Sex Pheromone of *Tortrix viridana*: (*Z*)-11-Tetradecenyl Acetate as the Main Component

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In electroantennographic studies on *Tortrix viridana* males, (*E*)-11-tridecenyl acetate displayed the highest activity of all straight-chain alkenyl acetates. However, evidence obtained by gas chromatography coupled to an electroantennographic detector or a mass spectrometer indicated that the ubiquitous pheromone compound (*Z*)-11-tetradecenyl acetate is the main component in the *T. viridana* female secretion. This compound and both (*Z*)- and (*E*)-11-tridecenyl acetate were found to be attractants for *T. viridana* males in the field. (*Z*)-9- and (*E*)-11-tetradecenyl acetate were not attractive and reduced male catches when added to either (*Z*)-11-tetradecenyl acetate or (*E*)-11-tridecenyl acetate. No evidence for synergistic effects was obtained.

(*Z*)-11-Tetradecenyl acetate (*Z*11 – 14 Ac) has been identified in the sex pheromone secretion of many species of Lepidoptera, notably of the tortricid family, and is an important component of many sex attractants discovered by field screening [1]. We have now obtained evidence that the chemical is also the main component of the sex pheromone of the European oak leaf roller or green tortrix moth, *Tortrix viridana* L. (Lepidoptera: Tortricidae), and a strong attractant for males of the species.

Outbreaks of the leaf roller moth near Freiburg/Br. permitted field collection of substantial numbers of insects in the pupal stage for electrophysiological and chemical investigation. Electroantennogram (EAG) and single receptor responses were recorded as reported in earlier studies with tortricid species [2]. Of the series of monounsaturated straight-chain alcohols, aldehydes and esters tested, acetates of a 13 or 14 carbon chain with a double bond in position 11 gave the highest responses. (*E*)-11-Tridecenyl acetate (*E*11 – 13 Ac) was the most effective test chemical, followed by *E*11 – 14 Ac (Table I). The findings were confirmed in gas chromatograms of acetate standards with electroantennographic detection [3] which showed the prominent signal obtained with *E*11 – 13 Ac at the expected retention time. In field tests in June 1977 at Freiburg/Br. 10 and 100 µg *E*11 – 13 Ac caught 7.0 and 44.3 *T. viridana* males per trap, respectively, while *E*11 – 14 Ac at the same test amounts was not attractive. These results suggested that this unusual C_{13} compound could be a key substance in the sex pheromone of *T. viridana*.

Extracts for chemical analysis were made by collecting calling females in methylene chloride and removing the bodies within a few seconds to avoid extracting too much fatty material. A temperature drop was found to be essential to induce calling in *T. viridana* females. At the end of a 16 h light phase the temperature was lowered from 20 to 15 ° and a light of 4 lux was maintained for observation. During the following 3 h, a substantial number of females was observed in calling position, and a wash of 76 females could be obtained in one session.

Gas chromatography of the female wash was carried out on a Silar 10 C glass capillary column; the effluent gas was split in a 10 : 1 ratio between the flame ionization (FID) and the electroantennograph-

Table I. Electroantennogram response values of male *Tortrix viridana* to (*Z*)- and (*E*)-alkenyl acetates. Values indicate equipotent stimulus amounts in a half log scale (0.01 represents a range from 0.0056 to 0.018 µg; 0.03, from 0.018 to 0.056 µg; etc.).

*Z*8 – 12 Ac	1	*E*8 – 12 Ac	0.3
*Z*9 – 12 Ac	0.3	*E*9 – 12 Ac	0.3
*Z*10 – 12 Ac	0.1	*E*10 – 12 Ac	0.1
*Z*9 – 13 Ac	0.3	*E*9 – 13 Ac	0.3
*Z*10 – 13 Ac	0.1	*E*10 – 13 Ac	0.1
*Z*11 – 13 Ac	0.03	*E*11 – 13 Ac	0.003
*Z*9 – 14 Ac	0.3	*E*9 – 14 Ac	0.3
*Z*10 – 14 Ac	0.1	*E*10 – 14 Ac	0.1
*Z*11 – 14 Ac	0.03	*E*11 – 14 Ac	0.01
*Z*12 – 14 Ac	0.1	*E*12 – 14 Ac	0.03
*Z*10 – 15 Ac	0.3	*E*10 – 15 Ac	0.1
*Z*11 – 15 Ac	0.1	*E*11 – 15 Ac	0.03
*Z*12 – 15 Ac	0.1	*E*12 – 15 Ac	0.1
*Z*11 – 16 Ac	1	*E*11 – 16 Ac	0.3
*Z*12 – 16 Ac	1	*E*12 – 16 Ac	1

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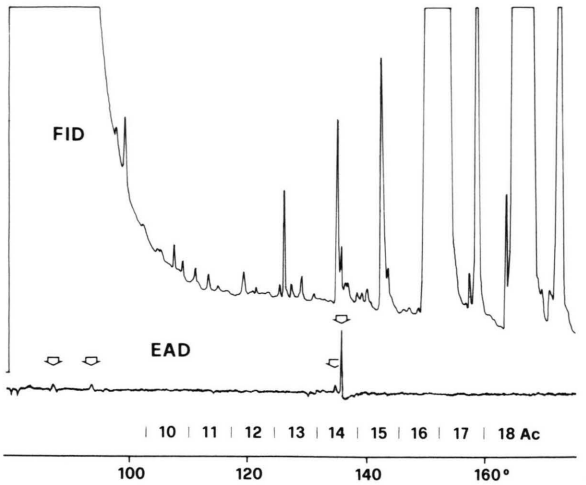

Fig. 1. Gas chromatogram of a *Tortrix viridana* female wash, recorded with flame ionization (FID)and electroantennographic detection (EAD). Arrows point at EAD peaks reproducible in multiple runs. Elution ranges of straight-chain acetates are indicated. Conditions: Silar 10 C, 22 m × 0.3 mm glass capillary column. Injection of 1 female equivalent at 70 °, after 2 min heated to 90 ° at 30 °/min, then heated at 6 °/min to end. The dip in the EAD trace following the main peak is caused by the electronic filter used to compensate for baseline drift.

ic detector (EAD) [3]. The filtered extract was introduced by splitless injection without prior cleanup. EAD recordings consistently showed a main peak at a retention time of *Z*11−14Ac which coincided with a small but sharp peak in the FID trace corresponding to an amount of a few nanograms per female (Fig. 1). The relative heights of the peaks obtained with the two detectors was approximately the same for the female wash and synthetic *Z*11−14Ac. Some chromatograms also showed a small EAD peak eluting just before *Z*11−14Ac, at a retention time similar to *E*11−14Ac or *Z*9−14Ac (Fig. 1). This peak appeared with the front slope of a major peak on the FID trace. Two other small EAD peaks were observed during elution of the solvent. These results indicate that other, unidentified compounds beside *Z*11−14Ac may be involved in the sex pheromone of *T. viridana*. No other EAD activity was observed within the retention range of 10 to 18 carbon acetates.

Further information on the main component was obtained by gas chromatography/mass spectrometry on a Finnigan 4000 GC-MS instrument equipped with a data system. Complete mass spectra (*m/e* 80 − 450, 1.4 sec/scan) were recorded using chemical ionization with iso-butane. After data acquisition, the spectra were searched for tri- and tetradecenyl acetates. The mass chromatogram at *m/e* 255 contained a major peak whose retention time and mass spectrum ($M+1 = 255$, $M+1-CH_3COOH = 195$) were as expected for a tetradecenyl acetate. The amount was estimated at 4 ng per female. No isomers were found at neighbouring retention times; if present, their amount would have been less than 5% of the main peak. No tridecenyl acetate was detect-

To allow an isomer assignment, mass fragmentograms were carried out at a slower temperature program (2 °/min), using the fragment of *m/e* 194 (M^+-CH_3COOH) obtained in electron impact ionization. Its main peak coincided with that of synthetic *Z*11−14Ac on two different columns. The Silar 10 C column separated *Z*11−14Ac from all other tetradecenyl acetates except from *E*12−14Ac and 13−14Ac; separation of these isomers from *Z*11−14Ac was achieved on OV-101. The above evidence indicated that *Z*11−14Ac is the main component of the *T. viridana* sex pheromone.

Extensive attraction studies were conducted in June 1978 near Freiburg/Br. in the same test area as in 1977. Since the middle of the 19th century the riparian woodland in the Upper Rhine Valley between Basel and Freiburg has changed into incomplete brushwood by ground water subsidence. Only a few solitary oaks (*Quercus robur* L.) overtop the xerophilous shrubs; their crowns often extend to the ground and are periodically infested with *T. viridana*.

The field tests included *Z*11 − 14Ac as the identified component, *E*11 − 13Ac as an attractant discovered in 1977, *Z*9- and *E*11 − 14Ac as candidate secondary components, and the analogues *Z*11 − 13Ac and *Z*11 − 14OH. Test compounds were at least 99% pure and contained less than 0.1% of the opposite geometric isomer. The chemicals were applied to the cavity of serum bottle caps (Tellergummikappen No. 90142, Auer Bittmann Soulié AG, Zürich) from hexane solutions. Tetra Traps with flaps [4] were hung from oak tree branches at eye level. Each test included a comparison of 6 treatments per tree in 6 replicates. Distances between treatments were 2 − 3 m, between replicates 20 − 200 m. Trap positions were systematically changed for each replicate. Catches were recorded weekly and the figures subjected to log $(x+1)$ transformation followed by two-way analysis of variance.

*E*11 − 13Ac, the compound showing highest EAG activity, was found to be a good attractant for *T. viri-*

Table II. *Tortrix viridana* catches with (*E*)- and (*Z*)-11-tridecenyl and tetradecenyl acetates, Freiburg, 13 to 20 June 1978. Totales of 6 replicates. Numbers in the same column followed by the same letter are not significantly different at the 95% probability level as indicated by Duncan's multiple range test.

Chemical and amount per dispenser		Test number			
		1	2	3	4
untreated		6 d	3 c	6 c	2 c
*E*11 – 13Ac	1 µg	59 c			
	10 µg	96 ab	111 b	356 b	84 b
	100 µg	185 a	179 b	728 a	121 b
	1 mg	105 ab			
*Z*11 – 13Ac	100 µg		372 a		
	1 mg		575 a		
	10 mg		537 a		
*E*11 – 14Ac	100 µg			11 c	
	1 mg			12 c	
	10 mg			14 c	
*Z*11 – 14Ac	100 µg				809 a
	1 mg				984 a

dana males (Table II), confirming the 1977 field results. The attractant threshold was below 1 µg per dispenser. *Z*11 – 13Ac and *Z*11 – 14Ac, which had not been tested previously, both attracted significantly more males than *E*11 – 13Ac when compared at a dispenser load of 100 µg. No significant catch improvement was obtained at dispenser loads above 100µg with any of these three compounds. The other test compounds, *E*11 – 14Ac (Table II), *Z*9 – 14 Ac and *Z*11 – 14OH (not shown) were not attractive to *T. viridana* males when tested at 100 µg to 10 mg. The alcohol attracted large numbers of *Choristoneura sorbiana*, confirming other reports [5, 7].

When 100 µg *E*11–13Ac was tested in combination with 2, 10 or 50 µg *Z*11–13Ac, no difference in attraction compared to the single compounds was observed (A 9 : 1 mixture of these two compounds, recently found to be attractive to *T. viridana* in field screening [6], was not included in our tests). Also, no synergistic or inhibitory effects were obtained with varying combinations of *E*11–13Ac and *Z*11–14Ac.

Binary mixtures of *Z*9 – 14Ac and *E*11 – 14Ac with attractants were tested since the GC-EAD data suggested their possible involvement as secondary components. *E*11 – 14Ac showed no synergistic effect when added to either *E*11 – 13Ac (Table III) or *Z*11 – 14Ac (Table IV); at 3% of the main product and above, it was inhibitory. Similarly, no synergism at low level, and a significant inhibition at 3% and above, was found for *Z*9 – 14Ac when combined with *Z*11 – 14Ac (Table IV). Thus, the specific attraction of *T. viridana* males to mixtures of *Z*11 – 14Ac and *Z*9 – 14Ac [7, 8] was not confirmed. A detailed report of the field data will be published elsewhere [9].

The detection of *Z*11 – 14Ac in the *T. viridana* female secretion represents the first sex pheromone identification in the Tortricini tribe of the subfamily Tortricinae. Synthetic sex attractants, found by field screening for various other Tortricini spp., include

*Z*11 – 14Ac for *Tortrix sinapina* [11],
E 11– 14Ac for *Croesia holmiana* [10, 6], *C. bergmanniana* [6] and *Acleris paradesana* [11],
*E*11- and *Z*11 – 14Ac 9 : 1 to 8 : 2 for *Acleris rhombana* [5, 6],
*Z*11- and *Z*9 – 14Ac 5 : 5 for *Spatalistis bifasciana* [11],

Table III. *Tortrix viridana* catches with (*E*)-11-tridecenyl acetate alone and in combination with (*E*)-11-tetradecenyl acetate, Freiburg, 13 to 20 June 1978. Totals of 6 replicates. Numbers followed by the same letter are not significantly different at the 95% probability level as indicated by Duncan's multiple range test.

Amounts per dispenser		
*E*11 – 13Ac [µg]	*E*11 – 14Ac [µg]	
0	0	3 d
10	0	117 b
100	0	238 a
100	3	85 bc
100	10	57 c
100	100	5 d

Table IV. *Tortrix viridana* catches with (*Z*)-11-tetradecenyl acetate (100 µg per dispenser) in combination with (*E*)-11 or (*Z*)-9-tetradecenyl acetate, Freiburg, 20 to 28 June 1978. Totals of 6 replicates. Numbers followed by the same letter are not significantly different at the 95% probability level as indicated by Duncan's multiple range test.

Amount added	Chemical added to *Z*11 – 14Ac	
	*E*11 – 14Ac	*Z*9 – 14Ac
0	189 a	190 a
0.1 µg	210 a	194 a
0.3 µg	137 a	250 a
1 µg	115 a	242 a
3 µg	26 b	53 b
10 µg	2 c	29 c

$Z11-14$Ac and $Z11-14$-aldehyde and 5 : 5 to 1 : 9 for *Croesia ascoldana* and *C. conchyloides* [11], and $E11$- and $Z11-14$-aldehyde 8 : 2 to 9 : 1 for *Croesia semipurpurana* [12].

Our results indicate that either the pheromone component $Z11-14$Ac or the parapheromones $E11$- and $Z11-13$Ac can be used as single compounds to monitor the *T. viridana* flight by sex traps.

An unusual discovery is the outstanding EAG activity of $E11-13$Ac, a chemical not found in the female moth. EAG activities of the test compounds (Table I) will be discussed further in the context of single receptor types in the olfactory system of the *T. viridana* male antenna [13].

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