solutions. In the test on Diazinon, fourteen of the Ayu tested died during the test (three died in the control group) and four of the Kawamutsu tested died (no deaths in the control group). In the Baytex test, three of the Ayu tested died on the eleventh day of the test and on the same day four died in the control group, while all of the Kawamutsu were alive during the test. In the Sumithion test, five of the Ayu treated and four of the control fish died, while all of the Kawamutsu were alive. From the results, it is suggested that Baytex and Sumithion are more suitable than Diazinon for the regular control of the blackfly larvae from the point of toxicity to fishes in the river.

Summary

The laboratory experiments were carried out for the purpose of finding out which insecticides would be more effective on blackfly larvae in a river and would have lower toxicity to the fishes. Fourteen insecticides were tested and the larvae used were *Simulium aokii* and *S. venustum* and the fishes used were *Plecoglossus altivelis*, *Salmo irideus* and *Sacco temmincki* (respectively the Ayu, the Nijimasu and the Kawamutsu in Japanese). From the results, it is suggested that of the insecticides tested under laboratory condition the wettable powder of Baytex and Sumithion are more suitable for regular control of the blackfly larvae living in a river.

Acknowledgment The writers are indebted to Dr. K. Uemoto of the Epidemic Prevent Center, Kyoto City and Dr. A. Yamaguchi of the Sakyo Health Center, Kyoto City, for furnishing materials of blackfly larvae and fishes from the fields.

References

1) Davis, A. N., Gahan, J. B., Fluno, J. A. and Anthony, D. W.: *Mosquito News*, **17**, 261 (1957).
2) Ferguson, D. E., Gardner, D. T. and Lindley, A. L.: *Mosquito News*, 26, 80 (1966).
3) Frempong-Boadu, J.: *Mosquito News*, 26, 562 (1966).
4) Jamnback, H.: *Mosquito News*, 22, 384 (1962).
5) Lea, A. O. and Dalmat, H. T.: *Jour. Econ. Ent.*, **47**, 135 (1954).
6) Lewallen, L. L. and Wilder, W. H.: *Mosquito News*, **22**, 369 (1962).
7) Matsuo, K. and Tamura, T.: *Scientific Pest Control*, **29**, 21 (1964).
8) Muirhead-Thomson, R. C.: *Am. J. Trop. Med. & Hyg.*, **6**, 920 (1957).
9) Nagahana, M., Matsuo, K., Tamura, T. and Uemoto, K.: *Sani. Injur. Insects*, 8, 1 (1964).
10) Shogaki, Y. and Yoshida, Y.: *Jap. Jour. Appl. Ent. Zool.*, 2, 157 (1958).
11) Suzuki, T., Ito, Y. and Harada, S.: *Jour. Exp. Med.*, **33**, 41 (1963).
12) Swabey, Y. H., Schenk, C. F. and Parker, G. L.: *Mosquito News*, **27**, 149 (1967).
13) Yoshida, Y. and Nakanishi, Y.: *Jap. Jour. Sani. Zool.*, **9**, 46 (1958).

An Apparatus for Bioassaying the Pheromones of Moths. Shozo Takahashi and Chikayoshi Kitamura (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto, Japan) Received October 7, 1970, *Botyu-Kagaku* **35**, 130, 1970.

18. ガ類の性フェロモンの生物検定装置について.

高橋正三, 北村実彬 (京都大学 農学部 農薬研究施設) 45. 10. 7. 受理.

ヤガ類の性フェロモンの定量的生物検定について, Shorey らは新しい装置を考案した. 我々は, この装置が他の種類のガの性フェロモンの定量的生物検定にも使用できるかを試し, 改良を加えた. それを用いて, カイコ, エリサン, スジマダラメイガについて, それぞれ性フェロモンに対する BR_{50} を求めた.

Introduction

An quantitative bioassay apparatus with completely closed air flow system was used for the sex pheromone of the noctuid moths by Shorey *et al.*[1] Availability of the apparatus in the bioassay of the other species of moth was tested for the silkworm moth, the eri-silkworm moth and the

almond moth. These three kinds of moth were chosen from their difference in wing length and behavior. The bioassay methods of the silkworm moth, *Bombyx mori* L., the eri-silkworm moth, *Philosamia cynthia ricini* Donovan (Saturniidae) and the almond moth, *Cadra cautella* Walker (Phycitinae) depending on a 'key response' of their mating behavior have been reported.[2,3,4] These methods were based mainly on the observation of wing vibration of the male moths when a glass rod impregnated with famale pheromone was inserted into the container in which the male moths had been confined. These assays, however, were hard to evaluate quantitatively the pheromone activity and occasionally caused contamination of the laboratory air.

An apparatus with a completely closed air flow system was devised for the quantitative assay of the sex pheromones of noctuid moths.[1] This method was adapted for the quantitative bioassay of the above three species of moths. Proper size of the cage for the male moths depending on the wing length affected to the responsiveness. The air flow rate was adjusted to obtain maximum response at the minimum amount of the pheromone. Responsiveness of the three species of moths tested to the pheromone were all not effected by light intensity and the time of the day.

Material and methods

Sex pheromones: The pupae of the silkworm moth and the eri-silkworm moth were segregated by sex and held separately in a natural diel light cycle at between 23 to 28°C. Pheromone glands of 2 days old females were extracted with methylene chloride. Decade dilutions of ether solutions were prepared after evaporation of methylene chloride.

The emerged almond moths reared on rice bran under the condition reported before[5] were collected every day and segregated by sex. The unmated females were picked out and dipped in methylene chloride. The test solutions were prepared by successive decade dilution of one female equivalent in 1m*l* of methylene chloride.

Males for bioassay: It has been reported that male and female *Bombyx mori* do not fly and are eager to mate immediately after emergence.[6] However one day old males often did not give the constant threshold to the pheromone. Therefore, 2 to 4 days old males were used for bioassay. As males of *Philosamia cynthia ricini* immediately after emergence were restless and did not show significant response to the pheromone, 2 to 4 days old males were used. Male moths of *Cadra cautella*, 1 to 2 days old, did not show clear response to the pheromone, and more than 90% of them died within a week and percent death increased after 4 days. Thus 3 days old moths were used.

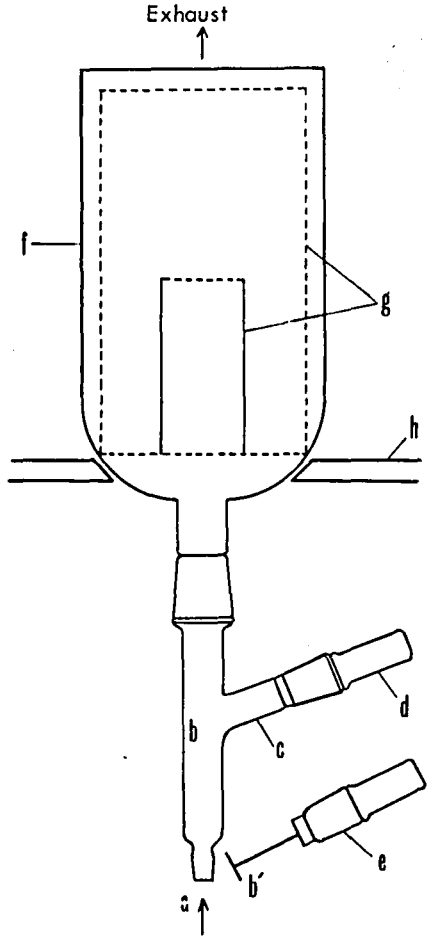

Fig. 1. A single unit of the assay apparatus a : air supply, b : position of sample application, c : side-arm, d : blank stopper, e : applicator stopper, f : assay compartment, g:cage, h : a part of turn table, b':sample applicator disk (1cm² round copper plate with cross-millings at 1mm intervals)

Bioassay : The bioassay apparatus and its accessaries are shown in Fig. 1. The male silkworm moths (a wingspread of 3.6 cm) were grouped in tens within cylindrical copper-wire mesh cages about 10 cm wide and 18 cm high. As for the male eri-silkworm moth (a wingspread of 10cm), same cages were used with five moths. The male almond moths (a wingspread of 1.6 cm) were grouped in tens within plastics pipes (4cm diameter, 8cm high) with copper-wire mesh in both ends.

The cages were loaded into the glass compartment and kept on turn-table. The assay was carried out under day light at between 25 and 28°C in the early afternoon after being acclimatized to the ambient condition. Air was passed to the each cage at the rate of 2.5 *l*/min for the silkworm moth and the eri-silkworm moth, and 0.1 *l*/min for the almond moth. The males were exposed to the stimulus once a day for the assay. The whole apparatus was installed under exhaust fan to prevent pheromone contamination.

Bioassay procedure was as follows; A given amount of the pheromone in female equivalent (F.E.) dissolved in ether was applied to the applicator disk (b' in Fig. 1). The solvent was allowed to evaporate before insertion to the side-arm (c). Basic moth activity (B) was recorded prior to the sample solution. The numbers of males responding at 15 sec and 30 sec after application of the stimuli were counted and averaged (R). Percentage response was calculated by the following equation.[7]

$$\frac{(R-B)\cdot 100}{10^{*}-B}$$

* 5 in case of the eri-silkworm moth

Result and discussions

Percent response was calculated at different F.E. of crude extract for 3 species. First, this was indicated in relation to the effect of male age to the different F.E. of pheromone of the silkworm moth and the eri-silkworm moth (Table 1). It is evident that males aged 2 to 4 days of these species have almost same responsiveness.

For the comparative, quantitative indication of pheromone activity, BR_{50} (biological response 50) has been proposed recently[8].

Table 1. Effect of age on male responsiveness

Silkworm moth

Pheromone concentration (Female equivalent)	Age of males (days)	Response (%)
0.1	2	81
	3	82
	4	75
0.01	2	38
	3	22
	4	20
0.001	2	21
	3	—
	4	4

Eri-silkworm moth

Pheromone concentration (Female equivalent)	Age of males (days)	Response (%)
0.001	2	87
	3	90
	4	95
0.0001	2	30
	3	27
	4	77
0.00001	2	6
	3	8
	4	20

This was obtained by measuring the various dosage-response curves at the 50% behavioral response level. Figs. 2, 3 and 4 show probit of percentage activation of males of three species of moths exposed to a dilution series of female sex pheromone. These figures give BR_{50} as 0.014 F.E., 0.00007 F.E. and 0.009 F.E. for the silkworm moth, the eri-silkworm moth and the almond moth respectively.

The apparatus showed its availability for the quantitative bioassay of the wide variety of moth. However, the size of cages for the male moths and the air flow rate affected to the responsiveness of the moths. Using the closed air flow system apparatus, air flow rate was one of the factors responsible for the constant response in our bioassay conditions. The air flow rate of 5 *l*/min used for the noctuid moths was rather too high for the moths in our assay. Higher air flow rate

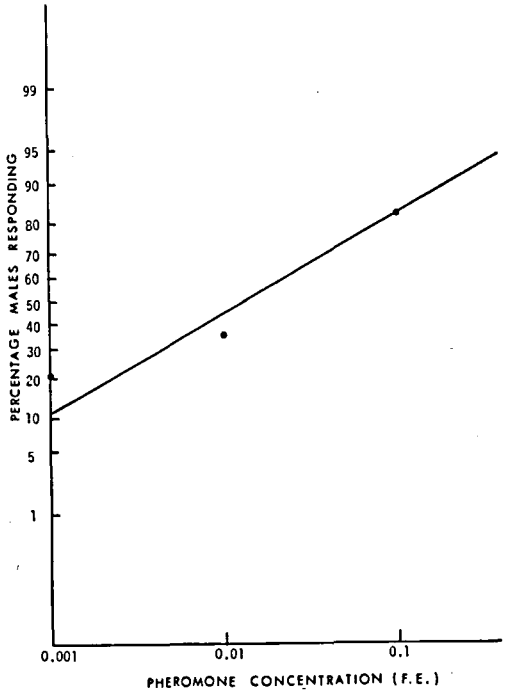

Fig. 2. Probit of percentage activation of the male silkworm moth aged 2 to 4 days to female pheromone

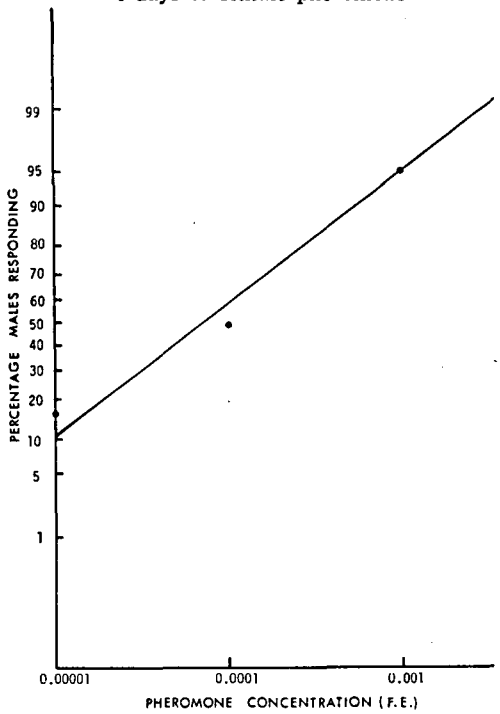

Fig. 3. Probit of percentage activation of the male eri-silkworm moth aged 2 to 4 days to female pheromone

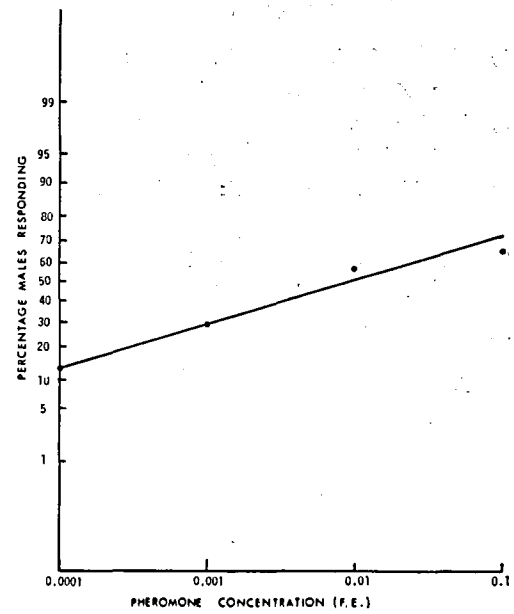

Fig. 4. Probit of percentage activation of the male almond moth aged 3 days to female pheromone

caused higher basic activity or no response at all at the exposure to the pheromone. We adopted different air flow rate depending on the male moths.

Accuracy of the assay was also increased by the use of proper size of cage according to the size of indicator moth.

Summary

A quantitative bioassay apparatus for the female sex pheromone of moths was devised. The apparatus, composed of glass compartments, cages and closed air flow system was satisfactorily used in the quantitative evaluation of sex pheromone activity of the silkworm moth, the eri-silkworm moth and the almond moth. The size of cages for the male moths and the air flow rate produced significant changes in the levels of the male moths responsiveness to the pheromone.

Acknowledgements The authors express their sincere thanks to Dr. K. Hayashiya, Kyoto University of Industrial Art and Textile Fibers for supplying the silkworm moth, and Dr. N. Ishizaki, Zoological Laboratory, Kyoto University for supplying the eri-silkworm moth.

Literature cited

1) Gaston, L.K. and H.H. Shorey: *Ann. Ent. Soc. Amer.* 57 779～780 (1964)
2) Karlson, P. and A. Butenandt: *Ann. Rev. Entomol.* 4 39～59 (1959)
3) Tomida, I. and S. Ishii: *Appl. Ent. Zool.* 3 103～106 (1968)
4) Kuwahara, Y., C. Kitamura, F. Takahashi and H. Fukami: *Botyu-Kagaku* 33 158～162 (1968)
5) Takahashi, F., C. Kitamura, Y. kuwahara and H. Fukami: *Botyu-Kagaku* 33 163～168 (1968)
6) Jacobson, M.: "Insect sex attractants" p. 75 John Wiley and Sons. (1965)
7) Bartell, R.J. and H.H. Shorey: *J. Insect Physiol.* 15 33～40 (1969)
8) Shorey, H. H. and L. K. Gaston: *Ann. Ent. Soc. Amer.* 57 775～779 (1964)

Behavior on and in Rice Plants of Diazinon Applied onto the Surface of Paddy Soil. Takeo Masuda* and Hideo Fukuda** (Kyushu Agricultural Experiment Station, Chikugo, Fukuoka) Received October 13, 1970, *Botyu-Kagaku* 35, 134, 1970.

19. 水田面に処理されたダイアジノンの水稲への移行と分解.

升田武夫・福田秀夫 (農林省九州農業試験場, 筑後市) 45. 10. 13 受理.

水稲を栽培したポットの土壌面に ^{32}P-ダイアジノン粒剤または乳剤を処理し, 薬剤の水稲地上部への移行と分解をトレーサー法により試験した. 田面水中のクロロホルム可溶 ^{32}P 物質の消失は, 乳剤区より粒剤区でゆるやかであった. 薬身のクロロホルム可溶 ^{32}P 物質は粒剤区では処理後12, 18日間連続的に増加したが, 乳剤区では処理1日後までに最高近くに達し, その後ほぼ同じ水準を保った. クロロホルム可溶 ^{32}P 物質は薬鞘より薬身で大きい傾向を示したが, これはクロロホルム可溶 ^{32}P 物質の薬鞘から薬身への移行が, 薬鞘への補給より速いことによるとみられた. 田面水中のクロロホルム可溶 ^{32}P 物質は実験期間中その大部分がダイアジノンであった. 薬身では処理後1日でダイアジノンは約50%となり, その後もほぼこの水準を保ち, 薬鞘ではこれよりやや低かった. 薬身の水可溶 ^{32}P 代謝物はジエチルりん酸>ジエチルチオりん酸>りん酸・チオりん酸の順で, 稈ではジエチルりん酸が主であった.

Diazinon (*O,O*-diethyl (-*O*-2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), is an excellent chemical for the control of various insect pests of crops. This insecticide is now used in Japan for the control of rice insects by "the paddy water application method". Granules and emulsion of Diazinon give simultaneous control of the rice stem borer and certain virus-transmitting leafhoppers when applied onto the standing water in rice fields. The method is very simple and has become widespread as a labor-saving practice. When applied by the above-mentioned method, an insecticide should reach into plant portions where insects live or into insects directly from applied zone to kill those insects. Two routes are postulated as pathways in penetration into rice plants of an insecticide applied onto paddy water or soil; one is through the root system which have been proved quantitatively by Tsukano and Suzuki (1962)[1] and Fukuda and Masuda (1965)[2] on γ-BHC and carbaryl, respectively, and the other is through the leaf sheath where an insecticide creeps up by capillarity and/or penetrates into tissues as pointed out by Ishii and Hirano (1962)[3], Tomizawa[4] and Hirano and Yushima (1969)[5] on γ-BHC, Carbaryl, and Diazinon, respectively.

Recent papers have shown that Diazinon was absorbed and translocated into plants from treated soil or irrigation water (Gunner *et al.*, 1967,[6] Onsager and Rusk, 1967,[7] Hirano and Yushima, 1969[5]) or sand (Lichtenstein *et al.*, 1967)[8] and from treated planting water (Miles *et al.*, 1967)[9] or culture solution though it is rapidly hydrolyzed in the foliage (Kansough and Hopkins, 1968)[10].

Present studies have been carried out in order

Present adress: *National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo, and **Plant Protection Division, Administration Bureau, Ministry of Agriculture and Forestry, Kasumigaseki, Chiyoda-ku, Tokyo