Eur. J. Entomol. **91**: 197–203, 1994
ISSN 1210–5759

Sex pheromone characterisation and field trapping of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), in South Moravia and Slovakia

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Pheromone, *Ostrinia nubilalis*, trapping

Abstract. The analysis of pheromone glands from individual females of the European corn borer, *Ostrinia nubilalis* (Hübner), originating in South Moravia and Slovakia showed that this population utilizes the "Z" pheromone system. The ratio of (Z)- and (E)-11-tetradecenyl acetates was found in the range of 98.5 : 1.5–99.5 : 0.5. Field experiments confirmed the identity of the local population as being predominantly of the Z strain. Individuals responding to E and hybrid blends were detected.

INTRODUCTION

The sex pheromone of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), has been identified as a mixture of geometric isomers of 11-tetradecenyl acetate. The proportion of the isomers has been found to vary according to the geographical origin of the moths. In North America and Europe, *O. nubilalis* occurs as a mosaic of populations with two distinct sex pheromone systems. The so-called Z strain has a 97 : 3 blend of (Z)-11- and (E)-11-tetradecenyl acetates (abbrev. Z11-14:Ac and E11-14:Ac) (Klun et al., 1973). The E strain has a 4 : 96 blend of Z11 and E11-14:Ac (Kochansky et al., 1975). The Z strain is widely distributed throughout the world, whereas the E strain occurs mainly in Italy, Switzerland and northeastern United States (Klun et al., 1975; Anglade et al., 1984). In the areas of sympatry, hybrids using an intermediate blend of the Z and E isomers in a ratio 35 : 65 (Klun & Maini, 1979; Anglade et al., 1984) were detected. A preliminary investigation of *O. nubilalis* populations in former Czechoslovakia indicated that the corn borers in this area probably belong to the Z strain. The attempts to use a synthetic pheromone for monitoring, however, were not efficient enough (Hrdý et al., 1986). In order to characterize a possible "pheromone dialect" of the local *O. nubilalis* population, female pheromone glands were chemically analyzed and males' ability to discriminate pheromone composition was investigated in field experiments.

MATERIAL AND METHODS

Insects: Experiments were performed on a laboratory colony of *Ostrinia nubilalis* (Hübner) established from individuals collected from corn fields as diapausing larvae. Further generations were reared on a semi-synthetic diet (Nagy, 1961) under a LD 16 : 8. The pheromone glands were analyzed during the scotophase in 3-day old virgin females, which are known to produce maximal amounts of pheromone (Borek & Kalinová, 1991). Males 2 to 3 days old were used for electroantennographic (EAG) recordings.

Pheromone analysis: The pheromone was analyzed by means of a solid-sample injection technique (Attygale et al., 1987) enabling gland analysis without solvent extraction. Capillary gas chromatography (GC), mass spectrometry (MS) and electroantennography (EAG) were combined during analysis.

Gland preparation: The VIII and IX abdominal segments of a calling female were detached. The intersegmental membrane was encapsulated in a thin-walled soda glass capillary (2 × 20 mm). The samples were either used immediately or stored at –20°C until use. The active pheromone glands were compared with the glands of decapitated females (24 hrs old), in which pheromone production is known to be suppressed due to the absence of a brain-released pheromonotropic hormone (Raina & Menn, 1987).

Gas chromatography: Capillary gas chromatography with flame ionisation detection (FID) was performed on a Hewlett Packard 5890A instrument equipped either with a SP 2340 or SE 52 polar columns (25 m × 0.22 mm i.d., Chrompack). The injector space was modified to adopt a solid sample injector (Attygale et al., 1987). Solid samples were injected splitless. N_2 supplied at a 2ml/min linear flow was used as a carrier gas. The temperature regime was set at 60°C to 195°C, at a rate of 4°C/min. The injector and detector temperatures were 220°C and 250°C, respectively.

Gas chromatography-mass spectrometry (GC-MS): Mass spectra of compounds in pheromone glands were obtained on a VARIAN 3400 gas chromatograph linked to a FINNINGAN MAT 90 mass spectrometr. In this system a SP 2340 and/or SE 52 column was directly coupled to the mass spectrometr. The solid-sample injection and temperature regime were the same as described above. Mass spectra were compared to those of authentic chemicals.

Gas chromatography with electroantennographic detection (GC-EAD): To obtain precise information about the retention times of gland compounds with olfactory activity, the EAG setup was directly coupled to the gas chromatograph. The antenna served as an additional (biological) GC detector (Arn et al., 1975). The outlet of the capillary column used for GC analysis was split into two parts (split ratio 1 : 1), one of which was connected to the FID detector, and the other of which led out of the oven into an airstream continuously blowing over the antennal preparation. Isolated antennae were used. Antennal responses were recorded by Ag/AgCl glass electrodes (Roelofs, 1984), amplified and registered simultaneously with the response of the FID detector.

Field experiments: Field experiments were performed in South Moravia (Velké Bílovice, Strážnice) and Slovakia (Nitra) in 1991 and 1992. Four replicated treatments were made at each locality. Traps were placed in single row along the edge of corn field, spaced 40 m apart at a height of 1 m. Traps were periodically rotated within each test series to minimize the possible position effect on trap efficiency. Captured moths were removed and recorded daily. The effectiveness of lure composition, dose, trap design (delta- and wing-type) and dispenser type was examined. Traps baited with a synthetic pheromone were compared to those baited with 3 virgin females (2 to 4 days old) placed in a small wire cage hung inside the trap. Females were provided with water, checked daily and regularly changed.

Dispenser preparations: Chemically pure (> 99%) Z and E11-14:Ac and 14:Ac were dissolved in hexane and GC analyzed, to ensure that no traces of attraction inhibitors (Z)-9-dodecenyl acetate or (Z)-9-tetradecenyl acetate (Struble et al., 1987) were present. Compounds were applied topically to dispensers in creating the various pheromone blends. After solvent evaporation, the dispensers were tightly sealed in aluminium-polyethylene foil and stored under –20°C until use. Three types of rubber and four types of polyethylene were tested as pheromone dispensers.

Data analysis: Analysis of variance (ANOVA) and Duncan's multiple range test ($P = 0.05$) were conducted to determine the influence of trap design, dispenser type, lure composition and pheromone dose on moth captures.

RESULTS

GC-MS: Complete mass spectra were obtained for the following components of the female glands: 14:Ac, Z11-14:Ac, E11-14:Ac, Z11-14:OH, methyl esters of hexadecanoic and octadecanoic acid. Of the hydrocarbons, saturated and unsaturated C22-27 were identified. All identified mass spectra were compared with those of standards. Compounds 14:Ac, Z11-14:Ac, E11-14:Ac were not present in inactive pheromone glands. The relative abundance of 14:Ac : Z11-14:Ac : E11-14:Ac was 14 : 100 : 1. In 10 analyzed females the

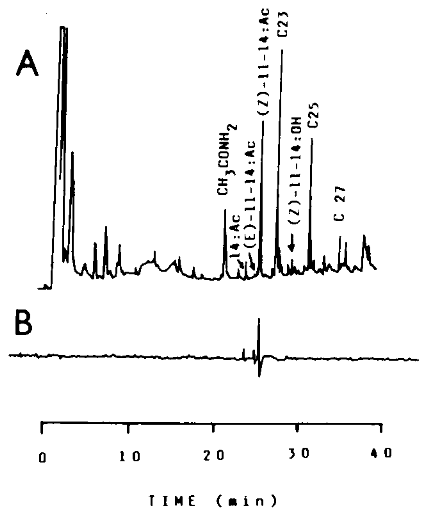


Fig. 1. GC-FID-EAD chromatogram obtained from active pheromone gland of female *Ostrinia nubilalis* on a 25 m x 0.22 mm fused silica capillary column SP-2340. Oven temperature was held at 60°C for 2 min and increased to 195°C at 4°C/min. 14:Ac – tetradecyl acetate, (E)-11-14:Ac – (E)-11-tetradecenyl acetate, (Z)-11-14:Ac – (Z)-11-tetradecenyl acetate, (Z)-11-14:OH – (Z)-11-tetradecenyl alcohol, C23 – tricosan, C25 – pentacosan, C27 – heptacosan. A: GC-FID responses B: GC-EAD responses to 14:Ac, (E)-11-14:Ac and (Z)-11-14:Ac.

Z:E ratio was determined to be in the range 98.5 : 1.5–99.5 : 0.5. Gland samples contained 3–5 ng of Z11-14:Ac per female.

GC-EAD: Reproducible responses to both Z and E isomers of 11-14:Ac were observed in all GC-EAD experiments (Fig. 1B). In several experiments antennae also responded to 14:Ac. No other EAD-active materials were detected in pheromone glands.

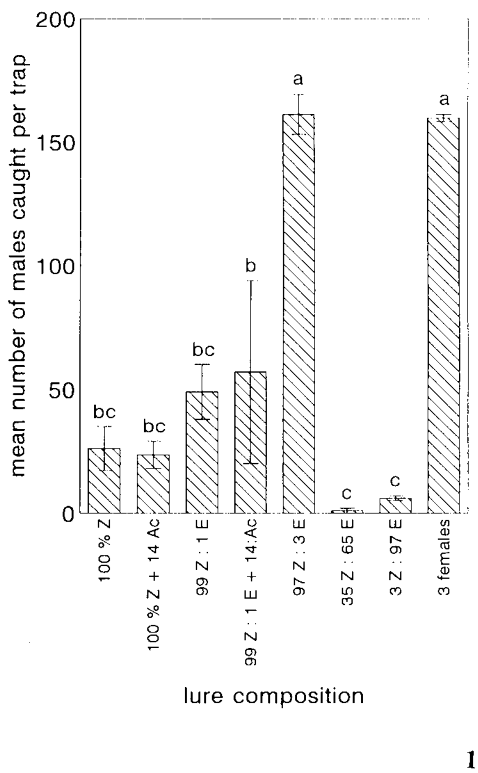


Fig. 2. Mean number of males of *O. nubilalis* caught by wing traps baited with 3 virgin females and 100 μg of various pheromone blends, loaded on red rubber septa. Strážnice, South Moravia, July 4–18, 1992. Columns signed by the same letter are not significantly different at P = 0.05.

Field experiments: The initial tests involved various lure compositions: 3 virgin females; 100% Z (100 μg); 99Z:1E (100 μg); 97Z:3E (100 μg); 99Z:1E (100 μg) + 10 μg 14:Ac; 35Z:65E (100 μg) and 3Z:97E (100 μg) loaded on red rubber septa (Struble et al., 1987). Male moths were most attracted to the "Z" blend and/or to virgin females (laboratory "Z" strain) (Fig. 2). The 99Z:1E blend trapped substantially fewer males than the 97Z:3E blend. The addition of 14:Ac had little effect in attracting moths to the 99:1 blend. The local population responded partially to pure Z isomer. In 1992 12 "E" and 2 "hybrid" males were caught in Strážnice and 2 "hybrid" males in Nitra (Fig. 2). The dose of 10 μg of the "Z" blend was the most effective, followed by 50, 100, and 500 μg (Fig. 3). The effectiveness of all doses, compared to that of virgin females, decreased gradually during experimentation, probably due to a slow pheromone degradation. Male captures in traps baited with virgin females did not decline during the experimental period, indicating an unchanged population density. The wing-type trap appeared to be more effective than the delta-type (Fig. 4). In all of the experiments mentioned above, red rubber septa (A.H. Thomas Co., Catalog No. 1780-J07) were used as pheromone releasers. The numbers of males caught in traps baited with the "Z" pheromone blend, loaded on other materials were substantially lower (Fig. 5).

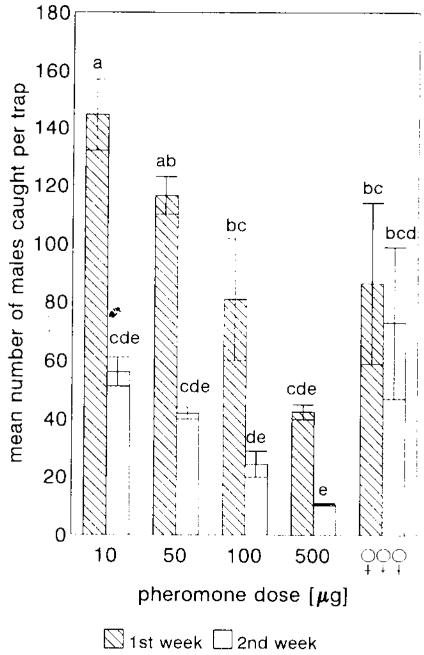


Fig. 3. Mean number of males of *O. nubilalis* caught by wing traps baited with 3 virgin females and by 10, 50, 100 and 500 μg of "Z" blend, using red rubber septa. Note the differences in moth catches of the 1st and 2nd week indicating pheromone degradation. Strážnice, South Moravia, July 4–18, 1992.

DISCUSSION

The results of this study generally confirm previous reports of the presence of the Z strain of *O. nubilalis* in the area. However, several "E" and "hybrid" individuals were detected.

In Europe, a survey by Klun et al. (1975) revealed the prevalence of the Z pheromone strain of the European corn borers. Analyses and field tests by Maini et al. (1978), Büchi at al. (1982), Anglade et al. (1984), Barbattini et al. (1985) and Peña et al. (1988) confirmed that the Z, E and hybrid strains are simultaneously present south of the Alps. The presence of the E strain in the northern areas was reported only in northern Germany (Langenbruch et al., 1985). This sporadic occurrence of individuals of the E and hybrid corn borers raises the question, whether the both strains are inherently present in some field population or if this isolated presence of E and hybrid moths could be explained otherwise, for instance by the migration from southern localities.

Fig. 4 (right). Mean number of males of *O. nubilalis* caught by delta- and wing-type traps baited with 100 μg of "Z" blend on red rubber septa. Velké Bílovice, July 1–14, 1991.

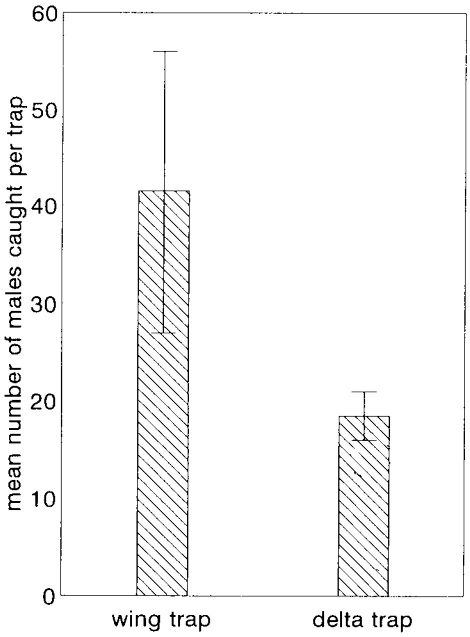
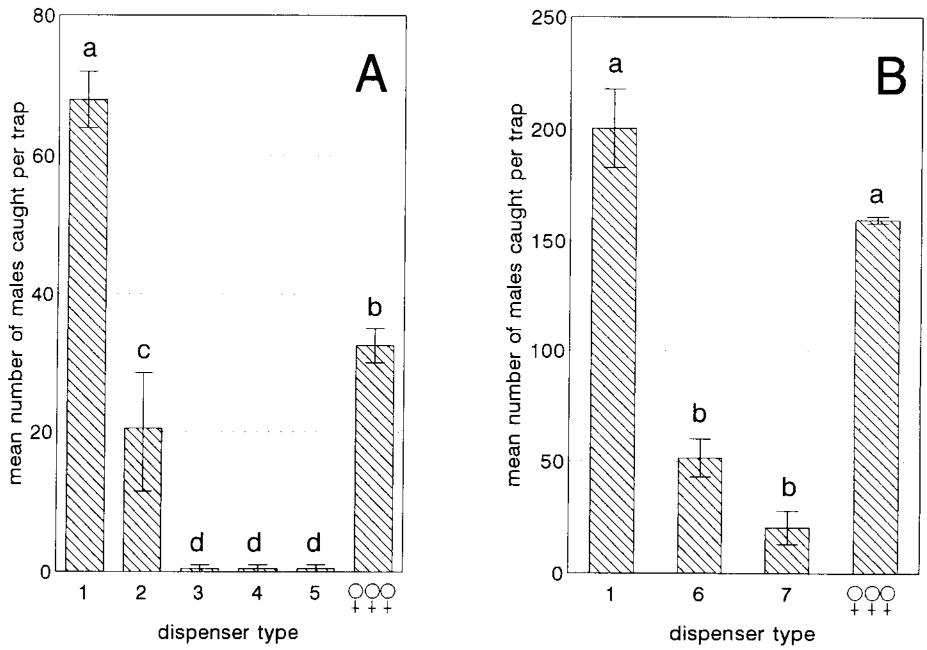


Fig. 5 (below). Mean number of males of *O. nubilalis* caught by wing-traps baited with 100 μg of "Z" blend loaded on various dispensers: 1 – red rubber (A.H. Thomas Co., Cat. No. 1780-JO7); 2 – "brown" rubber; 3 – red polyethylene; 4 – white polyethylene; 5 – transparent polyethylene I; 6 – transparent polyethylene II; 7 – "grey" rubber; ♀♀♀ – 3 virgin females. A – Velké Bílovice, 1991; B – Strážnice, 1992.



The present study did not prove the pheromone dialect of the local Z population. Although the isomer ratio found in pheromone glands slightly differs from those reported in other studies, 97:3 Z:E ratio was the most efficient in the field experiments. Besides the main acetates, the 14:Ac was considered as an additional pheromone component. However, field experiments did not support this possibility.

The presence of C23-C25 hydrocarbons in inactive pheromone glands and their low volatility seem to exclude the possibility that they could function as pheromones. However, in *Orgyia leucostigma* (Lepidoptera: Lymantriidae) alkanes, tricosane, tetracosane, pentacosane and heptacosane present in the female scales were found to improve copulation attemps and to serve as copulation behavior releasing pheromones, in spite of their low EAG activity (Grant at al., 1987). Wheather alkanes identified in female pheromone glands in *O. nubilalis* have a similar behavioral function remains to be seen.

The fact that trap design, pheromone dose and isomer ratio affect substantially the trapping efficiency has been repeatedly reported (McLeod & Starratt, 1978; Kennedy & Anderson, 1980). Similarly, for some moth species and pheromone systems, the type of dispenser used for pheromone release was found to be the most critical factor for maintaining the pheromone activity (Horák et al., 1989; Hrdý et al., 1986). Needed pheromone dispenser requirements are: pheromone stability, constant pheromone release and evaporation rate comparable to those produced by calling females. *O. nubilalis* appeared to be very sensitive to dispenser type. The reason for generaly low trap attractancy observed when other than red rubber dispenser were used is not clear. It could be caused by pheromone degradation. The autoxidation of the pheromone in the dispenser may lead to the formation of an inhibitory or repellent substance(s) (Starratt & McLeod, 1976) influencing the male behavior. These processes were perhaps responsible for our earlier, unsatisfactory results in trapping *O. nubilalis*, even when synthetic pheromone of high purity was used and the dispensers were prepared just prior to field use, as recommended by Struble et al. (1987).

ACKNOWLEDGEMENT. This research was supported in part under grant No. DHR-5600-6-00-1051-00, of the Program in Science and Technology Cooperation, of the Office of the Science Advisor, U.S. Agency for International Development.

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Received May 25, 1992; accepted April 30, 1993