INTRODUCTION

The behavior of sex pheromone release and calling behavior in female moths generally shows diel periodicity, which may be modulated by factors, such as, age, photoperiod, and temperature (Fatzinger, 1973; Nordlund and Brady, 1974; Webster and Cardé, 1982; Delisle and McNeil, 1987). The periodicity of the sex pheromone titer has been studied in several species of Lepidoptera. In many moths, the maximal pheromone titers in the pheromone gland are observed during the time of peak calling activities (Fatzinger, 1973; Pope et al., 1982; Raina et al., 1986) or precede calling peaks (Konno et al., 1980; Ono et al., 1990), but some moths show no distinct peak of pheromone titer (Schal et al., 1987).

Most moths use mixtures of several compounds as the sex pheromone and the proportion of the pheromone components is critical for the attractiveness of males. The temporal fluctuation patterns of multiple components in gland extracts or emitted blends have been reported for several species (Coffelt et al., 1978; Pope et al., 1982; Schal et al., 1987; Hunt and Haynes, 1990; Ono et al., 1990; Heath et al., 1991; Kamimura and Tatsuki, 1993). These previous results showed that in most cases blend ratios remain relatively constant but that the proportions among components that have different functional groups or different chain lengths may change.

The sex pheromone of Japanese populations of Cnaphalocrocis medinalis use (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) (Kawazu et al., 2000). The effectiveness of the sex pheromone as a monitoring tool or control agent may depend on the relative attractiveness of the synthetic sex pheromone as compared with that of naturally produced pheromones. Therefore, an understanding of the temporal variability in sex pheromone composition may have significant implications for monitoring tools or control agents. We report here the hourly and daily changes of both calling activity and titers of the four sex pheromone components in the pheromone glands.

Diel rhythms of calling behavior and temporal change in pheromone production of the rice leaffolder moth, Cnaphalocrocis medinalis (Lepidoptera: Crambidae)

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Abstract

Calling activity in Cnaphalocrocis medinalis showed a distinct diel periodicity. Moths showed high activity during 5–7 h into the scotophase. To clarify the temporal change of pheromone titers in the pheromone gland within 1 d, age 4 females were used. The changes in the calling activity and titer of the four sex pheromone 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), in the pheromone glands were roughly synchronous. At age 4, however, the pheromone components could already be detected at the onset of scotophase, while calling began at 3 h into the scotophase. Furthermore, calling was most active between age 3 and age 7, whereas the highest level of pheromone titer at 6 h after light off was observed from age 3 to age 5. These findings suggest the presence of different controlling mechanisms between calling behavior and pheromone production. The ratio of the four sex pheromone components remained relatively constant at all times of the day and all ages.

Key words: Cnaphalocrocis medinalis, sex pheromone, calling

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MATERIALS AND METHODS

Insects. The C. medinalis specimens used were from a stock culture originally collected in Osaka prefecture (Japan) in 1985. They were supplied by Sumitomo Chemical Company (Tokyo, Japan) and have been successively reared on an artificial diet (Silkmate 2(S), Nihon Nosan Kogyo, Yokohama, Japan) at 25±1°C under a long-day cycle (15L–9D). All experiments were carried out under the above conditions. Pupae were sexed and separately held in plastic-screen cages (30×30×30 cm). After emergence, female and male adults were kept separately under the same conditions as above and provided with 5% sucrose solution as food. A dimmed red lamp for visual observation was on throughout the dark period. Most moths emerged during 3–6 h into the scotophase. The age of the moths was designated as age 0 on the day of emergence and age 1, age 2, etc. on subsequent days.

Observation of calling behavior. Thirty age 0 virgin females were collected at 6 h after lights off and were individually confined in transparent plastic cups (11 cm dia.×10 cm ht.) and provided with 5% sucrose solution impregnated in pieces of cotton wool. The calling females exhibited a posture by extruding and bending the abdominal tip toward the dorsal surface. This calling posture is similar to that of other crambid moths, e.g. Chilo suppressalis (Tatsuki et al., 1975), Dichocrocis punctiferalis (Konno et al., 1980), Glyphodes pyloalis (Seol et al., 1986), Diaphania indica (Kinjo and Arakaki, 1997). The number of females that performed the calling posture was recorded at 1 h intervals from 1 h before the onset of scotophase to 1 h after the onset of the next photophase for 7 successive days. No calling activity was observed in the photophase.

Pheromone titer. Ovipositor tips were excised with a pair of fine scissors from virgin female moths. For each analysis ten ovipositors were extracted in 10 µl hexane containing 10 ng tridecyl acetate (13:Ac) as an internal standard for 10 min at room temperature. The ovipositors were rinsed twice with 10 µl hexane and the rinses were combined. The combined gland extract was concentrated to 2 µl under reduced pressure and injected onto a Shimadzu GC-17A gas chromatograph equipped with an FID and a splitless injector system. The column used was a chemically bonded fused silica capillary column, 30 m×0.25 mm ID, coated with DB-23 (J&W Scientific, CA, USA). The column temperature was initially set at 80°C for 2 min, then increased at 7°C/min to 220°C, and held at the final temperature for 18 min. The injector and detector were kept at 250°C. Nitrogen was used as the carrier gas at a column head pressure of 100 kPa. Each pheromone component in the extracts was identified from the relative retention time to the retention time of 13:Ac. Each pheromone component was quantified by comparing the peak area with that of 13:Ac. The proportions of the three minor components, Z11-18:Ald, Z11-18:OH and Z13-18:OH, to that of Z13-18:Ald were calculated.

To clarify the temporal changes in pheromone titer in the pheromone glands within 1 d, age 4 females were used and their ovipositor tips were extracted at −1, 0, 2, 4, 6, 8, 9 h after lights off. To investigate the daily changes in pheromone titer, every age of female between age 1 and age 7 were used and their ovipositor tips were extracted at 6 h after lights off. The test was replicated 3 times.

RESULTS

Observation of calling behavior

The results are shown in Fig. 1. In age 0 females, no calling was observed. In age 1 and age 2 females, calling was seldom observed throughout the dark period. Most moths emerged during 3–6 h into the scotophase. The age of the moths was designated as age 0 on the day of emergence and age 1, age 2, etc. on subsequent days.

Pheromone titer. Ovipositor tips were excised with a pair of fine scissors from virgin female moths. For each analysis ten ovipositors were extracted in 10 µl hexane containing 10 ng tridecyl acetate (13:Ac) as an internal standard for 10 min at room temperature. The ovipositors were rinsed twice with 10 µl hexane and the rinses were combined. The combined gland extract was concentrated to 2 µl under reduced pressure and injected onto a Shimadzu GC-17A gas chromatograph equipped with an FID and a splitless injector system. The column used was a chemically bonded fused silica capillary column, 30 m×0.25 mm ID,
tected. At the onset of the photophase, the amount of each component was approximately the same as that of corresponding components at 2 h after lights off. The amount of Z13-18:Ald was greater than those of other components. The ratio of the four components, Z11-18:Ald, Z13-18:Ald, Z11-18:OH and Z13-18:OH, was nearly constant at all sampling times (Fig. 3).

With respect to age, the pheromone titer of the four components increased gradually after age 2, reached a maximum at age 4 and then decreased (Fig. 4). At age 7, the amounts of the four components were approximately the same as those at age 2. The ratio of the four components, Z11-18:Ald, Z13-18:Ald, Z11-18:OH and Z13-18:OH, was nearly constant from age 1 to age 7 (Fig. 5).

DISCUSSION

Most C. medinalis virgin females maintained under 15L:9D started calling at age 3. Calling was active between age 3 and 7. The calling of C. medinalis shifted to earlier times with aging. A similar trend was also reported in some other species, e.g. Chilo suppressalis (Kanno, 1979), Glyphodes pyloalis (Seol et al., 1986) and Diaphania indica (Kinjo and Arakaki, 1997). Thus, it is suggested that sexually mature older moths are more competitive than their younger counterparts by calling and

Fig. 1. Daily calling activity of C. medinalis females under 15L–9D photoperiod. Numbers above the arrows indicate the mean onset time of calling and values followed by the same letters are not significantly different (Tukey-Kramer’s test, p<0.05). Solid bar under the abscissa indicates the scotophase.

Fig. 2. Temporal changes in the titers of the four sex pheromone components in age 4 females of C. medinalis. Values followed by the same letters in each component are not significantly different (Tukey-Kramer’s test, p<0.05). Vertical bars represent SE. Solid bar under the abscissa indicates the scotophase.
attracting males first (Swier et al., 1977).

The calling activity showed a distinct diel periodicity. At age 4, the changes in the calling activity and the titer of the four sex pheromone components, Z11-18:Ald, Z13-18:Ald, Z11-18:OH and Z13-18:OH, in the pheromone glands were roughly synchronous (Figs. 1 and 2). At other ages also, the four sex pheromone components were expected to show similar fluctuation patterns and both the calling activity and four components appear to be roughly synchronous. If so, this is the case for many moth species such as, *Pseudaletia unipuncta* (Delisle and McNeil, 1987), *Spodoptera frugiperda* (Ramaswamy et al., 1988), *Helicoverpa assulta* (Kamimura and Tatsuki, 1993). No calling was observed at 1 h before the onset of scotophase and 1...
h after the onset of the next photophase at all ages. No pheromone components could be detected in the pheromone gland in age 4 moths at 1 h before the onset of the scotophase. *C. medinalis* appears to produce sex pheromone only during the scotophase and releases the pheromone soon after production.

Calling was most actively performed from age 3 to age 7 (Fig. 1), whereas the pheromone titer at 6 h after lights off was maximal from age 3 to age 5 and thereafter decreased (Fig. 4). Thus, the peak age for calling behavior lasted 2 d longer than that for pheromone production. Furthermore, at age 2 females seldom performed calling and at age 7 they actively called, although they had approximately the same amount of pheromone components. At age 4, no calling activity was observed at the onset of scotophase, whereas a very small amount of the pheromone titer was detected. This result suggests that the starting of the pheromone production was affected by an endogenous rhythm. These findings suggest the presence of different controlling mechanisms between calling behavior and pheromone production as in *Chilo suppressalis* (Ohguchi et al., 1985).

The pheromone gland of *C. medinalis* contained a blend of Z11-18:Ald, Z13-18:Ald, Z11-18:OH and Z13-18:OH in a ratio of 11 : 100 : 24 : 36 (Kawazu et al., 2000). The fluctuation patterns of the four components were similar at all times of a day and all ages tested (Figs. 2 and 4) and thus the ratios of the four sex pheromone components were always close to that reported previously. This constant ratio is commonly seen in moth species such as *Heliothis virescens* (Heath et al., 1991), *Tri- choplusia ni* (Hunt and Haynes, 1990) and *Phthorimaea opercuella* (Ono et al., 1990).

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REFERENCES


