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18. We thank Mrs. Cathy Chvasta and Mrs. Jane Waldman for their help in the preparation of this manuscript.

25 February 1974; revised 22 April 1974 ■

Sex Pheromones and Reproductive Isolation of the Lesser Peachtree Borer and the Peachtree Borer

Abstract. *(E,Z)-3,13-octadecadien-1-ol acetate, and (Z,Z)-3,13-octadecadien-1-ol acetate, isolated from the female lesser peachtree borer,* Synanthedon pictipes *(Grote and Robinson), and the female peachtree borer,* Sanninoidea exitiosa *(Say), respectively, strongly attract the respective males of these species in field bioassays. These compounds are the largest pheromones isolated thus far from a lepidopterous species.* Sanninoidea exitiosa *males did not respond to the synthesized* (E,Z)*-isomer, and low concentrations of it in the synthesized* (Z,Z)*-isomer did not interfere with their response to the* (Z,Z)*-isomer. In contrast, even very low concentrations of the* (Z,Z)*-isomer (1 percent) in the* (E,Z)*-isomer significantly inhibited the response of* Synanthedon pictipes *males.*

At least three examples of reproductive isolation of closely related lepidopteran species by pheromones have been described. Male *Trichoplusia ni* (Hübner), the cabbage looper, and *Autographa californica* (Speyer), the alfalfa looper, respond to different concentrations of the same pheromone, (*Z*)-7-dodecen-1-ol acetate (*1*). Males of two tortricid moth species, *Adoxophyes orana* (F.v.R.) and *Clepsis spectrana* (Treitschke), respond to the different relative concentrations of (*Z*)-9- and (*Z*)-11-tetradecen-1-ol acetate produced by the females of these species (*2*). Females of the Indian meal moth, *Plodia interpunctella* (Hübner), and of the almond moth, *Cadra cautella* (Walker), both produce the same pheromone, (*Z,E*)-9,12-tetradecadien-1-ol acetate (*3*). However, the almond moth female produces a second compound, (*Z*)-9-tetradecen-1-ol acetate, that stimulates almond moth males and inhibits Indian meal moth males (*4*), and the Indian meal moth female produces a second compound, (*Z,E*)-9,12-tetradecadien-1-ol, that inhibits the response of almond moth males (*5*).

Release-recapture tests with the lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson), and the peachtree borer, *Sanninoidea exitiosa* (Say) (species that occur east of the Rocky Mountains in North America from the Great Lakes to Florida), have indicated that pheromones may be useful in controlling these pests (*6*). When marked *S. pictipes* males were released in an Indiana peach orchard, 82 percent were recovered in 2 days. The larvae of these species feed on the cambium tissue of the tree and severely damage peach trees, other fruit trees, and several woody ornamentals.

We now report the isolation, identification, and synthesis of the sex pheromones of the lesser peachtree borer and the peachtree borer. The synthesized pheromone of *S. pictipes*, (*E,Z*)-3,13-octadecadien-1-ol acetate, was strongly attractive to males of this species in field tests but did not attract *S. exitiosa* males. Indeed, when it was mixed in a 1 : 1 ratio with the synthesized pheromone of *S. exitiosa*, (*Z,Z*)-3,13-octadecadien-1-ol acetate, it appeared to inhibit the response of *S. exitiosa* males. Similarly, the synthesized *S. exitiosa* pheromone was strongly attractive to *S. exitiosa* males in the field. However, when the (*Z,Z*)-isomer was present in only 1 percent concentration in the *S. pictipes* pheromone, it significantly inhibited the response of *S. pictipes* males. Thus, the two species are isolated reproductively by the male responses to two geometrical isomers of the same compound. Additionally, the strong inhibition of the response of males of one species by the pheromone of the other species reinforces their isolation and may provide a useful mechanism (disruption of the communications system) for control of these insects (*7*).

The initial experiment, a bioassay conducted in a peach orchard in Byron, Georgia (*8*), indicated that the most active materials were obtained by clipping the ovipositors of 1- to 3-day-old unmated female *S. pictipes* or *S. exitiosa* and extracting the ovipositors with methylene chloride or pentane, respectively (*9*). All subsequent steps in the

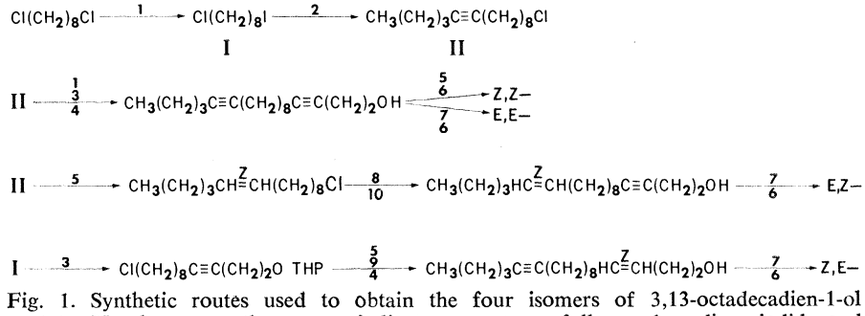

Fig. 1. Synthetic routes used to obtain the four isomers of 3,13-octadecadien-1-ol acetate. Numbers over the arrows indicate reagents as follows: 1, sodium iodide and acetone; 2, $CH_3(CH_2)_3C \equiv C^-Na^+$, liquid ammonia; 3, $THPO(CH_2)_3C \equiv C^-Na^+$, liquid ammonia (THPO, tetrahydropyranyl); 4, H^+, CH_3OH; 5, H_2, Pd; 6, acetic anhydride; 7, Na, liquid ammonia; 8, EDA · $CH \equiv CH^-Li^+$; 9, $CH_3(CH_2)_3-C \equiv C^-Li^+$; 10, ethylene oxide.

isolation procedure were monitored with this assay.

In brief, the ovipositors of 25,000 female *S. pictipes* and 800 female *S. exitiosa* were extracted by grinding them three times with the appropriate solvent in a tissue grinder; the extract was filtered to remove the solids; and most of the solvent was removed by distillation at atmospheric pressure through a 10-cm Vigreux column.

The concentrated extracts were purified by liquid chromatography on a silicic acid column eluted with a pentane-ether gradient (*10*). The fraction eluted with a mixture of pentane and ether (95.5) from both species when tested in the field showed activity equivalent to that of the original extract, and no increase in activity was obtained when all the fractions from the liquid chromatography were combined and assayed.

The active fraction from liquid chromatography was concentrated by distillation and then purified by sequential gas chromatography on SE-30, OV-101, and Carbowax 20M (*11*). One compound obtained from each species retained all the activity of the original extract, and each pheromone proved to be more than 99.5 percent pure when it was subsequently gas-chromatographed on SP-2300, a Dexsil capillary column (60 m), and a support-coated open, tubular diethylene glycol succinate (DEGS) column (45 m) (*11*).

The active compounds were identified by mass and infrared spectra and by chemical transformations. The methane ionization mass spectra of both compounds were identical and had the following diagnostic peaks: (M+1), 309; (M−1), 307; [(M+1)−60], 249; [(M−1)−60], 247; protonated acetic acid, 61; protonated ethyl acetate, 89; and a typical straight chain hydrocarbon series of peaks from *m/e* 67 to 207. Reduction of the *S. pictipes* pheromone with hydrogen and neutral palladium catalyst (*12*) in the inlet of the gas chromatograph produced a compound with the same retention time on the Carbowax 20M and Dexsil columns as octadecyl acetate; it also had a mass spectrum identical to that of octadecyl acetate. The infrared spectrum of the *S. pictipes* pheromone showed a strong absorption at 1745 cm^{-1} (C=O), 1235 cm^{-1} and 1035 cm^{-1} (C−O) ester, and 970 cm^{-1} (*trans* C=C) in addition to the normal hydrocarbon and olefinic bands. That of *S. exitiosa* was identical except for the 960 cm^{-1} band, which was absent. Thus, we had strong evidence that both pheromones were straight chain, C_{18} acetates with two double bonds. Additionally, both double bonds must be *cis* in the *S. exitiosa* and at least one must be *trans* in the *S. pictipes*.

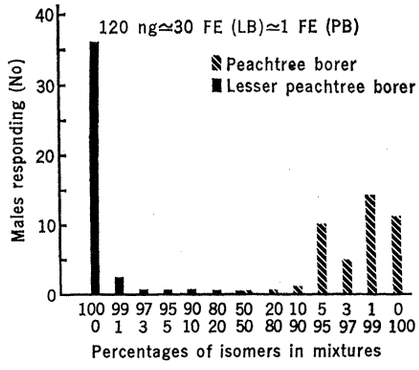

Fig. 2. Average male response (five replicates) to mixtures of the two synthetic pheromones in the mixture shown. *FE*, female equivalent; *LB*, lesser peachtree borer; *PB*, peachtree borer.

Microozonolysis (*13*) of the two pheromones in carbon disulfide at −78°C, reductive cleavage of the ozonide with triphenylphosphine, and gas chromatography on Carbowax 20 M yielded three peaks in each case. The first peak from both pheromones was identical in retention time and mass spectrum to valeraldehyde. The second could not be collected for mass spectroscopy because it decomposed. However, in later experiments with the synthetic pheromone, we produced enough of this product to obtain a mass spectrum that was consistent with the spectrum expected for 3-oxo-1-propanol acetate. The methane ionization mass spectrum of the third product from both pheromones showed the following diagnostic peaks: (M+41), 211; (M+29), 199; (M+1), 171; [(M+1)−18], 153; and [(M+1)−36], 135. Thus, the third ozonolysis product of the two pheromones was 1,10-decanedial; the *S. exitiosa* pheromone was (*Z,Z*)-3,13-octadecadien-1-ol acetate; and the *S. pictipes* pheromone was one of the other three isomers of this compound.

All four isomers of 3,13-octadecadien-1-ol acetate were synthesized by the routes outlined in Fig. 1. The isomers were purified by gas chromatography on OV-101, Carbowax 20M, and SP 2300 columns. This procedure yielded pure compounds (> 99.5 percent), except that each isomer contained about 3 to 5 percent of one or more of the other isomers that could not be removed by gas chromatography. Chemical analysis of the synthetic compounds proved that the (*E,Z*)-isomer and the (*Z,Z*)-isomer were identical in every way to the natural pheromones of *S. pictipes* and *S. exitiosa*, respectively. This analysis included gas chromatography on the support-coated DEGS column which separated all four isomers though only the (*E,E*)-isomer was completely resolved from the other three.

In the field in Byron, Georgia, bioassay of the isomers showed that equivalent amounts (1.2 μg, about ten female equivalents) of the (*Z,Z*)-isomer and the purified natural pheromone extracted from *S. exitiosa* females were identical in attractiveness to male *S. exitiosa*. However, the (*E,Z*)-isomer attracted an average of only two *S. pictipes* males though an equivalent amount (120 μg, about 30 female equivalents) of the purified natural *S. pictipes* pheromone attracted an average of 30 males during simultaneous testing.

The (*E,Z*)-, (*Z,E*)-, *and* (*Z,Z*)-isomers were further purified by liquid chromatography on a silica gel column treated with silver nitrate (*14*). Two passes of the compounds through this column produced isomers that were more than 99.5 percent pure. When these isomers were tested again in the field bioassay, the (*Z,Z*)-isomer was still as attractive as the *S. exitiosa* pure natural pheromone to male *S. exitiosa*; the (*E,Z*)-isomer was now as attractive as the *S. pictipes* pure natural pheromone to *S. pictipes* males; and neither isomer attracted the other species nor were the (*E,E*)- and (*Z,E*)-isomers attractive to either species.

Subsequently, binary mixtures of the (*E,Z*)-, (*Z,E*)-, and (*Z,Z*)-isomers were prepared and compared in the field with live females, the natural pheromone, and the 99.5+ percent pure isomers. The (*Z,E*)-isomer was not attractive to either species and had no effect on the attractiveness of the (*E,Z*)-isomer to *S. pictipes* males; its effect on the attractiveness of the (*Z,Z*)-isomer to *S. exitiosa* males was inconclusive because the test was made so late in the season when the population of *S. exitiosa* was low. However, the results of the tests of (*Z,Z*)- and (*E,Z*)-mixtures (Fig. 2) did show that the (*Z,Z*)-isomer, in concentrations as low as 1 percent, significantly reduced the response of *S. pictipes* males to

their pheromone. The (*E,Z*)-isomer apparently had less effect on the response of *S. exitiosa* males to their pheromone but it seemed to reduce the attractiveness of the (*Z,Z*)-isomer when the two were mixed in a 1 : 1 ratio.

The final test in this series, a trapping experiment, was conducted in October 1973, in a peach orchard near Byron. Thirty-six traps (Zoecon Pherocon 1C) were arranged in a complete randomized block. A glass petri dish containing 10 μg of (*E,Z*)-3,13-octadecadien-1-ol acetate was placed in the bottom of each of 12 traps; three virgin female *S. pictipes* were placed in each of 12 traps; and 12 traps were empty. Between 10:30 a.m. and 3:00 p.m. (E.D.T.) of the first day, the traps that contained the synthetic pheromone caught 1335 male *S. pictipes*; the traps containing females caught 114 males; and the empty traps caught no males. During the next 4 days, the pheromone traps caught an additional 263 males.

In view of the high percentage of recovery of released *S. pictipes* males in previous experiments, and the marked attractiveness of the synthetic pheromones to the males of both species, we anticipate that the synthesized materials will be useful in efforts to control these pests either by trapping or by disruption. It is possible that one compound or a mixture of the two may be useful in controlling both species via the communication disruption technique.

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9. Methylene chloride and pentane are suitable solvents for *S. pictipes* and *S. exitiosa* crude extracts, respectively [M. L. Cleveland and L. L. Murdock, *J. Econ. Entomol.* **57**, 762 (1964); C. R. Gentry, A. A. Sekul, C. E. Yonce, *J. Georgia Entomol. Soc.* **7**, 247 (1972)].
10. A glass column, 0.6 cm (inside diameter), packed with 28 g of Bio-Sil HA (Bio-Rad Laboratories), was eluted sequentially with 100 ml of pentane, 100 ml of a mixture of pentane and ether (95 : 5), 200 ml of a mixture of pentane and ether (90 : 10), and 200 ml of ether. The flow rate was maintained at 120 ml/hour, and 10-ml fractions were collected.
11. The following stainless steel gas chromatographic columns were used with the conditions noted; for SE-30, 5 percent on 80/100 mesh Chromosorb G-HP, 4 m by 4 mm (inside diameter), column temperature at 225°C, He flow at 80 cm³/min; for OV-101, 5 percent on 80/100 Chromosorb G-HP, 2 m by 2 mm (inside diameter), column temperature at 170°C, He flow at 20 ml/min; for Carbowax 20M, 5 percent on 80/100 Chromosorb G-HP, 1 m by 2 mm (inside diameter), column temperature at 180°C, He flow at 20 ml/min; for SP 2300, 3 percent on 100/120 Supelcoport, 8 m by 2.3 mm (inside diameter), column temperature at 230°C, He flow at 20 ml/min; for Dexsil, 60 m by 0.08 cm capillary (inside diameter), column temperature at 235°C, He flow at 22.5 cm/sec; and for DEGS, support-coated open tubular (Perkin-Elmer Corp.), 45 m by 0.05 cm, column temperature at 190°C, He flow at 23 cm/sec.
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14. A 25 cm by 0.62 cm (outside diameter) stainless steel column was packed with dry Adsorbosil-2-ADN (20 percent) (Applied Science Laboratories). This is silica treated with silver nitrate and is intended for use in thin-layer chromatography. The particle size ranges from about 1 to 11 μm. The column was eluted with benzene at a flow rate of 1 ml/min and a head pressure of 1000 pounds per square inch. The column effluent was monitored with a refractive index detector.

8 February 1974; revised 1 April 1974

Raman Spectroscopic Investigation of Gramicidin A′ Conformations

Abstract. *Gramicidin A′ is believed to form transmembrane channels in lipid bilayers and biological membranes. The first Raman spectroscopic study of gramicidin A′ is presented. Evidence is found for two types of conformation. One type is found in the powder and has a Raman spectrum similar to that of model polypeptides with β hydrogen bonding. The second type is found when gramicidin A′ is dissolved in dimethyl sulfoxide.*

The antibiotic gramicidin A (GA) is a linear pentadecapeptide (15 residues) with the sequence HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NH-CH_2CH_2OH (*1*). There is good evidence that dimers of GA can form transmembrane channels in lipid bilayers (*2–4*). This evidence includes the highly specific conductance (10^7 ohm^{-1} cm^{-2} $mole^{-1}$) of GA in lipid bilayers compared to carriers such as valinomycin (10^3 ohm^{-1} cm^{-2} $mole^{-1}$) (*2*), the second-order dependence of the ionic conductivity of these membranes on GA concentration (*3*), and the persistence of this conductivity when the lipid bilayer "freezes" (*4*).

Several possible structures for GA and its dimer have been proposed. On the basis of unpublished infrared spectra (*5*), Sarges and Witkop (*1*) have suggested that GA forms "side-by-side" dimers by intermolecular parallel β hydrogen bonding, similar to that found in β-keratin (*6*). This model, however, does not explain the membrane activity of GA.

More recently, on the basis of conformational energy calculations, Urry and co-workers have proposed a left-handed helical structure which they call a $\pi_{(L,D)}$ helix (*7, 8*). This structure is characterized by having C=O groups positioned alternately parallel and antiparallel to the helical axis, unlike the well-known α helix found inside most globular proteins, which has C=O groups only parallel (*6*). The $\pi_{(L,D)}$ helix is made possible by the existence of alternating L and D residues in the peptide chain. Urry (*7*) predicts that this type of helix can form "head-to-head" dimers by an *intermolecular* hydrogen bond pattern similar to the antiparallel β-pleated sheet. In contrast, the *intramolecular* hydrogen bonding of GA is postulated