Sex Pheromone Components of the Gamma Moth, *Autographa gamma* (L.) (Lepidoptera: Noctuidae)

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Z. Naturforsch. **38 c**, 1011 – 1014 (1983); received July 6, 1983

Sex Pheromone Components, *Autographa gamma*, *Z*-7-Dodecenyl Acetate, *Z*-7-Dodecenyl Alcohol

Z-7-Dodecenyl acetate and *Z*-7-dodecenyl alcohol have been identified as sex pheromone components of female *Autographa gamma*. This is the first time that *Z*-7-dodecenyl alcohol has been found in the natural pheromone of Plusiinae. When incorporated in pheromone traps of some Plusiinae species the alcohol is both synergist and inhibitor, thus being an important factor in sex isolation among sympatric Plusiinae species in Israel.

Introduction

The moth *Autographa gamma* (L.) is an important polyphagous pest in Europe. In Israel it is more harmful in the northern valleys but it is not considered as a major pest. There are two peaks in the adult populations, one in the spring and one in the autumn. The moth is known as a typical migrant, moving in swarms northward to Europe in the spring [1].

Pheromone release and male responsiveness to the natural pheromone have been studied recently [2]. Some field tests, using *Z*-7-dodecenyl acetate (*Z*7-12:OAc) as attractant in pheromone traps, were performed in Rumania [3]. An EAG screening study of male moths, including *A. gamma*, indicated the presence of five-component receptor sites on the male antenna for *Z*7-12:OAc, the corresponding alcohol and three additional unsaturated acetates [4].

This paper reports the isolation and identification of two components of the sex pheromone of *A. gamma* and their use in field traps.

Materials and Methods

Chemicals

*Z*7-12:OAc was purchased from Albany International, it contained 1 – 2% of the *E*-isomer; *Z*-7-dodecenyl alcohol (*Z*7-12:OH) was obtained from *Z*7-12:OAc by basic hydrolysis.

Derivatization:

*Z*7-12:OH and the alcohol fraction from gland extracts were converted to propionates (OPr) with propionyl chloride and to trifluoroacetates (TFA) with trifluoroacetic anhydride. A drop of reagent was added to 0.5 – 1 µg *Z*7-12:OH or sample of alcoholic fraction in 100 µl hexane or dichloromethane, left at room temperature in teflon sealed vials for 15 min. Solvent and excess reagent were evaporated with a stream of nitrogen, 10 µl hexane were added and the derivatives were ready for GC analysis. The trimethyl silane (TMS) derivatives were prepared in hexane solution with a drop of N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) reagent, left for 1 h at room temperature and analyzed directly by GC.

Gas chromatography (GC) and combined mass spectroscopy (GC-MS)

For analytical purposes, a Varian 3700 model, equipped with a FID detector and a splitless injector system, was used. Conditions of chromatography for all WCOT capillary columns were: injector and detector 250°, initial oven temperature relatively low, purge 45 s after injection, programming 3 min after injection. Column A=SP 2100 (glass, 60 m × 0.25 mm), for all samples injection at 100° and programming at 30°/min to 180°; He flow of 1 ml/min. Column B=PCP-sil (glass, 30 m × 0.25 mm); for gland extracts and OPr derivatives injection at 100° and programming at 30°/min to 150°; for TMS and TFA derivatives injection at 70° and programming at 10°/min to 120°; He flow of 2 ml/min. The GC-MS was performed on a Finigan

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* Contribution No. 765-E, 1983 series, from the Agricultural Research Organization, The Volcani Center, Institute of Plant Protection, Bet Dagan 50 250, Israel.

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0341-0382/83/1100-1011 $ 01.30/0

combination 4021 equipped with an OV 17 column (glass, 25 m × 0.25 mm); injection at 80° and programming at 7°/min to 160°; He inlet pressure of 1 psi. The mass spectra were recorded at an electron energy of 70 eV. For preparative purposes, a Varian 2400 model, equipped with a FID detector and an effluent splitter at a ratio of 1/25, was used. An OV 1; 3% column (glass 2 m × 2 mm) on Supelcoport 100–120 mesh was used at 170°, N_2 flow of 25 ml/min, injector and detector at 250°. Fractions were collected in cooled U shaped glass tubes.

Virgin females and extract preparation

Insects were reared on an artificial medium [5]. Pupae were sexed and placed in a room with a dark-light regime of 10:14 h. The abdominal tips of 3–5 day old virgin females were clipped, during their calling period, 3–5 h after the beginning of the dark period and extracted with hexane or dichloromethane. The extracts (batches of 30–100 glands) were filtered through a plug of glass wool and concentrated to a small volume on a Rotavapor and then further with a stream of nitrogen. The solutions were analyzed directly by GC, GC-MS or submitted to fractionation.

Field tests

Funnel traps were used for the field experiments. The traps were made of a protective wooden roof fixed 5–7 cm above a plastic funnel 20 cm in diameter, to which a 2-liter plastic container was attached. A screen cage, containing two virgin females, 3–5 days old, or the synthetic pheromone on a rubber septum, was hung from the center of the roof. Traps were located at the Bet Dagan farm, in a row 25 m apart and about 1 m above ground. The catches were recorded every 2–3 days, and the baits were rotated by one position at that time.

Results and Discussion

Chemical identification

Ten extracts, each comprising of 50–100 female abdominal tips, were analyzed by capillary GC (Fig. 1). Some of the extracts contained many interfering peaks and they were discarded. The largest identified peak correspond to *Z*7-12:OAc as analyzed on the non polar column A and on the polar column B and confirmed by GC-MS (third capillary column). The EI mass spectrum gave the

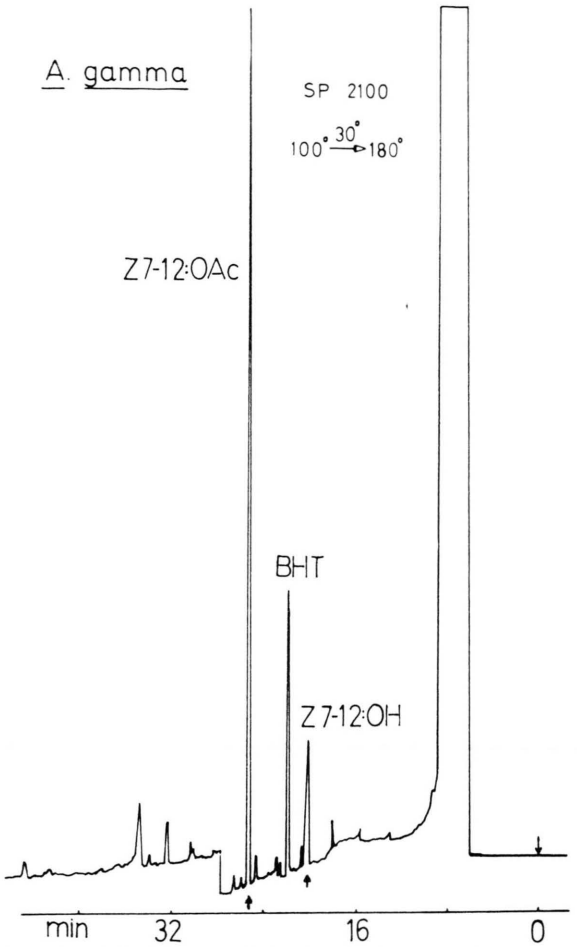

Fig. 1. SP 2100 high resolution WCOT column chromatography of crude extract from *Autographa gamma*.

highest mass at *m/e* 166 (M^+-60), 10%; and a diagnostic peak at *m/e* 61 ($CH_3COOH_2^+$), 9%; base peak at *m/e* 67. The analysis of the second peak, corresponding to *Z*7-12:OH, was more complicated. The amount of the alcohol was much lower and it gave a relatively broad peak, in particular on column B. A better chromatogram was obtained on column A (Fig. 1), in which one of the interfering peaks was identified as 2,6-di-*tert*-butyl-4-methylphenol (BHT). The amount of *Z*7-12:OAc per female was 2–3 ng and that of *Z*7-12:OH was 0.2–1 ng. Due to the small amount, the alcohol could not be analyzed by the GC-MS. To confirm its identity and that of *Z*7-12:OAc, a number of batches were combined and submitted to GC fractionation on a 2 m OV1 column. Two fractions were isolated, the first corresponding to *Z*7-12:OH (partially separated from

Table I. Trap catches of male *Autographa gamma* with synthetic chemicals.

Test No.	Bait	Number of traps	Number of nights	Total number of males
1	0.1 mg Z7-12:OAc	5	7	11[b]
	0.1 mg Z7-12:OAc + 0.01 mg Z7-12:OH	5	7	34[a]
2	0.1 mg Z7-12:OAc	4	4	11[a]
	0.1 mg Z7-12:OAc + 0.01 mg Z7-12:OH	4	4	16[a]
	2 virgin females	4	4	2[b]
3	0.2 mg Z7-12:OAc	4	3	5[b]
	0.2 mg Z7-12:OAc + 0.02 mg Z7-12:OH	4	3	15[a]

an unknown peak) and the second to Z7-12:OAc. The collected acetate was re-injected onto capillary column B and also co-injected with synthetic Z7-12:OAc. The first eluted fraction, containing the alcohol, was converted into three different derivatives for GC comparison with the same derivatives prepared from synthetic Z7-12:OH. The following derivatives were prepared: OPr ester, TFA ester and TMS ether. The two esters were analyzed on column B and the TMS ether was injected on both columns A and B. All tests showed that the three derivatives were identical to the corresponding Z7-12:OPr, Z7-12:TFA and Z7-12:TMS, thus confirming the identity of Z7-12:OH.

Field tests

In preliminary tests [6] it was found that a loading of about 0.1–0.2 mg of Z7-12:OAc per trap is optimal for trapping *A. gamma*. At higher doses the catches contain mainly *Trichoplusia ni*. It was also shown that high proportions of Z7-12:OH in the pheromone blend repel *A. gamma*. Therefore, in field tests conducted in the spring seasons (March–May) of 1981 and 1982, we used loadings of 0.1 and 0.2 mg of Z7-12:OAc each with 10% of Z7-12:OH (Table 1). The population of *A. gamma* in the central part of Israel (Bet Dagan) is generally low [1] therefore the number of *A. gamma* males caught in the traps was relatively small. In all tests the addition of 10% Z7-12:OH enhanced the catch of *A. gamma* males although in some experiments the difference was statistically nonsignificant. Interestingly, traps baited with two virgin females gave a very low catch and in several experiments no males were caught, although the females were replaced every 2–3 days (Table I).

The significant finding of this study is the identification of Z7-12:OH, in addition to the main component Z7-12:OAc, in the abdominal tip extracts of *A. gamma*. The acetate has so far been identified in all Plusiinae sex pheromones, in some being the only pheromone component [7–12]. The alcohol, Z7-12:OH, was found to act as a strong synergist for the capture of *Autographa californica* males in pheromone traps baited with Z7-12:OAc, however it was not detected in the gland extracts [13]. On the other hand it is known that Z7-12:OH is a potent inhibitor for *T. ni* [14] and *Plusia chalcites* [6]. In the present work the alcohol has been identified in the abdominal tip extracts of *A. gamma* and was found to enhance trap catches when added to Z7-12:OAc (Table 1). Occasionally, several *T. ni* were caught in traps baited with 0.1 mg or 0.2 mg Z7-12:OAc but not in traps baited with the acetate and 10% Z7-12:OH. If the amount of Z7-12:OH in the mixture was raised from 10% to 100%, the trap catch of *A. gamma* declined sharply [6] and at a ratio of 5:1 no *A. gamma* were caught; instead, a different Plusiinae species, namely *Syngrapha circumflexa* was captured (Table II).

Table II. Trap catches of male Plusiinae with synthetic chemicals.

Bait	Total number of males	
	Autographa gamma	*Syngrapha circumflexa*
0.1 mg Z7-12:OAc	11[a]	0
0.1 mg Z7-12:OAc + 0.01 mg Z7-12:OH	18[a]	0
0.1 mg Z7-12:OAc + 0.5 mg Z7-12:OH	1[b]	24

In four traps on ten nights.

Conclusions

Two components have been found in the abdominal tip extracts of *A. gamma: Z*7-12:OAc and the corresponding alcohol *Z*7-12:OH. Addition of 10% alcohol to the acetate onto the rubber septa used in traps resulted in an increase of male catch of *A. gamma.* The alcohol plays also an important role in the sexual isolation of some sympatric Plusiinae species; being both synergist or inhibitor at the appropriate concentrations for the different species.

Acknowledgements

We would like to thank Dr. B. Sklarsz from the Chemistry Department of Bar Ilan University for the mass spectra and Mrs. Myriam Harel and Mrs. Shlomit Levski for technical assistance.

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