

Pheromones, 88 [1]. Sex Pheromone Components of Female *Euzophera punicaella* M. (Lepidoptera, Pyralidae)

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Euzophera punicaella, Pyralidae, Sex Pheromone, (9*Z*,12*E*)-9,12-Tetradecadien-1-ol, (9*Z*,12*E*)-9,12-Tetradecadienyl Acetate

By means of GC, GC-MS and GC-combined EAG recordings (9*Z*,12*E*)-9,12-tetradecadien-1-ol (**1**) and (9*Z*,12*E*)-9,12-tetradecadienyl acetate (**2**) in a ratio of 4:1 were identified as the pheromone components of the female pyralid moth *Euzophera punicaella* M. originating from Armenia. Determination of EAG activity with male moth antennae and synthetic test chemicals revealed best responses with a mixture of both compounds.

Euzophera punicaella (syn. *bigella*) M. (Lepidoptera, Pyralidae, Phycitinae) is a serious pest of pomegranate, apple and quince in Europe, Caucasus, Middle Asia, India and Pakistan [2]. The larvae of this pyralid species develops in the fruits' flesh and can make unpalatable up to 80% of the harvest. In warm seasons the moth appears in a number of generations, and the flight of the last generation lasts to September. The larvae hibernate in cocoons underneath the trees' bark or in the fruits. To evaluate methods for a control of these insects based upon a biological method, the composition of sex pheromone of the female moths was analyzed.

Materials and Methods

Gland isolation

Larvae of *E. punicaella* were collected in a quince garden, Ararat district, Armenia, and sent to Erlangen. The larvae pupated within two weeks,

and the pupae were kept under a reversed photoperiod (14 h light:10 h dark) to eclosion two weeks later. Freshly hatched females were separated from males, and the insects were fed in the laboratory with 20% sucrose solution. At the time of calling, which was right at the change from scoto- to photoperiod, female moths were anaesthetized with CO₂, their intersegmental membranes between the eighth and ninth abdominal segment (pheromone gland) were dissected under a microscope and a) 4 glands each extracted with 10 µl of *n*-hexane or b) 6–8 glands sealed in glass capillaries ready for solid sampling GC analysis [3].

Electroantennography

Electroantennographic investigations were performed according to the method of Schneider [4] with excised antenna and filter paper as stimulus source, loaded with 1 ng–100 µg test chemicals each and 1 sec stimulation period.

Identification

GC-coupled electroantennography analyses were performed on a Packard United Technologies 48A instrument, equipped with a splitless injector, a flame ionization detector (FID) and a parallel electroantennogram detector (EAD) [5]. The volatiles were chromatographed on a 25 m × 0.25 mm FSCC SP2340 [5 min at 70 °C, 70–195 °C at 5°/min] and a 25 m × 0.25 mm FSCC SE30 [5 min at 70 °C, 70–260 °C at 6°/min], carrier gas N₂, 1 ml/min, injection port and detector temperatures 220 °C and 240 °C, respectively. GCMS analyses were conducted with a Finnigan MAT90 GC-mass spectrometer with data system in electron impact (EI) mode coupled with a Varian 3000 GC instrument, splitless injection, 25 m × 0.25 mm FSCC SE 52 [4 min at 60 °C, 60–260 °C at 6°/min], injector 220 °C, transfer line 220 °C, carrier gas He, 2 ml/min, 70 eV spectra, 1 sec/scan.

Results

Studying the female moths under natural light conditions, *E. punicaella* revealed a distinct calling behavior with a nearly vertically erection of the abdomen and rhythmically protruding of ovipositors (Fig. 1), which was observed at the begin of dawn

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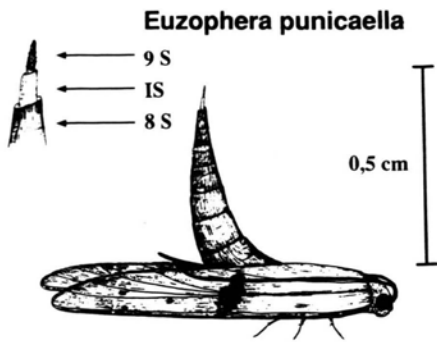
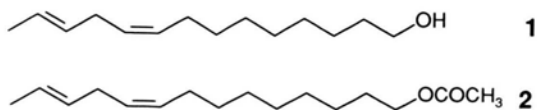


Fig. 1. Calling position of a female *Euzophera punicaella* M. (Lepidoptera, Pyralidae). **8S** eighth abdominal segment, **IS** intersegmental membrane, **9S** ninth abdominal segment.

and under laboratory conditions a few minutes after the start of the photophase, respectively.

Preceding the analysis, an electroantennogram [EAG] screening was carried out with saturated alcohols and acetates of various carbon chain length and revealed a C_{14} -chain as the most active molecular structure. Corresponding monounsaturated C_{14} -alcohols and -acetates resulted in the evaluation of a (*Z*)-9- and an (*E*)-12-configuration of double bonds to be the most effective. Furthermore, double unsaturated test compounds elicited stronger EAG responses than monounsaturated. As a conclusion, double unsaturated (9*Z*,12*E*)-9,12- C_{14} -alkadienols and -alkadienyl acetates were suspected to be constituents of the pheromone blend, as they are known as components of pheromone complexes in a series of pyralid species [6].

A GC analysis of the volatiles of the gland, using two columns of different polarities (SP2340 and SE30) and the hexane extract, was monitored with a male insect antenna as a species specific GC detector (EAG detector [5]), and two physiologically active components found (Fig. 2a), having the retention time of authentic (9*Z*,12*E*)-9,12-tetradecadien-1-ol [Z9E12-14:OH, **1**] and (9*Z*,12*E*)-9,12-tetradecadienyl acetate [Z9E12-14:Ac, **2**] (Fig. 2c and formula 1), which was



Formula 1.

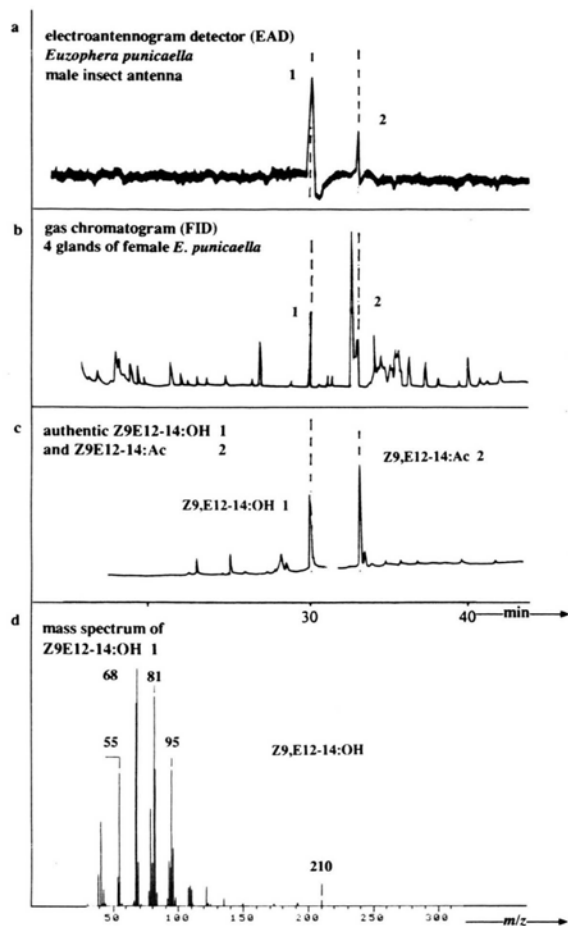


Fig. 2. Analysis of the sex pheromone of *Euzophera punicaella* (Lepidoptera, Pyralidae). **a** Electroantennogram detection (EAD) GC-analysis (SE30 column) of a hexane extract from pheromone glands; **b** gas chromatogram (SE30, hexane extract) of four glands; **c** GC of authentic Z9,E12-14:OH **1** and Z9,E12-14:Ac **2**; **d** EI GC-mass spectrum of Z9,E12-14:OH **1**.

proven by coinjection. With a subsequent GCMS analysis (Fig. 2b) of a solid sample probe, because of the minute amount of the acetate in the insects, the structure of the double unsaturated alcohol Z9E12-14:OH **1** only could be established with certainty by mass spectroscopy, its spectrum (Fig. 2d) being identical with that of the authentic sample. The amount of **1** was estimated to be 2 ng/insect because of RIC-peak size integration. Compound **2** represented about one fourth of this only, derived from the electroantennogram-GC recording. In addition, dodecanoic acid, tetradecanoic

acid, isopropyl tetradecanoate, pentadecanoic acid, hexadecenoic acid, hexadecanoic acid, pentadecan-2-one, heptadecan-2-one, octadecenoic acid, octadecanoic acid and a series of long chained C_{21} -, C_{23} - and C_{25} -hydrocarbons were found in the gland extract. None of these substances was physiologically active; they are frequently found as constituents of lipid material in insect tissues and hence not considered as pheromone components.

Comparative electroantennogram tests were carried out with synthetic alkadienol **1** and alkadienyl acetate **2** and with mixtures of the two substances in three different ratios, 1 μ g stimulus source loading each. Pure tetradecadienol **1** was electrophysiologically more effective than pure acetate **2** (Fig. 3), the highest efficacy was obtained from a 80:20 mixture of **1** and **2**. Mixtures of 95:5 and 90:10 showed slightly lower efficacies (Fig. 3 gives a diagram on the EAG activities of the test chemicals). Field trials to evaluate a monitoring system for this insect based upon pheromones will be carried out in Armenia in future seasons.

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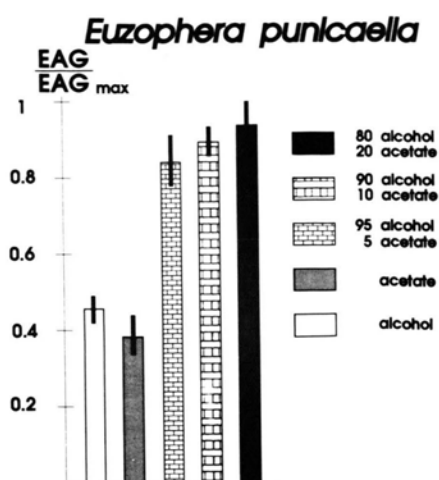


Fig. 3. Relative EAG-responses (EAG/EAG_{max}) of male *Euzophera punicaella* to tetradecadienol **1** and tetradecadienyl acetate **2**, respectively, and to three different mixtures of both test chemicals, stimulus source loading 1 μ g.

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