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Quantification and Bioassay of Components in the Sex Pheromone Gland of the Tussock Moth, Orgyia postica (Lepidoptera: Lymantriidae) in Taiwan 【Research report】

台灣小白紋毒蛾Orgyia postica (鱗翅目：毒蛾科) 性費洛蒙含量與生物活性檢定【研究報告】

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Abstract

Two components, trans-11S,12S-epoxy-(6,9)-heneicosadiene and (Z)-6-heneicosen-11-one, were identified from the sex pheromone glands of Orgyia postica (Walker) in Taiwan by gas chromatography-mass spectrometry. The average amounts of trans-11S,12S-epoxy-(6Z,9Z)-heneicosadiene and (Z)-6-heneicosen-11-one in individual virgin female glands were 30.7 ± 3.3 and 8.7 ± 0.6 ng/female, respectively, and the ratio of the amount of the two components was 3.6 ± 0.3. Using the single component of (Z)-6-heneicosen-11-one, no moths were caught in the field. Using the combination of trans-11S,12S-epoxy-(6Z,9Z)-heneicosadiene and (Z)-6-heneicosen-11-one at a ratio found in the gland extract of 4: 1, significantly more males were caught than with trans-11S,12S-epoxy-(6Z,9Z)-heneicosadiene alone.

摘要

用氣相層析質譜儀以及氣相層析儀鑑定台灣小白紋毒蛾的性腺體中兩種成分，trans-11S,12S-epoxy-(6Z,9Z)-heneicosadiene 與 (Z)-6-heneicosen-11-one，在處女蛾腺體的平均含量分別為 30.7 3.3 及 8.7 0.6 ng ，而二者的含量比例為 3.6 0.3. 單獨用trans-11S,12S-epoxy-(6Z,9Z)-heneicosadiene可以誘到雄蟲，單獨用 (Z)-6-heneicosen-11-one 做誘餌，無法抓到雄蛾，但是將兩種成分，用4: 1的比例做誘餌，誘到的雄蟲比單獨用trans-11S,12S-epoxy-(6Z,9Z)-heneicosadiene 誘到的多。

Key words: Orgyia postica, trans-11S,12S-epoxy-(6Z,9Z)-heneicosadiene, (Z)-6-heneicosen-11-one, EAG, bioassay

關鍵詞: 小白紋毒蛾Orgyia postica, trans-11S,12S-epoxy-(6Z,9Z)- heneicosadiene, (Z)-6-heneicosen-11-one, EAG, 生物檢定

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ABSTRACT

Two components, *trans*-11*S*,12*S*-epoxy-(6,9)-heneicosadiene and (*Z*)-6-heneicosen-11-one, were identified from the sex pheromone glands of *Orgyia postica* (Walker) in Taiwan by gas chromatography-mass spectrometry. The average amounts of *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one in individual virgin female glands were 30.7 ± 3.3 and 8.7 ± 0.6 ng/female, respectively, and the ratio of the amount of the two components was 3.6 ± 0.3. Using the single component of (*Z*)-6-heneicosen-11-one, no moths were caught in the field. Using the combination of *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one at a ratio found in the gland extract of 4: 1, significantly more males were caught than with *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene alone.

Key words: *Orgyia postica*, *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene, (*Z*)-6-heneicosen-11-one, EAG, bioassay

Introduction

The tussock moth, *Orgyia postica* (Walker), is an important defoliator of forests and orchards in Taiwan. *Trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene was recently reported as the single electroantennographically active component found in the pheromone gland extract from virgin females of *O. postica* in Okinawa, Japan (Wakamura *et al.*, 2001). *Trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene was also reported in a preliminary study on the sex pheromones of *O. postica* in Taiwan by Chow *et al.* (2001) who also found (*Z*)-6-heneicosen-11-one in the gland extract.

(*Z*)-6-Heneicosen-11-one has been found to attract several species of tussock moths, including *O. leucostigma* (J. E. Smith) (Grant, 1977), *O. thyellina* (Gries *et al.*, 1999), *O. antiqua* (L.) (Daterman *et al.*, 1976), *O. cana* Edwards (Daterman *et al.*, 1976), and *O. pseudotsugata* (McDunnough) (Gries *et al.*, 1997; Smith *et al.*, 1975). For *O. thyellina*, (*Z*)-6-heneicosen-11-one is the major component of the sex pheromone, and (*Z*)-6-

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heneicosen-9-one was identified as having a synergistic effect with it (Gries *et al*., 1999). For *O*. *pseudotsugata*, (6*Z*,8*E*)-heneicosadien-11-one synergized (*Z*)-6-heneicosen-11-one. In both cases, (*Z*)-6-heneicosen-11-one was the major sex pheromone component. However, there were also other components in gland extracts which had no synergistic effect, such as (6*Z*,9*Z*)-heneicosadien-11-one for *O*. *pseudotsugata* and (6*Z*,8*E*)-heneicosadien-11-one for *O*. *thyellina* (Gries *et al*., 1997, 1999).

The major component from the sex pheromone gland of *O*. *postica* in Taiwan was found to be *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene (Chow *et al*., 2001). A trace amount of (*Z*)-6-heneicosen-11-one was also present in the same gland. In order to understand the possible synergistic effect of (*Z*)-6-heneicosen-11-one, the following experiments were carried out, and the results are presented in this report.

1. EAG responses of the crude extract and of the fractions from HPLC separation of the crude extract were investigated.
2. Individual variations and the relative ratio of the two components, *trans*-11*S*, 12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one, in the gland were determined.
3. Field tests were carried out using individual compounds alone and different combinations of the two compounds.

Materials and Methods

Insects: An *O*. *postica* colony was started with larvae collected from tea plantations in Nankang, Taipei. Since females are wingless, they were put in an open box to mate with wild males in the field for each new generation. Larvae were reared in the laboratory on an artificial diet at 24±1°C with a photoperiod regime of 16L: 8D.

Artificial diet: The diet was composed of 2.5 g casein (Sigma, St. Louis, MO), 1.8 g fructose, 3 g α-cellulose (Sigma), 1 g salt max W (ICN Biomedical, Aurora, OH), 0.3 g cholesterol (Sigma), 0.12 g sorbic acid (Sigma), 0.5 g Na-alginate (Sigma), 0.1 g choline-chloride (Sigma), 2.5 g wheat germ, 2.5 g yeast powder, 2.5g agar, 30 μg linoleic acid (Sigma), 31 μg linoleic acid methyl ester (Sigma), 0.03 g streptomycin sulfate (Sigma), 0.5 g ascorbic acid (Riedel-deHaen), 1 g vitamin mix for insect (ICN Biomedical) and 80 ml of water (O'Dell and Rollinson, 1966). Larvae were reared in groups inside a petri dish, and the diet was added everyday.

Pheromone extraction and separation: Sexes were segregated according to different morphologies of the spinnerets as previously reported by Gu *et al*. (1992). Female tussock moths have a large sex pheromone gland at the intersegmental membrane between the 8th and 9th abdominal segments (Su, 1986). Abdominal tips with pheromone glands of calling females 1-2 days old were excised at the late photophase and placed in hexane. After 10 min, the solvent was withdrawn into a syringe, transferred to clean vials, and stored below –20°C. The extract from 1031 females was dried under a mild stream of nitrogen and dissolved in about 50 μl of 30% methylene chloride. The solution was subjected to HPLC on a 25-cm x 4.6-mm silica column (Spherisorb from Phase Sep, Deeside, UK), using 30% methylene chloride in hexane as the isocratic solvent system with a flow rate of 2 ml/min and UV detection (254 nm). The eluent was collected every 2 min.

Chemical analysis: Extracts and fractions of extracts were analyzed by splitless coupled gas chromatography-mass spectrometry (GC-MS) with a Thermo Quest Trace GC, interfaced with a Finnigan Trace mass spectrometer

(electron impact ionization, 70 eV). A DB-5ms (30 m x 0.25 mm) column was used. The GC was programmed at 40°C for 1 min, then 10°C/min to 300°C, with injector and transfer line temperatures of 200 and 250°C, respectively.

Component titer determination. For the component titer determination, pheromone gland extracts were prepared from virgin females which were 1, 2, and 3 days old, respectively. Individual glands were excised during the calling period (late photophase) and immersed in 100 μl of hexane for 5 min. Bezophenone (10 μl of a 50-ng/μl solution) was added to the extract as an internal standard after the gland was taken out. The extracts were quantified for *trans*-11*S*,12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one using a Varian 3400 GC with a DB-5ms column (30 m x 0.25 mm). The GC was programmed at 40°C for 1 min, then 10°C/min to 300°C, with an injector temperature of 200°C.

Chemicals: *Trans*-11*S*,12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one were provided by Dr. K. Mori of Science University of Tokyo, Japan (Muto and Mori, 2001).

EAG analysis. The EAG technique was similar to our previously described procedure (Ho *et al.*, 1996, 2002; Tsai *et al.*, 1999). Each EAG test was replicated with six different antennae. The ratio of the response to female extracts and clean air was used as the relative response.

Field bioassay. Field bioassays were conducted in a tea plantation near the Academia Sinica campus, Nankang, Taipei between March 16 and April 7, 2002. Winged traps (Jia-Fu Co., Taipei, Taiwan) (Tsai *et al.*, 1999) were used. Chemicals were dissolved in hexane, and 5 μl of chemicals in different ratios was applied inside the hollow 12-cm-long-polyethylene plastic capillary tube (I.D. 1 mm) as lures. Hexane (5 μl) was put inside the tubing and used as a control. Different ratios of *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one, including 4:0.1, 4:1, and 4:10, were used to compare the attractiveness of the different combinations in the field. Lures were placed under the upper cover of each sticky trap. Traps were hung 1.0~ 1.2m above the ground and 10 m apart from each other in a tea plantation. Traps were set up in the afternoon, and the number of the captured males was recorded every afternoon for 4 consecutive days; each set was repeated 3 times. Lures and controls were randomized after each count. The number of male moths captured in sticky traps baited with a single compound and a combination of two compounds were counted to assess the attractiveness of the lures. Data collected were entered into PROC GENMODE of SAS (generalized linear model) for analysis.

Results

Component titer determination: The average amounts (X ± SEM) of *trans*-11*S*, 12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one in each gland of all females of different ages were 30.7± 3.3 and 8.7±0.6 ng/female respectively. The ratio (X ± SEM) of the amount of *trans*-11*S*,12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one in individual females of all ages groups was 3.6±0.3 ng/female. The average component titers of individual females of different ages are listed in Table 1.

EAG response of male antennae to crude extract and fractions of female extract: The relative EAG responses (relative response ± SEM) of 1 female extract (FE) of abdominal tip crude extract, and HPLC fractions #4 and #5 were 7.0±1.7, 6.7±1.2, and 1.6±0.4, respectively, as shown in Fig. 1.

Field bioassay: Results are shown in Fig. 2. Significantly more male moths were captured with lures containing the

Table 1. Average quantity (ng/female) of *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene and (*Z*)-6-heneicosen-11-one produced by individual *O. postica* virgin females at 1, 2, and 3 days old, respectively

Age group		Mean component titer (ng/female, X ± SEM)		Mean ratio of epoxide/ketone
		epoxide[1]	ketone[2]	
1 day old	(n = 16)	40.0 ± 10.0[a]	9.4 ± 2.4 [a]	4.4 ± 1.1 [a, 3]
2 days old	(n = 9)	20.1 ± 5.4 [b]	6.9 ± 0.7 [a]	2.7 ± 0.5 [a]
3 days old	(n = 10)	25.4 ± 4.5 [ab]	9.1 ± 1.3 [a]	3.0 ± 0.5 [a]

[1]epoxide: *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene.

[2]ketone: (*Z*)-6-heneicosen-11-one.

[3] Values with different letters significantly differ at the 5% level (by Tukey's test).

4: 1 ratio of 11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene to (*Z*)-6-heneicosen-11-one than the other combinations (0.4: 1 and 40: 1) of the two compounds. The blend with the natural ratio (4: 1) found in the gland was thus more attractive than the single component, *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene. No male moths were caught when using the hexane control or (*Z*)-6-heneicosen-11-one alone.

Discussion

Wakamura *et al*. (2001) identified a single EAG-active component in the pheromone gland extract from virgin females of the tussock moth, *O. postica,* and field tests showed that male moths were attracted to the identified *trans*-11*S*, 12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene. Chow *et al*. (2001) found trace quantities of (*Z*)-6-heneicosa-11-one, in addition to *trans*-11*S*, 12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene, from abdominal tips of female tussock moths in Taiwan.

Geographic variations in sex pheromone blends are known to occur in other insects including the smaller tea tortrix moth *Adoxophyes* sp. (Kou *et al*., 1990), the Asian corn borer *Ostrinia furnacalis* (Kou *et al*., 1992), and the Lorey leafworm, *Mythimna loreyi* (Ho *et al*., 2002). Many sex pheromones consist of synergistic blends of compounds, such as those of *O. pseudotsugata* and *O. thyellina* (Gries *et al*., 1997, 1999). In a preliminary study, Chow *et al*. (2001) found that *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene alone can attract male *O. postica*, but (*Z*)-6-heneicosa-11-one alone attracted no male moths. So the role of (*Z*)-6-heneicosa-11-one in the sex pheromone gland of tussock moth was further investigated in this study.

In laboratory bioassays, fraction #4 (containing *trans*-11*S*,12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene) elicited the strongest responses from male antennae and fraction #5 (containing (*Z*)-6-heneicosa-11-one) also produced some response from male moths, so EAG responses from these fractions were compared with those from the crude extract. The results showed that the EAG responses from the crude extract were comparable to those from fraction #4, so synergism cannot be concluded from this result. However, the EAG response can only be used as a reference for an insect's behavior. So field bioassays were carried out to test the hypothesis that there is a synergistic effect between the two compounds.

The composition in the pheromone gland might not be the same as that of the released pheromone blend. In this study, field bioassays were conducted

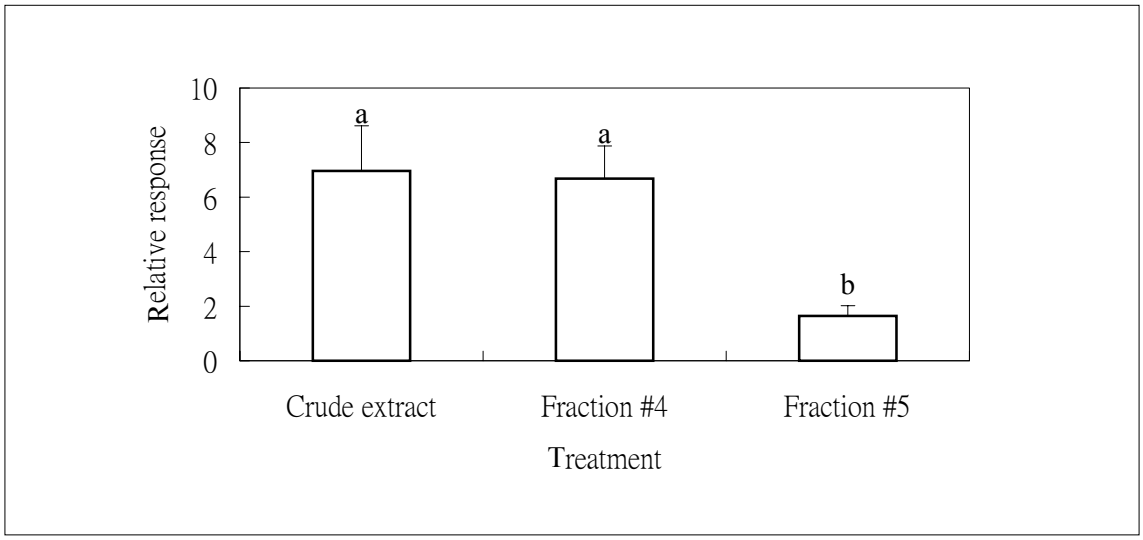

Fig. 1. EAG responses of crude extract and fractions separated by HPLC. Fraction #4 contains 11S,12S-epoxy-(6Z,9Z)-heneicosadiene and fraction #5 contains (*Z*)-6-heneicosa-11-one. Bars with the same letter do not significantly differ at the 5% level (by Tukey's test).

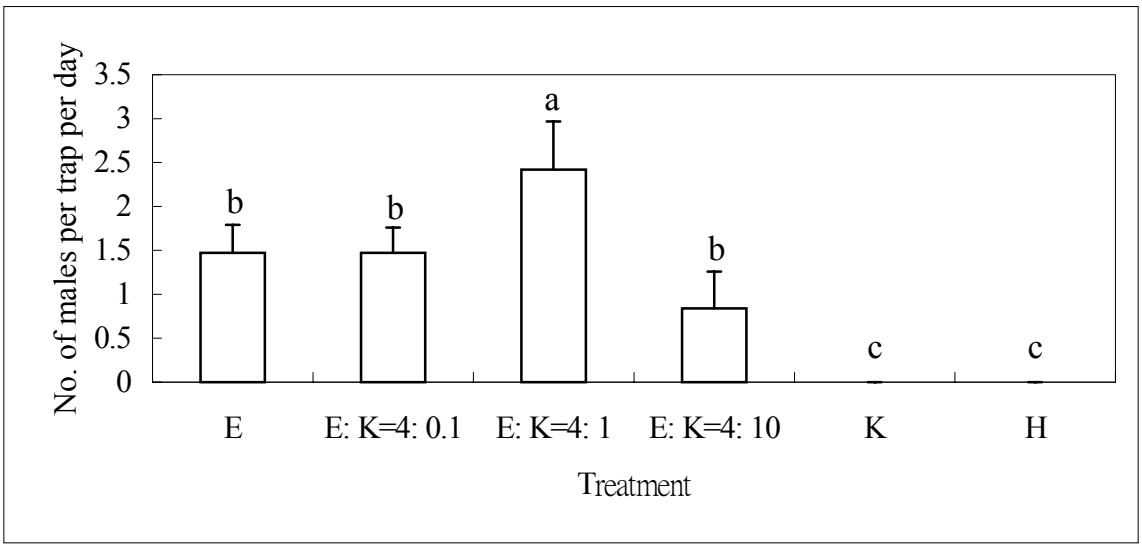

Fig. 2. Numbers of male *O. postica* captured (mean ± SEM) in traps baited with E: 10 μg epoxide, E: K = 4:0.1 (10 μg epoxide plus 0.25 μg ketone), E: K = 4:1 (10 μg epoxide plus 2.5 μg ketone), E: K = 4:10 (10 μg epoxide plus 25 μg ketone), K: 10 μg ketone, and H: 5 μl hexane in a tea plantation in Nankang, Taipei during March 16-April 7, 2002. Bars with the same superscript do not significantly differ. Epoxide: *trans*-11*S*,12*S*-epoxy-(6Z,9Z)-heneicosadiene and Ketone: (*Z*)-6-heneicosa-11-one. In total, 118 male moths were captured. PROC GENMOD of SAS was used for the statistical analysis.

using individual compounds, *trans*-11*S*, 12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene and (*Z*)-6-heneicosa-11-one, and two blends in different ratios. The results shown in Fig. 2 indicate that significantly more male moths were caught using the two-component blend in the ratio of 4:1 than with any of the other treatments. No moths were caught using (*Z*)-6-heneicosa-11-one alone. In this study, we demonstrated that two-component blend is better than *trans*-11*S*, 12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene by itself. Although *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene by itself did attract male moths, the fact that the presence of (*Z*)-6-heneicosa-11-one in the blend increased the attractiveness proves that the sex pheromone of the *O. postica* in Taiwan is composed of two components, *trans*-11*S*,12*S*-epoxy-(6*Z*, 9*Z*)-heneicosadiene and (*Z*)-6-heneicosa-11-one. The best formulation of the blend for attracting male moths in the field needs to be investigated further.

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台灣小白紋毒蛾 *Orgyia postica*（鱗翅目：毒蛾科）性費洛蒙含量與生物活性檢定

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摘 要

用氣相層析質譜儀以及氣相層析儀鑑定台灣小白紋毒蛾的性腺體中兩種成分，*trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene 與 (*Z*)-6-heneicosen-11-one，在處女蛾腺體的平均含量分別爲 30.7 ± 3.3 及 8.7 ± 0.6 ng ，而二者的含量比例爲 3.6 ± 0.3. 單獨用 *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene 可以誘到雄蟲，單獨用 (*Z*)-6-heneicosen-11-one 做誘餌，無法抓到雄蛾，但是將兩種成分，用 4: 1 的比例做誘餌，誘到的雄蟲比單獨用 *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene 誘到的多。

關鍵詞：小白紋毒蛾 *Orgyia postica*, *trans*-11*S*,12*S*-epoxy-(6*Z*,9*Z*)-heneicosadiene, (*Z*)-6-heneicosen-11-one, EAG, 生物檢定