

Individual Variation in the Sex Pheromone Components of the False Codling Moth, *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae)*

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Analysis of individual pheromone glands of the false codling moth *Cryptophlebia leucotreta* Meyr. originating from South Africa, was performed by capillary gas chromatography. During the calling period, the pheromones are concentrated in the dorsal part of the intersegmental membrane between the abdominal segments VIII and IX. The average amount of pheromones and related compounds present in the gland was 412 ng/♀. This total amount was highly variable from individual to individual (200–740 ng/♀). However, the relative amounts of (*Z*)- and (*E*)-8-dodecenyl acetates showed a narrow variance. The coefficients of variance values of these two components, 10 and 19 respectively, were significantly lower than those of other components present in the gland.

The results infer that the different isomer ratios of (*Z*)- and (*E*)-8-dodecenyl acetates reported for *C. leucotreta* cannot be due to individual variations. Hence, the presence of different geographical strains can be expected.

The false codling moth, *Cryptophlebia leucotreta* Meyr. (Lepidoptera: Tortricidae) is a polyphagous pest found throughout Africa, causing damage on various crops including macadamia [2]. The female sex pheromone of this moth has been reported as a mixture composed mainly of (*Z*)- and (*E*)-8-dodecenyl acetates (*Z*-8-12:Ac and *E*-8-12:Ac). While Persoons *et al.* [3] found approximately equal amounts of both isomers, Angelini *et al.* [4] and Zagatti *et al.* [5] reported that the pheromone emitted by the virgin females consisted of (*E*)- and (*Z*)-8-dodecenyl acetates together with dodecyl acetate (12:Ac), in a ratio of 69:23:8. In a recent paper Hall *et al.* [6] identified the two acetates in the washings of the ovipositors having the reverse ratio, *i.e.* 62:38 (*Z*)/(*E*)-isomer, together with 15.6% of the saturated C₁₂ acetate. A 1:1 mixture of the isomeric dodecenyl acetates has been used for field studies to monitor *C. leucotreta* in Malawi [7].

To clear this somewhat ambiguous results regarding the quantitative composition of the pheromone, we reinvestigated the female sex attractant by means of a solid-sample injection technique in conjunction with gas chromatography [8]. The main emphasis was focussed onto the quantification of the pheromone gland volatiles of single specimens in order to clarify whether the reported differences are a matter of individual variation of pheromone components or due to the existence of different geographical strains of the insects.

Materials and Methods

Insect material

Pupae of *Cryptophlebia leucotreta* Meyr. were provided by the Biologische Bundesanstalt, Darmstadt (Prof. F. Klingauf). The insects, originated from South Africa, were maintained in Germany as a laboratory colony for more than one year.

The pupae were sexed and kept at 22 °C in plastic boxes lined with moistened filter paper. A reversed 16 h light: 8 h dark cycle was maintained throughout the study. Eclosed adults were collected daily and transferred to plastic boxes containing wicks wetted with 5% sugar solution. To determine the calling period, the insects were observed under a red light during the scotophase.

Abbreviations: 10:Ac, decyl acetate; 12:Ac, dodecyl acetate; 14:Ac, tetradecyl acetate; 16:Ac, hexadecyl acetate; 18:Ac, octadecyl acetate; *E*-8-12:Ac, (*E*)-8-dodecenyl acetate; *Z*-8-12:Ac, (*Z*)-8-dodecenyl acetate; *E*-8-12:OH, (*E*)-8-dodecenol; *Z*-8-12:OH, (*Z*)-8-dodecenol.

* Pheromones 54 [1].

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Encapsulation of pheromone glands

The samples were prepared according to the method described by Attygalle *et al.* [8]. In order to determine the exact site where the pheromones are located, various parts of the abdominal tip of three-day-old female moths were excised during the period of maximum calling. The females were regarded as calling, when they clung to the walls, raised their wings and exposed the ovipositors. The samples were sealed in soda glass capillaries (2 cm × 2 mm) and used for immediate analysis or stored at -20 °C.

Gas chromatography with solid sample injection

Capillary gas chromatography with flame ionization detection was performed with a Packard United-Technologies 438A instrument equipped with a splitless injector and a Shimadzu Chromatopac C-R3A data system. GC-conditions: (A) Fused silica capillary column (25 m × 0.22 mm) SP-2340, 0.21 µm film thickness, oven temp. 60 °C for 2 min, 60–195 °C at a rate of 4 °C/min; (B) Fused silica capillary column (12 m × 0.22 mm) chemically bonded CP Sil 19 (≈OV-17), film thickness 0.2 µm, oven temp. 60 °C for 2 min, 60–270 °C, 10 °C/min.

Samples sealed in glass capillaries were chromatographed via a solid sample injection technique [8]. The quantification of pheromone components was performed by using a solution of Z-8-12:Ac as an external standard.

Gas chromatography – mass spectrometry

A Finnigan 9502 gas chromatograph, fitted with a Grob type injector linked to a Finnigan 3200E quadrupole mass spectrometer with a Data System 6000 was used: Fused silica capillary column SP-2340, 1 ml He/min, direct coupling, 70 eV EI spectra, 1–3 sec/scan.

Electrophysiology

Using the electroantennogram technique (EAG) [9], with male *C. leucotreta* antennae, the stimulus potentials of several test compounds were measured. The effluent from a fused silica capillary column (12 m × 0.2 mm, CP Sil 19) was split and the major part was directed over a male insect antenna [10]. By means of implanted capillary electrodes on the antenna, the retention times of the biologically active compounds present in the effluent were monitored (electroantennographic detection, EAD).

Results and Discussion

Linked GC-EAD analysis, of abdominal extracts from *C. leucotreta* females, on a CP Sil 19 fused silica capillary column showed only a single EAG response which occurred at a retention time corresponding to those of mono-unsaturated 12-carbon acetates.

Male *C. leucotreta* antennal responses for a series of dodecenyl acetates showed the antennae to be most responsive to (*E*)-8-dodecenyl acetate.

Under our laboratory conditions *C. leucotreta* females showed maximum calling activity ca. 4½ h into the scotophase. Various parts of the abdominal tip of three day old female moths were excised during the calling time and analysed for their pheromone content. The solid sampling technique [8] used, enabled the analysis of such pieces of tissue without the intervention of a solvent. The segments VIII (Fig. 1) and IX, and the ventral part of the intersegmental membrane contained only minute traces (less than 0.1%) of pheromone components. Almost all the sex pheromones were found in a small area located at the dorsal side of the intersegmental membrane between the segments VIII and IX (densely stippled area, Fig. 1). The traces of pheromone components found in the other parts of the abdominal tip may be due to contamination during the dissection procedure or to diffusion from the glandular area.

As shown by the chromatogram in Fig. 2, all the components identified were well resolved for accurate integration of peak areas. The positive identifi-

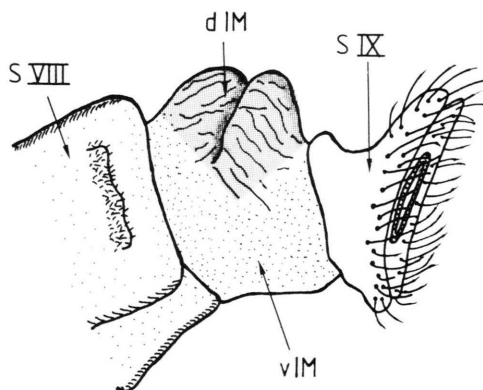


Fig. 1. An illustration of the abdominal tip of a female *C. leucotreta* (S VIII = 8th abdominal segment; S IX = 9th segment; dIM = intersegmental membrane, dorsal side; vIM = intersegmental membrane, ventral). The pheromones are located only in the dorsal area (densely stippled) of the intersegmental membrane.

cation of the chromatographic peaks was done by the comparison of retention times with those of authentic materials, GC-MS and mass chromatographic analysis. Further confirmation was afforded by the method described by Horiike and Hirano [11]. According to this method, the ratio of intensities of various ion clusters were compared to those obtained from a series of positional isomers of unsaturated acetates, under the same spectroscopic conditions. These ratios can be correlated with the double bond position.

C. leucotreta is a relatively small lepidopteran insect (~22 mg), nevertheless it produces remarkably high quantities of sex pheromones. The average amount of pheromones and related compounds (see Table I) found in the gland was 412 ng per insect. This total amount was highly variable from individual to individual (coefficient of variance, CV=45, Table I). In one extreme case, a female contained 740 ng in the gland. However, Hall *et al.* [6] could find only 5–10 ng per insect, by washing the ovipositors with heptane or dichloromethane. This exemplified the utility of a solid sampling technique in such quantification studies. Our results are essentially in agreement with the value of 800 ng per in-

sect, given as a personal communication of Frerot in Zagatti *et al.* [5].

The histogram presented in Fig. 3a illustrates the titres of E-8-12:Ac (open bars), Z-8-12:Ac (stippled bars), 12:Ac (hatched bars) and E-8-12:OH (black bars) in 15 individual females. It shows the wide variability of the absolute amount of each component considered, *e.g.* 117–516 ng for E-8-12:Ac (open bars in Fig. 3a).

However, when the relative amounts were compared to each other by normalizing them (E-8-12:Ac=100), a precise regulation of the (*E*) and (*Z*) isomeric ratio became evident (Fig. 3b). The proportion of Z-8-12:Ac to E-8-12:Ac shows only a narrow variance, ranging from 10–17% (E-8-12:Ac = 100%). This effect is further exemplified by their corresponding coefficients of variance (CV). The CV values for E-8-12:Ac and Z-8-12:Ac, 10 and 19 respectively, are significantly lower than those of the other compounds found in the gland (Table I).

From Fig. 3b, it can be seen, that the relative amounts of 12:Ac and E-8-12:OH do not exhibit such a rigorous control. The corresponding coefficients of variance are high, 46 and 64 respectively. Similarly, a high degree of variation of the relative

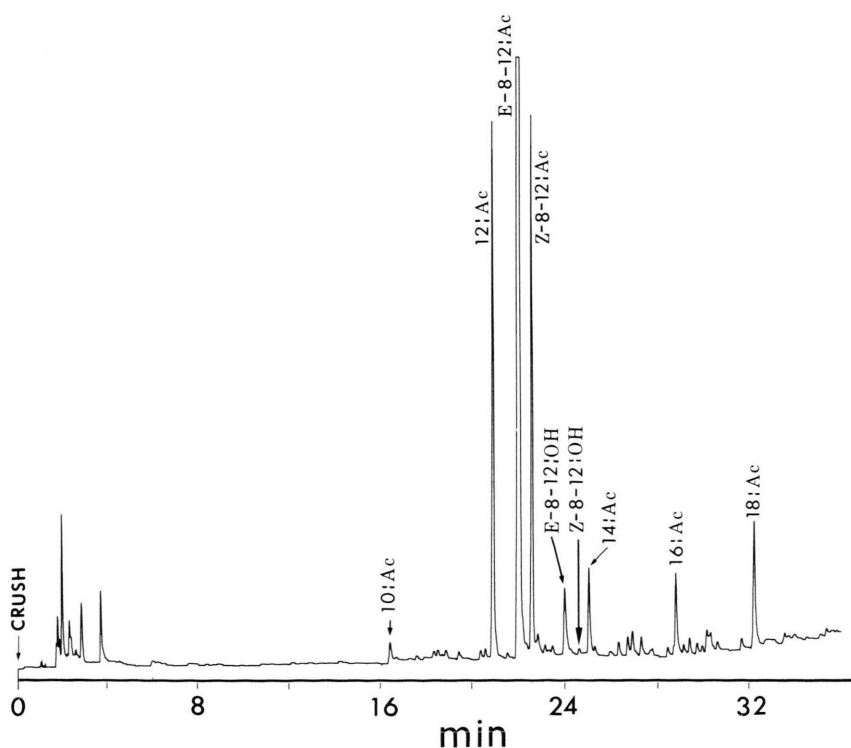


Fig. 2. A gas chromatogram obtained from a sex pheromone gland of a female *C. leucotreta*, by a solid sample injection technique on a 25 m × 0.22 mm fused silica capillary column coated with SP-2340. The oven temperature was held at 60 °C for two min and programmed to 195 °C at 4 °C/min (abbreviations see p. 1077).

amounts was also found for decyl acetate (10:Ac), (Z)-8-dodecenol (Z-8-12:OH), tetradecyl acetate (14:Ac), hexadecyl acetate (16:Ac), and octadecyl acetate (18:Ac); CV values of 71, 77, 44, 33 and 69,

respectively. (Table I gives the mean composition and percentage together with their coefficients of variance for the pheromone components and analogues found in 15 individual *C. leucotreta* glands).

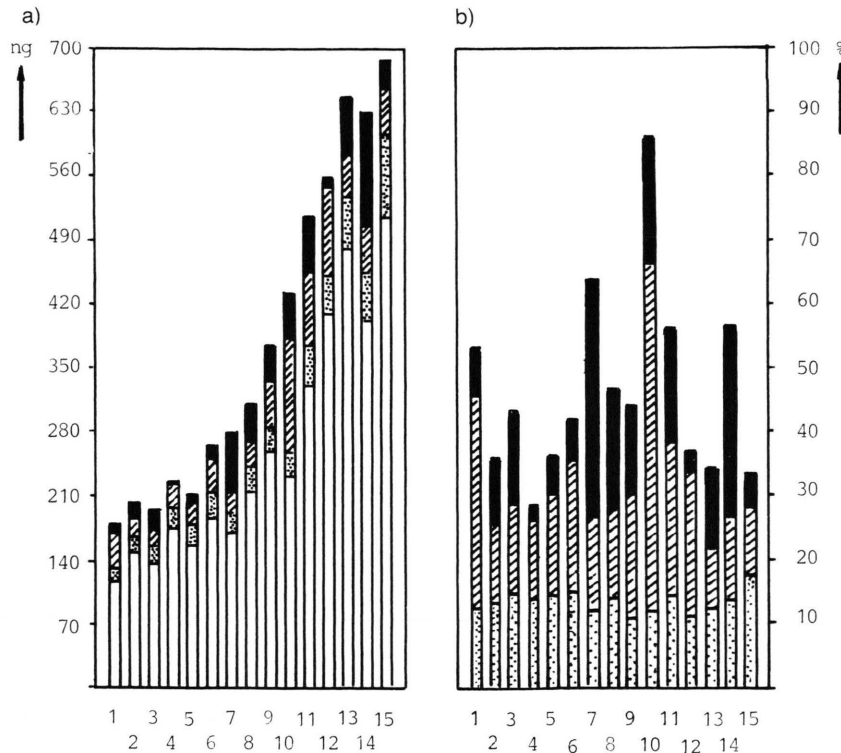


Fig. 3. a) Absolute amounts (ng/insect) of (*E*)-8-dodecenyl acetate (open bars), (*Z*)-8-dodecenyl acetate (stippled bars), dodecyl acetate (hatched bars) and (*E*)-8-dodecenol (black bars) present in the sex pheromone gland of 15 individual females of *C. leucotreta*. b) Relative amounts of (*Z*)-8-dodecenyl acetate (hatched bars), dodecyl acetate (stippled bars) and (*E*)-8-dodecenol (black bars) normalized to (*E*)-8-dodecenyl acetate (= 100%). In both figures, the individuals are arranged in order of increasing total pheromone and pheromone analogue titres. Fig. 3b shows that the ratio of *Z*-8-12:Ac (stippled bars) to *E*-8-12:Ac (normalized to 100%) remains essentially constant.

Table I. Average amount of pheromone components and analogues found in the intersegmental membrane between VIIIth and IXth abdominal segments of 15 *C. leucotreta* females [N = 15], standard deviation SD and coefficient of variance, CV.

Compound	Mean composition by weight [ng/♀]		Mean percentage by weight [%/♀]	
	$\bar{X} \pm \text{SD}$	CV	$\bar{X} \pm \text{SD}$	CV
decyl acetate	2 ± 2	93	0.5 ± 0.3	71
dodecyl acetate	47 ± 30	63	11.7 ± 5.4	46
(<i>E</i>)-8-dodecenyl acetate	262 ± 128	49	63.0 ± 6.0	10
(<i>Z</i>)-8-dodecenyl acetate	34 ± 20	57	8.2 ± 1.6	19
(<i>E</i>)-8-dodecenol	37 ± 31	83	8.6 ± 5.5	64
(<i>Z</i>)-8-dodecenol	4 ± 4	92	1.0 ± 0.7	77
tetradecyl acetate	9 ± 5	55	2.2 ± 1.0	44
hexadecyl acetate	8 ± 3	33	2.2 ± 0.7	33
octadecyl acetate	9 ± 4	48	2.6 ± 2.0	69
Total	412 ± 186	45		

$$\bar{X} = \frac{\sum_{i=1}^n X_i}{n}, \text{SD} = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n}}, \text{CV} = \frac{\text{SD}}{\bar{X}} \times 100$$

In the redbanded leaf roller moth, *Argyrotaenia velutinana*, Miller and Roelofs [12] found that the ratio of (*E*)/(*Z*)-11-tetradecenyl acetates shows only a very narrow variance ($CV=9.7\%$). Similarly, in the jack pine budworm, *Choristoneura pinus pinus*, although the total acetate content per female showed a broad spectrum of values (range 6.7–64.6 ng/gland), the (*E*)/(*Z*)-ratios of 11-tetradecenyl acetates showed only a minor variability with the (*Z*)-isomer at 13–15% of the (*E*)-isomer [13]. In the pyralid *Ostrinia nubilalis*, the so-called (*Z*)- and (*E*)-strains produce 97:3 and 4:96 ratios of (*Z*)- and (*E*)-11-tetradecenyl acetates respectively [14]. However, in the noctuid *Agrotis segetum*, for which the pheromone system does not consist of geometrical isomers, there is a wide variation in the proportions of the pheromone components between individuals [15].

Our results on *C. leucotreta* exhibit the existence of a rigorous control of the ratio of the geometrical pheromone isomers. Although a more comprehensive study would be necessary for a final conclusion, the present results imply the appearance of geographical polymorphism within this insect species. The existence of “pheromone dialects”, depending on the geographical origin of the moths, is well documented at least for a few species [16, 17]. When compared with those of the “Malawi strain” (mean ratio $Z/E = 62:38$, varying between 72:28 and

52:48), the dodecenyl acetate proportions of the *C. leucotreta* strain investigated in our laboratory are almost opposite (mean ratio $Z/E=88.5:11.5$). Our results somewhat resemble the ratios reported for the insects in ref. [4] and [5]. Although sometimes significant differences in the pheromone composition between laboratory and wild populations of moths have been found, these differences never exceeded more than a few percents and thus cannot account for the various different isomer ratios observed in *C. leucotreta*.

Although it is difficult to generalize with the little data available, it appears that at least for some lepidopterous species there is a more precise regulation in ratios when geometrical isomers are being utilized as behaviourally active pheromone components. This phenomenon may be restricted to members of certain insect families or subfamilies, but also it may be a consequence of a greater demand of selection pressure and isolation control for certain moths, brought about by the presence of closely related and sympatrically occurring species.

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