:Ac and *Z*11-14:Ac, found in *Xestia triangulum* and *X. ditrapezium*, also occurred in further species of the genus studied electrophysiologically [10]. Of Central European species this included *X. rhomboidea* (Esp.), *X. ashworthii* (Doubl.) and the spotted cutworm *X. c-nigrum* (L.). Again, in field trapping tests, each species responded to its particular attractant blend: the *rhomboidea* males to a 100/5 mixture of *Z*5-14:Ac / *Z*9-14:Ac [13] and the *ashworthii* and *c-nigrum* males to 10/100 and 1/100 blends of *Z*5-14:Ac / *Z*7-14:Ac [10, 14]. Notwithstanding the homogeneity of the male's sensory repertoires, the behavioural data thus point to a broad diversity in the composition of female sex pheromones among species of this moth genus.

[1] E. Priesner, in: Chemical Ecology: Odour Communication in Animals (F. J. Ritter, ed.), p. 57, Elsevier, Amsterdam 1979.
[2] E. Priesner, Z. Naturforsch. **39c,** 845 (1984), and references therein.
[3] E. Priesner, Ann. Zool. Ecol. anim. **11,** 533 (1979).
[4] E. Priesner, in: Insect Neurobiology and Insecticide Action (Neurotox 79), p. 359, Soc. Chem. Ind., London 1980.
[5] E. Priesner, Z. Naturforsch. **38c,** 874 (1983).
[6] E. Priesner, Z. Naturforsch. **35c,** 990 (1980).
[7] W. Steck, E. W. Underhill, and M. D. Chisholm, J. Chem. Ecol. **8,** 731 (1982).
[8] Y. Tamaki, in: Handbook of Naturally Occurring Pesticides, **Vol. III** (Insect Sensory Substances), CRC Press, Boca Raton, Florida 1985.
[9] D. L. Struble, Environ. Entomol. **10,** 167 (1981).
[10] E. Priesner, unpublished results.
[11] H. J. Bestmann, T. Brosche, K. H. Koschatzky, K. Michaelis, H. Platz, O. Vostrowsky, and W. Knauf, Tetrahedron Lett. **21,** 747 (1980).
[12] J. Vrkoč, V. P. Konyukhov, and B. G. Kovalev, Acta entomol. bohemoslov. **80,** 184 (1983).
[13] E. Priesner, Z. Naturforsch. **40c,** 943 (1985).
[14] W. Steck, E. W. Underhill, M. D. Chisholm, and B. K. Bailey, Ent. exp. appl. **31,** 328 (1982).