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Sex Pheromone Composition of the Diamondback Moth, *Plutella xylostella* (L.) in Korea

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Abstract The mating behavior of the diamondback moth (DBM), *Plutella xylostella*, was actively observed from 2 h post-scotophase and maintained up to 2 h post-photophase. The pheromone glands of DBM populations in Korea were extracted with hexane and gas chromatograph (GC) analysis showed that three major components of sex pheromone, (Z)-11-hexadecenal (Z11-16:Al), (Z)-11-hexadecenyl acetate (Z11-16:Ac) and (Z)-11-hexadecenyl alcohol (Z11-16:OH), were identified at the ratio of 8:3:2. To contrast, Z11-16:OH was not detected from Solid Phase Microextraction for the sex pheromone emitted during the scotophase. Both Z11-16:Al and Z11-16:Ac elicited the antennal response of male DBM in GC- electroantennographic detection and electroantennogram (EAG) assay. EAG assay revealed that the ratio of major components, Z11-16:Al and Z11-16:Ac, in the blend of 5 : 5 and 7 : 3 strongly attracted DBM male adults. The minor components, 0.5% of Z11-16:OH or 5 to 10% of (Z)-9-tetradecenyl acetate (Z9-14:Ac) also elicited the EAG response when mixed with major components blends at the ratio of 10:10 between Z11-16:Ac and Z11-16:Al, suggesting these components affect the activity of major components of sex pheromone.

Key words electroantennogram, *Plutella xylostella*, sex pheromone, Z11-16:Ac, Z11-16:Al, Z11-16:OH

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) is one of the most destructive insect pest to cruciferous plants throughout the world (Talekar and Shelton, 1993). Spray of chemical insecticides has been a major method of controlling for the last few decades. However the action mechanism based on neurotoxicity of those chemicals has also caused several side effects such as toxicity to other non-target organisms including human beings, development of insecticide resistance in pests and residual toxicities in the agricultural products and environment. Since the resistance to chlorinated insecticides was first reported (Ankersmit, 1953), DBM has developed resistance to more than 46 commercial formulations of insecticide, including even that to *Bacillus thuringiensis* toxins (Sun *et al.*, 1986; Pérez *et al.*, 2000). Therefore, strategies of integrated pest management (IPM) to reduce or minimize those side effects have been adopted for controlling several key pests. Detailed knowledge about bionomics and behavior of pests are pre-requisites for IPM. And insect sex pheromone that is species-specific and highly active at low concentrations has been practically used as a tool for investigating or controlling insect behavior in IPM. A number of studies on insect sex pheromone have been done since bombykol of silkworm moth was first identified (Butenandt *et al.*, 1959), and then population monitoring, mass trapping and mating disruption with insect sex pheromone have been successfully employed for several insect pests (Cameron *et al.*, 1974; Glen and Brain, 1982).However, the use of insect sex pheromone has not been applied to all pests, but has been successfully adopted only for the crop pests that spray of insecticides is difficult or environmentally-undesirable, and that less than 100% control can be adopted (Boo, 1995). In some cases, furthermore, even in one insect species, the composition of its sex pheromone is quite different depending on its distribution regions (Boo, 1998). Therefore the sex pheromone of a target insect in a region should be identified separately and the conditions to use be analyzed for successful use.

Major components of DBM sex pheromone have been identified as (Z)-11-hexadecenal (Z11-16:Al) and (Z)-11-hexadecenyl acetate (Z11-16:Ac) (Tamaki *et al.*, 1977). And it was also reported that the attractiveness of mixtures of the main compounds to males was enhanced synergistically when minor components were added such as (Z)-11-hexadecen- 1-ol, (Z)-9-tetradecenyl acetate and (Z)-9- tetradecen-1-ol, (Koshihara and Yamada, 1980; Chisholm *et al.*, 1983).

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In Korea, activities of compounds organo-chemically-synthesized have been tested with field- and electroantennographic (EAG)-responses (Kang *et al.*, 1988, 1990). However because the tests have used only one chemical or one composition of compounds known without identification processes, components and composition of sex pheromone of Korean DBM population is unclear yet. In this study, therefore, we clarified the uncertainty through successive experiments of gas chromatograph and EAG tests using sex pheromone gland extract, female effluvia and synthetic chemicals.

Materials and Methods

Insects

The diamondback moth (DBM), *Plutella xylostella* larvae and pupae were collected in Chinese cabbage fields of Cheongsong and Seoguipo, Korea. Some of insects were also obtained from Dr. B.R. Choi (Rural Development Administration, Suwon, Korea). Hatched larvae were reared on the leaves of a rape under the photoperiod of 16L:8D at 25 ± 1℃, 60~80% R.H. Adults were sexed and transferred to transparent acrylic cages (36×30×30 ㎝) and fed with 10% sugar solution.

Observation of mating time

Two hundred DBM pupae were transferred to two acrylic cages (100 pupae each cage) and allowed to be emerged. The mating behaviors of adults were examined every hour and the number of mating pairs was recorded. Experiment was performed under the photoperiod of 16L:8D. For observation during the scotophase, a flash-light covered with a Kodak Wrattern 29 red gelatin filter (Kodak, USA) was used to prevent their response to light.

Chemicals

All synthetic sex pheromone components used in this experiment were purchased from Research IPO-DLO (Wageningen, Netherlands).

Analysis of pheromone components

Preparation of extracts. One-day-old adults were collected from the virgin female at the peak time of mating and anesthetized with CO_2 gas. The terminal abdominal segments were forcefully everted with blunt forceps and the tip containing sex pheromone gland was dissected with microscissors. The dissected tip was soaked for at least 1 hr in 30 ㎕ of *n*-hexane. Tips of fifteen females were extracted to be combined and applied to analyses.

Collection of female effluvia. Ten virgin females of one-day-old were placed in a 500 ml Erlenmeyer flask at 1 hr before the scotophase. Emitted pheromone components were collected with solid phase micro-extraction (SPME) (SUPELCO, Bellefonte, USA) during the scotophase and analyze gas chromatograph-electroantennographic detector.

Gas chromatograph analysis. The pheromone gland extracts and effluvia collected from females were analyzed with a GC system (HP6890, Hewlett Packard) equipped with a split/splitless capillary injector and a flame ionization detector (FID). Separation of sex pheromone components was conducted through a fused silica capillary column (DB-Wax, 30 m × 0.25 ㎜ ID, 0.25 ㎛ film thickness; Alltech Co., PA, USA) coated with polyethylene glycol. Temperature of column oven was programmed as follows: initial temp. 90℃, holding for 4 min, increase to 120℃ at 10℃/min, holding for 4 min, increase to 160℃ at 10℃/min, increase to 170℃ at 5℃/min, holding for 11 min, increase to 210℃ at 10℃/min, holding for 2 min. Injector temperature was 220℃ and detector temperature was 220℃. Helium was used as the carrier gas with the constant flow rate of 10 ml/min.

GC- Electroantennographic detector (GC- EAD). HP6890 GC (Hewlett Packard) was equipped with a fused silica capillary column (DB-225, 30 m × 0.25 ㎜ ID, 0.25 ㎛ df; Alltech Co., PA, USA) coated with 50% cyanopropyl − 50% phenylmethyl polysiloxane. Temperature of column oven was programmed as follows: initial temp. 120℃, holding for 4 min, increase to 190℃ at 10℃/min, holding for 5 min, increase to 210℃ at 10℃/min, holding for 10 min. Injector temperature was 220℃ and detector temperature was 220℃. Helium was used as the carrier gas with the constant flow rate of 10 ml/min. Samples were injected in a splitless mode. Effluent split gas was passed through the silica fused capillary column and was added with make-up gas from the Y-connector. The gas was allowed to flow simultaneously into FID and the EAD.

Electroantennogram (EAG). Electrical response of each DBM male adult to pheromone components was examined three times with an EAG apparatus (Syntech, Netherlands). The antenna of 1~3-day old

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