Chemotaxonomy of the Cotton Leaf-Roller (Lepidoptera: Pyralidae) in Japan with Special Reference to Differences in Sex Pheromones

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Male responses to virgin females' signals, pheromone gland extracts and synthetic pheromone blends, as well as pheromone profiles, were compared among four populations of the cotton leaf-roller collected from the Chinese bottle tree (CBT), the linden tree (LND), the cotton-rosemallow (CRM) and the hibiscus (HBS). Neither virgin female moths nor the sex pheromone gland extracts of the female CBT and LND had any cross-attractiveness for the male CRM and HBS. Highly unsuccessful cross mating and genetic incompatibility were also observed between the CBT and the CRM. (*E*, *Z*)- and (*E*, *E*)-10,12-16: ALDs were detected in the pheromone gland of the female CBT and LND in a ratio of 77.0:23.0 and 78.5:21.5, respectively, but only (*E*, *Z*)-10,12-16: ALD was found in the female CRM and HBS. These analytical results agreed well with the results of bioassays performed with synthetic compounds in a cage. The male CBT and LND responded well to 7:3 and 8:2 mixtures of synthetic (*E*, *Z*)- and (*E*, *E*)-10,12-16: ALDs, as did the male CRM and HBS to 9:1 and 10:0 mixtures of these isomers. The first two populations of the cotton leaf-roller seemed to have been already reproductively isolated from the last two. From these results, and with reference to the morphological and biological differences reported on earlier, we confirmed that the cotton leaf-roller in Japan is comprised of *Notarcha basipunctalis* (BREM.) (CBT and LND) and *N. derogata* (FAB.) (CRM and HBS).

Key words: *Notarcha derogata* (FAB.), *N. basipunctalis* (BREM.), the cotton leaf-roller, chemotaxonomy, sex pheromone, (*E*, *Z*)-10,12-hexadecadienal, (*E*, *E*)-10,12-hexadecadienal

INTRODUCTION

To date, the cotton leaf-roller, *Notarcha derogata* (FAB.) (Lepidoptera: Pyralidae) has been recorded to feed on some 60 species of plants belonging to 10 families including Marvaceae and Sterculiaceae (SILVIE, 1990). In Japan, for half a century, most authors have treated all populations of the cotton leaf-roller as *N. derogata* (FAB.) (ESAKI et al., 1957; ISSIKI, 1969; INOUE et al., 1982). The first author of the present paper

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found, however, that the cotton leaf-roller larvae from the Chinese bottle tree, *Sterculia simplex* refused to feed on okra, *Hibiscus esculentas* (HONDA, unpublished data) which had been reported as a suitable host of this species. Recently, IWAMOTO and YOSHIYASU (1990) reported that the cotton leaf-roller in Japan is comprised of two groups which feed on the Chinese bottle tree, *S. simplex* or linden tree, *Tilia miqueliana*, and rose-mallow, *H. mutabilis* or hibiscus, *H. rosasinensis*, respectively. They also reported some morphological and biological differences between these two groups. INOUE (1990) suggested that the cotton leaf-roller of Japan is comprised of *N. derogata* (FAB.) and *N. basipunctalis* (BREM.), which corresponds to the two groups reported by IWAMOTO and YOSHIYASU (1990) (Fig. 1).

HIMENO and HONDA (1992) identified (*E,Z*)-10,12-hexadecadienal ((*E,Z*)-10,12-16:ALD) and (*E,E*)-10,12-hexadecadienal ((*E,E*)-10,12-16:ALD) as the major sex pheromone components of the cotton leaf-roller from the Chinese bottle tree. Whether the female moths of these feeding types exhibit any difference in their sex pheromone profile, which acts as a reproductive isolation between two populations, is an ecologically and taxonomically interesting question.

Here, we compare sex pheromone components of female and male responses to synthetic pheromone blends among 4 different local populations of the cotton leaf-roller from different host plants, and also discuss the taxonomic status of each population.

MATERIALS AND METHODS

Insects. Four populations of the cotton leaf-roller were used for this study. The first population (CBT) was obtained from the Chinese bottle tree, *S. simplex* in Tokyo. The second (CRM) and third one (LND) were from rosemallow, *H. mutabilis*, and linden trees, *T. miqueliana*, respectively, in Kyoto, and the last one (HBS) was from the hibiscus, *H. rosa-sinensis* in Okinawa. Larvae were reared on leaves of each host plant as described by HONDA (1991). Pupae and adults of each population were sexed and caged under $23 \pm 1^\circ\text{C}$ and 15L9D photoperiod.

Hybridization. For the cross-test of the two populations, CBT and CRM, 20

Fig. 1. Female adults of *Notarcha basipunctalis* (BREM.) (a) and *Notarcha derogata* (FAB.) (b) of the cotton leaf-roller. They correspond to the CBT or LND, and the CRM or HBS, respectively.

separate pairs of CBT females and CRM males, and CRM females and CBT males were placed into plastic cups (9 cm in diameter and 4.5 cm in depth), supplied with a 10% sucrose solution in a cotton pad and allowed to mate and oviposit for 7 days. All insects used in this experiment were collected in Kyoto in July, 1991. Ten control pairs of CBT and CRM were treated in the same way.

Chemical analysis. Female pheromone glands were excised from 25 to 100, 4-day-old virgin females after 3–5 h of lights-off and then extracted with 100 μ l of *n*-hexane for 5 min. Each extract was concentrated up to 0.5FE (female equivalent)/ μ l to 2FE/ μ l with dry N_2 immediately before chemical analysis. All synthetic standard chemicals used were generously supplied by Shin-Etsu Chem. Co. Ltd. (Tokyo, Japan) and Dr. T. ANDO, Tokyo University of Agriculture and Technology (Tokyo, Japan). Capillary GC, EI- and CI-MS analyses were performed, as described earlier (HIMENO and HONDA, 1992).

Laboratory bioassay. Specificity of male responses to sex pheromones was evaluated using the following as pheromone sources: two 4-day-old virgin females in a ball mesh (8 cm dia.); 2FE of the gland extract; 100 ng of a synthetic pheromone component or a blend of synthetic pheromones. The gland extract, or the synthetic pheromone components, were placed onto strips of filter paper (1 cm $\times$ 2 cm). Each bait was suspended from the top of a screen cage (32 cm $\times$ 22 cm $\times$ 30 cm) into which five 5-day-old males of CBT, LND, CRM or HBS were introduced. The male responses to pheromone source were observed for 3 min and the observation was repeated 4 times at 5-min intervals. The number of males which were attracted to and were in contact with these baits was recorded. The bioassays were conducted 3–5 h after lights-off under the same rearing conditions as mentioned above. Statistical analysis of data was done by a multiple comparison based on the KRUSKAL-WALLIS test (HOLLANDER and WOLFE, 1973).

RESULTS

Cross attraction and hybridization

Virgin females and pheromone gland extracts were used for reciprocal attraction tests among three populations (Table 1). Males of the three populations were apparently attracted to virgin females and to gland extracts of their own population. In cross attraction tests among CBT, CRM and HBS populations, both the males of HBS and CRM were also attracted to virgin females and to gland extracts of the CRM and the HBS, but the male CBT was not attracted to the virgin females nor by gland extracts of the CRM and the HBS.

In the hybridization test between the CBT and the CRM, no mating was observed in any parental combinations, while in the control cross-test, 40% (4 pairs) and 60% (6 pairs) mated normally. However, a few cases of cross-mating between the CBT and CRM had been noted in a stock culture in the last author's lab, but the sex-ratio of adults obtained in F_1 generation was extremely unbalanced (male > female).

Chemical analysis

Sex pheromone components of the CBT were described in detail by HIMENO and HONDA (1992). Briefly, a 77.0:23.0 mixture of (*E,Z*)-10,12-16:ALD and (*E,E*)-10,12-16:ALD was identified as a major component, with possible minor candidates

Table 1. Reciprocal cross-response of males to different pheromone sources among three populations which have different host plant specificity

Females	Pheromone sources	Males attracted (%) ^a		
		CBT	CRM	HBS
CBT	Virgin females ^b	98.8 a	0.0 c	0.0 c
	Gland extract ^c	98.8 a	0.0 c	0.0 c
CRM	Virgin females	0.0 b	83.8 a	77.5 b
	Gland extract	0.0 b	86.3 a	78.3 b
HBS	Virgin females	0.0 b	77.5 b	92.5 a
	Gland extract	0.0 b	76.3 b	86.3 a

CBT: the Chinese bottle tree feeder; CRM: the Cotton-rosemallow feeder; HBS: the Hibiscus feeder.

^a Means followed by the same letter within the same column are not significantly different ($p=0.05$ in KRUSKAL-WALLIS test).

^b 2 virgin females in a boll mesh.

^c 1FE (female equivalent).

Fig. 2. Capillary GC traces of sex pheromone gland extracts of the cotton leaf-roller from four different host plants. a: Chinese bottle tree (CBT), b: Linden tree (LND), c: Cotton-rosemallow (CRM), d: Hibiscus (HBS).

being (*E,Z*)-, (*E,E*)-10,12-16: OH and *n*-16: ALD (Fig. 2a). Two components (Peak 2, $t_{R\text{ DB-23}}$ 30.99 min and Peak 3, $t_{R\text{ DB-23}}$ 31.28 min) were similarly detected in the gland extract of LND (Fig. 2b). Retention times of these two peaks were identical to those of (*E,Z*)-10,12-16: ALD and (*E,E*)-10,12-16: ALD. Electron impact mass spectra of Peak 2 and 3 were quite similar to each other, and giving the following fragment ions: m/z (%) 236 (M^+ : $C_{16}H_{28}O^+$, 5%), 123 ($C_9H_{15}^+$, 3%), 109 ($C_{18}H_{13}^+$, 5%), 95 ($C_7H_{11}^+$, 20%), 81 ($C_6H_{19}^+$, 41%), 67 ($C_5H_7^+$, 100%), 54 ($C_4H_6^+$, 31%), which was identical to those of the synthetic (*E,Z*)-10,12-16: ALD and (*E,E*)-10,12-16: ALD. CI-mass spectra of these two components were also consistent with those of the synthetic compounds (HIMENO and HONDA, 1992). The ratio of these components in the gland extract of the female LND was 78.5:21.5. A trace of (*E,Z*)-10,12-16: OH and (*E,E*)-10,12-16: OH was also found in the LND extract (Fig. 2b). In the gland extract of the female CRM and HBS, the (*E,Z*)-10,12-16: ALD (Peak 2) was detected as a major component, whereas the (*E,E*)-10,12-16: ALD was not (Fig. 2c and d). A trace of *n*-16: ALD was detected in the gland extracts of the CRM and the HBS females, but neither (*E,Z*)- nor (*E,E*)-10,12-16: OH was found. No other compounds that could be considered as sex pheromone candidates has been found in the gland extracts analyzed.

Male responses to pheromone blends

Based upon the results of chemical analysis of the gland extracts, the attraction properties of individual compounds and various binary blends of synthetic (*E,Z*)-10,12-16: ALD, and (*E,E*)-10,12-16: ALD were tested in the screen cage (Fig. 3). The male CBT and LND were attracted to 7:3 and 8:2 blends of the two geometrical isomers of 10,12-16: ALD, but showed significantly lower response to other blends tested. The male CRM and HBS were attracted to 9:1 and 10:0 blends of (*E,Z*)- and (*E,E*)-10,12-16: ALD but not to other blends, including the blends that were attractive to the male CBT and LND. *n*-16: ALD did not attract any of the males

Fig. 3. Attractiveness of two synthetic pheromone components and their blends to the male cotton leaf-rollers from four different host plants in a cage. Open rectangle: Chinese bottle tree (CBT), solid circle: Linden tree (LND), solid rectangle: Cotton-rosemallow (CRM), and open circle: Hibiscus (HBS).

tested. Also, no synergistic and/or additive effect was observed when *n*-16:ALD was mixed with (*E,Z*)-10,12-16:ALD.

DISCUSSION

The results of the reciprocal attraction tests suggest that the sex pheromone of the CBT is qualitatively different from that of the CRM and the HBS. This was supported by the GC and GC-MS analyses of the female gland extracts and also by the attraction tests using synthetic pheromone blends. In addition, the gland extract components of the LND female were not significantly different from those of the CBT female, suggesting that the LND and CBT males may be attracted to either type of these females. The LND and CBT male response spectra to synthetic pheromone blends were quite similar. These two facts provide evidence that no reproductive isolation is provided by the pheromonal differences between the LND and the CBT.

A similar relationship was observed between the CRM and the HBS whose gland extracts exhibited cross-attraction to their males. GC profiles of the components in the CRM gland extracts, as well as male response spectra to pheromone blends, were very similar to those of the HBS. Although the male CRM and HBS showed no difference in their responses to synthetic pheromone blends, the attraction of the virgin females and of the gland extracts of their own populations was slightly higher than that of the other population. This difference in attraction suggests that there may be some unknown factors present in the pheromone components probably resulting from a geographic allopatry between the CRM and the HBS (Okinawa is ca. 1,100 km from Kyoto).

As a result, these 4 populations of the cotton leaf-roller should be tentatively classified into at least 2 groups: group-1, CBT and LND, and group-2, CRM and HBS. The third author found several morphological and biological differences between the CBT or the LND and the CRM or the HBS of the cotton leaf-roller (IWAMOTO and YOSHIYASU, 1990). The unsuccessful cross mating between the CBT and the CRM and the F_1 progenies with unbalanced sex-ratio suggest a post-mating reproductive isolation and genetic incompatibility (MAYER, 1975; HALDANE, 1922).

INOUE (1990) reported that some populations which had been recognized as *N. derogata* (FAB.) in Japan are *N. basipunctalis* (BREM.). Considering the reproductive isolation and genetic incompatibility between the CBT and the CRM, they may not be intra-specific local variations. The last author confirmed that the groups 1 and 2 are morphologically identical to *N. basipunctalis* (BREM.) and *N. derogata* (FAB.), respectively.

Pheromonal heterogeneity was well known in the European corn borer, *Ostrinia nubilalis* (HÜBNER) (KLUN et al., 1973; KOCHANSKY et al., 1975), but the hybrid progeny of their pheromone strains exhibited no great genetic deficiencies (LIEBHERR and ROELOFS, 1975). In sharp contrast, the *Adoxophyes orana* complex was revised into two different species by genetic incompatibility and a difference in the sex pheromone (HONMA, 1972; TAMAKI and HONMA, 1976). Also, the fruit-feeding type and the Pinaceae-feeding type of the yellow peach moth, *Conogethes punctiferalis* (GUENÉE), were compared and classified into two different species through biosystematic studies (e.g. KONNO et al., 1981; HONDA, 1986 a, b; HONDA and MATSUMOTO, 1987; HONDA and MITSUHASHI, 1989).

We would like to stress that a given population should be correctly identified before sex pheromones are applied to control the cotton leaf-rollers. It is possible that many tropical Asian and African pyralid pests tentatively identified as a species are actually species complexes. Sex pheromone chemotaxonomy may contribute to the revision of these closely related species.

From our results, and with reference to the morphological and biological differences reported earlier, we conclude that the cotton leaf-roller in Japan is comprised of *N. derogata* (FAB.) and *N. basipunctalis* (BREM.). In addition, CBT population of the cotton leaf-roller previously identified as *N. derogata* (FAB.) by HIMENO and HONDA (1992) must be considered *N. basipunctalis* (BREM.). Therefore, sex pheromone identification for *N. derogata* (FAB.) by HIMENO and HONDA (1992) should be revised as for *N. basipunctalis* (BREM.).

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