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IDENTIFICATION OF A FOUR-COMPONENT SEX PHEROMONE OF THE FEMALE ORIENTAL FRUIT MOTH, *Grapholitha molesta* (LEPIDOPTERA: TORTRICIDAE)[1]

A.M. CARDÉ, T.C. BAKER, and R.T. CARDÉ

Department of Entomology and Pesticide Research Center
Michigan State University, East Lansing, Michigan 48824

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Abstract—The female-emitted pheromone of *Grapholitha molesta* (Busck), the Oriental fruit moth, was collected by holding females in glass flasks during calling. Flask washes were found to contain four pheromone components: (*Z*)-8-dodecenyl acetate and (*E*)-8-dodecenyl acetate in a 100:7 ratio, and (*Z*)-8-dodecen-1-ol and dodecanol in a 100:20 ratio. The ratio of (*Z*)-8-acetate to (*Z*)-8-dodecen-1-ol was approx. 100:30. Approximately 0.1–0.2 ng of pheromone was recovered per female per hour of calling.

Key Words—*Grapholitha molesta*, Oriental fruit moth, attractant, pheromone, (*Z*)-8-dodecenyl acetate, (*E*)-8-dodecenyl acetate, dodecyl acetate, (*Z*)-8-dodecen-1-ol, dodecanol.

INTRODUCTION

One component of the female sex pheromone of the Oriental fruit moth, *Grapholitha molesta* (Busck), was characterized as (*Z*)-8-dodecenyl acetate (Z8–12:Ac) by Roelofs et al. (1969). A number of congeners of Z8–12:Ac have been reported as contributing to male trap catch when these analogs were emitted simultaneously with Z8–12:Ac. Trap catch has been reported to be elevated by the addition of (*E*)-8-dodecenyl acetate (*E*8–12:Ac) by Beroza et al. (1973), dodecanol (12:OH) by Roelofs et al. (1973), and (*Z*)-8-dodecen-1-ol (Z8–12:OH) by Cardé et al. (1975). However, other than

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*Z*8–12:Ac, the female's natural blend has remained undefined. We report here the identification of three new components of the female's pheromone.

METHODS AND MATERIALS

The laboratory culture of *G. molesta* was derived from material collected in apples from abandoned orchards in East Lansing in the fall of 1975. The insects were maintained on thinning apples in a regime of 16:8 hr L:D, 25°C, and 75% relative humidity. Adults for pheromone collection were obtained by segregation of pupae by sex; females were held as adults in 30 × 30 × 40 cm screen cages with access to water.

Female-emitted pheromone was obtained by placing 50 2-to-6-day-old females in stoppered 250-ml round-bottom 24/40 flasks just prior to the initiation of calling, similar to the technique of Weatherston et al. (1971). After approx. 3 hr of calling, the flasks were cooled to −5° for 5 min and the females were dumped out of the flasks. Flasks were given 3 consecutive rinses with 30 ml of redistilled hexane. The hexane was filtered through glass wool, concentrated to approx. 1 ml volume, and transferred to an ampoule where it was evaporated to dryness with N_2. Immediately 100 μl of redistilled methylene chloride was added, and the extracts were stored at −5°C until analyzed.

Electroantennograms (EAGs) of GLC fractions of airborne pheromone were conducted according to the procedures of Roelofs (1977). Analytical GC columns were glass, 2 mm × 1.8 m, and were packed with either 3% OV-1 on Gas-Chrom-Q (100/120 mesh) or 10% XF-1150 on Chromosorb DMCS AWW (100/120 mesh). A 4-mm × 1.8-m OV-1 column was utilized for initial purification of the airborne material. Mass spectra were obtained on a LKB 9000.

RESULTS

Approximately 0.1–0.2 ng of pheromone was recovered per female per hour of calling. The crude airborne pheromone (10,000 female hours) was collected from the OV-1 column at 132°. The 5.4–6.8 min area, which would contain saturated and unsaturated 12-carbon alcohols (standard *Z*8–12:OH = 5.9 min), elicited a 6.6-mV EAG response, somewhat above the response of adjacent fractions (5.6 mV), whereas the 10–14 min area, representing saturated and unsaturated 12-carbon acetates (standard *Z*8–12:Ac = 11.2 min, 12:Ac = 11.7 min), gave good EAG activity, eliciting a 7.0 mV (adjacent fractions 3.2 mV) with a very slow 10-sec return to baseline.

Analyses of the Acetate Region. The acetate region was reinjected on an OV-1 column at 135° (standards: *Z*- and *E*8–12:Ac = 10.0, 12:AC = 10.5

min) and gave a single broad peak at 9.8 min. Injection of this material on an XF-1150 column at 120° (standards: 12:Ac = 10.0, *E*8–12:Ac = 11.1, *Z*8–12:Ac = 12.1 min) showed components with retention times of 10.0, 11.0, and 12.1 min in relative quantities of 5:8:100. These components were isolated by collection from XF-1150 under the same conditions.

Dodecyl Acetate Region. An aliquot of the area collected at 9.4–10.4 min, which would contain dodecyl acetate and which showed low EAG activity, was reinjected on XF-1150 at 130° (standards: 12:Ac = 8.2, *E*8–12:Ac = 9.0, *Z*8–12:Ac = 9.9 min) and showed a single peak at 8.25 min. Injection of an aliquot of this material on OV-1 at 125° showed one peak at 17.7 min (standards: *Z*8–12:Ac = 16.8, 12:Ac = 17.7 min). Microozonolysis of this material (Beroza and Bierl, 1967) and reinjection on OV-1 at 125° (standards: 12:Ac = 18.1, 8-acetoxyoctanol = 6.1) gave the original peak at 18.2 min, indicating the material is the saturated 12-carbon acetate.

(E)-8-Dodecenyl Acetate Region. An aliquot of material collected from 10.5–11.4 min, which would contain the *E*8–12:Ac and which showed EAG activity was reinjected on XF-1150/130° (standards: 12:Ac = 8.2, *E*8–12:Ac = 9.0, *Z*8–12:Ac = 9.9) and showed 1 peak at 9.0 min. Injection of the same material on OV-1 at 125° (standards *Z*8–12:Ac = 16.8, 12:Ac = 17.7) gave one peak at 16.6 min. Microozonolysis of the component and reinjection on OV-1 at 125° [standards: *Z*8–12:Ac = 17.0, 8-acetoxyoctanal (obtained from ozonolysis of standard *Z*8–12:Ac) = 6.1] gave a peak at 6.1 min and no discernable peak at 17.0, indicating that this 12-carbon acetate is unsaturated in the 8-position.

(Z)-8-Dodecenyl Acetate Region. The *Z*8–12:Ac area (11.4–13 min) which showed EAG activity was reinjected on XF-1150 at 120° and gave a major peak at 11.9 with a 1.5% impurity at 11.0 min (standards: 12:Ac = 9.9, *E*8–12:Ac = 11.0, *Z*8–12:Ac = 12.1 min). Injection of the same material on OV-1 at 125° gave 1 peak at 16.7 (standards: *Z*8–12:Ac = 16.8, 12:Ac = 17.7 min). A mass spectrum of approx. 500 ng of this component was identical with a mass spectrum of synthetic *Z*8–12:Ac. Microozonolysis and subsequent injection on OV-1/125° [standards: *Z*8–12:Ac = 16.8, 8-acetoxyoctanal (from ozonolysis of standard *Z*8–12:Ac) = 6.05 min] did not give any peak at 16.8 and showed a new peak at 6.05 min, indicating unsaturation in the 8-position and confirming the presence of *Z*8–12:Ac.

Analyses of the Alcohol Region. Reinjection of an aliquot of the alcohol region on a preparative OV-1 column at 135° gave a major peak at 5.4 min and a small one at 6.4 min (standards: *Z*8–12:OH, *E*8–12:OH and 12:OH = 5.4 min). The same material on XF-1150 at 135° (standards: 12:OH = 6.7, *E*8–12:OH = 7.3, *Z*8–12:OH = 8.1) showed two major peaks at 6.7 and 8.2 min, respectively, in a ratio of 33:67. Acetylation of the material and subsequent injection on OV-1/135° (standards: *Z*8–12:Ac and *E*8–12:Ac = 10.0, 12:Ac = 10.6 min) gave a new peak at 10.1 min with a

shoulder at 10.6, in addition to the two small peaks with retention times of 5.4 (possibly unreacted 12-carbon alcohols) and 6.4 min. Injection of the acetylated material on XF-1150 at 135° showed 2 major peaks at 6.0 and 7.3 min in a relative ratio of 40:60 (standards: 12:OH = 6.7, *E*8–12:OH = 7.3, *Z*8–12:OH = 8.1, 12:Ac = 6.0, *E*8–12:Ac = 6.7, *Z*8–12:Ac = 7.3 min). To remove any unreacted alcohols from this product, it was again collected from OV-1 at 135° (standards: *Z*8–12:OH = 5.5, *Z*8–12:Ac = 10.3). The acetate area from OV-1 (9.5–12 min) showed EAG activity and two discrete areas were collected from XF-1150 at 130° (standards: 12:Ac = 6.3, *E*8–12:Ac = 6.9, *Z*8–12:Ac = 7.55) in an effort to isolate the presumed two different acetates.

Dodecyl Acetate Region (of the Acetylated Alcohol). An aliquot of the material collected from 5.9–6.6 min on XF-1150 gave no appreciable EAG activity, and upon reinjection of XF-1150/120° only a single peak of 6.3 min was noted. The same material on OV-1 at 125° gave a single peak at 17.7 min (standards: *Z*8–12:Ac = 16.5, 12:Ac = 17.7, 8-acetoxyoctanal = 6.05 min). After microozonolysis the product showed no additional peaks on OV-1 and gave the same original peak at 17.7, indicating that there is no unsaturation in this acetate. Therefore the original 12-carbon alcohol component was saturated.

(Z)-8-Dodecenyl Acetate Region (of the Acetylated Alcohol). The region collected from 7.3 to 8.5 min on XF-1150 gave appreciable EAG activity, and an aliquot of this material reinjected on XF-1150/130° gave a single peak at 7.5 min. On OV-1 at 125° [standards: *Z*8–12:Ac = 16.5, 8-acetoxyoctanal (obtained from ozonolysis of standard *Z*8–12:Ac) = 6.0 min] the material showed a peak of 16.4 min. After microozonolysis this product gave no peak at 16.4 min and a fragment showed up at 6.0 min (8-acetoxyoctanal) indicating a double bond in the 8-position in the 12-carbon acetate. Thus, the original 12-carbon alcohol was unsaturated in the 8-position.

DISCUSSION

The characterization of *Z*8–12:Ac, *E*8–12:Ac, *Z*8–12:OH and 12:OH as pheromone components represents the second, 4-component, female-emitted pheromone system in the Lepidoptera. Dodecyl acetate was found to be emitted in relatively low amounts by the female but, because it is not known to elicit a pheromone-related response, this compound cannot be termed a pheromone. Previously, *Archips argyrospilus* (Walker) was shown to be maximally attracted to a mixture of (*Z*)-11-, (*E*)-11- and (*Z*)-9-tetradecenyl acetates and dodecyl acetate (Cardé et al., 1977).

Other *Grapholitha* species may use attractant pheromones that share components in common with *G. molesta*. Males of *G. prunivora* (Walsh) have

been reported to be attracted to mixtures of *Z*8–12:Ac and *E*8–12:Ac in a 100:2 ratio (Roelofs and Cardé, 1974). Males of *G. funebrana* (Treitschke) appear to be maximally attracted to a 100:4 ratio of *Z*8–12:Ac and *E*8–12:Ac, whereas the maximal trap catch of male *G. janthiana* (Duponchel) is elicited by a 100:27 ratio of these compounds (Biwer, 1977). These species are broadly sympatric with *G. molesta* and reproductive isolation among these species may be achieved by differences in secondary pheromone components, in close-range courtship behaviors (Baker and Cardé, 1979), and in time of sexual activity (Biwer, 1976).

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