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 premating 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.

References

1) Kaburaki, T. *et al.*: Study on rice borers III. (Nozikairyosiryo No. 140) (1939).
2) Tatsuki, S. and J. Fukami: *Kontyū*, 40, 203 (1972).
3) Sato, Y.: *Appl. Ent. Zool.*, 3, 6 (1964).
4) Uchiumi, K.: *Rika Gaku Kenkyūsho Hōkoku*, 50, 70 (1974).
5) Kamito, A. and S. Kurihara: *Ōyō-Dōbutsugaku-Zasshi*, 1, 55 (1929).
6) Tamaki, Y. *et al.*: *Botyu-Kagaku*, 34, 97 (1969).
7) Ono, T.: *Botyu-Kagaku*, 39, 93 (1974).

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 UCHIUMI, 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，内海基一，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-ジクロルエタン粗抽出物も，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きらかになった．

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 females[1]. 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 seen[2]. 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-

tion.

Materials and Methods

Field test: Almost the same way as that already described[1] was adopted except traps were set at about 30 cm above the ground of footpaths between rice fields. A crude extact solution of the sex pheromone was soaked in a rolled filter paper (9 cm in d. m., Toyo Roshi No. 1) and after evaporation of the solvent it was hung in the center of the trap.

Laboratory investigations: Insects used for the laboratory investigations were reared and treated in the same manner as those in the previous report[2]. Under dark condition, observations were carried out with a aid of red darkroom lamp and a flashlight with red filter. All the preconditionings of the insects and bioassays were conducted at ca. 25°C.

Preparation of a crude extract solution of the sex pheromone: The last few abdominal segments of virgin females were cut off and squashed in 1,2-dichloroethane with a glass rod. After filteration, the solution was appropriately diluted to a concentration required for bioassay except, for the field tests, the solution was used before filteration.

Results and Discussion

1. Field test

Table 1 shows that the crude extract as well as virgin females attracted male moths. This indicates the presence of the sex pheromone which attracts male moths. Isolated abdomens of virgin females which had been freshly prepared also attracted males. On the other hand, virgin females without the apical abdominal portion did not show any attractiveness though the moths were still alive. These facts suggest that the sex pheromone gland of this insect is located in the apical abdominal segments like many other lepidopterous females[3].

Mated females were not attractive for at least 2 days after mating.

2. Laboratory bioassay

We have reported that the female sex pheromone seemed to release the characteristic mating dance of male moths from the observations of adult moths confined in glass containers[2]. This led us to design a laboratory bioassay method for the sex pheromone using the mating dance as a criterion.

Male moths within the mating period sometimes show the 'premating activity'[2]. Therefore, the male moths used for bioassay were placed into flasks (30m*l*) one by one to avoid any interference among individuals. The flasks were then kept under designated conditions in a ventilated room for bioassay.

Male response to crude extract: At first we examined whether the crude extract released the mating dance of male moths. In this case, preconditioning for the male moths was as follows; From the pupal stage to 1-3 days after emergence the insects had been kept under continuous light (LL). Then, they were transferred to the flasks and were held in darkness for a few hours.

The bioassays were conducted using only the

Table 1. Attractiveness of various sources to native males of the rice stem borer moth.

Attractive sources	No. of males caught/trap/night*
Virgin females**(V.F.) (1-3 days after emergence)	10.5
Mated females**	0.0
V.F.** (without apical abdominal segments)	0.0
V.F.**(isolated abdomens)	2.0
Crude extract of V.F.**	5.9

* mean values of at least three replicates, carried out in June, 1972 at Omagari-shi, Akita.

** 4 to 5 female equivalent in each trap.

moths in the still posture. Five μl of crude extract were put on a piece of a filter paper (Toyo Roshi, No. 2) and the solvent was allowed to evaporate. The filter paper was then inserted into the flask as near as about 1 cm to the moth. In response to the crude extract, they performed the typical mating dance and sometimes showed vigorous search movement with the dance on the filter paper. This fact demonstrated that the mating dance is also released by the sex pheromone.

Presence of male response was checked during 10 seconds from the insertion of the filter paper in consideration of premating activity of the moths. When the moth showed walking or wing fluttering during 10 seconds, the observation was continued for further 10 seconds from the initiation of the response. The intensity of male response were degreed as follows (Fig. 1). (−): no response or the case in which only antennal movement or/and intermittent walkings was found. (±): continuous walking or/and brief wing fluttering. (+): typical mating dance including vigorous search movement on the filter paper.

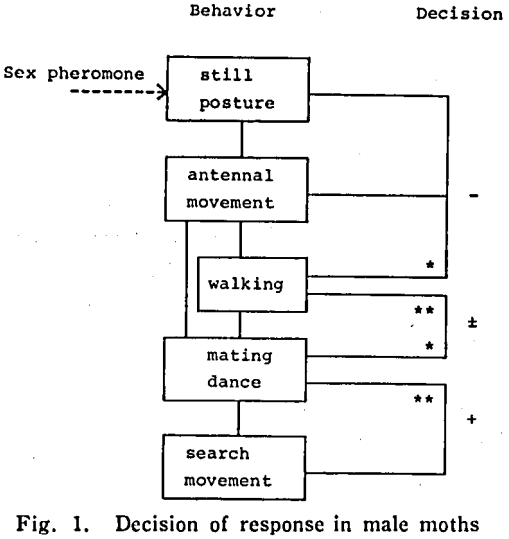

Fig. 1. Decision of response in male moths exposed by the crude extract of the sex pheromone.
*: intermittent response
**: continuous response

Factors influencing the responsiveness in preconditioning of male moths: To conduct the most effective bioassay, we tested several factors which seemed to have effects to the male responsiveness. Generally, as TAMAKI *et al.* already pointed out[4], it is desirable that a bioassay can be carried out at any time of a day with high sensitivity to the pheromone. For this reason, the male moths which had been kept under LL and did not show any rhythmisity in mating activity were used for the present study.

Suitable time for bioassay; In the previous report[2], it was shown that most of mating in the moths which had been held under LL occurred within 0 to 2 hours after light-off. Indeed, Fig. 2 shows the relatively high responsiveness from one hour after light-off and then it was kept for several hours. However, especially for 1 to 2

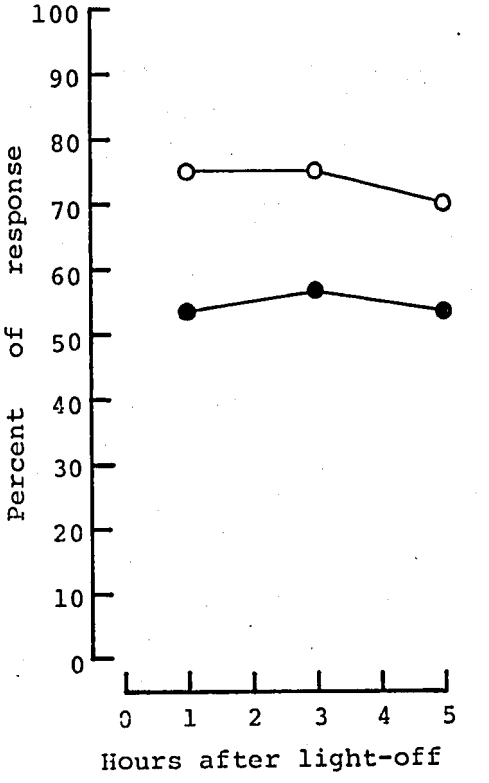

Fig. 2. Effect of time of bioassay on the responsiveness of male moths to the sex pheromone. Score was based on an average of 6 assays (total 60 males). Open circle indicates ± plus + and shaded circle indicates +. Amount of the crude extract applied per male was 5×10^{-3}F. E.

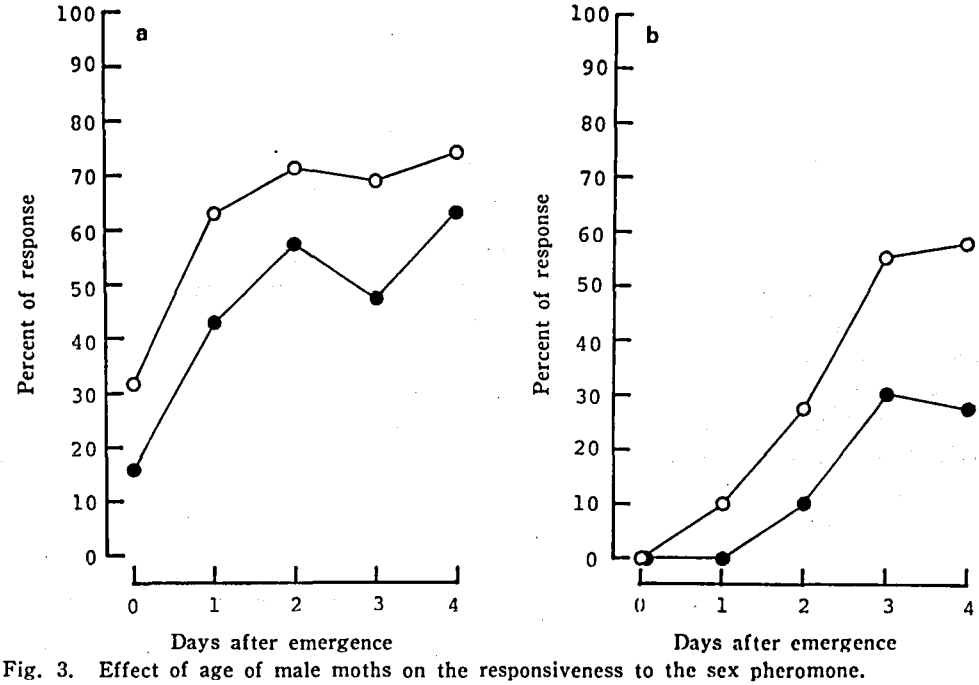

Fig. 3. Effect of age of male moths on the responsiveness to the sex pheromone.
a. Assayed under dark condition. b. Assayed under light condition. Score was based on an average of 4 assays (total 70 males for a, 40 males for b; except * 38 males). Open circle indicates ± plus + and shaded circle indicates +. Amounts of the crude extract applied per male were 5×10^{-3}F.E. for a. and 5×10^{-2}F.E. for b. respectively.

hours after light-off many moths were in an active phase (=premating activity), so that bioassay was sometimes difficult to conduct. Therefore, suitable time for assay was from about 2 to 5 hours after light-off.

Age of male moths; It has been generally known that age of male moths is an important factor for the responsiveness to the female sex pheromone[3]. In the rice stem borer, the moths which had emerged within 24 hours (0-day-old) only showed relatively low level and two- or more-day-old moths had stable and high responsiveness under the dark condition within 3 to 4 hours after light-off (Fig. 3a). Under light condition, male responsiveness was low till 2 days after emergence, and even in 3- and 4-day-old moths the response of the 'plus' to 10-fold higher concentration of the crude extract (5×10^{-2} F.E.) was lower than those conducted under dark condition (Fig. 3b).

These data show that suitable assay can be carried out in darkness after 2 to 5 hours of

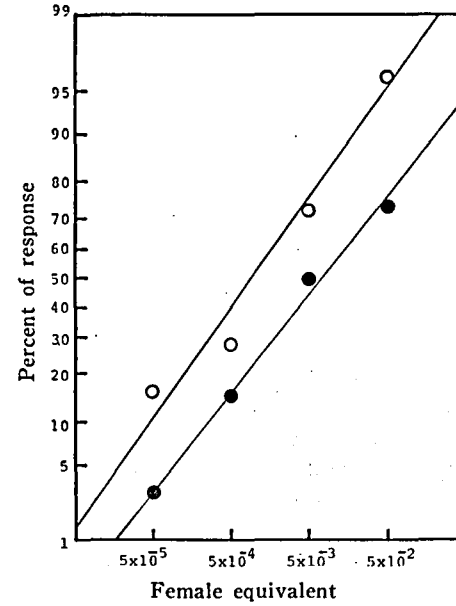

Fig. 4. Percentage of response of male moths exposed to a dilution series of the crude extract of the sex pheromone. Score was based on an average of 5 assays (total 50 males). Open circle indicates ± plus + and shaded circle indicates +. Regression lines were fitted by eye.

light-off with 2- to 4-day-old moths which had been kept under continuous light similar to that reported on *Spodoptera litura*[4].

By this method, a concentration—response line was obtained (Fig. 4) and above 5×10^{-5} F.E. of the crude extract could be detected.

Summary

The functions of the female sex pheromone of the rice stem borer moth, *Chilo suppressalis* W., were investigated both in the field and in the laboratory. Attractiveness of the pheromone to male moths, which had been suggested earlier, was ascertained by the field test. Moreover, in the laboratory observations, the 'mating dance' of the male prior to the copulation was also proved to be released by the pheromone.

A laboratory bioassay method for the sex pheromone using the male mating dance as a criterion was designed and some factors influencing the responsiveness to the pheromone were examined.

It was found that suitable assay could be carried out in darkness after 2 to 5 hours after light-off with 2- to 4-day-old male moths which had been held under LL at ca. 25°C.

References

1) Tatsuki, S. and J. Fukami: *Kontyū*, 40, 203 (1972).
2) Tatsuki, S. *et al.*: *Botyu-Kagaku*, 40, 143 (1975).
3) Jacobson, M.: Insect Sex Pheromone, Academic press, N.Y. and London (1972).
4) Tamaki, Y. *et al.*: *Botyu-Kagaku*, 38, 147 (1973).

On the Mechanisms of Resistance in Malathion Resistant Sapporo Strain of Houseflies. Akifumi HAYASHI*,** Rokuro KANO** and Sadami ISHIBASHI*** (Section of Medical Zoology, Public Health Laboratory, Chiba Prefecture*, Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University**, and Entomological Laboratory, Tokyo University of Agriculture and Technology***, Tokyo, Japan) Received June 20, 1975. *Botyu-Kagaku*, 40, 154, 1975. (with English Summary 158)

29. 札幌系イエバエの malathion 抵抗性の作用機構について

林 晃史*,**, 加納六郎**, 石橋定己*** (千葉県衛生研究所医動物研究室*, 東京医科歯科大学医学部医動物学研究室**, 東京農工大学農学部植物保護学科***) 50. 6. 20 受理

Malathion 抵抗性イエバエ（札幌系，首里系）の *in vitro* での各種殺虫剤の分解量を測定した．抵抗性イエバエ群の札幌，首里の両系統が malathion，acethion および PAP に対して高槻系に比較して顕著な活性を示すが三崎系ではその差異が認められなかった．なお，ホモジネートの上清部と沈澱部で各基質の酵素活性を調べたところ，沈澱部に活性の高いことがわかった．また，malathion 抵抗性イエバエの札幌系と三崎系は酵素活性の面でも明かに差異のあることがわかった．

林ら[1]は malathion 抵抗性の札幌系イエバエの *in vitro* における malathion の分解率は感受性系統に比較して高いが，カルボキシエステラーゼ阻害剤である TOCP を混用した場合，その分解率が顕著に低下することを明かにした．また，林ら[2]は P^{32}-malathion を用いた *in vitro* の実験で，札幌系の malathion 抵抗性機構の一つは malathion を基質とするカルボキシエステラーゼの増大に起因することを報告している．さらに札幌系の malathion 抵抗性の遺伝は単一の遺伝子に支配されていることも明かにされた[2]．

林[3]は malathion 抵抗性イエバエを札幌型と三崎型に分けているが，これについて酵素レベルでも検討して置く必要があるので，malathion 抵抗性系統である札幌系，首里系および三崎系を用いて *in vitro* でエステラーゼ活性について比較検討を行なった．

脚注）本研究の一部は昭和50年度文部省科学研究費による．

実験材料および方法

供試昆虫：実験に用いたイエバエ *Musca domestica* Linné, 1758は感受性系統の高槻系，malathion 抵抗性の札幌系，首里系および malathion，sumithion 抵抗性の三崎系で，いずれも当研究室で累代飼育中の個