

が急速に減少し、高濃度側では顕著な減少を示さなかった。変曲点以上は肉眼的にも食害を判定し得ず有効な濃度であった。

(5) 以上の結果から実用上の処理濃度は、保管状態や耐洗たく性などを考慮して、Dielmoth 0.3%, Eulan U₃₃, Eulan NKF, Mitin FF は3%, Eulan CNA は5%以上を必要とする。

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Summary

In this study, the feeding damage of clothes moth on the woolen fabrics treated with proofing

agent such as Dielmoth, Eulan U₃₃, Eulan NKF and Mitin FF, in varying concentration was examined, and then the concentration for practical use of these proofing agents was determined.

Results were as follow.

- 1) The killing effect of these proofing agents increased with the concentration of these agents and this effect was higher at 30°C than at 20°C.
- 2) Two different straight lines were observed in the relationship between amount eaten by clothes moth larvae and concentration of proofing agents when the concentration of these proofing agents was transformed into logarithm. The concentration for practical use of these proofing agents was at high concentration side from an inflection point.
- 3) The concentration for practical use of these proofing agents was determined 0.3% for Dielmoth, 3% for Eulan U₃₃, 3% for Eulan NKF and 3% for Mitin FF, respectively.

Sex Pheromone of the Rice Stem Borer Moth, *Chilo suppressalis* WALKER (Lepidoptera: Pyralidae) I. Laboratory Mating Behavior. Sadahiro TATSUKI, Shinji ATSUSAWA, Kyoichi UCHIUMI, Masaaki KURIHARA and Jun-ichi FUKAMI (The Institute of Physical and Chemical Research, Wako-shi, Saitama, 351 Japan) Received April 11, 1975. *Botyu-Kagaku*, 40, 143, 1975.

27. ニカメイガの性フェロモン I. 室内における配偶行動 田付貞洋, 阿津沢新二, 内海恭一, 栗原政明, 深見順一 (理化学研究所, 埼玉県和光市) 50. 4. 11 受理

ニカメイガの配偶行動を、室内のコントロールされた条件下で観察し、照明条件が、羽化と交尾のタイミングに強く影響することを明らかにし、また、行動のパターンを野外の場合²⁾と比較しながら、くわしく記載した。

12L:12Dの条件下では、羽化と交尾はそれぞれ消灯直後と数時間後に集中するが、連続照明下では、羽化は特定の時間帯に集中せず、交尾は抑制されるが、任意の時刻に消灯すればただちに行動がなれることがわかった。

個々の行動のパターンは、野外で観察されたものと基本的には変わらないが、オスの「探雌飛翔」に代って「交尾前活動」がみられた。メスのコーリング姿勢には、静止姿勢に近いものから、典型的なものまで種々の段階が認められた。配偶行動は、メスのコーリング; オスの交尾前活動、メーティングダンス、および交尾行動; メスの交尾受容行動; 交尾の順に行なわれ、それらのうち、おそらくオスのメーティングダンスだけが、メスの性フェロモンによって開始される。

The rice stem borer, *Chilo suppressalis*, is a well-known insect pest to rice plants of Japan and other east and south-east Asia.

Although attractancy of female moths to male moths has already been suggested in the report

described by KABURAKI *et al.* in 1939¹⁾, this phenomenon had scarcely been regarded until recent years. The authors previously reported that in this insect the sex pheromone could have played an important role in attracting males from

the result of the field test using sticky traps baited with virgin female moths²⁾.

To design a laboratory bioassay method for the sex pheromone for the purpose of its isolation and characterization, it is necessary to take knowledges on the mating behavior in the laboratory, especially on the timing of mating and the behavioral pattern of the male elicited by the sex pheromone.

This paper reports the results of observations and some experiments on the mating behavior of *Chilo suppressalis* under the laboratory conditions from the viewpoint mentioned above.

Materials and Methods

The insects used for the observations were from the stock colony which had been reared on rice seedlings as the larval diet. The essential method of rearing was reported by SATO³⁾, and practical techniques modified in our laboratory was described by UCHIMU⁴⁾. Rearing of the larvae were carried out under 16L:8D photoperiod at ca. 28°C and 50-60% R.H.

Pupae were separated by sex, and male and female insects were kept respectively in glass containers (9 cm in diameter and 6-9 cm high; for adult emergence and all the observations) or in clear plastic boxes (15×15×15 cm; only for adults) with moist cotton balls under the experimental light regimen for at least 3 days

until use. For the preconditioning and the observations, room temperature was throughout ca. 25°C.

Fluorescent lamps were used for the illumination under light conditions. In darkness, observations were made with the aid of a red darkroom lamp and a flashlight with red filter.

Results

1. Timing of mating

The timing of mating was thought to be affected by the timing of adult emergence especially in 0-day-old moths. Therefore, adult emergence was also recorded under the same conditions as those for the observations of mating.

Light conditions adopted for the preconditioning were 12L:12D and continuous light (LL) respectively. Emergence and mating were checked during the scotophase under 12L:12D, and after light-off of LL at every one hour. A female and a male moth were put together just before light-off.

(1) 12L:12D

Emergence of a small numbers of adults were found from 5 or 6 hours before light-off with a slight increase. Most of moths emerged within one hour after light-off with a definite peak followed by abrupt decrease (Fig. 1a). There was no apparent difference in the timing of

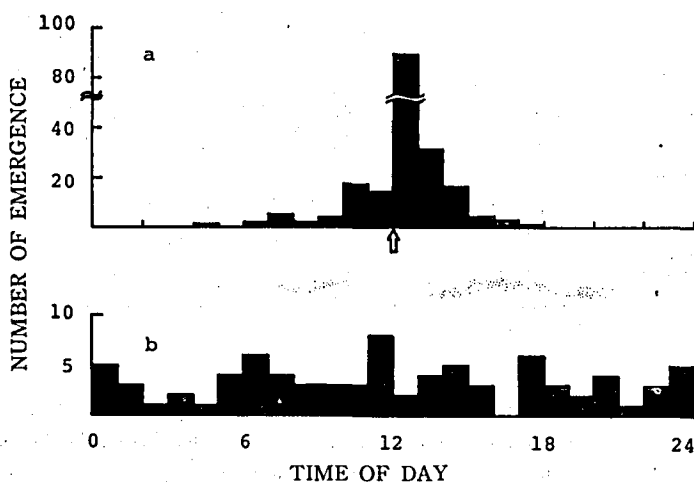


Fig. 1. Timing of adult emergence under two different light regimen. a. under 12L:12D (the arrow indicates light-off). b. under LL.

emergence between the sexes.

Mating in 0-day-old adults which had emerged within one hour after light-off (=maximum period of emergence) began about 2 hours after, then mostly occurred between 3 and 4 hours after light-off and then gradually decreased (Fig. 2a). One-day-old pairs which had emerged between 12 and 24 hours before the initiation of observation showed slightly early timing pattern in mating (Fig. 2b). In this case, matings were found from soon after light-off with a peak between 2 and 3 hours after.

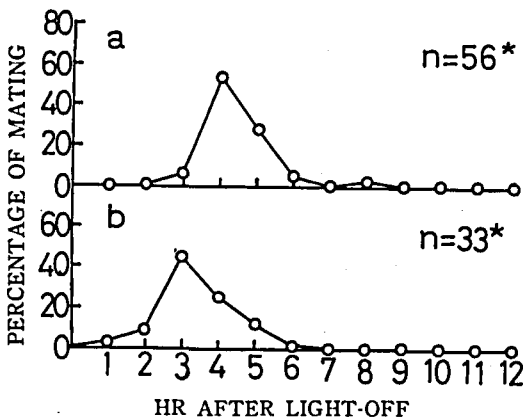


Fig. 2. Timing of mating under 12L:12D.
a. 0-day-old. b. 1-day-old.
*: total number of mating pairs.

(2) LL

Under continuous light from pupal stage, the daily rhythm of adult emergence disappeared and moths emerged at random (Fig. 1b).

Fig. 3a-c show the patterns of the timing of mating shown by the pairs that had emerged within 12 (a), 12-24 (b) and 24-72 hours (c) respectively before the initiation of the observation (=light-off).

In both the pairs of b and c, most of matings occurred within 2 hours after light-off with a peak at the first one hour. On the contrary, the pair of 'a' showed a different pattern which had a peak between 2 and 3 hours after light-off similar to that found under 12L:12D.

2. Behavioral pattern

The mating behavior in glass containers under the laboratory conditions was recorded. Most of

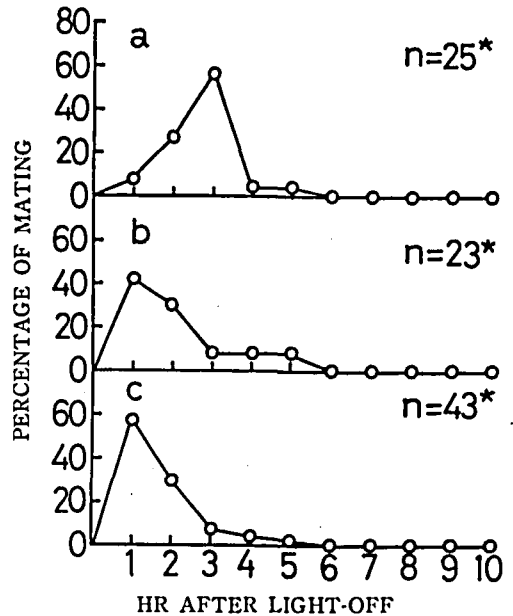


Fig. 3. Timing of mating in darkness after LL.
a. 0-12 hours after emergence.
b. 12-24 hours after emergence.
c. 24-72 hours after emergence.
*: total number of mating pairs.

the observations were made successively in the scotophase under 12L:12D. Fig. 4a-c show the examples given by individual females, individual males and pairs of a male and a female respectively.

(1) Individual females

Almost all females of both 0- and 1-day-old assumed the typical "calling position"²⁾ of which posture had the wings slightly opened and abdomen curved upward between them with extrusion of the apical segments (Fig. 5d). In 1-day-old moths calling positions were found from about 2 hours after light-off, but in the moths which had emerged after light-off they were delayed for one to two hours likewise with timing of mating (Fig. 4a).

(2) Individual males

They kept their still posture till a few hours after light-off, then many of them became active with intermittent wing fluttering and random crawling alternatively or simultaneously (Fig. 4b). The timing pattern of these behaviors almost coincided with that of mating under the same

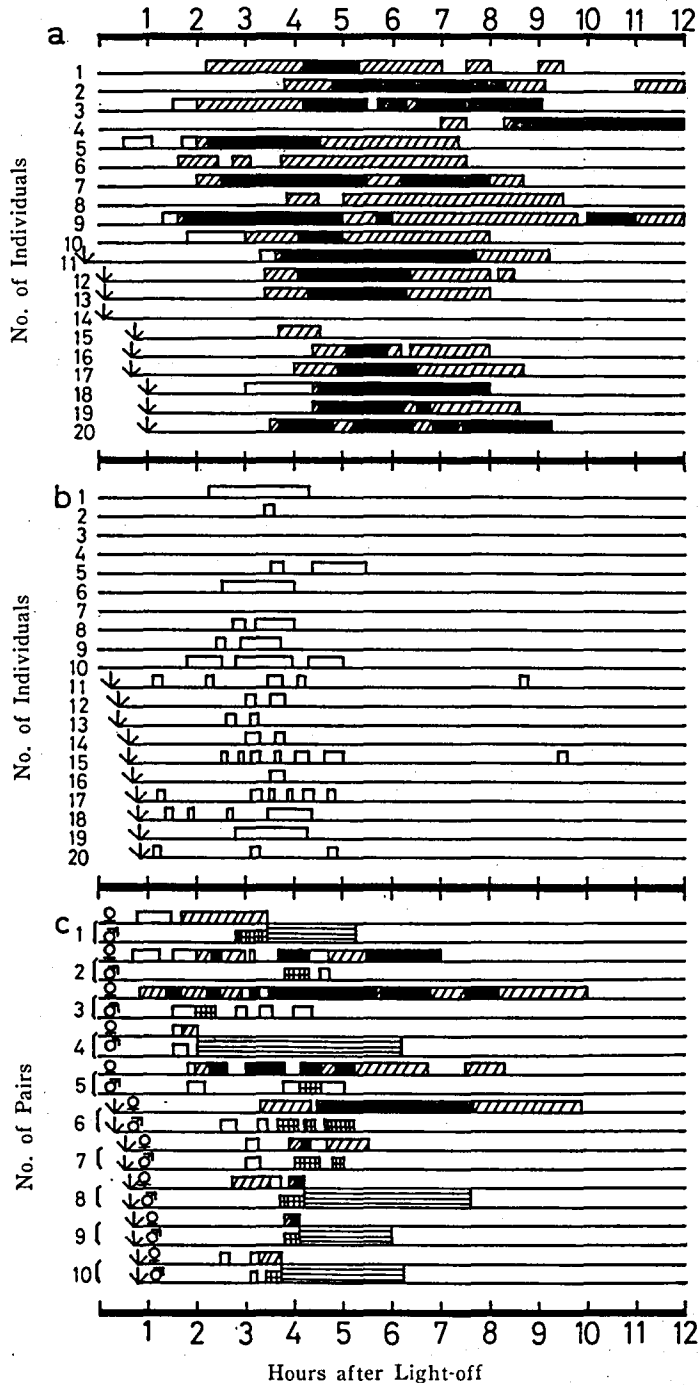


Fig. 4. Behavioral patterns of individual and paired adults in the scotophase of 12L:12D. a. individual females, b. individual males and c. paired adults. No.1-10 individuals of a. and b., and No. 1-5 pairs of c. had emerged on the previous day. (↓: adult emergence, —: still posture, □: premating activity, //: calling position only with extrusion of apical abdominal segments, ■: typical calling position, ▤: mating dance, —•—: copulation)

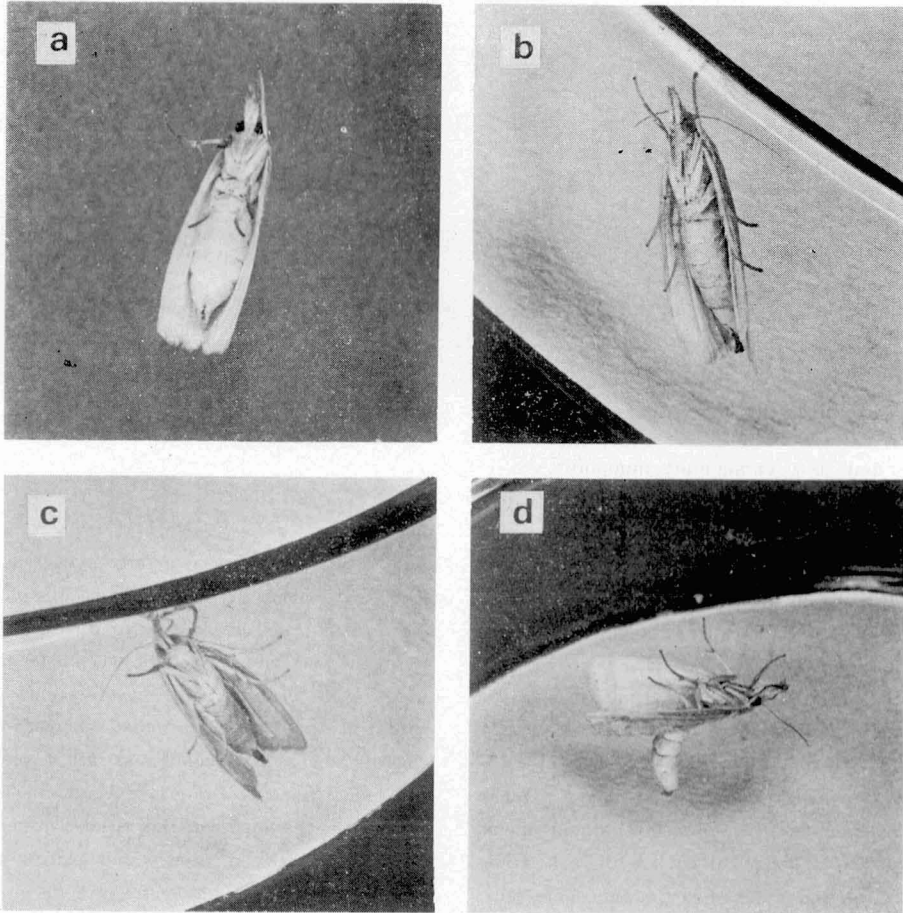


Fig. 5. Calling position of female moths. a. still posture (non calling). b. and c. only with extrusion of apical abdominal segments. d. typical posture.

conditions (Fig. 2). Similar behaviors were sometimes found in females before the calling position with less activity than in males. Hereafter, these behaviors are called "premating activity".

(3) Pairs of a female and a male

1) Calling position

Although female moths which mated later also assumed the calling position with the same timing as in individuals, most of them did not show the typical posture but only with extrusion of their apical abdominal segments (Fig. 5b, c). On the other hand, even in pairs when females did not succeed in copulation or copulated after a relatively long period, almost all assumed the typical calling position. It was also found that most of the callings were only with extrusion at

the early stage and gradually went on to the typical posture (Fig. 4a, c).

2) Male behavior

In addition to the behaviors observed in individuals, only in pairs the "mating dance"²⁾ of males were found simultaneously with or after the calling position of females (Fig. 4c). Mating dance in glass containers, which was basically the same as that in the field, composed of continuous wing fluttering while slow walking on the inner surface to any directions with the abdomen curved upward and extruding and often opening the clasper (Fig. 6). Flights were rarely observed and the walking was sometimes ceased. On the bottom of containers, the male drew small circlic shapes. Moreover, in contrast to the case

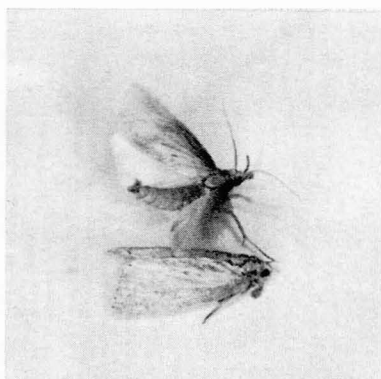


Fig. 6. Mating dance of the male moth (above). The insect under the male is a female moth immobilized by finger pressure.

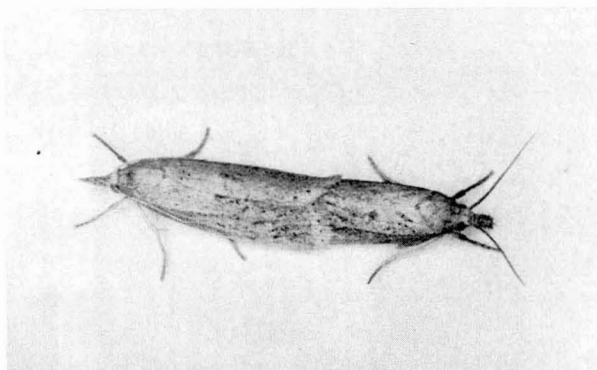


Fig. 7. Copulating adults. left : female, right : male.

in the field, it was noteworthy that the mating dance was begun not always by contact with females and was mostly continued for a longer period, i. e. for scores of seconds to a few minutes in containers though usually for only a few seconds in the field.

3) Copulation

When the male in the mating dance touched the calling female, he became excited and moved in front of her, then went back while continuing mating dance and touched her again. This backward movement often repeated twice or more while the male sometimes curved his abdomen downward (copulatory attempt). If the female did not refuse, she raised her abdomen upward usually with short and slow wing fluttering when touched by the male. Then the male stopped his movement on her back, curved his abdomen downward and copulated with her. He immediately turned 180° and the bottom half of his body was covered by her wings (Fig. 7).

Mating sequence in the laboratory is schematized in Fig. 8 comparing with that in the field.

Discussion

1. Timing of mating

The fact that mating of this species is found in the first half of night have been well known. On the observations in the laboratory, KAMITO and KURIHARA⁵⁾ and KABURAKI *et al.*¹⁾ long ago reported that in both first and second brood

moths adult emergence mainly occurred between 6:00 and 9:00 p. m., and mating took place from soon after the emergence to about 11:00 p. m. with the maximum period between 8:00 and 9:00. The result of our field investigations at Akita pref. in the first brood also showed that the mating behavior began just after sunset and continued for 2 to 3 hours²⁾. Under the artificial conditions adopted in this study, especially in 12L : 12D, both emergence and mating occurred on the whole with the same timing process as those described above.

On the adult emergence, it was reported that males were apt to emerge a little earlier than females¹⁾. The same tendency was also found under 12L : 12D in the present study, however, there was no apparent difference and both sexes showed to have a distinct peak of emergence at the first one hour after light-off. Therefore, under the artificial light condition adult emergence of this species is thought to be strongly promoted by light-off stimulus.

Even in the same age adults (1-day-old), two or more hours were needed from light-off to the peak of mating under 12L : 12D (Fig. 2b), on the contrary, the peak was found just after light-off in the LL moths (Fig. 3b). This difference suggests that an endogenous factor may be involved in the daily rhythm of mating activity observed under 12L : 12D. Under LL, activity rhythm of the moths might be suppressed by

continuous light. But the moths seemed to keep through relatively high mating activity, so that light-off stimulus acted as a direct trigger for raising the activity level. In addition, in the preliminary observation under LL 2- or more-day-old adults sometimes showed the mating behavior even in a light room. This may also support the above remarks. On the rhythms of emergence and mating further experiments should be given.

Timing of mating seems also to be a little affected by the adult maturity. Under 12L:12D 1-day-old pairs mostly mated between 2 and 3 hours after light-off. On the other hand, 0-day-old pairs that had emerged soon after light-off showed the maximum of mating after 3 hours (Fig.2a, b). Pairs that had emerged before 0-12 hours of light-off under LL had a delayed peak of mating compared with that shown by the pairs of 12 hours or more old (Fig.3a-c). It may also be explained by the adult maturity.

2. Behavioral pattern

Mating sequence was also found in the laboratory similar to that observed in the field²⁾ (Fig.8). But male flight searching for females could not be observed in the glass containers probably because of their limited space. Instead, many males became active called premating activity with almost the same timing as of mating. So, it may be caused by raised mating activity due to the light-off stimulus and also to the endogenous factor.

In the calling position of females, there were various degrees from the still posture only with extrusion of the apical abdominal segments to the typical posture. Probably, the latter shows stronger mating activity than the former. TAMAKI *et al.* discussed the relationship between the mating activity and the degrees of calling posture in the smaller tea tortrix moth, *Adoxophyes fasciata*⁹⁾.

The mating dance may be released by the female sex pheromone because it was not found without female moth. The dance is characterized by several concurrent behaviors, especially continuous wing fluttering, slow walking and clasper extrusion. Therefore, it should be possible to distinguish the mating dance from other behaviors such as the premating activity. In the containers,

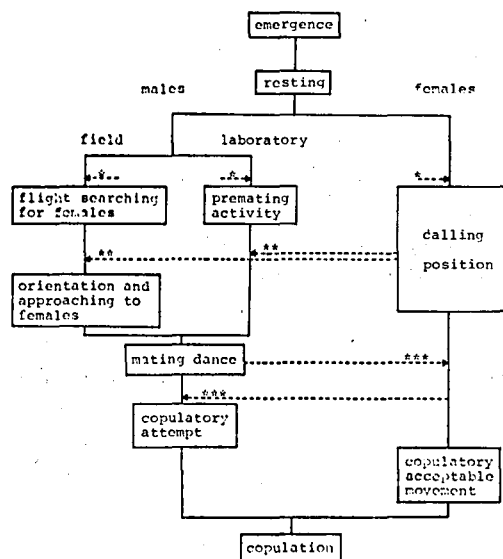


Fig. 8. Schematic mating sequence of *C. suppressalis*. Dotted arrows indicate possible releasers for respective behaviors.
*: environment and endogenous factors,
: olfactory stimulus (the sex pheromone), *: physical and/or contact chemical stimuli.

mating dance was continued for a longer period than in the field and sometimes interrupted. These may be due to the difficulty of the male moth to orientate to the female because of accumulation of the sex pheromone in the containers. Male moths did not seem to use any visual cues but only might have relied on the olfactory cue for orientation to females in the containers. The fact that the same behavior was found under light condition also supports the importance of the olfactory cue. But whether this is just or not in the field should be investigated.

Copulatory attempt of males and acceptable movements of females are next steps of the sequence probably released by physical (and/or contact chemical) stimuli. What releasers act in these behaviors is a interesting problem. Ono recently reported that male copulatory attempt in the potato tuber moth, *Phthorimaea operculella*, was physically released by wing scales of both sexes of this species and even of other species⁷⁾.

Acknowledgement: The authors wish to express their sincere thanks to Dr.K.Okui of the

University of Kitazato, for his kindness in informing the precious suggestion.

Summary

The mating behavior in the laboratory of the rice stem borer moth, *Chilo suppressalis*, was observed with special reference to the timing and the behavioral patterns of mating. Under 12L:12D at 25°C, both adult emergence and mating showed the daily rhythms of which maximum periods were 0-1 and 2-4 hours after light-off respectively. On the contrary, emergence rhythm disappeared under LL at 25°C and mating mostly occurred soon after light-off probably because the endogenous rhythm of mating had been suppressed by continuous light.

The almost same mating sequence as that in the field was observed though the male flight searching for females was replaced by the pre-mating activity. The sequence consists of female

calling position, male premating activity, male mating dance and copulatory attempt, female copulatory acceptable movement and copulation. Among these behaviors, only mating dance of males may be released by the female sex pheromone.

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Sex Pheromone of the Rice Stem Borer Moth, *Chilo suppressalis* WALKER (Lepidoptera: Pyralidae) II. A Laboratory Bioassay Method for the Sex Pheromone. Sadahiro TATSUKI, Masaaki KURIHARA, Shinji ATSUSAWA, Kyoichi UCHIMI, Jun-ichi FUKAMI (The Institute of Physical and Chemical Research, Wako-shi, Saitama, 351, Japan) and Ken-ichi KISHINO (Tohoku National Agricultural Science Station, Omagari-shi, Akita, 014-01, Japan) Received June 16, 1975. *Botyu-Kagaku*, 40, 150, 1975.

28. ニカメイガの性フェロモン II. 性フェロモンの室内生物検定法 田付貞洋, 栗原政明, 阿津沢新二, 内海恭一, 深見順一(理化学研究所) 岸野賢一(農林省東北農業試験場) 50. 6. 16 受理

ニカメイガ雌の性フェロモンの存在を確認し、その機能を解明するために野外および室内で調査を行なった。野外においては、処女メスと同様に、処女メスの腹部末端部の1,2-ジクロロエタン粗抽出物も、オスを誘引することから、性フェロモンの存在が示された。同様の粗抽出物は、室内では、交尾に先立ってみられる、オスの「メーティングダンス」を惹起することがわかった。そこで、メーティングダンスを指標とする室内の性フェロモン検定法を考案し、さらにその際用いるオス成虫の性フェロモン感受性に影響する2, 3の条件を検討した。その結果、蛹期から、25°C、全照明下においた羽化後2~4日のオスを1頭ずつ小型の三角フラスコに入れて消灯し、2~5時間後に暗黒下で検定するのが最適であることが明らかになった。

We have previously reported that the male of the rice stem borer moth, *Chilo suppressalis* W., approached to the female moth from the leeward and mated with her and that many male moths were captured in the sticky traps baited with virgin females¹⁾. In a laboratory, when a male and a female moth were put together in a glass container, the male performed the 'mating dance' prior to the copulation. On the contrary, when

a male moth was alone under the same conditions, the mating dance could not be seen²⁾. These facts enable us to conceive that the sex pheromone may attract male moths and may also release the mating dance.

This paper deals with further experiments to ascertain the functions of the sex pheromone and with a laboratory bioassay method for the sex pheromone necessary for its chemical identifica-