Identification of the Female Sex Pheromone of the Rice Green Caterpillar

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The rice green caterpillar, *Naranga aenescens* Moor (Lepidoptera: Noctuidae, Eustrotiinae), is one of the most harmful pest insects of rice plant in eastern Asia. The effective synthetic sex pheromone of this insect is required in integrated pest control programs, either as a monitoring tool for timing insecticide application or as an insect control tool.

In our preliminary study, it was confirmed that the virgin female produces the sex pheromone which attracts the male moths in the field, and the following chemical nature of the pheromone was disclosed by the combination of preliminary chemical reactions and field tests. The attractive activity of the crude extract was lost after treatment with methanolic potassium hydroxide, but treatment of the crude saponified mixture with Ac_2O -pyridine restored the original activity. Hydrogenation of the pheromone over PtO_2 eliminated the attractive activity. Thus the pheromone was suggested to be acetate of unsaturated alcohol.

In our field sex attractant-tests for lepidoptera using synthetic compounds, about one-hundred monoene alcohols (C_{10} – C_{18}) and their acetates were tested¹⁾ but none of them could attract *N. aenescens* when tested in a single source. On the other hand, in the assay by the electroantennogram (EAG) technique²⁾ this

insect clearly responded to several acetates. This suggests that the sex pheromone of *N. aenescens* may have multi-components and/or polyene nature. On the basis of these findings, we decided to isolate and characterize EAG-active components at first and then to conduct field tests on these components to confirm whether they are necessary to attract the male moth or not.

The abdominal tips excised from 1200 virgin female moths were soaked in *n*-hexane for thirty minutes. The crude extract obtained by evaporation of the solvent was subjected to thin-layer chromatography (TLC) (silica gel; solvent: *n*-hexane–benzene (1:3)). EAG-active zone (R_f 0.4~0.5) was fractionated by gas-liquid chromatography (GC) (OV-1 2%, 3 mm $\times$ 120 cm, 140°C) to give two EAG-active fractions (Fractions A and B). Fraction A was collected at 3.6~5.6 min and Fraction B at 9.9~13.2 min. (In this GC condition, retention times of tetradecanyl acetate and hexadecanyl acetate were 4.2 min and 10.4 min, respectively.)

Gas liquid chromatography combined with mass spectroscopy (GC–MS) of Fraction A on a PEG 20 M column showed one major peak which possessed the following fragment ions: m/e 194 (13%, $\text{C}_{14}\text{H}_{26}^+$), 166 (2%, $\text{C}_{12}\text{H}_{22}^+$), 152 (3%, $\text{C}_{11}\text{H}_{20}^+$), 138 (6%, $\text{C}_{10}\text{H}_{18}^+$), 124 (8%, $\text{C}_9\text{H}_{16}^+$), 110 (18%, $\text{C}_8\text{H}_{14}^+$), 61 (16%) and 43 (100%, base ion peak), indicating that the peak should be a tetradecenyl acetate. Similarly the mass spectrum of the major peak of Fraction B was shown to be a hexadecenyl acetate.

To determine the location of the double bond in these compounds, ozonolysis was conducted after a further purification of Fractions A and B by the GC fractionation with a PEG 20 M column. GC–MS of the ozonolysis product of the purified Fraction A showed that one major component is 9-acetoxynonal in the respect of the retention time and the mass spectrum, while that of the purified Fraction B gave two peaks, which were shown to be due to 9-acetoxynonal (minor component) and 11-acetoxundecanal (major component).

FIG. 1. EAG Activity to Monoene Acetates, 10 µg/cartridge.

TLC of the crude extract on 20% AgNO₃/silica gel plates using benzene as developing solvent and subsequent EAG analysis showed activity only at Z-monoene acetate zone (*R_f* 0.4~0.5). (E-monoene; *R_f* ca. 0.6, E, Z-diene; *R_f* ca. 0.3.)

Thus the EAG-active components in *N. aenescens* virgin female were characterized as Z-9-tetradecenyl acetate (compound I), Z-9-hexadecenyl acetate (compound II) and Z-11-hexadecenyl acetate (compound III). By the GC analysis, it was observed that the extract contained compounds I, II and III in the ratio of 1:1:4, respectively.

EAG activities of several isomers of tetradecenyl acetates and hexadecenyl acetates are shown in Fig. 1. Synthetic specimens of compound I~III showed clear EAG responses, furnishing the strong support for the above identification. Further, stronger responses of Z-isomers than E-isomers are in good agreement with our assignment of geometry of the double bond.

Field trials to the synthetic pheromones are in progress. However, in preliminary experi-

ment it has been demonstrated that only the mixture of the three compounds could attract the male moths. The optimum ratio of the attractant in the field is also being examined.

Compounds I and III have been known to be the sex pheromone of some other species in the family Noctuidae, but compound II has been identified as the pheromone for the first time.³⁾

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