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Sex pheromone composition of *Ascotis selenaria* (Lepidoptera: Geometridae) and its regional variation in Korea

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ABSTRACT

This study was conducted to investigate sex pheromone composition of *Ascotis selenaria* (Lepidoptera: Geometridae) in Korea. Two sex pheromone compounds such as (*Z,Z*)-6,9-*cis*-3,4-epoxynonadecadiene (6*Z*,9*Z*-*cis*-3,4-epoxy-19:H) and (*Z,Z,Z*)-3,6,9-nonadecatriene (3*Z*,6*Z*,9*Z*-19:H) were identified in the glands of *A. selenaria* females by gas chromatography–mass spectrometry analysis. However, the component 3*Z*,6*Z*,9*Z*-19:H neither elicited an electroantennogram response nor increased the attractiveness for *A. selenaria* males in the field. The role of 3*Z*,6*Z*,9*Z*-19:H seems to be as an antagonistic signal for mating behavior of *A. selenaria* males.

The blend ratios of two 6*Z*,9*Z*-*cis*-3,4-epoxy-19:H isomers such as, 6*Z*,9*Z*-*cis*-3*R*,4*S*-epoxy-19:H and 6*Z*,9*Z*-*cis*-3*S*,4*R*-epoxy-19:H, were critical to attract *A. selenaria* males. The blend ratios of the two isomers showing peak catch of *A. selenaria* males had large variations among the locations investigated. *A. selenaria* populations in Gunwi showed peak activity at ratios of 0.9:0.1 and 0.8:0.2, whereas the populations in Goheung, Yeongam, and Jeju (Aewol and Harye) showed peak activity at a 0.5:0.5 ratio. In Changnyeong, the peak activity occurred in a bimodal form at ratios of 0.7:0.3 and 0.4:0.6. Such variation was partially explained by geographical isolation due to mountain ranges. Consequently, the results of our study should be useful for designing a region-specific pheromone lure for successful *A. selenaria* monitoring.

Introduction

Ascotis selenaria is an important pest of avocado in Israel, coffee in Kenya, and tea in Georgia, USSR and also causes severe damage to orange, peanut, alfalfa, apple, lemon, and pecan (Wysoki, 1982). It has been reported as a major pest in citrus orchards in Korea, causing direct damage to fruit such as gnawed and deep scars or wide holes as well as feeding on citrus leaves (Kim et al., 2000; Choi et al., 2011b). This pest occurs in apple, bean, and carrot fields in Korea (Kim and Beljaev, 2001). Thus, an effective population monitoring tool is required for successful management of *A. selenaria*.

A. selenaria sex pheromones have been classified in the type-2 pheromone group based on hydrocarbons with a polyene and/or epoxide functional group that are typically found in four large moth families: Geometridae, Noctuidae, Arctiidae, and Lymantriidae (Millar, 2000; Byer, 2006). (*Z,Z*)-6,9-*cis*-3,4-epoxynonadecadiene (6*Z*,9*Z*-*cis*-3,4-epoxy-19:H) and (*Z,Z,Z*)-3,6,9-nonadecatriene (3*Z*,6*Z*,9*Z*-19:H) have been identified in the sex pheromone glands of *A. selenaria* females in Japan (Ando et al., 1997) and Israel (Becker et al., 1990; Cossé et al., 1992). However, 3*Z*,6*Z*,9*Z*-19:H did not evoke any behavioral response in *A. selenaria* in a wind tunnel (Cossé et al., 1992) and had no attractive activity in field tests (Becker et al., 1990; Ando et al., 1997). In contrast, the two 6*Z*,9*Z*-*cis*-3,4-epoxy-19:H isomers such as 6*Z*,9*Z*-*cis*-3*S*,4*R*-epoxy-19:H (SR) and 6*Z*,9*Z*-*cis*-3*R*,4*S*-epoxy-19:H (RS), attracted *A. selenaria* males in the field. Japanese populations are frequently attracted to RS, whereas Israel populations prefer SR. It seems that the optimum pheromone blend of *A. selenaria* populations varies by geographical region. The sex pheromone compositions of several agricultural insect pests in Korea differ from those reported in the neighboring countries of Japan and China (Boo, 1998; Boo and Park, 2005). Therefore, it is necessary to thoroughly examine the sex pheromone composition of native *A. selenaria* populations to apply field monitoring in Korea.

The objective of this study was to elucidate the sex pheromone composition of *A. selenaria* in Korea in terms of the blend ratios and regional variations. A series of studies was carried out, including mating behavior, sex pheromone biosynthesis, electroantennogram (EAG) responses of male antennae, and field tests of pheromone blends.

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Materials and methods

Insects and chemicals

Ascotis selenaria larvae, collected from a Jeju citrus orchard in 2007, were reared on a semi-artificial diet (Choi et al., 2011a) in the laboratory at 25 ± 1°C under 16:8 h (L:D) conditions. Pupae were sexed, and emerged adults were provided with a 10% sucrose solution as food. All synthetic sex pheromones (purity > 95%), were purchased from Chemtech B. V. (Amsterdam, the Netherlands).

Emergence and mating rhythm

The emergence rhythms of male and female adult moths were examined individually (♀: 86, ♂: 114). Pupae were placed in a plastic cage (40 × 30 × 40 cm), and the number of moths that emerged was checked at 1 h intervals for 24 h.

Groups of 10 females with the same age (0, 1, 2, and 3-d-old) were placed in each cage (25 × 25 × 25 cm) and calling behavior was observed at 1 h intervals after lights-off. To examine mating rhythm, a single female at different ages (0, 1, 2, and 3-d-old) was placed individually in a cage (15 × 15 × 15 cm) with two males (1–3-d-old), and mated pairs were counted at 30 min intervals after lights-off. This experiment was replicated nine times.

Pheromone analysis

Seventy-three females (1–2-d-old) were anesthetized with CO_2 and their abdominal tips were excised at 5 h after lights-off. The sex pheromone components were extracted by soaking the abdominal tips in 100 μl of *n*-hexane (HPLC grade) for 15 min. The extract was analyzed by gas chromatography–mass spectrometry (GC–MS) (Shimadzu QP-2010) using a capillary column (30 m × 0.32 mm I.D., 0.25 um thick; Rtx5MS). Injector temperature was set to 250 °C. Oven temperature was set at 80 °C for 1 min, raised 10 °C/min to 200 °C, and maintained at 200 °C for 13 min. One microliter of the extract was injected in the splitless mode. Helium was used as the carrier gas and flowed at 2 ml/min into the column. Effluents were ionized by electron impact mass spectrometry while the temperatures of the ion source and interface were maintained at 200 and 250 °C respectively. The peaks were then analyzed by comparing the retention time and ionization patterns with those of authentic compounds.

To measure the antennal responses to the sex pheromone components in the extract, GC–electroantennographic (EAD) tests were conducted with an HP 7890 GC coupled with Syntech EAD system, using a capillary column (30 m × 0.32 mm I.D., 0.25 μm thick; HP-5). The end of the column was split into two paths at a 1:1 ratio using a Y-splitter (Agilent Technologies, Santa Clara, CA, USA). One of the paths was connected to a flame ionization detector, whereas the other path was connected to an EAD system through heated transfer. The temperature of the injector, detector, and interface was set to 250 °C. Nitrogen was used as the carrier gas. One microliter of extract was injected in splitless mode. Oven temperature was set to 80 °C for 1 min, increased to 170 °C at 15 °C/min, raised to 200 °C at 10 °C/min, and then held at the final temperature for 15 min. To measure EAG responses, antenna of unmated male adults (1–4-d-old) with both ends cut out, was mounted between the reference and recording electrode of an EAG probe (PRG-2) using an electroconductive gel (Parker Laboratories Inc., Fairfield, NJ, USA). The mounted antenna was then placed close to the outlet of a glass tube (length, 120 mm; I.D., 8 mm). Charcoal-filtered and moisturized air was continuously blown (about 600 ml/min) through the glass tube during GC–EAD. EAG signals were recorded using the Syntech EAG 2000 program (Hilversum, The Netherlands) on a personal computer that included a probe/micromanipulator (MP-15), a data acquisition interface box (serial IDAC-232), and a stimulus air controller (CS-55).

Pheromone quantification

To quantify the amount of sex pheromone produced in the female gland during scotophase, the abdominal tips of eight female adults (1-d-old) were excised and soaked individually for 15 min in 20 μl *n*-hexane containing 50 ng/μl (*Z,Z,Z*)-3,6,9-octadecatrienyl acetate (3*Z*,6*Z*,9*Z*-18:Ac) as an internal standard at every hour after lights-off. In addition, gland extracts were prepared from 10 females (each of 0, 1, 2, 3, and 4-d-old) at 5 h after lights-off using the same method described above for the sex pheromone analysis study. Each 1 μl of extract was injected into the GC–MS, and pheromone quantification was conducted under the same GC–MS conditions described above.

EAG response

Five *A. selenaria* males (3-d-old) were used in the EAG tests. The antenn preparation method was the same as for GC–EAD. A charcoal-filtered air stream was blown continuously over the antennae via a stainless steel pipe during the EAG test. All compounds used in the EAG test were dissolved in *n*-hexane and applied to a filter paper strip (2 × 80 mm, Whatman No.1), which was finally inserted into a Pasteur pipette (15 cm long). The pipette was connected to the stimulus air

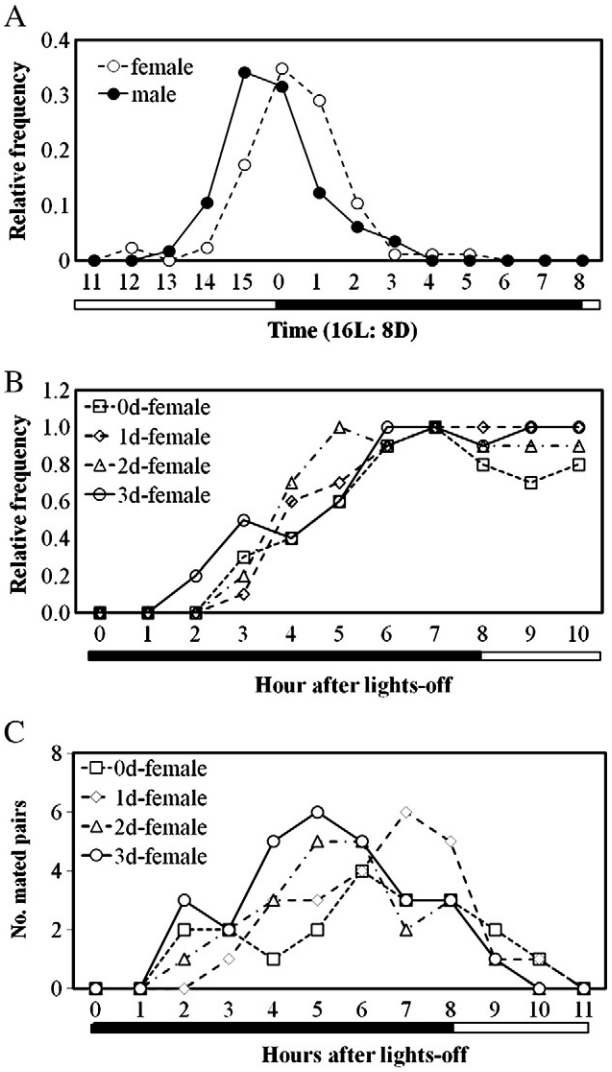

Fig. 1. Emergence, calling, and mating rhythms in *Ascotis selenaria*. (■: scotophase, □: photophase) (A) Emergence rhythms (♀: 86, ♂: 114). (B) Calling frequency of 10 females in different age groups. (C) Mating rhythms of nine females in different age groups.