Sex Attractant System in *Polia pisi* L. (Lepidoptera: Noctuidae)

Ernst Priesner

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

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Electrophysiological analysis of olfactory hair sensilla in male *P. pisi* has revealed four different types of presumed pheromone receptor cells, maximally responsive to (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac), (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac), (*Z*)-11-hexadecenyl acetate (*Z*11-14:Ac) and (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac), respectively. These four compounds were tested, singly and in various combinations, for efficacy in attracting *P. pisi* males in the field. High trap catches were obtained with mixtures of *Z*11-14:Ac/*Z*9-14:Ac in the ratio 100/100, whereas the 100/30 and 30/100 mixtures of the two compounds were only slightly attractive. No male *P.* pisi were captured by single chemicals or binary combinations of *Z*11-14:Ac/*Z*11-16:Ac, *Z*11-14:Ac/*Z*7-12:Ac, *Z*9-14:Ac/*Z*11-16:Ac, *Z*9-14:Ac/*Z*7-12:Ac, or *Z*11-16:Ac/*Z*7-12:Ac. Various compounds, including *Z*11-16:Ac and *Z*7-12:Ac, were tried as third chemicals in addition to 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac but none increased trap catches over the basic lure.

Effective sex attractant lures for male Lepidoptera have in some cases been established by single receptor analysis combined with field trapping studies. This approach is based upon a systematic survey of the different types of receptor cells present in the trichoid hair sensilla (generally known to contain the pheromone-sensitive cells in male moths), followed by field trials of the "key compounds" of these receptors for their optimally attractive combination(s). The method has prooved particularly successful with some Noctuidae and Tortricidae test spp. (see [1–5]) and is applied in this study to the broom moth *Polia* (*Ceramica*) *pisi* L. (Noct., Hadeninae), a minor pest of agricultural crops in Europe and Asia.

Electrophysiology

Earlier electroantennogram (EAG) studies in this species have shown [6, 7] that (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac) is the most stimulatory compound in the series of mono-unsaturated acetates, alcohols, and aldehydes. This compound, a common pheromone constituent in noctuid moths, was thus considered to be a possible sex pheromone component in *P. pisi.* Further information resulted from the responses of single receptor cells. As in previous studies with other noctuid species [1–4], nerve impulse activity was monitored via the cut end of a male hair sensillum (*S. trichodeum*), a technique which maintains the responsiveness of individual receptor cells for up to several hours. A total of 200 synthetic compounds (acetates, alcohols, aldehydes) were tested. These were derived from known noctuid pheromones by systematic alteration in chain length and the olefinic double bond system and thus defined the maximally effective ("key") compounds for the different types of receptor cells present in these sensilla.

Results of these studies indicate four different cell types in *P. pisi.* The A cells (largest nerve impulse amplitude) responded maximally to (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac); the B cells, to *Z*9-14:Ac (the compound maximally effective in the EAG measurements, mentioned above); the C cells, to (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac); and the D cells, to (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac). No evidence for a receptor cell sensitive to an alcohol or an aldehyde component was obtained. Specialist cells for *Z*11-14:Ac, *Z*9-14:Ac, *Z*11-16:Ac, or *Z*7-12:Ac are known to occur, combined with various further cell types, in the pheromone receptor systems of some other noctuid species (see [1–4]); however, the combination of these four cell types within the same system has not been reported previously.

Field Studies

A field test programm on the four "key compounds" began in 1978. Included were some analogous chemicals not having corresponding male recetor types, such as the (*E*) isomers or the alcohol

Reprint requests to Dr. E. Priesner.

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and aldehyde derivatives corresponding to the key compounds. The test chemicals, all ≧ 99% pure, were soaked into rubber cups (serum bottle caps, Auer Bittmann Soulié AG) as solutions in hexane. Tetratraps [8] were generally used. They were set at vegetation level in randomized blocks comprising 4 to 7 different treatments in 4 to 6 replicates. Traps were spaced 2–4 m within blocks whereas distances between replicates were at least 50 m. The traps were checked and rerandomized at 3–5 day intervals and the results submitted to log $(x + 1)$ transformation followed by analysis of variance. Additional trials were conducted as "choice tests" between two traps, baited with a different chemical formulation (*e. g.*, a different mixture of the same compounds) and positioned 1.0 to 1.2 m apart. The field tests were carried out, from May to early July, in the woodland area ranging between Seewiesen and Starnberg, Upper Bavaria.

An initial test series included the following chemical formulations: the four key compounds as single chemicals in the amounts of 10 µg, 100 µg, and 1000 µg; the 24 binary combinations between these compounds in the ratio of 100 µg + 10 µg; and the 12 binary combinations in the ratio of 100 µg + 100 µg. In these initial tests, the mixture of 100 µg *Z*11-14:Ac with 100 µg *Z*9-14:Ac was the only lure that captured *P. pisi* males.

In 1979, the same synthetic formulations were used again, supplemented by the 100/30 and 30/100 mixtures of *Z*11-14:Ac with *Z*9-14:Ac. In these tests, the single compounds again were ineffective in capturing *P. pisi* males, as were all test combinations between *Z*11-14:Ac/*Z*11-16:Ac, *Z*11-14:Ac/*Z*7-12:Ac, *Z*9-14:Ac/*Z*11-16:Ac, *Z*9-14:Ac/*Z*7-12:Ac, and *Z*11-16:Ac/*Z*7-12:Ac (some of which caught effectively other Noctuidae spp.). The same holds for the 100/10 and 10/100 combinations of *Z*11-14:Ac/*Z*9-14:Ac. Most of the traps baited with a 100/30 or a 30/100 mixture of these two componds caught a few *P. pisi* males. Considerable catches of this species, ranging up to 31 males par trap/week, were consistently recorded in all traps baited with 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac, thus confirming the results obtained in 1978. The strong preference for the 100/100 mixture of these two compounds over other mixture ratios, apparent from Table I, also occurred in choice tests with pairs of traps (see above) baited with the 100/100 *vs.* the 100/30 or the 30/100 mixture.

A further test series was aimed at the optimally attractive dose for combinations of *Z*11-14:Ac with *Z*9-14:Ac in the 1 : 1 ratio. Total amounts of 2 µg, 20 µg, 200 µg, and 2000 µg were compared (Table II). In these tests the 2 µg dose was apparently below the attraction threshold, whereas up to 6 males per trap were taken with the 20 µg dose. Trap catches by 200 µg and by 2000 µg were consistently higher, in all replicates, and not significantly different between these two lures (Table II). Similarly in choice experiments with trap pairs, catches by 200 µg *vs.* 2000 µg did not differ more than 2 fold.

The receptor studies reported above (p. 990) had revealed, in addition to the specialist cells for *Z*11-14:Ac and *Z*9-14:Ac, the presence in the olfactory system of male *P. pisi* of two further cell types, specific to *Z*11-16:Ac and *Z*7-12:Ac, respectively. These two compounds, singly or in combination with *Z*11-14:Ac or *Z*9-14:Ac, had failed to attract *P. pisi* males (see above, p. 991). Possible synergistic or inhibitory effects of these chemicals on male

Table I. Captures of *Polia pisi* males in tetratraps baited with combinations of *Z*11-14:Ac and *Z*9-14:Ac in various ratios. Seewiesen, May 26 to June 12, 1979; six replicates.

Amount of chemical [µg/trap]		$\bar{X}$ males/trap *
*Z*11-14:Ac	*Z*9-14:Ac	
100	0	0 c
100	10	0 c
100	30	2.5 b
100	100	23.8 a
30	100	4.0 b
10	100	0 c
0	100	0 c

* Capture means followed by the same letter are not significantly different at $P = 0.05$ (Duncan's multiple range test).

Table II. Captures of *Polia pisi* males in tetratraps baited with 1 : 1 mixtures of *Z*11-14:Ac + *Z*9-14:Ac in various lure doses. Seewiesen, June 6 to 19, 1979; four replicates.

Amount of chemical [µg/trap]		$\bar{X}$ males/trap *
*Z*11-14:Ac	*Z*9-14:Ac	
1	1	0 c
10	10	3.5 b
100	100	17.5 a
1000	1000	16.0 a

* Capture means followed by the same latter are not significantly different at $P = 0.05$.

Table III. Captures of *Polia pisi* males in tetratraps baited with 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac as the basic lure and *Z*11-16:Ac as a third component. Seewiesen, June 7 to 19, 1979; five replicates.

Amount of added *Z*11-16:Ac [µg]	$\bar{X}$ males/trap *
0	7.8 a
0.1	7.2 a
1	9.8 a
10	11.6 a
100	0.4 b

* Capture means followed by the same letter are not significantly different at $P = 0.05$.

Table IV. Captures of *Polia pisi* males in tetratraps baited with 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac as the basic lure and *Z*7-12:Ac as a third component. Seewiesen, June 7 to 19, 1979; five replicates.

Amount of added *Z*7-12:Ac [µg]	$\bar{X}$ males/trap *
0	10.4 a
0.1	12.8 a
1	9.4 a
10	2.6 b
100	0 c

* Capture means followed by the same letter are not significantly different at $P = 0.05$.

Table V. Captures of *Polia pisi* males in tetratraps baited with 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac as the basic lure and *E*11-14:Ac, *E*9-14:Ac, *E*11-16:Ac, or *E*7-12:Ac as a third component. Seewiesen, June 13 to July 2, 1980; four replicates.

Third chemical, amount	$\bar{X}$ males/trap *
none (control)	7.2
*E*11-14:Ac, 10 µg	9.2
*E*11-14:Ac, 100 µg	5.7
E 9-14:Ac, 10 µg	8.2
E 9-14:Ac, 100 µg	10.0
*E*11-16:Ac, 100 µg	7.0
E 7-12:Ac, 100 µg	5.5

* None of the values is significantly different from the control ($P = 0.05$).

catches by the 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac lure were investigated by adding to this lure *Z*11-16:Ac or *Z*7-12:Ac at amounts ranging from 0.1 µg to 100 µg (Tables III and IV).

In these tests, *Z*11-16:Ac at doses of 0.1 µg, 1 µg, or 10 µg did not significantly enhance or reduce the catch rate over the basic lure, whereas after addition of 100 µg *Z*11-16:Ac no single male *P. pisi* was caught (Table III). Similarly with *Z*7-12:Ac, the lower doses had no significant synergistic or inhibitory effect, whereas the addition of 10 µg drastically reduced catches and 100 µg abolished any catch (Table IV).

Table VI. Captures of *Polia pisi* males in tetratraps baited with 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac as the basic lure and *Z*11-14:OH, *Z*9-14:OH, or *Z*11-16:OH as a third component. Seewiesen, June 12 to July 2, 1980; four replicates.

Third chemical, amount	$\bar{X}$ males/trap *
none (control)	9.5
*Z*11-14:OH, 10 µg	11.0
*Z*11-14:OH, 100 µg	8.5
Z 9-14:OH, 10 µg	8.7
Z 9-14:OH, 100 µg	5.7
*Z*11-16:OH, 10 µg	7.2
*Z*11-16:OH, 100 µg	10.5

* None of the values is significantly different from the control ($P = 0.05$).

In a further test series (results not shown in Tables), both *Z*11-16:Ac and *Z*7-12:Ac were added. Again, compared to catches by the 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac mixture alone, the addition of 0.1 + 0.1 µg or 1 + 1 µg of both compounds had not significant synergistic or inhibitory effect; the mixture containing 10 µg *Z*11-16:Ac + 10 µg *Z*7-12:Ac caught only a few males (comparable to the results obtained previously by addition of *Z*7-12:Ac, see above); and the quarternary mixture comprising 100 µg of each compound was essentially unattractive.

The trapping results of further chemicals tried in this manner are listed (Tables V and VI) for the (*E*) isomers and the alcohol analogous corresponding to the key compounds. Evidently, up to the high dose of 100 µg, these compounds had no pronounced modifying effect on catches by 100 µg *Z*11-14:Ac + 100 µg *Z*9-14:Ac. This same was found to hold for the aldehyde analogues (*Z*11-14:Ald, *Z*9-14:Ald, *Z*11-16:Ald, *Z*7-12:Ald) and three further acetates tested (*Z*9-12:Ac, *Z*7-14:Ac, *Z*9-16:Ac).

Concluding Remarks

Sexual attraction of *P. pisi* males as established by the present study appears to rely on the simultaneous activation of both the A and the B type cells of the male receptor system. Similar conclusions have been reached for some other noctuid species (see [1–4]). However, with *P. pisi*, a ratio of close to 1 : 1 of the two synergistic compounds (*Z*11-14:Ac, *Z*9-14:Ac) is apparently essential. Furthermore, two further cell types (C, D) present in the receptor system in this species do not seem to convey messages resulting in increased trap catches; activation of these receptors by higher doses strongly reduced catches. Various additional test compounds, not having corresponding specialist cells in the male sensory system, did not show marked effects upon trap catches at doses of up to 100 μg.

This kind of attractant system resembles the pattern shown by the tomato moth *Mamestra* (*Polia*) *oleracea*. The female sex pheromone in this species had been identified as a 60/40 to 50/50 blend of *Z*11-16:Ac with *Z*11-16:OH [9–11], compounds highly attractive to *M. oleracea* males at mixture rates close to 1 : 1 [9–11]. In this species too, the pheromone receptor system of the male antenna has been found to include two further cell types [1] although in field studies the addition of various third chemicals had little effect on male captures by the appropriate *Z*11-16:Ac/*Z*11-16:OH mixture [12], comparable to the results reported here for *P. pisi*. In this context it should be emphasized that other species from this taxonomic group (the *Mamestra*/*Polia* complex) evidently use a basically different attractant system. For example, in the cabbage moth *Mamestra* (*Barathra*) *brassicae*, trap catches using the main component of the female sex pheromone, *Z*11-16:Ac [13–18], were abolished by the addition of ≧ 0.1% of *Z*11-16:OH [19] or ≧ 1% of *Z*9-14:Ac [10, 19], compounds not found in the female pheromone secretion [13–18] but perceived by types of specialist receptor cells present on the male antenna [1]. A similar relationship of receptor *vs.* behavioural responses to test compounds applies to the bertha armyworm moth *Mamestra configurata* [1, 20–22]. Hence, it appears that within a taxonomic group of moths some species use a sex attractant system based upon a 1 : 1 combination of two pheromone components, hardly modified by third compounds, whereas in other species the attraction response to a single pheromone component is highly affected even by minute amounts of further chemicals.

During the course of the present study, males of other noctuid species (representing the genera *Panolis, Aplecta, Oligia, Cucullia, Parastichtis* and *Axylia*) were effectively captured by mixtures of *Z*11-14:Ac with *Z*9-14:Ac at the ratios of 9 : 1 or 1 : 9. However, very few of these were taken in the traps baited with the 1 : 1 combination of the two compounds, which attracted almost only male *P. pisi* (a result confirmed by field tests in Switzerland [23] and Eastern Austria [24]). Also, none of the various further synthetic lures operating in the test area during the same time caught any *P. pisi* male. Thus, at least for the biotopes and activity periods represented in these tests, *P. pisi* appears to be reproductively isolated from other moth species as a result of its unique sex attractant system.

An intriguing question, yet unanswered, concernes the possible functions of *Z*11-16:Ac and *Z*7-12:Ac, compounds perceived by specialist cells on the *P. pisi* male antenna but not markedly effective in the field trapping tests. It is necessary to state that all conclusions presented here were based upon the numbers of males caught in sticky traps, which leaves to laboratory behavioural studies the analysis of possible close-range functions of additional compounds. On the other hand, considering the interspecific relationships of pheromone receptor responses in this moth family [1–3], further conclusions may derive from a closer investigation of more taxonomically related species. So far, field attraction data have been reported for only one other species from the present *Polia* subgenus, *Ceramica*: male zebra caterpillar moths, *Polia* (*Ceramica*) *picta*, were captured in numbers by traps baited with *Z*11-14:Ac alone [25] or mixtures of *Z*11-14:Ac with *Z*11-14:Ald [26], lures unattractive to *P. pisi*.

No attempt to identify the actual composition of the female sex pheromone was made in the present study. Recent chemical analysis of washes from pheromone glands of calling *P. pisi* females has established the presence of two major components which in GC retention times corresponded with *Z*11-14:Ac and *Z*9-14:Ac, respectively [27].

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