# Analysis of a Sex-Attractant System in the Noctuid Moth *Rhyacia baja* Schiff

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Binary combinations of (*Z*)-7-dodecenyl acetate and (*Z*)-9-tetradecenyl acetate in a 1/10 ratio were highly attractive to *Rh. baja* males in field trapping tests conducted in southern Germany. Trap captures increased further on adding 0.1 or 0.3% of (*Z*)-5-dodecenyl acetate to this lure; higher amounts of this chemical were strongly inhibitory, as were additions of > 3% of (*Z*)-11-hexadecenyl acetate. The trapping data are considered in terms of electrophysiological responses of five types of antennal receptor cells.

The combination of (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac) with its longer-chain homologue, (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac) is a common theme in sex pheromone systems of noctuid moths (Lepidoptera: Noctuidae) [1, 2]. In the subfamily Noctuinae, the black cutworm moth *Agrotis ypsilon* Hufn. and the turnip moth *Scotia segetum* Schiff. have been reported to use combinations of these two chemicals; the sex pheromone emitted by virgin *A. ypsilon* females is a 5/1 mixture of *Z*7-12:Ac/*Z*9-14:Ac [3, 4], and that of *S. segetum* is a multicomponent blend including these two compounds along with a shorter-chain homologue, (*Z*)-5-decenyl acetate (*Z*5-10:Ac) [5–10]. This report provides evidence that the 1/10 combination of *Z*7-12:Ac/*Z*9-14:Ac, with trace amounts of (*Z*)-5-dodecenyl acetate (*Z*5-12:Ac) admixed as third component, is highly attractive to males of another Noctuinae species, the dotted clay moth *Rhyacia* (=*Amathes*, *Xestia*) *baja* Schiff. Results of field trapping tests will be specified here and related to electrophysiological data obtained from the pheromone receptor system on *Rh. baja* male antennae.

Single receptor responses were monitored from male *Sensilla trichodea* as in previous work on other noctuid species [11]. The test chemicals included the usual series of mono- and di-unsaturated, $C_{10}$ to $C_{18}$ acetates, alcohols, and aldehydes. Four different types of acetate receptor cells were discovered in these recordings and each shown to be specifically responsive to a particular (*Z*)-alkenyl acetate, *viz.* *Z*5-12:Ac, *Z*7-12:Ac, *Z*9-14:Ac, and *Z*11-16:Ac. A fifth type of cell responded highly-sensitively to an alcohol, (*Z*)-7-dodecen-1-ol (*Z*7-12:OH). No evidence for any further cell type present in these sensilla was obtained.

For each cell type the graded responses to selected analogous chemicals were determined and expressed as equipotent stimulus amounts relative to the single "key compound" of the cell, as in other moth species [12–15]. Sections of response spectra obtained on the four acetate receptors of male *Rh. baja* are presented in Table I.

Field trapping studies on potential attractive and inhibitory effects of these five compounds to wild *Rh. baja* males were conducted at Seewiesen, Upper Bavaria. Trap types, odour dispensers and trap placement procedures were as in previous work on other noctuid species [11]. The tests continued from 1978 through 1983 and were generally conducted from late July to late August, which is the main flight period of *Rh. baja* in this area as indicated by yearly light trap records.

On initiation of these tests, field work on other lepidopteran species had already revealed occasional captures of *Rh. baja* males in traps baited with formulations containing both *Z*7-12:Ac and *Z*9-14:Ac. These two compounds were therefore preselected as potential primary attractant components. As shown by a first test series, the *Rh. baja* males did respond to a wide range of different mixture ratios of these two compounds, with highest captures occurring to *Z*7-12:Ac/*Z*9-14:Ac mixtures of the ratios of 30/100, 10/100, and 3/100 (Table II). This pattern was confirmed by subsequent test series (results not specified here) which again revealed no difference ($P < 0.05$) in captures obtained by these three mixtures. A 0.3/100 mixture also caught some males whereas pure *Z*9-14:Ac alone was totally unattractive. In tests using the 1/10 mixture ratio but in different total amounts, captures increased with increasing lure dose, from 1 + 10 μg towards 100 + 1000 μg. The combination of 10 μg *Z*7-12:Ac + 100 μg *Z*9-14:Ac was chosen as the standard lure used in all further series.

The three remaining "key compounds" were accordingly studied as third components added to this standard lure. A 10 μg amount of either *Z*5-12:Ac or *Z*11-16:Ac totally abolished captures whereas the *Z*7-12:OH did not show any modifying

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Table I. Stimulatory effectiveness of selected alkenyl acetates on 4 types of receptor cells located in antennal hair sensilla of male *Rhyacia baja*.

| Test compound [a] | Effect [b] on cell type | | | |
|---|---|---|---|---|
| | A | B | C | D |
| *Z*5-10:Ac | 100 | > 1000 | 30 | > 1000 |
| *E*5-10:Ac | 1000 | | 100 | |
| *Z*7-10:Ac | 30 | > 1000 | 300 | > 1000 |
| *E*7-10:Ac | 100 | | > 1000 | |
| *Z*5-12:Ac | 100 | > 1000 | **1** | > 1000 |
| *E*5-12:Ac | 300 | > 1000 | 30 | |
| *Z*6-12:Ac | 30 | 1000 | 100 | |
| *E*6-12:Ac | 300 | | 100 | |
| *Z*7-12:Ac | **1** | 100 | 300 | 1000 |
| *E*7-12:Ac | 30 | | 1000 | > 1000 |
| *Z*8-12:Ac | 30 | 300 | 300 | > 1000 |
| *E*8-12:Ac | 300 | > 1000 | > 1000 | > 1000 |
| *Z*9-12:Ac | 300 | 100 | 1000 | 1000 |
| *E*9-12:Ac | 1000 | 300 | > 1000 | > 1000 |
| *Z*10-12:Ac | 1000 | 1000 | > 1000 | > 1000 |
| *E*10-12:Ac | 1000 | | | > 1000 |
| 11-12:Ac | 1000 | > 1000 | > 1000 | 1000 |
| *Z*5-14:Ac | > 1000 | 300 | 1000 | > 1000 |
| *E*5-14:Ac | > 1000 | | > 1000 | > 1000 |
| *Z*7-14:Ac | 100 | 100 | 1000 | 1000 |
| *E*7-14:Ac | 1000 | 300 | | 1000 |
| *Z*9-14:Ac | 100 | **1** | > 1000 | 100 |
| *E*9-14:Ac | 300 | 10 | | |
| *Z*11-14:Ac | > 1000 | 100 | > 1000 | 100 |
| *E*11-14:Ac | > 1000 | 1000 | | 300 |
| *Z*7-16:Ac | > 1000 | > 1000 | > 1000 | 1000 |
| *E*7-16:Ac | > 1000 | > 1000 | | 1000 |
| *Z*9-16:Ac | 1000 | 300 | > 1000 | 300 |
| *E*9-16:Ac | > 1000 | 1000 | | 1000 |
| *Z*11-16:Ac | 1000 | 100 | > 1000 | **1** |
| *E*11-16:Ac | > 1000 | 1000 | | 10 |
| *Z*13-16:Ac | > 1000 | 1000 | | 300 |
| *E*13-16:Ac | | > 1000 | | > 1000 |

[a] The compounds are listed with first the configuration and position of the olefinic double bond and secondly the number (– 10 till – 16) of C atoms in the alcohol moiety of the esters.
[b] The values indicate equipotent stimulus amounts referred to a half-decade scale (see [12–15]).

Table II. Captures of *Rhyacia baja* males in tetratraps baited with binary combinations of *Z*7-12:Ac and *Z*9-14:Ac. Seewiesen, August 4 to September 2, 1979; three replicates.

| Amount [μg/trap] of | | $\bar{X}$ males/trap |
|---|---|---|
| *Z*7-12:Ac | *Z*9-14:Ac | |
| 100 | 0 | 0 |
| 100 | 1 | 0 |
| 100 | 3 | 0 |
| 100 | 10 | 0 |
| 100 | 30 | 0.6 |
| 100 | 100 | 5.0 |
| 30 | 100 | 14.3 |
| 10 | 100 | 17.0 |
| 3 | 100 | 15.6 |
| 1 | 100 | 8.6 |
| 0 | 100 | 0 |

Table III. Captures of *Rhyacia baja* males in tetratraps baited with 10 μg *Z*7-12:Ac + 100 μg *Z*9-14:Ac as the basic lure and *Z*7-12:Ac, *Z*11-16:Ac, or *Z*7-12:OH as third components. Seewiesen, August 2 to 28, 1980; four replicates.

| Third chemical, amount [μg] | | $\bar{X}$ males/trap [a] |
|---|---|---|
| none | | 13.25 b |
| *Z*5-12:Ac, | 0.1 | 31.0 a |
| | 1 | 4.25 c |
| | 10 | 0 d |
| *Z*11-16:Ac, | 0.1 | 12.5 b |
| | 1 | 7.75 b, c |
| | 10 | 0 d |
| *Z*7-12:OH, | 0.1 | 15.0 b |
| | 1 | 10.25 b |
| | 10 | 11.5 b |

[a] Capture means followed by common letters are not significantly different (P = 0.05) by Duncan's multiple range test.

effect up to this test amount (Table III). However, the addition of 0.1 μg of the *Z*5-12:Ac significantly raised captures above the basic lure (Table III), suggesting synergistic properties of this chemical at very low amounts. This supposition was confirmed by various further test series conducted in 1981–83. In the experiments illustrated in Table IV, the *Z*5-12:Ac doses of 0.1 and 0.3 μg caused a 2–3 fold increase in captures, whereas the lower (0.01, 0.03 μg) doses did not show this effect. Again, 1 μg or more of the *Z*5-12:Ac drastically reduced captures.

In analogous series of experiments the *Z*11-16:Ac did not show synergistic effects at any test amount. Additions of 0.01–3 μg of this compound did not significantly alter captures beyond those obtained with the basic lure alone, whereas higher doses were again inhibitory (Table V). Additional tests on the effects of *Z*7-12:OH as a third component failed to

Table IV. Captures of *Rhyacia baja* males in tetratraps baited with 10 µg Z7-12:Ac + 100 µg Z9-14:Ac as the basic lure and Z5-12:Ac as a third component. Seewiesen, July 28 to August 25, 1981 (series A) and July 24 to August 20, 1983 (series B); four replicates per series.

| Amount [µg] of added Z5-12:Ac | $\bar{X}$ males/trap [a] | |
|---|---|---|
| | Series A | Series B |
| 0 | 10.5 b, c | 18.0 b |
| 0.01 | 8.75 b, c | 22.5 b |
| 0.03 | 14.75 b | 20.25 b |
| 0.1 | 26.0 a | 45.75 a |
| 0.3 | 22.75 a | 37.0 a |
| 1 | 3.50 c | 6.5 c |
| 3 | 0.5 c, d | 2.0 c |
| 10 | 0 d | 0 d |
| 30 | 0 d | 0 d |

[a] In each column, captures means followed by common letters do not differ significantly (P = 0.05).

Table V. Captures of *Rhyacia baja* males in tetratraps baited with 10 µg Z7-12:Ac + 100 µg Z9-14:Ac as the basic lure and Z11-16:Ac as a third component. Seewiesen, July 28 to August 25, 1981; four replicates.

| Amount [µg] of added Z11-16:Ac | $\bar{X}$ males/trap |
|---|---|
| 0 | 7.5 |
| 0.01 | 11.0 |
| 0.03 | 8.75 |
| 0.1 | 12.75 |
| 0.3 | 10.0 |
| 1 | 8.75 |
| 3 | 12.5 |
| 10 | 3.25 |
| 30 | 0 |

show any marked influence of this compound upon trap captures, up to the test amount of 100 µg. The same held for a number of acetates and alcohols which in the receptor recordings (Table I) did not reveal any corresponding types of specialist cells. These compounds included: Z5-10:Ac, *E*7-12:Ac, Z9-12:Ac, Z5-14:Ac, Z7-14:Ac, *E*9-14:Ac, Z11-14:Ac, Z9-16:Ac, Z5-12:OH, and Z9-14:OH.

Based on the present results, the Z7-12:Ac and Z9-14:Ac may both be classified as essential "primary" components of the sex-attractant system of *Rh. baja;* the Z5-12:Ac, as a "co-attractant"; and the Z11-16:Ac, as an "attraction-inhibitor". The Z7-12:OH, perceived by its own type of specialist receptor cell, had no apparent behavioural effect as reflected by trap captures. The female sex pheromone of *Rh. baja* is as yet unidentified chemically but from the present data a mixture of Z5-12:Ac/Z7-12:Ac/Z9-14:Ac of a ratio of close to 0.1/10/100 is most likely. The ternary combination of these three compounds appears to be as yet unknown from Noctuidae but has recently been reported from sex-attractant systems of *Zygaena* moths (Zygaenidae) [16].

The synergistic effect of the Z5-12:Ac on the 10 + 100 µg standard mixture was limited to test amounts of 0.1 and 0.3 µg. The failure of lower Z5-12:Ac doses to show significant trapping synergism is conceivable considering the reciprocal stimulatory effects caused by the "key compounds" of the other cell types on the Z5-12:Ac receptor (type C in Table I). The Z7-12:Ac is effective on this cell type at approx. 300 fold the stimulus amount of the Z5-12:Ac, thus rendering a Z7-12:Ac/Z5-12:Ac mixture of a ratio of 100/≦ 0.3 indistinguishable from pure Z7-12:Ac alone. Evidently the behavioural synergism of Z5-12:Ac in *Rh. baja* is limited to a certain level of stimulation of the Z5-12:Ac receptor cell slightly above that produced by the minor primary attractant component (Z7-12:Ac) alone.

There are various examples in the literature of sex-attraction synergists in Lepidoptera that were initially described as "inhibitors" due to the usage of an overdose test amount. In *Rh. baja*, the Z5-12:Ac would likely have been classified as a strong "attraction-inhibitor" had it been only tested in amounts of > 3% of the Z7-12:Ac. Detection of "trace-coattractants" [17] in lepidopterous sex-attractant systems should be greatly facilitated by electrophysiological analysis of the pheromone receptor cells on male antennae. This analysis not only reveals the different cell types involved in each species but also permits a rough estimate of the minimum recognizable amount of a potential minor attractant component in a multi-chemical blend.

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