Attraction of *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae) males in Southeast Asia to female sex pheromone traps: Field tests in southernmost China, northern Vietnam and southern Philippines with three synthetic pheromone blends regarding geographic variations

Kei KAWAZU,1,*,† Yoshito SUZUKI,2 Yutaka YOSHIYASU,3 Efieda B. CASTILLON,4 Hiroshi ONO,5 Pham Thi VIUONG,6 Feng-Kuan HUANG,7 Taro ADATI,8 Takehiko FUKUMOTO9 and Sadahiro TATSUKI1

1 Graduate School of Agricultural and Life Sciences, The University of Tokyo; Tokyo 113–8657, Japan
2 National Agricultural Research Center; Tsukuba, Ibaraki 305–8666, Japan
3 Graduate School of Agriculture, Kyoto Prefectural University; Kyoto 606–8522, Japan
4 Bohol Agricultural Promotion Center; Dao District, Tagbilaran City, The Philippines
5 Appropriate Agriculture International Co., Ltd.; Machida, Tokyo 194–0013, Japan
6 Ministry of Agriculture and Rural Development, Vietnam National Institute for Plant Protection; Dong Ngac, Tu Liem, Hanoi, Vietnam
7 Institute of Plant Protection Guangxi Academy of Agricultural Sciences; Daxue Road 44, 530007, Nanning, P. R. China
8 International Institute of Tropical Agriculture; Kano Station, Kano, Nigeria
9 Specialty Chemicals Research Center, Shin-Etsu Chemical Industry Co., Ltd.; Joetsu, Niigata 942–8601, Japan

(Received 9 August 2004; Accepted 31 March 2005)

Abstract
Field tests of three types of synthetic sex pheromone blend (Japanese blend: Z11-18:Ald (55 μg), Z13-18:Ald (500 μg), Z11-18:OH (120 μg) and Z13-18:OH (180 μg), Indian blend: Z11-16:Ac (50 μg) and Z13-18:Ac (500 μg) and Philippine blend: Z11-16:Ac (500 μg) and Z13-18:Ac (10 μg) based on geographic variations in sex pheromones so far reported) for the rice leaffolder moth, *Cnaphalocrocis medinalis* Guénée (Lepidoptera: Crambidae), were performed at Nanning, in southernmost China, Hanoi, in northern Vietnam, and on Bohol, an island in the southern Philippines. In all of these tests, only the Japanese blend attracted significant numbers of male *C. medinalis*, while neither the Indian nor the Philippine blend showed any attractiveness to the males. The results at Nanning and Hanoi, from which the Japanese population may originate from, support the current inference about the migration routes of *C. medinalis*. On the contrary, the results on Bohol, in the Philippines, were unexpected and showed the presence of a population responding to the Japanese blend, but not to the Philippine blend.

Key words: Rice leaffolder; *Cnaphalocrocis medinalis*; geographic variation; sex pheromone

INTRODUCTION
The rice leaffolder, *Cnaphalocrocis medinalis*, is a migratory rice pest widely distributed in humid tropical to temperate countries of Asia, Oceania and Africa (Khan et al., 1988). In Japan, *C. medinalis* fails to over-winter and the early summer adults are thought to be overseas migrants, mostly from the rice double-cropping areas of southern China, such as the Fujian and Guangdong provinces, as with rice planthoppers (Wada et al., 1980; Kamiwada et al., 1994).

In the Asian populations of *C. medinalis*, geographic variations in the female sex pheromone blend have been reported: Sex pheromone blend of Indian and pheromone-like components of Philippine populations consist of (Z)-11-hexadecenyl acetate (Z11-16:Ac) and (Z)-13-octadecenyl
acetate (Z13-18:Ac) at ratios of 1–6:20 (Indian blend) (Ganeswara Rao et al., 1995) and 98:2 (Philippine blend) (Ramachandran et al., 1990). On the contrary, a blend of a Japanese population consists of different components, (Z)-11-octadecenal (Z11-18:Ald), (Z)-13-octadecenal (Z13-18:Ald), (Z)-11-octadecen-1-ol (Z11-18:OH) and (Z)-13-octadecen-1-ol (Z13-18:OH) at a ratio of 11:100:24:36 (Kawazu et al., 2000). Field tests in Japan using the above three synthetic blends have shown that only the Japanese blend had significant attractiveness to male C. medinalis (Kawazu et al., 2000, 2001, 2002). Also, in field tests at Hangzhou in the lower Yangtze Valley, China, with the same three blends, only the Japanese blend was significantly attractive (Kawazu et al., 2002). These results are consistent with the inference described earlier, since the insect populations in the lower Yangtze Valley may also originate from the annual migrants from the rice double-cropping areas of southern China (National Coordinated Research Team, 1981; Chen et al., 1989; Suzuki and Wada, 1994; Sogawa, 1995). Furthermore, since C. medinalis in the rice double-cropping areas cannot over-winter, they also may originate from migrants from southernmost China (areas to the south of 22°N latitude) and/or northern Vietnam where C. medinalis can breed all year round (National Coordinated Research Team, 1981; Chen et al., 1989). It is therefore interesting to examine the responsiveness of males to the sex pheromones in populations in southernmost China and northern Vietnam included in the putative primary emigration area of C. medinalis populations migrating to Japan.

On the other hand, it may also be possible that C. medinalis originating from islands in Southeast Asia, including the Philippines, migrate to Japan with the aid of seasonal southerly and south-westerly winds (Mills et al., 1996). In such a case, male moths responding to the Philippine blend would appear in Japan. But, until now, no significant attraction of C. medinalis males by the Philippine blend has been recorded in Japan (e.g., Kawazu et al., 2001), suggesting no migration routes of C. medinalis to Japan from the Philippines. In the report regarding the identification of the Philippine blend, no behavioral activity has been demonstrated; only EAG activity was reported (Ramachandran et al., 1990). In our analyses, neither Z11-16:Ac nor Z13-18:Ac were detected in the crude extract of Japanese populations (Kawazu et al., 2000).

For the background described above, we examined the responsiveness of male C. medinalis to sex pheromone using synthetic Japanese, Philippine and Indian blends in paddy fields at Nanning, in southernmost China; Hanoi, in north Vietnam; and on Bohol Island, in the Philippines.

MATERIALS AND METHODS

Chemicals. Synthetic monounsaturated aldehydes, alcohols and acetates were synthesized by Shin-ETSu Chemical Company (Tokyo, Japan). The compounds were purified by column chromatography in the same manner as reported in our previous report (Kawazu et al., 2000). Gas chromatographic (GC) analyses showed that the purified compounds contained <0.1% of the corresponding E-isomers (Kawazu et al., 2000). The GC analyses were conducted on a Shimadzu GC-17A gas chromatograph equipped with a FID and a splitless injector system. The column used was a chemically bonded, fused silica capillary column coated with DB-23 (30m×0.25 mm ID, J&W Scientific, CA, USA).

Field tests. Baits were prepared using gray halobutyl isoprene blend elastomer septa (West Co., Singapore). Each pheromone blend containing the required amounts and ratios of the synthetic compounds mixed with 200μl hexane was poured onto the inner surface of a rubber septum. After evaporation of the solvent, the baits were placed in aluminum-laminated polyethylene bags and stored at −20°C until use. The composition and amount of the synthetic pheromone components used for each septum were as follows: Japanese blend: Z11-18:Ald (55μg), Z13-18:Ald (500μg), Z11-18:OH (120μg) and Z13-18:OH (180μg); Philippine blend: Z11-16:Ac (500μg) and Z13-18:Ac (10μg); and Indian blend: Z11-16:Ac (50μg) and Z13-18:Ac (500μg).

Tests were conducted in paddy fields in Nanning (22°48′N, 108°20′E), China (May 31–June 16, 2002), Hanoi (21°02′N, 105°51′E), Vietnam (Aug. 29–Sep. 22, 2002) and on Bohol Is. (9°50′N, 124°10′E), the Philippines (Aug. 7–Oct. 2, 2001 and Mar. 14–May 2, 2002). Cone traps slightly modified from those reported by Kawasaki and Sugie (1990) were used. The traps were arranged in two blocks, each with traps containing a set of
baits (Japanese, Indian and Philippine blends, and control). Pheromone traps were set at the boundaries of the paddy fields and at the height of the top of the rice plants. The distance between traps within a block was greater than 10 m and the distance between blocks was greater than 10 m. When checking the number of trapped moths, the trap locations were moved one position within a block. The number of trapped moths was checked every 3 or 4 d. The captured moths in the Philippines and Vietnam were morphologically identified.

For statistical analyses, the total number of trapped males for each treatment on a checking day were transformed to SQRT (X+0.5) and differences among means were tested for significance by Tukey's test.

**Morphology species identification.** The genus *Cnaphalocrosis* contains about 35 species in the world (Robinson et al., 1994). The species of the genus share similar wing markings and a bifurcated uncus in the male genitalia.

A rice pest, *Cnaphalocrosis patralis*, described and recorded from the Philippines (Bradley, 1981), is the most closely related species to *C. medinalis* in terms of wing markings and genitalic characteristics. However, the former species is easily separated from the latter by a displaced post-medial line in the forewing and the lack of the harpe on the vulva (=spur-like projection in Bradley, 1981) seen in *C. medinalis* in the male genitalia (Kawazu et al., 2000), and other characteristics. *C. medinalis* is also distinguished from another related species, *Cnaphalocrosis pilosa* (Warren, 1896), in the ovate and simpler vulva, together with a straight post-medial line in the forewing.

**RESULTS**

In the present tests at Nanning, China, Hanoi, Vietnam and Bohol Is., the Philippines, only the Japanese blend showed significant attractiveness to male *C. medinalis* moths, while neither the Indian nor the Philippine pheromone blends attracted males (Fig. 1).

At Nanning, despite the short testing periods, the Japanese blend attracted a large number of male moths (9.9 moths/trap/d), while the Indian and Philippine blends attracted no moths (Fig. 1a). At Hanoi, the Japanese blend attracted a number of male moths (3.4 moths/trap/d), both the Indian and

![Fig. 1](image_url)
Philippine blends attracted a small number of males of *C. medinalis*, yet the number was not significantly different from the control (Fig. 1b). On Bohol Island, a relatively small number of trap catches (0.3 moths/ trap/d in the wet season and 0.4 moths/trap/d in the dry season) were obtained (Fig. 1c). This was probably due to very low population densities, since almost no moths or injury to rice leaves by the larvae could be observed in the testing field. Each of the Indian and Philippine blends attracted one female of *C. patinalis*, a closely related species to *C. medinalis*, in 2002 in the Philippines. Neither the Indian nor Philippine blends attracted *Cnaphalocrocis pilosa* males in our field test. This may have been due to a low population density of *C. pilosa*.

**DISCUSSION**

The present results obtained from all of the test sites during the specified periods in southernmost China, north Vietnam and the southern Philippines are quite similar to those obtained from various sites in Japan and Hangzhou, China. This suggests that *C. medinalis* populations, of which males respond to the Japanese blend, are widely distributed from eastern Asia through Southeast Asia.

The primary emigration areas of *C. medinalis* migrating to the east Asian region have been inferred to be in southernmost China and/or northern Vietnam, like the rice planthoppers, *Nilaparvata lugens* (Stål) and *Sogatella furcifera* (Horvarth) (Hemiptera: Delphacidae), of which the seasonal occurrence pattern in eastern Asia is quite similar to that of *C. medinalis* (National Coordinated Research Team, 1981; Chen et al., 1989; Suzuki and Wada, 1994; Sogawa, 1995). The results at Nanning, China, and Hanoi, Vietnam, in which only the Japanese blend among the three blends tested was attractive, support the above inference as well as the validity of the use of the Japanese blend for monitoring *C. medinalis* in Japan.

On the other hand, the results on Bohol were unexpected. Contrary to our expectations that male moths would be attracted to the Philippine blend, here also only the Japanese blend showed significant attractiveness, whereas the Philippine blend did not. Also, the very low number of the trap catches (Philippine blend) may have been due to a very low population density and/or differences in the period of moth occurrences.

*C. medinalis* may originate from migrants from southernmost China (areas to the south of 22°N latitude) and/or northern Vietnam to the rice double-cropping areas of southern China from April to May with the aid of southwesternly monsoons. Thereafter, *C. medinalis* in the rice double-cropping areas of southern China may migrate to Japan, the middle of China and Korea from June to July with the aid of the low-level jet streams (Fig. 2). On the other hand, from the results in the Philippines, it is also possible that *C. medinalis* moths originating from the Philippines migrate to Japan in spring and summer with the aid of seasonal southerly and southwest winds (Fig. 2).

The Philippine blend used in the present tests was prepared based on Ramachandran et al. (1990). It is possible that geographic variations in the pheromone system are present within the Philippines as previously reported with the sex pheromones of other species such as *Agrotis segetum* (Tóth et al., 1992), if Ramachandran et al. used a population of another locality in the Philippines for their pheromone identification. This also might indicate the occurrence of pheromone-based assertive mating or random mating as has been
found with population using different pheromone blends of *Ostrinia scapulalis* (Takanashi et al., 2005). Since, however, in their report descriptions about the collection locality of the insects used and the behavioral activity of the pheromone components are lacking, verification of the female sex pheromone system of this species in the Philippines may be of interest.

The EAG-active components of *C. patanalis* were reported to be Z13-18:Ac and Z11-16:Ac at a 96:4 ratio (Ramachandran et al., 1990), which is very close to the Indian pheromone blend. In this research, however, the Indian blend attracted no male *C. patanalis* moths in the Philippines. This may have been due to a low population density. It will be necessary to conduct pheromonal research on the *C. patanalis* over a wide area of the Philippines.

*C. medinalis* is regarded as a member of the *Cnaphalocrocis* species, to which many related species exist. Various populations of *C. medinalis* depend on the habit. It is notable that the carbon molecule structure, Z13-octadecen, is common to all, and there are various types of the functional groups, such as alcohol, aldehyde and acetate in the sex pheromone of *C. medinalis* (Ramachandran et al., 1990; Ganeswara Rao et al., 1995; Kawazu et al., 2000). It is possible that *C. medinalis* select a different functional group and its combination of sex pheromone components as a consequence of the sex pheromone system of the cohabiting related species (e.g., *C. patanalis* and *C. patanalis*).

Research on the sex pheromone system in *C. medinalis* over wide areas of humid tropical and temperate Asia may still be important to establish an effective pheromone monitoring system.

**ACKNOWLEDGEMENTS**

We express our thanks to Sankei Chemical Company (Tokyo, Japan) for kind supplying the rubber septa. This research was partly supported by the Research Project on the Development of Integrated Pest Management Systems to Reduce Environmental Load, the Ministry of Agriculture, Forestry and Fisheries, Japan, and by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 13460021).

**REFERENCES**


Suzuki, Y. and T. Wada (1994) Rice cropping and occur-
Takanashi, T., Y. Huang, K. R. Takahashi, S. Hoshizaki, S.
Tatsuki and Y. Ishikawa (2005) Genetic analysis and
population survey of sex pheromone variation in the
adzuki bean borer moth, Ostrinia scapulalis. Biol. J.
Linn. Soc. 84: 143–160.
Tóth, M., C. Löfstedt, B. W. Blair, T. Cabello, A. I. Farag, B.
S. Hansson, B. G. Kovalev, S. Maini, E. A. Nesterov, I.
Pajor, A. P. Sazonov, I. V. Shamshev, M. Subchev and G.
Szöcs (1992) Attraction of male turnip moths Agrotis
segetum (Lepidoptera: Noctuidae) to sex pheromone
components and their mixtures at 11 sites in Europe, Asia
Wada, T., M. Kobayashi and M. Shimazu (1980) Seasonal
changes of the proportions of mated females in the field
population of the rice leaf roller, Cnaphalocrocis medi-
nalis Guenée (Lepidoptera: Pyralidae). Appl. Entomol.
Zool. 15: 81–89.