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Sex pheromone composition of the cotton caterpillar, *Palpita indica* (Lepidoptera: Pyralidae), in Korea

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ABSTRACT

This study was conducted to investigate the sex pheromone composition of *Palpita* (=*Diaphania*) *indica* (Saunders) (Lepidoptera: Pyralidae) in Korea. Two sex pheromone components, *E*11-16:Al and *E,E*-10,12-16:Al, were identified by GC and GC-EAD analysis of sex pheromone gland extracts. The mean ratios of the two components, *E*11-16:Al and *E,E*-10,12-16:Al, were about 6.5:3.5 and 7.5:2.5 in gland extracts and in SPME collection, respectively. In field bioassays, maximum attraction occurred in traps baited with a 7:3 ratio of *E*11-16:Al and *E,E*-10,12-16:Al. The head extracts of *P. indica* stimulated the sex pheromone production of *P. indica,* as well as Hez-PBAN and PssPT, indicating that a PBAN-like substance exists in the head extracts of *P. indica*. Whole-mount immunocytochemistry showed that three groups of neurosecretory cells showed PBAN-like immunoreactivity in the SEG of *P. indica*. The PBAN-like immunoreactivity connected to the Corpora Cardiaca, a neurohemal organ. Also, PBAN-like immunoreactivity was found in the brain and in the thoracic and third/fourth abdominal ganglia. The addition of sex pheromone components of *Peridroma saucia* to the sex pheromone of *P. indica* greatly improved the attraction of *P. indica* males. The mixing of the sex pheromone components of *S. exigua* did not significantly increase the catches of *P. indica*, while the sex pheromone of *H. armigera* completely inhibited the attractiveness.

Introduction

The cotton caterpillar, *Palpita (=Diaphania) indica* (Saunders) (Lepidoptera: Pyralidae), is a worldwide pest and is found in Japan, Korea, China, Taiwan, Africa, The Netherlands, Pacific Islands, the Australian region, and USA (Inoue, 1982; Peter and David, 1991). The larvae feed primarily on leaves of cotton, mulberry, and many cucurbitaceous crops (Inoue, 1982; Anonymous, 2000). Occasionally, serious damage occurs on fruits when the pest feeds on and punctures the skin of young fruits (Patel and Kulkarny, 1956). In Korea, *P. indica* was first recorded in 1932 on cotton. Since then, this pest had been a minor or non-pest in most crops till the 1990s. However, it has become an important pest of cucurbitaceous crops in fields and greenhouse since early 1990s (Pesticide Information, 1999).

Currently, the control of *P. indica* relies largely on chemical insecticides. Intensive chemical control can induce many side effects, such as toxicity to non-target organisms, development of insecticide resistance, and insecticide residues on agricultural products and in the environment. To minimize such effects, alternative control strategies are required. One of the alternatives is using sex pheromone. This method can be used in population monitoring, mass trapping and mating disruption, all of which can reduce the population density of a pest.

Three sex pheromone components of *P. indica* were identified in Japan (Wakamura et al., 1998): two major components, (*E*)-11-hexadecenal (*E*11-16:Al) and (*E,E*)-10,12-hexadecadienal (*E,E*-10,12-16:Al), and a minor component, hexadecanal (16:Al). *P. indica* males are attracted to a synthetic mixture of *E,E*-10,12-16:Al and *E*11-16:Al in the field, although it was inferior as an attractant compared to three 2-d-old virgin females (Wakamura et al., 1998). The sex pheromone compositions of several insect pests in Korea differ from those reported in the neighboring countries of Japan and China (Boo, 1998; Boo and Park, 2005). Therefore, we thoroughly reexamine the sex pheromone composition of *P. indica* in Korea.

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The objective of this study was to elucidate the sex pheromone composition of *P. indica* in Korea. A series of studies were conducted, including mating behavior, sex pheromone biosynthesis, and EAG response of male antennae.

Materials and methods

Insects rearing

Cotton caterpillar, *P. indica*, pupae were collected from watermelon fields in Kochang, Korea in 2000. Subsequent generations were used to analyze sex pheromones and to examine their mating and calling behavior. In addition, larvae and pupae of *P. indica* were supplied by the Bio-Organic Science Division of the Korea Research Institute of Chemical Technology in 2001 to quantify pheromones. Insect colonies were maintained at 25±1 °C, RH 60–80%, and a photoperiod of 16L:8D. Adults were sexed immediately after emergence and provided with 10% sucrose solution as food. Larvae were reared on leaves and fruits of cucumber or pumpkin.

Observation of the emergence and mating behavior

The emergence rhythms of male and female moths were checked on an individual level (♀: 172, ♂: 159). Pupae were placed in a rearing cage and the number of moths emerged was checked at 1 h intervals for 24 h.

Twenty 1-d-old virgin females, nineteen 2-d-old virgin females, and twenty 3-d-old virgin females were placed in a plastic cage with thirty 1 to 3-d-old unmated males, and 10% sucrose solution was provided as food source. Their mating behavior was observed at 30 min intervals for 24 h, including 8-h scotophase. During scotophase, a flash light covered with a Kodak Wrattern 29 red gelatin filter (Kodak, USA) was used so as not to interfere with their mating behavior.

Pheromone identification and quantification

To identify the sex pheromones of *P. indica*, sex pheromone glands of 10 virgin females (1-d-old) were dissected during peak mating time and extracted individually in 20 μl of n-hexane (HPLC grade) for 1 h. Extracts were stored at −20 °C until gas chromatography (GC) and GC-EAD analysis.

Four 1-d-old virgin females were placed in a 500 ml Erlenmeyer flask 30 min before scotophase. Volatile pheromone components were collected with a SPME (Solid Phase Micro Extraction: Supelco, Bellefonte, USA) fiber cleaned at 200 °C for 2 min before use. Pheromones on the fiber were subjected immediately to a GC analysis.

The extracts and SPME collections were analyzed with a GC (HP6890, Agilent Technologies, Wilmington, DE, USA) equipped with a split/splitless capillary injector and a flame ionization detector (FID). Separation of compounds was conducted using a fused silica capillary column (60 m×0.25 mm id, Rtx®Restek) coated with 50% cyanopropyl–50% phenylmethyl polysiloxane. Oven temperature was set at 80 °C for 2 min, raised 8 °C/min to 150 °C, maintained at 150 °C for 5 min, raised 5 °C/min to 200 °C, and maintained at 200 °C for 1 min. The temperature of the injector and detector was 200 °C and 250 °C, respectively. Helium (He) was used as the carrier gas with flow rate of 40 ml/min. Samples were injected in the splitless mode.

The extract was also analyzed with a GC linked with an electroantennographic detector (GC-EAD). The GC (Shimadzu GC-14A Japan) was equipped with a fused silica capillary column (30 m×ID 0.32 mm DB225). The end of the column was split into two paths with a Y splitter (Agilent) at a 1:1 ratio to connect to a FID and an EAD. The oven temperature was programmed at 80 °C for 2 min, raised 10 °C/min to 200 °C, and maintained at 200 °C for 2 min. The temperature of the injector and the detector was 200 °C. Helium was used as the carrier gas. Samples were injected in a splitless mode. The split valve was opened for 1 min after injection. The gas was allowed to flow simultaneously into the FID and the EAD. The EAG system and antenna setting are described below. The retention times at EAG-active signal were confirmed with those of authentic sex pheromone components of *P. indica*.

To quantify the sex pheromone of *P. indica*, the pheromone glands of 2-d-old females were dissected and extracted individually in 20 μl of n-hexane with 200 ng of (*Z*)-9-tetradecenal (*Z*9-14:Al) as an internal standard. Dissections occurred at 2-h intervals from 2-h before lights-off, through scotophase, to 8-h after lights-on. Quantification was conducted by GC (HP 6890) analysis of 1 μl of each extract by comparing peak areas of two sex pheromone components, *E*11-16:Al and *E*,*E*-10,12-16:Al, and the internal standard *Z*9-14:Al. GC conditions were the same as those described above.

PBAN studies

Experiments were conducted to determine the existence of a pheromonotropic factor (PBAN, Pheromone Biosynthesis Activating Neuropeptide) in the head of *P. indica*. Extracts of ten heads of 1-d-old adult males and females were prepared using the same protocol of Choi et al. (2004). Two reported PBAN compounds in other insect species, Hez-PBAN in *Helicoverpa zea* and PssPT in *P. separate* were applied for the comparison.

Then 1 μl of Hez-PBAN (Peninsula Laboratories, Inc, Belmont, CA, USA), PssPT (Sigma Chemical Co., Steinheim, Germany), or the prepared head extracts in insect saline solution (10 pmole) was injected into the female abdomen between the 4th and 5th segment. To remove the effect of neuropeptides that had already been secreted in the head of *P. indica*, females (1-d-old) were decapitated during the photophase before one day of the injections. Insect saline solution (1 μl) was injected as the control. Two hours after each injection, the sex pheromone glands were excised and extracted in 20 μl of n-hexane with 200 ng of (*Z*)-9-tetradecenol (*Z*9-14:OH) as an internal standard for 15 min to extract the sex pheromone and 1 μl of the extract was subjected to GC analysis using the same method as the quantification of the sex pheromone.

An experiment was also conducted to determine the time required for pheromone production after injection of Hez-PBAN and its persistence time. First, 1-d-old females were anesthetized with CO_2 and decapitated during the photophase. The next day, 1 μl of Hez-PBAN in the insect saline (10 pmole) was injected between the 4th and 5th abdominal segments of the females. The pheromone glands were extracted at 0, 1, 2, 3, 4, 5, 6, and 7-h after injection, and GC analysis was conducted using the method described above.

Whole-mount immunocytochemistry

Whole-mount immunocytochemistry was used to observe the localization of PBAN-like immunoreactivity in the central nervous system (CNS) of *P. indica,* as described in Ma and Roelofs, 1995 and

Table 1
Sex pheromone composition of four pest insect species.

Species	Sex pheromone	
	Components	Ratio
Palpita indica	(*E*)-11-hexadecenal (*E*11-16:Al),	7
	(E,E)-10,12-hexadecadienal (E,E-10,12-16:Al)	3
Peridroma saucia	(Z)-11-hexadecenyl acetate (Z11-16:Ac),	6
	(Z)-9-tetradecenyl acetate (Z9-14:Ac)	4
Heicoverpa armigera	(Z)-11-hexadecenal (Z11-16:Al),	9
	(Z)-9-hexadecenal (Z9-16:Al)	1
Spodoptera exigua	(Z,E)-9,12-tetradecadienyl acetate (Z,E-9,1-14:Ac),	7
	(Z)-9-tetradecenol (Z9-14:OH),	3
	(Z)-11-hexadecenyl acetate (Z11-16:Ac)	1