

A Potent Sex Attractant of the Male Tea Tussock Moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae) in Taiwan: Field and EAG Responses

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Ru-Shiow Tsai, En-Cheng Yang, Chin-Yih Wu, Hsin-Kuang Tseng and Yien-Shing Chow (1999) A potent sex attractant of the male tea tussock moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae) in Taiwan: field and EAG responses. *Zoological Studies* 38(3): 301-306. 10,14-Dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) was identified from extracts of pheromone glands of the female tea tussock moth, *Euproctis pseudoconspersa* (Strand) by use of gas chromatography in Taiwan. EAG records showed that (*R*)- and (*S*)-enantiomers, and pheromone extract could elicit male antennal responses. Crude pheromone extract elicited a larger EAG response than did (*R*)-10Me14Me-15:iBu, but the EAG response to the (*S*)-enantiomer was smaller than that to crude extract or to the (*R*)-enantiomer. In field tests, the (*R*)-enantiomer showed greater attraction than did either the (*S*)-enantiomer or racemic mixtures in July 1998. However, later in the season, the (*R*)-enantiomer and racemic mixtures showed a similar attractancy, and both were better than the (*S*)-enantiomer. Traps baited with 20 µg of racemic mixtures and those with 2 virgin females showed similar attractancies; however, more males were caught with 80-µg baited traps.

Key words: *Euproctis pseudoconspersa*, 10,14-Dimethylpentadecyl isobutyrate, Enantiomers, Sex pheromone.

Tea tussock moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae, also known as the two-star tussock moth, *E. conspersa* Butler in Taiwan [Yi 1965]) is one of the major pests of tea, *Thea sinensis* in Taiwan. However, in Japan, *E. pseudoconspersa* mostly attacks camellia plants, including *Thea sinensis*, *Camellia sasanqua*, and *C. japonica*. The larva of this insect is notorious for its irritating hairs, which cause an allergic reaction on human skin due to a de-erythrocyte substance (Bleumink et al. 1982). The major component of *E. pseudoconspersa* sex pheromone was identified to be (*R*)-10,14-dimethylpentadecyl isobutyrate (*R*-10Me14Me-15:iBu) in Japan (Wakamura et al. 1994 1996, Ichikawa et al. 1995). However, in moths, geographical variation in pheromone blends of the same species is commonly encountered (Klun and Maini 1979, Hansson et al. 1990). Therefore, in this re-

search the synthetic pheromone (*R*)- and (*S*)-enantiomers of 10Me14Me-15:iBu and racemic mixtures were tested for male attractiveness with traps in a tea plantation in Taiwan. Electrophysiological responses of male antennae of Taiwan-collected individuals were also investigated to see whether variations in sex pheromone systems exist between Japanese and Taiwanese populations. Field studies were conducted to investigate the possibility of the use of a potent attractant to control or monitor this notorious pest in Taiwan.

MATERIALS AND METHODS

Insects

Euproctis pseudoconspersa larvae collected

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from a field planted with *Thea sinensis* at the Puhsin Tea Plantation were reared in the laboratory on tea leaves from the same plantation until pupation. Then pupae were stored individually in glass tubes (O.D. 2 x 5 cm).

Preparation of pheromone extracts

Pheromone glands were carefully excised from 2-d-old virgin females and extracted with n-hexane for 2 min at room temperature. Extracts were pooled and stored below -20°C until use.

Gas chromatography

Gas chromatographic (GC) analysis was performed with a Varian 3400 gas chromatograph equipped with DB-23, DB-225, or DB-5MS fused silica capillary columns (30 m x 0.25 mm I.D., 0.25 μm film thickness, J & W Scientific, Folsom, CA, USA). The oven temperature program (TP) for DB-23 was set at 40°C initially, raised at $10^{\circ}\text{C}/\text{min}$ to 120°C , then at $2^{\circ}\text{C}/\text{min}$ to 200°C , and maintained at the final temperature for 5 min (abbreviation for this TP: 40[0]-10-120[0]-2-200[5]). For the DB-225 and DB-5MS columns, TPs were 150(10)-5-200(5) and 160(10)-10-280(10), respectively. The sample was injected in splitless mode for 0.50 min.

Chemicals

(*R*)- and (*S*)-enantiomers and racemic mixtures of 10Me14Me-15:iBu were provided by Dr. S. Wakamura of the National Institute of Entomological and Sericultural Science, Tsukuba 305-8634, Japan. (*R*)- and (*S*)-enantiomers were originally synthesized by Dr. A. Ichikawa (Ichikawa et al. 1995), and racemic mixtures were provided by Dr. Fukumoto of Shin-Etsu Chem. Co. (Fukumoto et al. Patent).

Electroantennogram (EAG)

In EAG experiments (Roelofs 1984, Ho et al. 1996), the dissected antenna from a 2-d-old male which had been kept separated from females was continuously flushed with moistened, charcoal-filtered air (100 ml/min). Stimulus cartridges consisted of a Pasteur pipette containing a piece of filter paper (2 x 20 mm) on which the pheromone solution was applied. In the dose-response tests, a series of dilutions (0.01 FE, 0.1 FE, and 1 FE of crude pheromone extract [FE: female equivalent]), and 0.01, 0.1, 1, and 10 ng of 10Me14Me-15:iBu in 10 μl of n-hexane) were applied from low to high concentrations. In

EAG recording, Ag/AgCl electrode was used to couple the electrical responses of the antenna to the headstage of a DC preamplifier (NL102, Neuro Log System, Digitimer Ltd.). Signals were monitored on an oscilloscope screen (Gould 4305), and simultaneously digitized, stored by an on-line computer (IBM compatible PC 586) with data acquisition system (Digidata 1200 & Axoscope 1, Axon Instruments, Inc.).

Field test

Sticky traps (with a green upper cover and white bottom 26.5 x 22 cm, Cha-Fu Co., Taiwan) were used to evaluate the attractancy of (*R*)-, (*S*)-enantiomers, and racemic mixtures of 10Me14Me-15:iBu, as well as virgin females in the field. Aliquots of 20 μg of chemicals were applied to a 12-cm-long polyethylene plastic capillary tube (I.D. 1 mm) as a lure. Then 1 or 4 lures were placed under the upper cover of each sticky trap. Two virgin (1- to 2-d-old) females were confined in a small cylindrical net cage (ca. 6 cm high and 6 cm diameter) and placed in the middle of the sticky trap. Traps were set 1.0 ~ 1.2 m above the ground and 10 m apart from each other in a tea plantation. Traps were baited with chemicals or

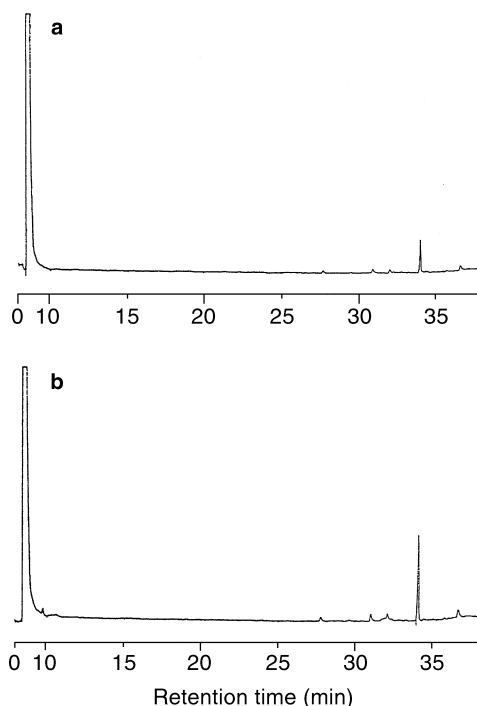


Fig. 1. (a) Gas chromatogram of the extract of pheromone glands of female *Euproctis pseudoconspersa*. (b) Coinjection of the crude pheromone extract plus 10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu).

virgin females in the morning, and the number of captured males was recorded every morning. The field test was conducted at Longtan and Puhsin Tea Plantations in Taiwan in Oct. 1997 and July to Sep. 1998. Data collected were submitted to two-way analysis of variance. The means were then compared by Tukey's multiple range test.

RESULTS

Gas chromatography

When the female pheromone extract of *E. pseudoconspersa* in Taiwan was analyzed by gas chromatography on a DB-23 column, 1 major peak occurring at a retention time 34.20 min (Fig. 1a) was observed. This peak coincided with the retention time of synthetic 10Me14Me-15:iBu. Coinjection of female pheromone extract and synthetic 10Me14Me-15:iBu gave a single peak at the same time (Fig. 1b). The retention times of the pheromone extract analyzed by DB-225 and DB-5MS also were very close to that for synthetic 10Me14Me-15:iBu (Table 1). All these data indicate that the major component of the pheromone gland of the female moth of *E. pseudoconspersa* in Taiwan is 10Me14Me-15:iBu.

EAG

Typical antennal responses showed a quick negative deflection followed by a slow recovery phase when female pheromone extract, synthetic (*R*)- and (*S*)-10Me14Me-15:iBu were applied to the male antenna (Fig. 2a).

EAG responses to the (*S*)-enantiomers of 10Me14Me-15:iBu were obviously smaller than those to pheromone extract and (*R*)-enantiomer (Fig. 2b). Dose responses of (*R*)-10Me14Me-15:iBu showed that the EAG response was saturated at a dosage of 50 ng. However, at a higher dosage (100 ng) of all chemicals stimulated, the EAGs showed a reduced response. Since 1 FE of crude pheromone

Table 1. Retention times of *Euproctis pseudoconspersa* female pheromone gland extract and authentic compound on different GC columns

Chemical	Retention time (min)		
	DB-23	DB-225	DB-5MS
Pheromone extract	34.20	33.89	21.24
(<i>R</i>)-10Me14Me-15:iBu	34.20	33.88	21.23

extract contained 10 ng of 10Me14Me-15:iBu, 1 FE elicited a larger EAG response than did the same amount of (*R*)-10Me14Me-15:iBu.

Field test

In the field, sticky traps (Fig. 3a) baited with 20 µg racemic mixture of 10Me14Me-15:iBu captured a similar number of males as those baited with 2 virgin females during 23-24 Oct. 1997 at Longtan. However, the male catch was greatly increased when the trap was baited with 80 µg of racemic mixture (Table 2a,b). Besides, the male catch fluctuated daily; on 22 Oct. it was significantly larger than those of other days ($p < 0.0001$).

The attractancies of (*R*)-, (*S*)-enantiomers, and racemic mixtures of 10Me14Me-15:iBu were also compared in the Puhsin Tea Plantation from July to Sept. 1998. A few male moths were captured during 15-18 July and during 2-4 Aug. 1998, and no moths were captured on other days, which were not indicated. On these days, however, (*R*)-10Me14Me-15:iBu captured more moths than did either the racemic mixture or (*S*)-enantiomer (Table 3a). More males were caught during 12-16 Aug. 1998. Statisti-

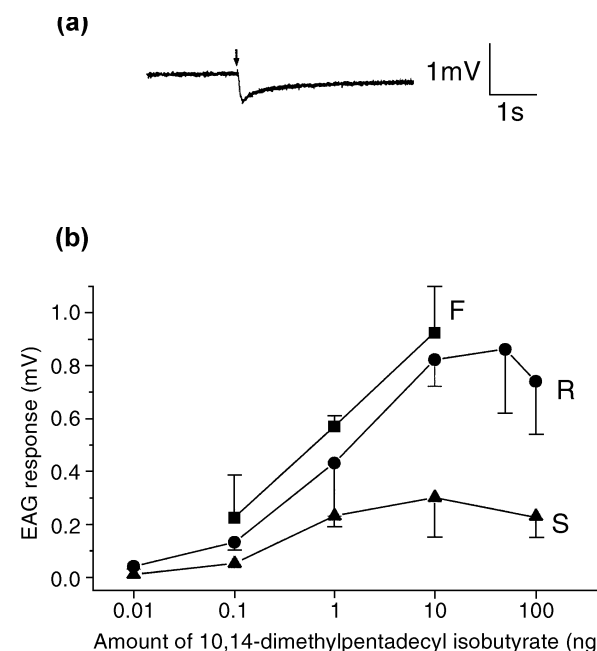


Fig. 2. (a) Typical EAG response of male antenna to 1 female equivalent. Arrow indicates the onset of the stimulation lasting for 200 ms. (b) EAG responses (\pm SE) of *Euproctis pseudoconspersa* male antenna to the female pheromone extract and to synthetic (*R*)- and (*S*)-10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu). F: female pheromone extract; R: (*R*)-10Me14Me-15:iBu; S: (*S*)-10Me14Me-15:iBu.

cal analysis of all data collected from 15 July to 16 Aug. showed that male catches of traps baited with (*R*)-enantiomer and racemic mixture of 10Me14Me-15:iBu showed no significant differences, but both were significantly greater than that of (*S*)-enantiomer (Table 3b). And both date and chemicals significantly influenced the male catch ($p < 0.05$).

During the experimental period, male moths of *E. pseudoconspersa* collected from the field showed dimorphism in coloration (Esaki et al. 1958). In autumn and spring most male moths were yellow, like the female moths, and only a few were dark brown. However, in summer (June, July, and Aug.) only dark brown male moths were caught in the field in Taiwan (Fig. 3b). Similar seasonal dimorphism was observed in male moths collected from the field but which emerged in the laboratory. Any difference in attractiveness between these 2 types was not considered to be present.

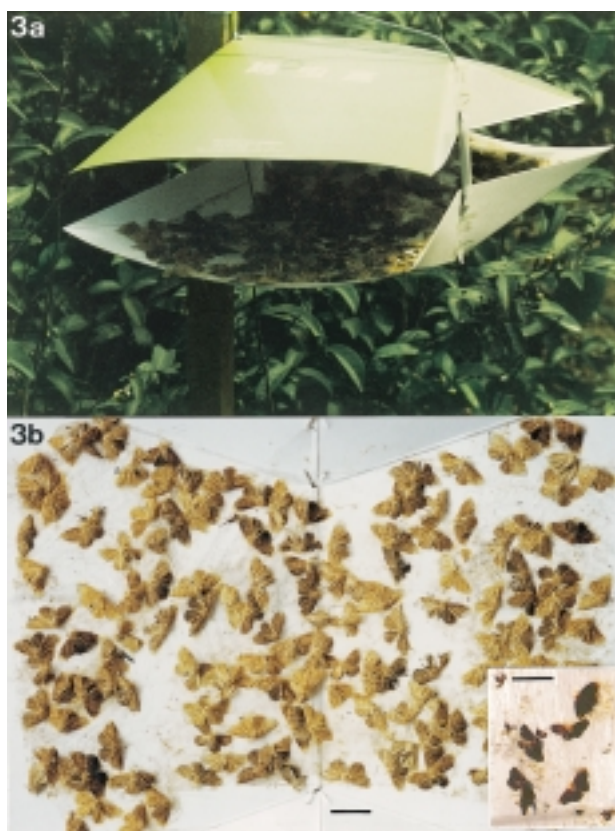


Fig. 3. (a) Photograph showing a green sticky trap baited with 10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) having caught many male *Euproctis pseudoconspersa*. (b) Most male moths showed yellow color in Oct., but only dark-brown ones were found in July and Aug. (inset). Bar: 2 cm.

DISCUSSION

The attractiveness of 10Me14Me-15:iBu to male *E. pseudoconspersa* had been evaluated in Japan (Wakamura et al. 1994 1996), and results indicated that 24 μg of the synthetic pheromone had a similar attractancy as did 80 μg when treated on a filter paper pad. Both 24- and 80- μg doses were far better than traps baited with 2 virgin females. However, in this study, the field test showed that 80 μg of the synthetic pheromone was a more attractive lure than either 20 μg or 2 virgin females. Different kinds of dispenser may influence the evaporation rate of the pheromone chemicals. This has been investigated for pheromone compounds of the diamondback moth (Mottus et al. 1997). Therefore, it was thought that differences obtained by these 2 studies may have been caused by the different kind of lures being used. In Japan, synthetic pheromone was applied to strips of filter paper, while in our present research it was applied to plastic capillary tubes. The evapora-

Table 2a. Trap catches of *Euproctis pseudoconspersa* males with 10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) and virgin female (Oct. 1997)

Source of variation	ANOVA* table			Treatment	No. of males caught-trap ⁻¹ .d ⁻¹
	DF	Fs	p		Mean \pm S.E. ⁺ (23-24 Oct.)
Treatment	2	6.50	0.0310	20 μg	24.5 \pm 21.5 ^b
Date	1	17.03	0.0062	80 μg	83.5 \pm 49.9 ^a
				2 virgin females	41.3 \pm 41.8 ^b

*Significance ($p < 0.05$).

+Tukey's multiple range test was used after ANOVA. Means followed by the same letter are not significantly different.

Table 2b. Trap catches of *Euproctis pseudoconspersa* males with 10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) (Oct. 1997)

Source of variation	ANOVA** table			Treatment (μg)	No. of males caught-trap ⁻¹ .d ⁻¹
	DF	Fs	p		Mean \pm S.E. ⁺ (21-24 Oct.)
Treatment	1	67.14	0.0001	20	40.2 \pm 25.0 ^a
Date	3	31.23	0.0001	80	105.2 \pm 60.9 ^b

**Significance ($p < 0.01$).

+Tukey's multiple range test was used after ANOVA. Means followed by the same letter are not significantly different.

tion from 4 tubes (20 µg x 4) was assumed to be 4 times greater than that from 1 tube. Besides, our results showed that virgin females had great attractiveness and could attract a larger number of males; this is different from the results obtained by Wakamura et al. (1994). The different degrees of attractivities between these 2 reports could be due to the age of the virgin females used. In Taiwan 1- to 2-d-old virgin females were used and the experiment lasted for only 2 days, but in Japan 3- to 4-d-old virgin females were used. Or differences could be caused by other unidentified pheromone components, which were released by the virgin female.

The attractiveness of (*R*)-, (*S*)-enantiomers, and racemic mixtures of 10Me14Me-15:iBu did not differ in field tests conducted in Japan and Xiangxi, China (Wakamura et al. 1996, Zhao et al. 1998). Although

this differs from our results, it implies that there might be some variation in this species. However, more detailed examination would be necessary for testing geographical variation in the sex pheromone system in *E. pseudoconspersa*.

The EAG response to the female pheromone extract was larger than that elicited by the same quantity of (*R*)-10Me14Me-15:iBu. Any minor compound such as 14-methylpentadecyl isobutyrate as identified by Wakamura et al. (1994) might cause this difference in Taiwan. However, further detailed evaluation of the latter compound is necessary in order to check whether any synergistic influence existed in this sex pheromone study.

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Table 3a. Trap catches of *Euproctis pseudoconspersa* males with (*R*)- and (*S*)-10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) and racemic mixture (20 µg/trap)

	No. of males caught.trap ⁻¹ .d ⁻¹ (mean ± S.E.) ⁺
Date	July 15-18, Aug. 2-4 [#]
Chemical	
<i>R</i> -enantiomer	1.6 ± 0.8 ^a
<i>S</i> -enantiomer	0.0 ± 0.0 ^b
Racemic mixture	0.4 ± 0.8 ^b

[#]Data between 18 July and 4 Aug. were excluded because no males were trapped.

⁺Data were transformed to the square root of (x + 0.5) and submitted to analysis of variance. Means followed by the same letter in the same column are not significantly different (*p* < 0.05 by Duncan's multiple range test).

Table 3b. Trap catches of *Euproctis pseudoconspersa* males with (*R*)- and (*S*)-10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) and racemic mixture (20 µg/trap)

Source of variation	ANOVA ^{**} table			Chemical	No. of males caught/night
	DF	F _s	<i>p</i>		Mean ± S.E. ⁺ (Jul. 15-Aug. 16)
Chemical	2	8.05	0.0008	<i>R</i> -enantiomer	1.80 ± 3.0 ^a
Date	31	3.42	0.0001	<i>S</i> -enantiomer	0.06 ± 0.3 ^b
				Racemic mixture	1.30 ± 2.6 ^a

^{**}Significance (*p* < 0.01).

⁺Tukey's multiple range test was used after ANOVA. Means followed by the same letter in the same column are not significantly different.

REFERENCES

- Bleumink E, MCJM DeJong, F Kawamoto, GT Meyer, AJ Kloosterhuis, IJ Slijper-Pal. 1982. Protease activities in the spicule venom of *Euproctis* caterpillars. *Toxicon* **20**: 607-613.
- Esaki T, S Issiki, A Mutuura, H Inoue, M Ogata, H Okagaki, H Kuroko. 1958. Icones heterocerorum Japonicorum in coloribus naturalibus. Japan: Hoikusha Press, 34 pp.
- Hansson BS, M Toth, C Lofstedt, G Szocs, M Suche, J Lofqvist. 1990. Pheromone variation among eastern European and western Asian population of the turnip moth, *Agrotis segetum*. *J. Chem. Ecol.* **16**: 1611-1622.
- Ho HY, YT Tao, RS Tsai, YL Wu, HK Tseng, YS Chow. 1996. Isolation, identification, and synthesis of sex pheromone components of female tea cluster caterpillar, *Andraca bipunctata* Walker (Lepidoptera: Bombycidae) in Taiwan. *J. Chem. Ecol.* **22**: 271-285.
- Ichikawa A, T Yasuda, S Wakamura. 1995. Absolute configuration of sex pheromone for tea tussock moth, *Euproctis pseudoconspersa* (Strand) via synthesis of (*R*)- and (*S*)-10,14-dimethyl-1-pentadecyl isobutyrate. *J. Chem. Ecol.* **21**: 627-634.
- Klun JA, S Maini. 1979. Genetic basis of an insect communication system: the European corn borer. *Environ. Entomol.* **8**: 423-426.
- Mottus E, V Nomm, IH Williams, I Liblikas. 1997. Optimization of pheromone dispensers for diamondback moth *Plutella xylostella*. *J. Chem. Ecol.* **23**: 2145-2159.
- Roelofs WL. 1984. Electroantennogram assays: rapid and convenient screening procedures for pheromones. In HE Hummel, TA Miller, eds. *Techniques in pheromone research*. New York: Springer-Verlag, pp. 131-139.
- Wakamura S, A Ichikawa, T Yasuda, N Arakaki, T Fukumoto. 1996. EAG and field responses of the male tea tussock

- moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae) to (*R*)- and (*S*)-enantiomers and racemic mixture of 10,14-dimethylpentadecyl isobutyrate. Appl. Entomol. Zool. **31**: 623-625.
- Wakamura S, T Yasuda, A Ichikawa, T Fukumoto, F Mochizuki. 1994. Sex attractant pheromone of the tea tussock moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae): Identification and field attraction. Appl. Entomol. Zool. **29**: 403-411.
- Yi ST. 1965. Economical entomology. Taiwan: National Institute for Compilation and Translation, pp. 120-121.
- Zhao CH, JG Millar, KH Pan, CS Xu. 1998. Responses of tea tussock moth, *Euproctis pseudoconspersa*, to its pheromone, (*R*)-10,14-dimethylpentadecyl isobutyrate, and to the *S*-enantiomer of its pheromone. J. Chem. Ecol. **24**: 1347-1353.

臺灣茶毒蛾 (*Euproctis pseudoconspersa*) 性費洛蒙之嗅覺 電位反應與田間誘蟲試驗

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臺灣茶毒蛾 (*Euproctis pseudoconspersa*) 的性費洛蒙經過氣相層析、嗅覺電位反應 (EAG) 的比較、及田間誘蟲試驗後，證實其性費洛蒙的主要成分為 *R*-10,14-dimethylpentadecyl isobutyrate ([*R*]-10Me14Me-15:iBu)。在 EAG 的反應中雄蛾對雌當量的反應比對合成性費洛蒙 (*R*)-10Me14Me-15:iBu 的反應為大。光學異構物 (*S*)-10Me14Me-15:iBu 亦能引起雄蛾反應，但其 EAG 反應較 (*R*)-異構物及雌當量小。田間誘蟲的結果顯示 20 µg 劑量的合成性費洛蒙誘蟲效果與 2 隻處女雌蛾相同；但 80 µg 劑量則明顯比 20 µg 和處女蛾的誘蟲效果好。

關鍵詞：臺灣茶毒蛾，性費洛蒙。

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