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


Institut für Organische Chemie, FAU-Universität Erlangen-Nürnberg, Henkestraße 42, D-W-8520 Erlangen, Bundesrepublik Deutschland

E. V. Babayan, and Sh. O. Badanyan

Institute of Organic Chemistry, Armenian Academy of Sciences, Kamo str. 167-a, 375094 Erevan, Armenia

Z. Naturforsch. 48c, 110–112 (1993); received September 15/December 16, 1992

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

By means of GC, GC-MS and GC-combined EAG recordings (9Z,12E)-9,12-tetradecadien-1-ol (1) and (9Z,12E)-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 develop 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 scotophase 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 48 A 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 He, 2 ml/min, 70 eV spectra, respectively. GCMS analyses were conducted with a Finnigan MAT 90 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.
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 (9Z,12E)-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 SE 30) 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 (9Z,12E)-9,12-tetrade- adien-1-ol [Z9E12-14:OH, 1] and (9Z,12E)-9,12-tetrade- adienyl acetate [Z9E12-14:Ac, 2] (Fig. 2c and formula 1), which was proved 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 µg 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.

Acknowledgements

We are thankful to Dr. G. Asaryan (Institute of Plant Protection, Mezzavan, Armenia) for supplying biomaterial and are indebted to the A. v. Humboldt Foundation for awarding a fellowship to G. G. Melikyan. D. Schäfer thanks the Studienstiftung des Deutschen Volkes. Financial support from the Deutsche Forschungsgemeinschaft and the Volkswagen-Stiftung is greatly acknowledged.