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SEX PHEROMONE COMPONENTS OF THE OMNIVOROUS LEAFROLLER MOTH, *Platynota stultana*[1]

ADA S. HILL and WENDELL L. ROELOFS

Department of Entomology
New York State Agricultural Experiment Station
Geneva, New York

Abstract—A mixture of *trans*- and *cis*-11-tetradecenyl acetates have been found in omnivorous leafroller moth female tip extracts in a ratio of 88 : 12, respectively. In the field they are the most attractive to male omnivorous leafroller moths in a ratio of 94 : 6. Field attractancy can be increased by addition of small quantities (0.2–2.0%) of mixtures of *trans*- and *cis*-11-tetradecenyl alcohols, indicated to be present in female tip extracts in a ratio of 88 : 12, respectively.

Key Words—sex pheromones, sex attractants, omnivorous leafroller, *Platynota stultana*.

INTRODUCTION

The omnivorous leafroller moth (OLR), *Platynota stultana* (Walsingham) is a major tortricid vineyard pest in California, with potential for becoming a significant pest of other crops in the warmer climates. In the eastern United States, it is a problem primarily in greenhouses, feeding on a wide variety of plants. Clearly, the development of an effective synthetic sex pheromone could be very useful in integrated pest control programs, either as a monitoring tool for timing insecticide sprays or as an insect control tool (Tette, 1974).

A sex pheromone emitted by the female to attract males has been demonstrated for this insect (AliNiazee and Stafford, 1971). This paper reports the identification of the various pheromone components.

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METHODS AND MATERIALS

GLC columns (glass, 2 m × 4 mm or 4 m × 2 mm) were packed with 3% OV-1 (methyl silicone) on 100–120 mesh Gas-Chrom Q, 3% CHDMS (cyclohexanedimethanol succinate) on 100–120 mesh Gas-Chrom Q, or 3% PDEAS (phenyldiethanolamine succinate) on 100–120 mesh Chromosorb W-AW-DMCS; a hydrogen flame ionization detector was used. The mass spectrometer was a Hitachi RMU-6E interfaced with an Aerograph 1740–10 gas chromatograph (CHDMS column).

Moths were reared continuously in a greenhouse on fava beans (Glass and Hervey, 1962). Excised female tips were extracted with methylene chloride. Chemical analyses of tip extracts were carried out essentially as described elsewhere (Hill et al., 1974; Roelofs et al., 1971*b*). Solvents were distilled prior to use. Diethyl ether (dry, Mallinckrodt reagent) was used without distillation.

Electroantennograms (EAG) were carried out as previously described (Roelofs and Comeau, 1971*a*); they were used for assaying GLC collections of female tip extracts (Roelofs et al., 1971*a*) and for determining normalized response profiles of male antennae to a series of long-chain acetates, alcohols, and aldehydes (Hill et al., 1974).

RESULTS

Analysis of Female Tip Extracts

Aliquots (ca. 5 female equivalents) of female tip extracts in carbon disulfide were collected from OV-1 and CHDMS columns in one-minute fractions. Assay by EAG showed one major area of activity (10–12 minutes) from OV-1 (170°C) at the retention of *trans*-11-tetradecenyl acetate (t11-14:Ac; 11.05 minutes) (Figure 1), and one major area of activity (7–8 minutes) from CHDMS (170°C) also at the retention of t11-14:Ac (7.35 minutes). GLC tracings of female tip extracts on OV-1 showed 2 major peaks, A and B, with the retentions of *trans*-11-tetradecen-1-ol (t11-14:OH) and t11-14:Ac, respectively (Fig. 1). The ratio of A to B varied among all the extracts examined, with a relatively low ratio (1:14) observed with an extract of virgin females and higher ratios (1:1.4 to 1:0.7) observed with extracts of females from mixed populations in which some mating was known to have taken place. Although component A did not show EAG activity in the GLC collections, it was still considered a potential pheromone component, because (a) it was the only other major component found in the extract, and (b) minute quantities of secondary components eliciting reduced EAG activity are often overlooked.

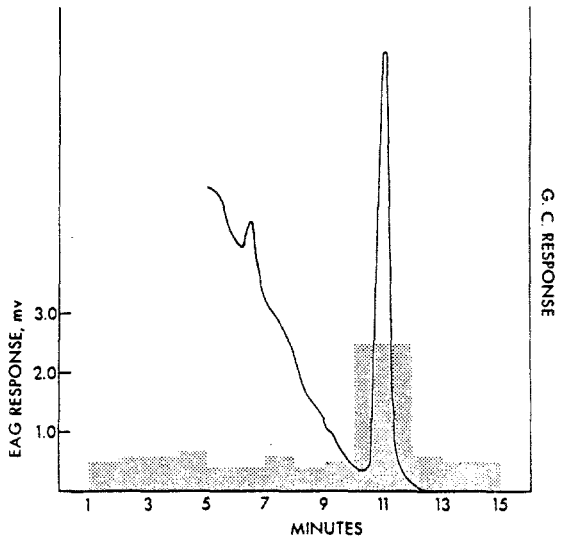

FIG. 1. OLR virgin female tip extract: EAG response of 1-minute collections and GLC tracing, using an OV-1 column at 170°C. The retention times for t11–14 : OH and t11–14 : Ac under these conditions were 6.55 and 11.05 minutes, respectively.

Chromatography of crude female tip extract was carried out on Florisil® using petroleum ether (30–60°C), 2, 5, 10, 25, and 50% diethyl ether in petroleum ether, and diethyl ether as eluants, followed by examination of the recovered materials using GLC on OV-1 and EAG assay of OV-1 collected samples. Component A was eluted with 50% diethyl ether (the alcohols fraction) and component B with 25% diethyl ether (the acetates fraction).

Analysis of A

A was collected from OV-1 for all analyses. Acetylation and subsequent saponification of A resulted in the GLC behavior on OV-1 expected for t11-14 : OH, including the appearance of EAG activity (10–11 minutes) after acetylation at the retention of t11-14 : Ac (10.9 minutes at 169°C, with t11-14 : OH at 6.35 minutes) and the disappearance of this activity after saponification. A apparently has no EAG activity at the nanogram level used in this test, although the 10 μg standard does elicit antennal responses (see section on EAG standards).

Microozonolysis of A and of t11-14 : OH produced fragments with similar retentions on OV-1 (6.55 and 6.5 minutes, respectively, at 165°C) confirming the presence of a double bond at the 11- position.

TLC of acetylated A on silver nitrate-impregnated silica gel-G resulted in recovery of an EAG-active material from the *trans* area. This EAG-active material was collected from CHDMS, 180°C at 5–6 minutes, which coincides with the retention time of t11-14 : Ac (5.7 minutes).

Examination of A on PDEAS (174°C) revealed three peaks with the retentions of tetradecyl alcohol (14 : OH; 12.5 minutes), t11-14 : OH (15.0 minutes), and *cis*-11-tetradecenyl alcohol (c11-14 : OH; 15.9 minutes) in the ratios of 6 : 83 : 11, respectively, or a *trans-cis* ratio of 88 : 12. The only other 14-carbon alcohol that can be converted to 11-hydroxyundecanal on ozonolysis is 12-methyl-11-tridecenyl alcohol (12Me-11-13 : OH); on PDEAS (175°C) the retention of 12Me-11-13 : OH was 0.5 minutes longer than that of t11-14 : OH.

These data confirm the presence of t11-14 : OH in female OLR tip extracts and strongly suggest the presence of c11-14 : OH and 14 : OH.

Analysis of B

Unless stated otherwise, B was collected from OV-1 for all analyses.

Saponification and reacetylation of B resulted in the GLC behavior on OV-1 expected for t11-14 : Ac, including the disappearance and subsequent reappearance of EAG activity (6–7 minutes at 180°C) at the retention of t11-14 : Ac (6.4 minutes at 180°C, with t11-14 : OH at 3.95 minutes).

The presence of only one double bond was confirmed by TLC of the acetoxymercuri-methoxy adduct of B on silica gel-G (Mangold and Kammereck, 1961). The crude extract as well as the acetate-containing fraction from Florisil chromatography were used in this procedure. EAG activity (8–9 minutes) at the GLC retention time of t11–14 : Ac (8.25 minutes) on OV-1 (180°C) was recovered only from the monoeneoates TLC area after regeneration of the free acetate.

TLC of B on silver nitrate-impregnated silica gel-G followed by recovery, collection from CHDMS (180°C), and subsequent EAG analysis resulted in recovery of EAG activity from both *trans* and *cis* areas, with most of it present in the *trans* area; all activities (5–6 minutes) coincided with the GLC retention of t11-14 : Ac (5.74 minutes). GLC of the recovered samples on PDEAS (170°C) revealed a material with the retention of t11-14 : Ac (9.3 minutes) from the *trans* TLC area and another with the retention of *cis*-11-tetradecenyl acetate (c11-14 : Ac; 9.8 minutes) from the *cis* TLC area.

On PDEAS (174°C), B was resolved into three peaks having the retentions of tetradecyl acetate (14 : Ac; 12.35 minutes), t11-14 : Ac (14.45 minutes), and c11-14 : Ac (15.3 minutes) in the ratios 10 : 80 : 11, respectively, or a *trans-cis* ratio of 88 : 12.

Location of the double bond at the 11- position was confirmed by micro-

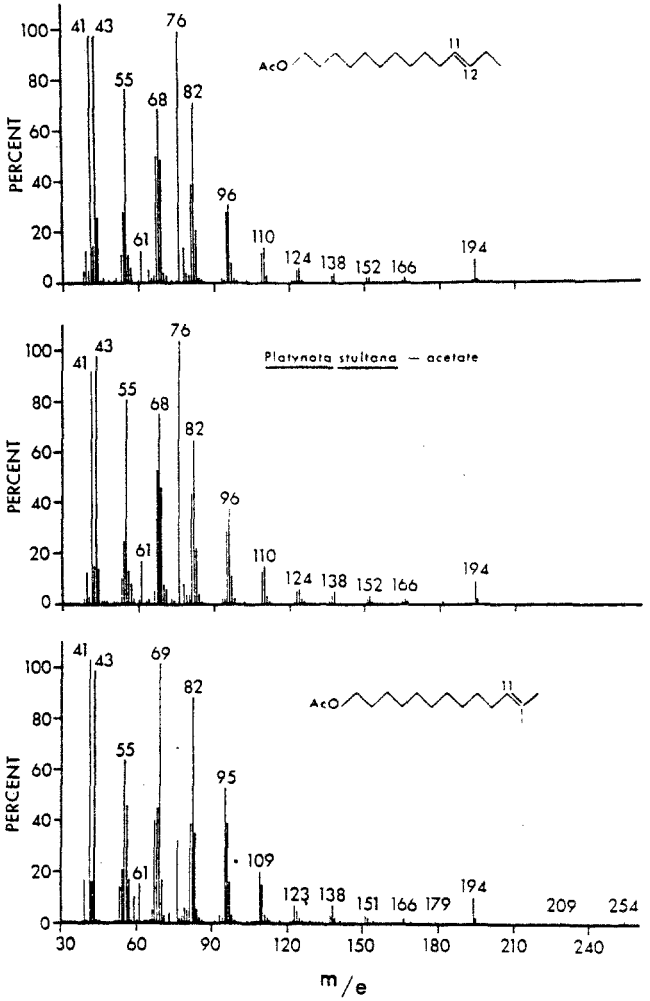

FIG. 2. Mass spectra of OLR acetates, t11–14 : Ac and 12Me-11–13 : Ac.

ozonolysis. An ozonolysis product of B and authentic 11-acetoxyundecanal produced from t11-14 : Ac had similar retentions on OV-1 (10.95 and 11.0 minutes, respectively, at 165°C) and on CHDMS (13.4 and 13.2 minutes, respectively, at 165°C).

A mass spectrum of B was identical to that of t11-14 : Ac, and differed somewhat from that of 12-methyl-11-tridecenyl acetate (12Me-11-13 : Ac; Figure 2), which was a plausible potential structure for B. In addition,

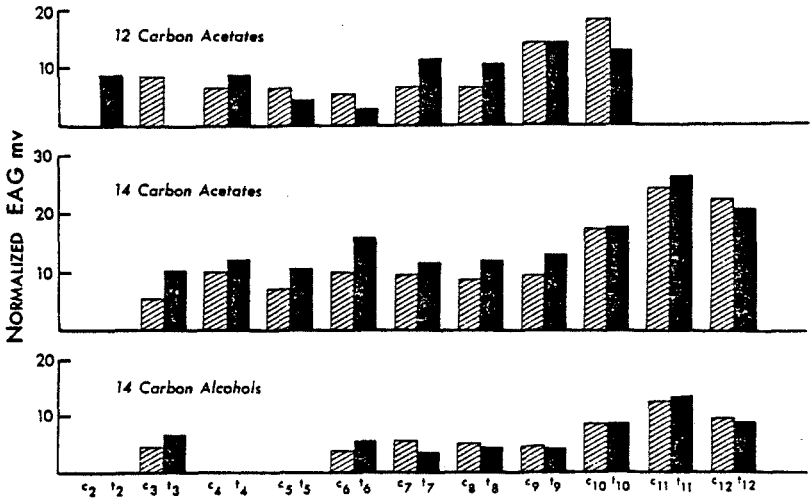

FIG. 3. Normalized EAG responses of male OLR antennae to 14-carbon alcohols and 12- and 14-carbon acetates using *cis*-6-tetradecenyl acetate as the standard. Normalization consisted of multiplying the average value of the EAG determinations (amplitude in mV) for each compound by 10 and dividing by the corresponding value of the standard.

12Me-11-13:Ac also had a retention time on PDEAS (170°C) that differed from that of the major component of B (9.8 and 9.25 minutes, respectively).

These data confirm the presence of t11-14:Ac and c11-14:Ac in the ratio of 88:12 in female OLR tip extracts, and strongly indicate the presence of 14:Ac.

Approximately 5 ng per female tip of peak B was found when assayed by GLC on OV-1.

EAGs of Standards

The normalized EAG responses on male OLR antennae of 14-carbon monounsaturated acetates and alcohols (Figure 3) show that t11-14:Ac elicits the greatest antennal response, with that from c11-14:Ac being almost as intense. While t11-14:OH and c11-14:OH elicit the greatest responses in the alcohol profile, these are only about half as intense as those from the corresponding acetates. None of the normalized responses for the 14-carbon aldehydes, 16-carbon acetates, alcohols, and aldehydes, and 12-carbon alcohols and aldehydes tested had values greater than 10. These data support the assignment of t11-14:Ac and c11-14:Ac as pheromone components.

DISCUSSION

The two compounds isolated from female OLR in an 88 : 12 ratio and identified as t11-14 : Ac and c11-14 : Ac, respectively, were shown in field tests to be the essential pheromone components of this species for attraction of males to females (Baker et al., 1975). These tests showed that t11-14 : Ac containing 4–9% c11-14 : Ac is a powerful attractant for OLR males, with optimum attractancy obtained with ca. 6% c11-14 : Ac. In most tests the synthetic lures were more than 5 times as attractive as virgin females. We have no explanation for the slight difference in the *trans-cis* ratio found in the female and the optimum ratio required for attraction of males in the field. Collection and analysis of the pheromone actually emitted by the female may resolve this discrepancy. Neither isomer by itself is attractive, and mixtures containing 20% or more of the *cis* isomer also are essentially non-attractive. The requirement of a specific geometric isomer ratio in a sex pheromone to obtain attraction of males has been reported for other tortricids, such as the redbanded leafroller moth *Argyrotaenia velutinana* (Walker) (Klun et al., 1973), the oriental fruit moth *Grapholitha molesta* (Busck) (Beroza et al., 1973; Roelofs and Cardé, 1974), the fruit tree leafroller moth *Archips argyrospilus* (Walker) (Roelofs et al., 1974), and the fruit tree tortrix moth *Archips podana* (Scopoli) (Persoons et al., 1974); for a pyralid, the European corn borer moth *Ostrinia nubilalis* (Hübner) (Klun et al., 1973; Kochansky et al., 1975); and for a gelechiid, the pink bollworm moth *Pectinophora gossypiella* (Saunders) (Hummel et al., 1973).

Field tests in which the 11-tetradecenyl acetates (11-14 : Acs) were mixed with the 11-tetradecenyl alcohols (11-14 : OHs) in approximately the same *trans-cis* ratios as the acetates (10–11% and 16–17% in two tests) showed that the alcohols increase trap catches when present as less than 3% of the mixture, optimally 0.2–2%, but decrease catches when present as greater than 20% of the mixture (Baker et al., 1975). Since females can contain the 11-14 : OHs in proportions that have been found to be inhibitory in field tests, it is possible that they function in several roles with this species. They could be biosynthetic precursors to the acetates, secondary pheromone components when released at a very low ratio with respect to the acetates, or inhibitors when released at higher ratios, possibly resulting from accumulation after mating. This last possibility would agree with the observation that the highest alcohols : acetates ratios were observed with extracts of females collected from mixed populations, while the lowest ratios were found in virgin female extracts.

Compounds found in female gland extracts which are closely related to the primary pheromone component and might even interact strongly with male antennae in EAG tests, are not necessarily pheromone components involved in attraction. It has been found that such compounds (a) can be inhibitory

to the attraction of males of the same species, e.g., 2-methyl-*cis*-7-octadecene is found in sex pheromone glands of the female gypsy moth *Porthetria dispar* (L.) (Bierl et al., 1970), but it inhibits the attraction of gypsy moth males to the synthetic pheromone 2-methyl-*cis*-7,8-epoxyoctadecane and to calling females (Cardé et al., 1973); or (b) can be inhibitory to the attraction of males of other species with no apparent effect on conspecific male attraction. An example of the latter is *cis*-9,*trans*-12-tetradecadien-1-ol, a compound emitted by females of the Indian meal moth *Plodia interpunctella* (Hübner). This compound has no apparent effect on conspecific male attraction to the pheromone *cis*-9,*trans*-12-tetradecadienyl acetate (c9,t12-14:Ac), but it does inhibit the response of male almond moths *Cadra cautella* (Walker) to c9,t12-14:Ac, which is a pheromone component common to both species (Sower et al., 1974).

Should synergism of the type described here for OLR, with compounds emitted at very low rates relative to the primary pheromone component, be a general phenomenon, it will add another factor to the task of defining these systems. Compounds found to be inactive or inhibitory for male attraction in the field at a level greater than 5% could be disregarded as pheromone components, however they could well be an integral part of the pheromone system at a very low level. These subtle components could provide the fine tuning used in species recognition, similar to individual recognition reported for higher animals (Müller-Schwarze et al., 1974; Thiessen et al., 1974).

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