

Multicomponent Sex Pheromone in *Agrotis segetum*: Preliminary Analysis and Field Evaluation

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Sex Pheromone Sex Attractant, *Agrotis segetum*, Turnip Moth, (Z)-5-Decenyl Acetate

Abdominal rinses of calling females of the turnip moth, *Agrotis segetum*, were analyzed by capillary gas chromatography combined with flame ionization detection, electroantennographic detection and mass spectrometry. Traces of a compound showing the retention time and high electrophysiological activity of (Z)-5-decenyl acetate could be detected only by electroantennography. In addition, (Z)-7-dodecenyl acetate, (Z)-9-dodecenyl acetate, dodecyl acetate and a compound resembling (E)-5-dodecenyl acetate were found. A mixture of these 5 compounds was as attractive in the field as were virgin females, while (Z)-8-dodecenyl acetate, which was also found in the female, was a strong inhibitor. Wind tunnel and field experiments indicate that (Z)-5-decenyl acetate, (Z)-7-dodecenyl acetate and probably (Z)-9-dodecenyl acetate are essential for attraction.

The amount of pheromone an insect produces and the number of insects available for analysis are often so small that rigorous chemical identification of the components is a hopeless task. But sometimes the analytical data obtained give clues which can be substantiated by behavioural test and lead to a product useful for insect monitoring in the field. A study which was started on a handful of insects has led us to use a five-component mixture which attracts males of the turnip moth *Agrotis segetum* in similar numbers as do traps baited with living females.

Pupae of the turnip moth, a pest of a Danish Laboratory culture [1], were brought to Wädenswil in the spring 1978. Laboratory observations showed that the females emit pheromone during the last

three hours of a 6 hour scotophase. Calling females were held with the ovipositor extruded and the latter rinsed with a few drops of methylene chloride. These abdominal rinses were concentrated and analyzed without further cleanup.

Chemical Analysis

Gas chromatography was carried out on a 22 m × 0.3 mm Silar 10 C glass capillary column at a temperature program from 90 to 160° with the effluent split 10:1 between a flame ionization detector (FID) and an electroantennographic detector (EAD) [2] using an *A. segetum* male antenna. Chromatograms of abdominal rinses showed the presence of an EAD-active component in the retention range of the 10 carbon acetates. No signal was obtained on the FID trace at the retention time, indicating that the amount of EAD-active product was considerable less than 1 ng per female. Tests

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with synthetic standards available showed that Z5-10Ac ((Z)-5-decenyl acetate) elicited a very high EAD response. Detection threshold with a good antenna preparation was in the range of 1 pg or below. Furthermore, the chemical co-chromatographed exactly with the EAD-active female component. From this information, we strongly suspected that the female component was Z5-10Ac. Meanwhile this compound has been identified in *A. segetum* by Bestmann *et al.* [3].

One rinse obtained from 7 females gave an excellent response at the retention time of Z5-10Ac and showed a second EAD peak in the retention range of the 12 carbon acetates with a matching signal on the FID trace, corresponding to a few nanograms of chemical per female. On co-injection, this peak eluted with Z8-12Ac ((Z)-8-dodecenyl acetate). A mass spectrum confirmed the structure of a dodecenyl acetate (*i.e.*, M^+ : $m/e = 226$, $M^+ - CH_3COOH$: $m/e = 166$). At the same time it was found that three other dodecenyl acetates were present: one in a larger amount than the previous one and of a retention time corresponding to Z9-12Ac, and traces of two others, tentatively assigned from the retention time as Z7-12Ac and E5-12Ac.

Three of the above assignments were confirmed using the electro-antennographic detector with male antennae of different species which each selectively respond to one compound: *Agrotis ipsilon* for Z7-12Ac [4], *Grapholitha funebrana* for Z8-12Ac [5] and *Eupoecilia ambiguella* for Z9-12Ac [6]. In each case, the chromatogram of the female rinse gave a peak at the retention time of the compound in question, and its amplitude was approximately of the size that could be expected from the corresponding FID signal. The use of the male antenna of one species to detect minor pheromone constituents in an other species was first exemplified in the case of *Euxoa ochrogaster* [7]. Finally an MS search revealed that the *A. segetum* female rinse also contained a substantial amount of 12Ac (dodecyl acetate).

Field Tests 1978

On the basis of this information, preliminary field tests were carried out in Denmark and Switzerland in 1978 with only one trap per treatment in each location (Table I). Treatment 1 contained the 6 components in approximately the same ratio as

Table I. *Agrotis segetum* catches with various chemicals in 1978.

Treatment No.	Composition of attractant: amount of chemical per dispenser [μg]							No. males caught per location*			
	Z5-10Ac	12Ac	E5-12Ac	Z7-12Ac	Z8-12Ac	Z9-12Ac	Z7-12OH	CH1	CH2	DK	total
1 (17)	1	100	10	2	20	100		1	3	0	4
2 (18)	10	100	10	2	20	100		18	9	1	28
3 (19)	1	100	10	2	20	100	1	10	5	1	16
4 (16)		100	10	2	20	100		0	0	0	0
5 (17)	1		10	2	20	100		1	3	0	4
6 (12)	1	100		2	20	100		2	3	0	5
7 (13)	1	100	10		20	100		0	0	0	0
8 (14)	1	100	10	2		100		8	31	7	46
9 (15)	1	100	10	2	20			3	1	0	4
10 (10)	1		10	2	20			1	1	0	2
11 (9)			10	2	20			0	0	0	0
12 (8)	1		10		20			0	0	0	0
13 (7)			10		20			0	0	0	0
14 (4)	1				20			0	1	0	1
15 (5)	10				20			0	0	0	0
16 (6)	100				100			0	0	0	0
17 (1)	1							1	0	1	2
18 (2)	10							1	0	0	1
19 (3)	100							1	1	0	2
20 (20)	blank							0	0	0	0

* One trap of each treatment in the following locations: CH1 = Dällikon (Switzerland), 20. 6. – 18. 7. 78, CH2 = Basadingen (Switzerland), 20. 6. – 14. 7., DK = Bonderup (Denmark), 30. 6. – 12. 7. Pherocon I C trap (Zoecon Corp., Palo Alto, California) with a serum bottle rubber cap (no. 90142, Auer Bittmann Soulié Zürich) as dispenser. Trap sequence randomized between locations.

found in the female, except for Z5-10Ac whose proportion in the mixture was probably higher than in the female. This complex mixture attracted only a few moths, but substantial catches were obtained when either the amount of Z8-12Ac was increased or when Z8-12Ac was omitted. A catch improvement was also obtained when Z7-12OH ((Z)-7-dodecen-1-ol) was added. The latter compound was included in the tests due to its activity in single cell recordings (E. Priesner, personal communication). Catches were nil when Z5-10Ac was omitted and only sporadic in the absence of Z7-12Ac, even at higher loads of Z5-10Ac. The tests indicated that Z5-10Ac and Z7-12Ac were essential that Z7-12OH was synergistic and Z8-12Ac inhibitory.

Wind Tunnel Experiments

Additional information was obtained from laboratory observations. *A. segetum* males were released in a wind tunnel of 50 × 50 × 170 cm at a wind speed of 30 cm/sec, and approaches to a filter paper held at the upwind end were recorded. With the scotophase placed between 11 and 17 h, observations were made from 14 to 16 h when response was optimal; a light of 4 lux was used for observation. Test samples were changed every 15 minutes. Standard stimulus was the fine-component mixture (treatment 8) which had been attractive in the field, with Z5-10Ac at 100 pg and the other components added in the proportions given in Table I. In a typical experiment with 9 males present in the tunnel, between 10 and 30 upwind flights could be observed per 15 min interval. About 20% of the approaching males landed on the filter paper for a few seconds; the others left the plume in an upward swing 10 to 30 cm before the source. Approaches and landings were both reduced to near zero when the amount of stimulus was dropped by a factor of 10; no significant increase was obtained when the amount was 10 times the standard. A mixture containing only the two components, Z5-10Ac and Z7-12Ac at 100 and 200 pg, respectively, gave similar numbers of approaches and landings as the standard mixture. The proportion of Z7-12Ac could be reduced by a factor of 4 and the total amount of the mixture increased by a factor of 5 without any apparent change in biological activity. Beyond this range, activity was reduced. These data confirmed the importance of both Z5-10Ac and Z7-12Ac.

Field Tests 1979

The above observations were in part confirmed by field trapping experiments in 1979 (Table II). With the binary mixtures, a catch optimum was observed in Denmark with Z7-12Ac present at 0.1 to 2 times the amount of Z5-10Ac. In France, on the other hand, Z5-10Ac alone was as attractive as any of the mixtures. This raises the question whether there are true geographical differences between populations. The insects used in the wind tunnel were of Danish origin. In all locations, higher amounts of Z7-12Ac were inhibitory.

The importance of the additional components has not yet been clarified. In a test carried through both flights in France (Table III) and in the results obtained in Switzerland in an other test (Table IV), the standard mixture was significantly more attractive than the two-component blend, which could

Table II. *A. segetum* catches with (Z)-5-decenyl acetate (1 µg per cap) in combination with various amounts of (Z)-7-dodecenyl acetate.

Z 7-12 Ac added [µg]	No. males caught in 6 traps per location *			
	DK 1	DK 2	CH	F
none	4 de	27 g	5 i	38 k
0.1	12 cd	62 f	20 i	26 k
0.5	23 c	52 f	12 i	27 k
2	5 de	51 f	13 i	6 l
10	0 e	22 g	5 i	5 l
50	2 e	14 h	7 i	0 m

* DK 1: Samsø, 20. 6.–2. 7. 79, DK 2: Frederikssund, 2. 7.–23. 7., CH: Basadingen, 19. 6.–25. 7., F: Avignon 17. 7.–12. 9. 79. Tetra traps with flaps [8], dispensers as in Table I. Numbers followed by the same letter are not significantly different at the 95% probability level as indicated by analysis of variance and Duncan's multiple range test.

Table III. *A. segetum* catches with various mixtures and virgin females.

Composition of attractant: Amount of chemical per dispenser						No. males caught *
Z5-10 Ac	12 Ac	E 5-12 Ac	Z 7-12 Ac	Z 9-12 Ac	Z 7-12 OH	
1	100	10	2	100		131 a
1			2			74 b
1			2		1	80 b
virgin females						152 a

* Total of 4 replicates, Montfavet 27. 4.–15. 10. 79. INRA traps, serum bottle rubber caps as dispensers, replaced in 6 week intervals.

Table IV. *A. segetum* catches with various mixtures.

Composition of attractant: Amount of chemical per dispenser [μ g]					No. males caught *				
Z 5- 10Ac	12Ac	E 5- 12Ac	Z 7- 12Ac	Z 9- 12Ac	DK 1	DK 2	CH	F	
—	—	—	2	—	2 p	1 r	2 st	0 w	
1	—	—	2	—	20 no	14 q	0 t	24 v	
1	100	—	2	—	11 o	13 qr	5 st	24 v	
1	—	10	2	—	27 no	14 qr	9 s	32 v	
1	—	—	2	100	38 n	25 q	43 u	32 v	
1	100	10	2	100	36 no	33 q	31 u	27 v	

* Total catch of 6 replicates in each of the following locations: DK 1 = Samsø, 20. 6.–25. 7. 79, DK 2 = Lyngby, 2. 7.–23. 7., CH = Diessenhofen, 19. 6.–25. 7., F = Montfavet, 17. 7.–12. 9. 79. Tetra traps [8], dispensers as in Table I. Meaning of letters see Table II.

indicate that at least a third component is essential. From the Swiss results it appears that addition of Z9-12Ac to the binary mixture is sufficient to obtain the full attraction of the standard mixture, while 12Ac and E5-12Ac have no effect. The

synergism of Z7-12OH could not be confirmed in a test in which it was added to the binary mixture (Table III).

In the trapping experiments (Table III) the five-component mixture appears to be as effective as the living females. However, field and wind tunnel observations seem to suggest that the synthetic product does not fully elicit the precopulatory behaviour of the males. More thorough studies will be necessary to define the true pheromone blend emitted by *A. segetum* females, including the occurrence and function of Z8-12Ac which appears to be an inhibitor and whose electroantennogram activity is higher than that of Z7-12Ac. For the time being, the attractant mixtures defined in this study seem to provide a basis for monitoring this pest and for further behavioural studies. The best formulation to be recommended from this study is a rubber cap containing 1 μ g Z5-10Ac, 2 μ g Z7-12Ac, 100 μ g Z9-12Ac, 100 μ g 12Ac and 10 μ g E5-12Ac although it appears that the latter two components can be omitted without loss of attractiveness.

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