Receptors for Di-Unsaturated Pheromone Analogues in the Male Summerfruit Tortrix Moth

Ernst Priesner

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

Z. Naturforsch. **38 c**, 874–877 (1983);
received June 30, 1983

Sex Pheromones, Inhibitors, Tetradecadienyl Acetates, Olfactory Receptors, *Adoxophyes*, Tortricidae

The antennal *Sensilla trichodea* in male *Adoxophyes orana* (F. v. R.) contain specialist receptor cells for 3 reported pheromone components (*Z*9- and *Z*11-tetradecenyl acetates and *Z*9-tetradecen-1-ol) and, additionally, the analogues *Z*9,*E*11- and *Z*9,*E*12-tetradecadienyl acetate. Possible behavioural functions of these dienes are briefly considered.

A decade ago, Den Otter and his co-workers started an electrophysiological analysis of pheromone perception mechanisms in the tortricid moth *Adoxophyes orana* F. v. F. (= *reticulana* Hb.; summerfruit tortrix). The authors recorded action potentials from individual hair sensilla (*S. trichodea*) on *A. orana* male antennae and demonstrated that these sensilla contained mainly two types of receptor cells, the one (cell type B) specifically responsive to (*Z*)-9-tetradecenyl acetate, and the other (type A) to a positional isomer, (*Z*)-11-tetradecenyl acetate [1–5]. These two compounds (*Z*9-14:Ac, *Z*11-14:Ac) are the reported major constituents of the *A. orana* female sex pheromone [6–8]. In field trapping studies, neither compound alone was effective in capturing male moths but a combination of the two chemicals in the female-produced ratio of *Z*9-/*Z*11-14:Ac of 9:1 proved to be highly attractive to native male moths both in Central Europe and Japan [6–8]. These data permitted the conclusion that "... the attractiveness of the pheromone should be determined in the central nervous system by comparison of the responses from the A and B cells" [1–3].

Recently Guerin *et al.* [9] identified a number of additional constituents at levels of a few % or less of the main component (*Z*9-14:Ac) in pheromone gland extracts of *A. orana* females. They included 6 acetates and 2 alcohols, *viz.* dodecyl acetate (12:Ac), (*Z*)-9-dodecenyl acetate (*Z*9-12:Ac), tetradecyl acetate (14:Ac), (*E*)-9- and (*E*)-11-tetradecenyl acetate (*E*9-14:Ac, *E*11-14:Ac), (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac), and (*Z*)-9- and (*Z*)-11-tetradecen-1-ol (*Z*9-14:OH, *Z*11-14:OH). Preliminary field trapping data presented by these authors indicated an increase in captures due to the addition of minor components, particularly the alcohols, to the 9:1 *Z*9-/*Z*11-14:Ac mixture [9].

Den Otter *et al.* had already provided morphological and electrophysiological evidence for a third type of receptor cell present in the *A. orana* male hair sensilla [5, 10, 11]. However, various compounds tested by these authors, including *E*9- and *E*11-14:Ac, and 10-methyldodecyl acetate (a pheromone constituent of another *Adoxophyes* sp., discussed further below), all failed to elicit substantial responses in this cell [11]. The adequate ("key") stimulus for this "third cell" thus remained unknown.

The data reported here were collected in the course of an electrophysiological survey of receptor systems of male *Sensilla trichodea*, conducted throughout European representatives of the Tortricinae: Archipini tribe. For the *A. orana* male hair sensilla these studies revealed the presence of three further cell types in addition to the A and B cells. Closer investigation of response spectra to synthetic compounds indicated that two of these cells were each specific to a particular tetradecadienyl acetate, whereas the remaining cell responded to one of the female-produced alcohols.

Nerve impulse responses of individual receptor cells were monitored via the cut ends of randomly-selected *Sensilla trichodea*, as in previous work on *A. orana* [3, 5] and other tortricid moths by this author [12–15]. Standard procedures of recording, stimulation, and data evaluation were applied to a three-stage analysis:

First, potential "key" stimulants for further cell types were searched for by presenting various test chemicals in comparison to the same amount of *Z*9-14:Ac or *Z*11-14:Ac, thereby evaluating the spike frequencies elicited over all cells active in a given preparation. The test compounds included the minor constituents identified from the *A. orana* female pheromone secretion (see above), the various pheromone and attractant components reported from other tortricid species (see [16]), and approx. 100 further, structurally-related chemicals.

0341-0382/83/0900-0874 $ 01.30/0

The results of these studies showed that there were indeed a number of acetates, and also some alcohols, that were equally or even more stimulatory than Z9-14:Ac or Z11-14:Ac with some preparations.

Differentiation between individual receptor cells showed that responses to acetate analogues that were more stimulatory than Z9-14:Ac or Z11-14:Ac were attributable to two cell types; the one responding maximally to (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac), and the other to the (Z, E)-9,11-isomer (Z9,E11-14:Ac). Nerve impulse responses to test alcohols were found to originate from a receptor cell specific to Z9-14:OH. No evidence for any cell type more responsive to a test compound other than Z9-14:Ac, Z11-14:A, Z9,E12-14:Ac, Z9,E11-14:Ac or Z9-14:OH was obtained.

Table I. Stimulatory effectiveness of olefinic acetates on 4 types of receptor cells located in antennal hair sensilla of male *Adoxophyes orana* [19].

Test compound [a]	Effect [b] on cell type			
	A	B	C	D
Z7-12:Ac	1000	100	> 1000	1000
E7-12:Ac	> 1000	1000		> 1000
Z9-12:Ac	30	30	300	100
E9-12:Ac	1000	300	> 1000	
11-12:Ac	30	1000	> 1000	> 1000
Z9,11-12:Ac	300	1000	30	1000
E9,11-12:Ac	1000	> 1000	1000	
Z7-14:Ac	1000	300	> 1000	> 1000
E7-14:Ac	> 1000		> 1000	
Z8-14:Ac	1000	100	> 1000	> 1000
E8-14:Ac	> 1000	1000	> 1000	
Z9-14:Ac	300	**1**	300	100
E9-14:Ac	1000	30	> 1000	> 1000
Z10-14:Ac	30	100	> 1000	1000
E10-14:Ac	300	300		> 1000
Z11-14:Ac	**1**	300	1000	300
E11-14:Ac	100	> 1000	1000	1000
Z12-14:Ac	30	1000	> 1000	> 1000
E12-14:Ac	100	> 1000		1000
Z9,E11-14:Ac	300	100	**1**	1000
E9,Z11-14:Ac	30	300	30	1000
E9,E11-14:Ac	1000	1000	100	> 1000
Z9,E12-14:Ac	300	10	1000	**1**
E9,Z12-14:Ac	300	300	> 1000	100
E9,E12-14:Ac	1000	1000	> 1000	100
Z9-16:Ac	1000	300	1000	1000
Z11-16:Ac	100	100	> 1000	> 1000
Z13-16:Ac	100	1000	> 1000	
(R)-(−)-10me-12:Ac	> 1000	1000	30	1000
(S)-(+)-10me-12:Ac	> 1000	> 1000	300	> 1000

[a] For abbreviations see text.
[b] The values indicate equipotent stimulus amounts referred to a half-decade scale (see [12–14]).

The third stage of analysis investigated the specificity of the five cell types as reflected by their dose-response relationships to structurally-related compounds. For each cell type these measurements resulted in a "response spectrum" of equipotent stimulus amounts for a set of test chemicals, derived from the cell's "key compound" along various lines of structural modification. Sections of these spectra are illustrated in Table I.

The response spectra obtained for the A and B type cells of *A. orana* closely resembled those reported for Z9-14:Ac and Z11-14:Ac receptors in some other moths [12–14]. The type C and D cells showed response specificities similar (see Table I for selected stereoisomers and monoenic analogues) to those of Z9,E11-14:Ac and Z9,E12-14:Ac receptor cells in moth species known to use these dienes as pheromone components; typical examples being some *Spodoptera* spp. (Noctuidae) which also have both receptor cells combined in their male hair sensilla [12]. The response spectra thus support the supposition that the two dienes, attributed here to receptors C and D, should be the "true key compounds" for these cells [18]. The response spectrum of the alcohol receptor (not listed) closely resembled that reported for Z9-14:OH receptor cells in other tortricid species [13]. Results of these specificity studies will be detailed elsewhere [19].

From the response spectra, inferences may also be made as to the reciprocal effects elicited by the five "key compounds" on the other cell types of the system. These effects evidently limit the perception of lower amounts of the other compounds when presented in mixtures. As shown by Table I, the Z9,E12-14:Ac (key stimulus for cell type D) also has a considerable effect on the Z9-14:Ac receptor (the additional unsaturation lowering stimulatory potency by not more than 10 fold [20]), whereas with all other cell type combinations the reciprocal responses were in the order of 1/1000 to 1/100 that of the proper key stimulus.

The alcohol Z9-14:OH, key stimulant for cell type E, is among the constituents identified from the *A. orana* female secretion [9]; and in field tests a 0.5% addition of Z9-14:OH to the two major components (Z9-/Z11-14:Ac, 9:1) improved captures [9, 21]. This compound thus meets all criteria for a

secondary pheromone component. On the contrary, the two dienes, *Z*9,*E*11- and *Z*9,*E*12-14:Ac, were not among the reported *A. orana* gland constituents [9] (and are unknown from the sex pheromones of other tortricid moths as well; see [16]). Also in further analyses, employing gas chromatography linked to compound-specific antennographic detectors, these compounds were not found in *A. orana* sex gland extracts at a detection limit 0.001% of the main component [21].

However, field data show that these dienes do have effects on attraction of male summerfruit tortrix moths to pheromone sources. In two independent test series, conducted in the Netherlands [22] and in Switzerland [21], a 2–5% addition of *Z*9,*E*12-14:Ac to the binary standard (*Z*9-/*Z*11-14:Ac, 9:1) resulted in an increase in captures, whereas higher doses appeared to be inhibitory; tests with the conjugated diene, *Z*9,*E*11-14:Ac, indicate inhibition at levels of 2% or higher of the main component [21].

The effect of *Z*9,*E*12-14:Ac on male attraction has been studied more closely in another *Adoxophyes* sp., the smaller tea tortrix. This species, in earlier work [23–28] referred to as *A. fasciata* Wals. [29], uses the same two major pheromone constituents as *A. orana* but in *Z*9-/*Z*11-ratio of 2:1 [23]. Of a series of minor components identified from the smaller tea tortrix pheromone secretion [30, 31], only 10-methyldodecyl acetate (10me-12:Ac) and *E*11-14:Ac were considered as effective secondary pheromone constituents [30–32] (the others probably representing behaviourally-inert by-products of the pheromone biosynthesis; see [30]). Again, no dienic acetate was detected in the female extract. However, in this species too, when *Z*9,*E*12-14:Ac was added to the *Z*9-/*Z*11-14:Ac standard mixture it significantly increased captures [27] although the relative amounts of *Z*9,*E*12-14:Ac needed were higher than in the case of the summerfruit tortrix. Moreover, in subsequent field work it was found that a binary mixture of *Z*9,*E*12-14:Ac with *Z*11-14:Ac in a 2:1 ratio was even more attractive to smaller tea tortrix males than the 2:1 *Z*9-/*Z*11-14:Ac standard [28]. However, the *Z*9,*E*12-14:Ac samples used in these tests were later found to contain a branched analogue, 11-methyl-(*Z*)-9,12-tridecadienyl acetate (11me-*Z*9,12-13:Ac), to which part of the synergistic effects could be assigned since *Z*9,*E*12-14:Ac samples free of this branched analogue showed only poor attraction synergism [33, 34].

In the present analysis of single cell responses in *A. orana*, particular effort was directed at detecting receptor cells specialized for 10me-12:Ac. This compound is apparently absent from the *A. orana* female pheromone ([9]; detection limit, 0.5% of major component) but has been isolated, and assigned pheromone function, in the smaller tea tortrix [30–32], as mentioned above. On recording from *A. orana* hair sensilla it was repeatedly observed that stimulation with 10me-12:Ac increased spike frequency, the (*R*)-(−) isomer [35, 36] generally being more active than the (*S*)-(+) antipode. However, on stimulation with various other test compounds the responding unit was consistently found to be cell type C – the specialist receptor for the conjugated diene: the (*R*)-(−)-10me-12:Ac acted on this cell as a "mimic", requiring approx. 30 fold the stimulus amount of the key compound, *Z*9,*E*11-14:Ac (Table I). Comparative single cell data on the smaller tea tortrix are not yet available. In the case of the latter, it is noteworthy that 10me-12:Ac occurs in pheromone extracts at 2% of the major constituents [30] whereas in field trapping tests a 10–100 fold higher relative amount was required in order to obtain synergistic effects [32]. Such a pattern appears to be in favour of a mimic, rather than a receptor key compound, suggesting a similar mode of perception for this compound as found in *A. orana*.

Similarly with 11me-*Z*9,12-13:Ac (another attraction synergist reported for the smaller tea tortrix, mentioned above), moderate spike responses were frequently obtained from cell type C. No specialist cells for any of the other constituents isolated from the *A. orana* pheromone secretion (including *Z*9-12:Ac, *E*11-14:Ac, *Z*11-16:Ac, and *Z*11-14:OH) were detected in the present study. Specialist receptors for *E*9-14:Ac were anticipated due to its inhibitory effects on *A. orana* male attraction in field studies [22, 37–39]; however, as in previous work [3, 5, 11], no *E*9-14:Ac receptor cells were found.

The possible behavioural functions of the two dienic acetates, *Z*9,*E*11-14:Ac and *Z*9,*E*12-14:Ac, reported here as "receptor key compounds" in male *A. orana*, remain obscure. An intraspecific role in *A. orana* is unlikely considering the total absence of these dienes from the gland extracts investigated.

Alternatively, a possible function of these compounds in species isolation should be considered. Reproductive isolation between the summerfruit tortrix and the smaller tea tortrix has been studied in areas of sympatry of the two species in southern Japan, and shown to rely primarily upon sex pheromone specificity [25, 40–42]. In choice experiments the males strongly preferred calling female moths of their own species, the presence of smaller tea tortrix females even suppressing mating activities in summerfruit tortrix males [41, 42]. The various components thus far identified from the smaller tea tortrix pheromone can hardly account for these inhibitory effects, thus pointing to yet unidentified (trace) gland constituents. A possible candidate structure is provided by the conjugated diene, *Z*9,*E*11-14:Ac, which in field experiments effectively suppressed attraction responses of summerfruit tortrix males when added to sources of synthetic pheromone [21].

Acknowledgements

The test chemicals used in this study were generously supplied by Drs. H. Arn (Wädenswil), C. Descoins (Paris), D. Hall (London) and S. Voerman (Wageningen). I am particularly greatful to H. Arn and P. M. Guerin for valuable comments and their permission to quote unpublished experimental data. I also thank P. J. Charmillot (Nyon) for continuous supply with test insects.

[1] C. J. den Otter, Abstract, 1st ECRO Congr., Paris 1974.
[2] C. J. den Otter, Abstract, 2nd ECRO Congr., Reading 1976.
[3] C. J. den Otter, J. comp. Physiol. **121,** 205 (1977).
[4] C. J. den Otter, Abstract, 3rd ECRO Congr., Pavia 1978.
[5] C. J. den Otter, H. A. Schuil, and A. Sander-Van Oosten, Ent. exp. appl. **24,** 370 (1978).
[6] F. J. Ritter, Meded. Rijksfak. Landbouwwetensch. Gent **36,** 874 (1971).
[7] Y. Tamaki, H. Noguchi, T. Yushima, C. Hirano, K. Honma, and H. Sugawara, Kontyu **39,** 338 (1971).
[8] G. M. Meijer, F. J. Ritter, C. J. Persoons, A. K. Minks, and S. Voerman, Science **175,** 1469 (1972).
[9] P. M. Guerin, H. Arn, H. R. Buser, and P. J. Charmillot, Symp. Int. sur les Médiateurs Chimiques (Colloques de l'INRA **7**), p. 267, INRA Publ. 1982.
[10] C. J. den Otter and G. Thomas, Preference in an Ecological Context (J. H. A. Kroeze, ed.), p. 171, IRL, London 1979.
[11] C. J. den Otter and J. W. Klijnstra, Ent. Exp. Appl. **28,** 15 (1980).
[12] E. Priesner, Chemical Ecology: Odour Communication in Animals (F. J. Ritter, ed.), p. 57, Elsevier, Amsterdam 1979.
[13] E. Priesner, Ann. Zool. Ecol. anim. **11,** 533 (1979).
[14] E. Priesner, Insect Neurobiology and Insecticide Action (Neurotox 79), p. 359, Soc. Chem. Ind., London 1980.
[15] E. Priesner, H. Bogenschütz, and H. Arn, Z. Naturforsch. **35c,** 390 (1980).
[16] W. L. Roelofs and R. L. Brown, Ann. Rev. Ecol. Syst. **13,** 395 (1982).
[17] Den Otter's terminology of A and B type cells in *A. orana* is followed here for continuity. Usually with insect olfactory sensilla, the cell with the largest spike amplitude is referred to as type A.
[18] The *Z*9,*Z*11-isomer was not available in a steric purity permitting closer determination of its effect on the *Z*9,*E*11-14:Ac receptor. There was no evidence for receptors specific to *Z*,*Z*-, *E*,*Z*-, or *E*,*E*-unsaturated pheromone analogues.
[19] E. Priesner, in preparation.
[20] Strong stimulatory effects of *Z*9,*E*12-14:Ac on *Z*9-14:Ac receptors were similarly obtained with various other moth species studied. Consequently, there appears to be no reported example of a moth sex pheromone, consisting of *Z*9,*E*12-14:Ac as the major component combined with *Z*9-14:Ac as an essential minor one.
[21] P. M. Guerin *et al.*, in preparation.
[22] S. Voerman, Symp. Int. sur les Médiateurs Chimiques (Colloques de l'INRA **7**), p. 55, INRA Publ. 1982.
[23] Y. Tamaki, H. Noguchi, T. Yushima, and C. Hirano, Appl. Ent. Zool. **6,** 139 (1971).
[24] Y. Tamaki, T. Ishiwatari, and M. Osakabe, Jap. J. appl. Ent. Zool. **19,** 187 (1975).
[25] Y. Tamaki, K. Yamada, and K. Honma, Appl. Ent. Zool. **11,** 209 (1976).
[26] Y. Hirai, Y. Tamaki, and T. Yushima, Nature **247,** 231 (1974).
[27] T. Negishi, T. Ishiwatari, and S. Asano, Appl. Ent. Zool. **12,** 178 (1977).
[28] T. Ishiwatari, T. Negishi, and S. Asano, Appl. Ent. Zool. **12,** 211 (1977).
[29] T. Yasuda's (1975) revision of Japanese Tortricinae moths (Bull. Univ. Osaka Pref., Ser. B, **27,** 79) refers to *fasciata* Wals. as the eastern subspecies ("Japanese summerfruit tortrix") of *A. orana* F. v. R. According to this revision the scientific name for the smaller tea tortrix is presently pending.
[30] Y. Tamaki, H. Noguchi, H. Sugie, R. Sato, and A. Kariya, Appl. Ent. Zool. **14,** 101 (1979).
[31] Y. Tamaki, Chemical Ecology: Odour Communication in Animals (F. J. Ritter, ed.), p. 169, Elsevier, Amsterdam 1979.
[32] Y. Tamaki, H. Noguchi, H. Sugie, A. Kariya, S. Arai, M. Ohba, T. Terada, T. Suguro, and K. Mori, Jap. J. appl. Ent. Zool. **24,** 221 (1980).
[33] T. Negishi, T. Ishiwatari, M. Uchida, and S. Asano, Appl. Ent. Zool. **14,** 478 (1979).
[34] T. Negishi, T. Ishiwatari, S. Asana, and H. Fujikawa, Appl. Ent. Zool. **15,** 113 (1980).
[35] T. Suguro and K. Mori, Agric. Biol. Chem. **43,** 869 (1979).
[36] Optical isomers of 10me-12:Ac supplied by Dr. K. Mori to Dr. H. Arn.
[37] A. K. Minks and S. Voerman, Ent. exp. appl. **16,** 541 (1973).
[38] A. K. Minks, 5e Symp. Lutte Integrée en Vergers, OILB/SROP, p. 295 (1975).
[39] A. K. Minks, S. Voerman, and W. M. Herrebout, Crop Protection Agents (N. R. McFarlane, ed.), p. 223, Academic Press 1977.
[40] K. Honma, Appl. Ent. Zool. **9,** 143 (1974).
[41] K. Honma and Y. Tamaki, Appl. Ent. Zool. **11,** 202 (1976).
[42] R. Sato and Y. Tamaki, Appl. Ent. Zool. **12,** 50 (1977).