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SEX PHEROMONE OF THE DIAMONDBACK MOTH (LEPIDOPTERA: PLUTELLIDAE)[1,2]

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ABSTRACT

Y. S. Chow, Y. M. Lin and C. L. Hsu (1977). *Sex Pheromone of the Diamondback Moth (Lepidoptera: Plutellidae).* Bull. Inst. Zool., Academia **16**(2): 99-105. Sex pheromone of the diamondback moth *Plutella xylostella* (L.) has been confirmed to be the mixture of Z-11-hexadecenyl acetate and Z-11-hexadecenal by using chromatographical isolation techniques (LC. and GLC.) and field attraction experiments. The best attractive ratio of the above two compounds in field tests is from 1:1 to 3:1. Parapheromones such as Z-9-tetradecenyl acetate and Z-9-hexadecenyl propionate alone can cause partial male response in laboratory, but all failed to attract insect in field work. Because chemically synthesized pheromonal mixture can trap two thirds of the male moths attracted by four live virgin females, the mixture in 1:1 ratio is proposed as the sampling tool in surveys of populations, or mass trapping agent for controlling of the diamondback moth.

Because the diamondback moth, *Plutella xylostella* (L.) developed resistance to various pesticides considerably faster than other insects, it became an important cabbage pest in Taiwan recently. Although we have isolated the sex pheromone from female moths, the structure of the true attractant pheromone has not been elucidated. Parapheromone compound such as Z-9-tetradecenyl acetate could cause stimulating response of male in laboratory, but was not attractive in field conditions. So the problem was reinvestigated and the results were presented in this paper.

METHODS AND RESULTS

1. Field attraction experiment

The diamondback moth was reared on common cabbage (*Brassica oleracea* var. *vapitata*) in the laboratory. Male and female were segregated before emergence. Virgin females were placed in a small nylon screen cage first, then the nylon cage was placed at the center of a plastic tube trap coated with a sticky gum compound[(2)]. Each sticky trap was placed over cabbage leaves about 60 cm above the ground before night fall. The traps baited with different numbers of female were examined every

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day, and the attracted males were removed and counted. The results are presented in Table 1.

TABLE 1

Male moths captured by trap of virgin females in the cabbage field (10 × 10 meter) at experimental station of Taiwan Agricultural Research Institute, Taipei.

Date 1974	Weather condition	Number of Female					
		0	2	6	10	15	20
April							
10	Sunny	0	0	0	0	0	0
13	Sunny	0	4	5	3	0	3
15	Sunny	1	5	5	13	0	3
21	Sunny	0	0	0	0	0	0
22	Rainy	0	0	0	0	0	0
23	Sunny	0	21	32	0	31	27
May							
23	Sunny	0	0	0	0	0	0
24	Sunny	0	12	8	12	15	—
25	Sunny	0	13	0	12	17	—
26	Sunny	0	39	17	110	56	—
28	Sunny	0	35	59	56	30	—

In the table above, there is no doubt that the virgin female moth can secrete a very powerful male attractant pheromone. In this first attraction experiment we also found that more males were attracted in dry weather than on a rainy day, and in some days, more males were attracted at downwind positions than at any other locations. So the natural pheromone was extracted from 5000 virgin females with methylene chloride and fractionated on a silicic acid column according to our previous method[1]. After bioassaying each fraction (F_1-F_{10}) with laboratory caged males, fractions F_3 and F_4 gave good response of the males. In the previous work, fractions F_2 and F_3 gave the best response[1]. So F_3 and F_4 were combined and mixed with antioxidant 2,6-di-tert-butyl-4-methylphenol. When the population of diamondback moth in field was high, measured quantity of this mixture was placed in a polyethylene cap for field test. Parapheromones such as Z-9-tetradecenyl acetate etc. obtained from Farchan Div. of Story Chem. Corp. were also used for comparison. Results were tabulated in Table 2.

TABLE 2

Attractancy of the pure chemicals and female extracts in the cabbage field in the vicinity of the Institute of Zoology, Nankang, in April, 1975.

Chemicals or Extracts	24 hours	48 hours
Z-9-C_{14} Acetate 50 μg	1	0
Z-9-C_{14} Acetate 100 μg	0	0
Z-9, E-12-C_{14} Acetate 100 μg	3	0
Z-9-C_{14} AC: Z-9, E-12-C_{14} AC=1:2 50 μg	0	0
Palmitic Acetate 10 μg	2	0
Crude Extract 12 F. E.	0	0
F_3+F_4 5 F. E.	1	2
F_3+F_4 30 F. E.	10	15
F_3+F_4 60 F. E.	17	26
Control, 2 virgin female	39	59

Average number of 5 replicates. F. E., female equivalent.

From Table 2, we can see that female extracts have attractancy in the field as the live virgin females do, whereas the pure chemicals tested did not.

2. Refined laboratory techniques and experiments

Since our former pipette bioassay method gave only very low response percentage toward the caged males, we tried to see whether the lighting condition or the cyclic change of day and night had any effect on the males response. So we tested the response of the unmated males by pipette method at different time of the day, and the results obtained were shown in Fig. 1. There were two response peaks in one day, a small peak around 3 o'clock in the early morning and a large peak at 19 o'clock in the evening. On the other hand, in our histological

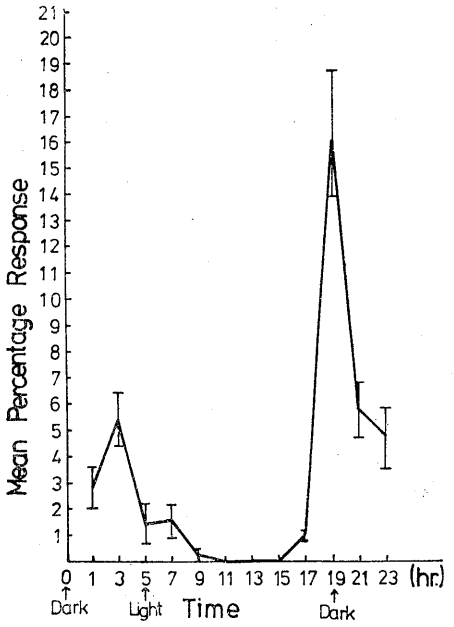

Fig. 1. Mean percentage response of the male diamondback moth to female sex pheromone (1 F. E.) at 25°C. The bars represent standard errors.

work we have located the sex pheromone gland at the female abdominal tip[3]. So 500 virgin female abdominal tips were excised and extracted with methylene chloride. The methylene chloride solution (50 F. E.) was then injected directly into a gas chromatograph (Varian 2800) and the column eluate bioassayed with caged males at around 19 o'clock in the evening. The relative retention time of the pheromone confirmed our previous finding that it was very close to that of n-hexadecenyl acetate on either SE-30 or NPGS column, but longer than n-hexadecenyl acetate on a DEGS column[1]. Because Z-9-hexadecenyl acetate does not give response in the laboratory, close relatives of the compound such as Z-9-tetradecenyl propionate, Z-9-tetradecenyl butyrate, Z-9, E-12-tetradecadienyl propionate, Z-9-hexadecenyl propionate, and their corresponding alcohols were synthesized in laboratory and bioassayed against caged male moths at 19 o'clock of the day. Only Z-9-hexadecenyl propionate at a concentration of 1 mg/ml gave good response. At a dose as small as 5 μg directly applied to a pipette tip, the response was twice as much as that given by the same smount of Z-9-tetradecenyl acetate, and the response given by the compound on the pipette tip persisted for 2 weeks. The data were presented in Fig. 2.

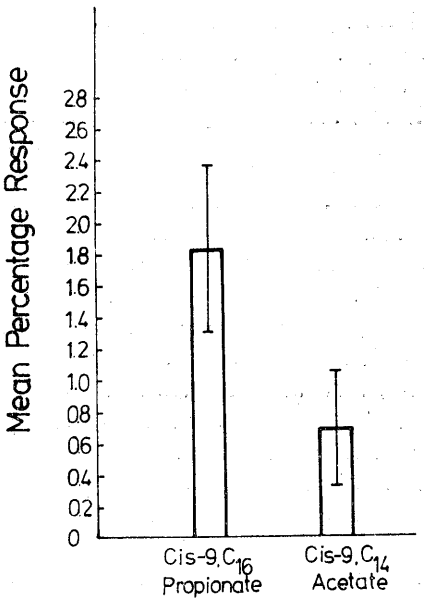

Fig. 2. Mean response percentage between Z-9-hexadecenyl propionate and Z-9-hexadecenyl acetate by pipetting method. The bars stand for standard errors.

But when the compound was tested in the field condition, no male was attracted again. This is not surprising, because when one compares the results of Fig. 1 and Fig. 2, Z-9-hexadecenyl propionate can only give 2% male response whereas the female extract can give almost 20% response or 10 times more than the synthetic chemicals. So we call these low response chemicals as stimulation pheromones.* In order to elucidate the true pheromone structure, compounds with a double bond at positions other than 9 must be tried. We obtained or

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synthesized Z-7-hexadecenyl acetate, Z-7-hexadecenyl propionate and Z-12-pentadecenyl acetate, no response was obtained with these compounds. In order to obtain an ester with a double bond at the position 11 of the alcohol moiety, 1 gram of Z-11-hexadecenal (Virelure) was purchased from Chem. Samp. Co. (Columbus, Ohio 43220 U. S. A.). The aldehyde was reduced to the corresponding alcohol and then acetylated to Z-11-hexadecenyl acetate in good yield (70%). When tested in the laboratory, it gave a better response than Z-9-hexadecenyl propionate, but failed again in the field test.

3. Confirmation work

Recently, we were informed by Dr. Tamaki[6] that they had identified Z-11-hexadecenal and Z-11-hexadecenyl acetate as the sex pheromone of the diamondback moth by using gas chromatograph-mass spectrometer and electroantennogram techniques. In the short communication[6], they also reported that as compared with 4 virgin females, one fourth strength of male attraction was found when 0.1 to 1 μg of the compounds in the ratio of 1:1 to 4:6 (aldehyde: acetate) were tested in the field condition. In order to confirm their results and compare our results with theirs, we have isolated the sex pheromone from 3000 virgin females again according to our previous method and the fraction 3 (F_3), which showed good response when bioassayed in laboratory, were injected into a μ-porasil column of a high speed liquid chromatograph (Waters Associates M-6000A). The chromatograms of F_3 and pure Z-11-hexadecenyl acetate and Z-11-hexadecenal were shown in Fig 3. Since our UV monitor and refractive index (R. I.) detector were connected in series, and the compound were passed through the UV monitor first and then to the R. I. detector, the UV peak of Z-11-hexadecenal in Fig. 3 was ahead of its R. I. peak. We have tried many solvent systems, but the 2 pheromones still could not be separated into components. After we collected the eluent from the μ-porasil column separately, only fraction 2 (corresponding exactly

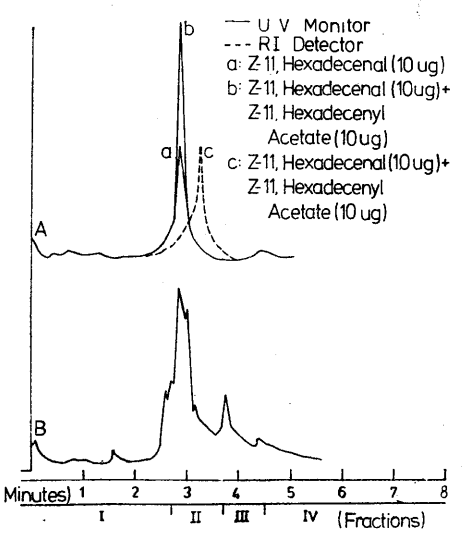

Fig. 3. High speed liquid chromatograms of the pure pheromonal chemicals (A) and the female extract (B) of the diamondback moth. Solvent system was *n*-hexane: Chloroform: methanol=90:10:2. I, II, III and IV were collected fractions.

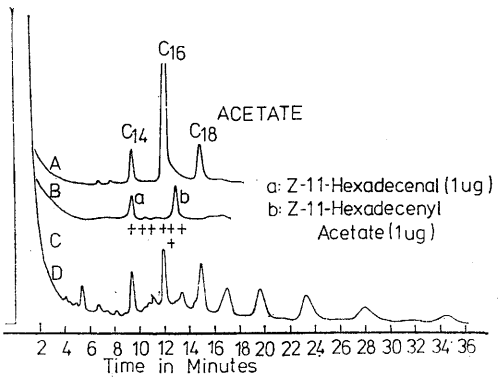

Fig. 4. Gas chromatograms of pure synthetic chemicals (A and B), active fractions (D) from a liquid chromatograph and behavioral biological activity (C, represented as +) with a 10% SP-1000 on 80/100 Supelcoport column, Varian 2800, F. I. D. programing from 120°C-220°C, 8°C/min.

to the retention time of Z-11-hexadecenyl acctate and Z-11-hexadecenal) gave good bioassay response. Then the fraction was concentrated under reduced pressure and injected

TABLE 3
Relative retention times of the sex pheromone of the diamondback moth compared to known synthetic pure pheromonal chemicals and analogs.

Compounds	Chromatographic Columns			
	12% DEGS[a]	10% SE-30[b]	2% NPGS[c]	SP-1000[d]
n-Hexadecanyl Acetate	1.00	1.00	1.00	1.00
Cis-9-Tetradecenyl Acetate	0.87	0.81	0.81	0.79
Cis-11-Hexadecenal	0.84	0.55	0.89	0.78
Cis-11-Hexadecenyl Acetate	1.07	0.97	1.02	1.08
Cis-9-Hexadecenyl Propionate	1.11	0.95	1.20	1.33
Sex pheromone	1.09	1.00	0.97	1.07

a. 110-190°C 8°C/min, b. 130-250°C 8°C/min, c. 100-220°C 8°C/min,
d. isothermal at 199°C.

into a SP-1000 column of a gas-chromatograph (Varian 2800). The different retention times for each related compound studied were shown in Fig. 4 and Table 3. From these results, we can see that Z-11-hexadecenal and Z-11-hexadecenyl acetate can be separated easily by gas chromatography. But by using our behavior response as a bioassay indicator, only Z-11-hexadecenyl acetate could be responsive. In our gas-chromatographical bioassay, the Z-11-hexadecenal eluted from the column gave only slight irritation response of males; no definate sex response was obtained.

For field tests, pherocon TM 1C traps baited with pure pheromone chemicals which were mixed in polyethylene caps at the ratios (acetate: aldehyde) of 1:0.5, 1:1, 1:1.5, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, (total 10 μg) were used. The results for 40 replicates were shown in Fig. 5. Our data confirm those of Japanese; mixtures of Z-11-hexadecenyl acetate and Z-11-hexadecenal in ratios from 1:1 to 1:3 do attract male diamondback moth in the field.

DISCUSSION

When Takahashi *et al.* (1971)[5] identified the sex pheromone of the almond moth (*Cadra cautella* Walker) as Z-9, E-12-tetradecadien-1-ol acetate, they also reported that, in laboratory, Z-7-tridecenyl acetate and Z-9-tetradecenyl acetate caused the sexual excitement to the male almond moth. In the diamondback moth, we also found that Z-9-tetradecenyl acetate and Z-9-hexadecenyl propionate could give male excitement but did not attract insect in the field condition. These pheromone analogs only caused male activity. This may be due to the resemblance of the aliphatic chains between the true pheromone molecule and those of pheromone analogs. In this research, although we have not been able to isolate Z-11-hexadecenyl acetate and Z-11-hexadecenal from the moth, the sex pheromone molecules identified by Tamaki *et al.*[6] could be confirmed by laboratory and field tests as the pheromones of diamondback moth. It is interesting to note that these compounds have been reported as pheromones for other insects[4]. The natural pheromone fraction isolated by us behaved almost identically to the synthetic pheromones by chromatographical methods (Fig. 3, 4 and Table 3). In field tests, instead of 0.1-1 μg of pheromone used by Tamaki *et al.*[6], we used 10 μg to bait each trap. So more males were attracted by our traps. In our experience virgin females will attract more males in their early days of its life span after emergence, and then their pheromone secretion declines[3]. So, if you used aged virgin females as a control, in many cases, the

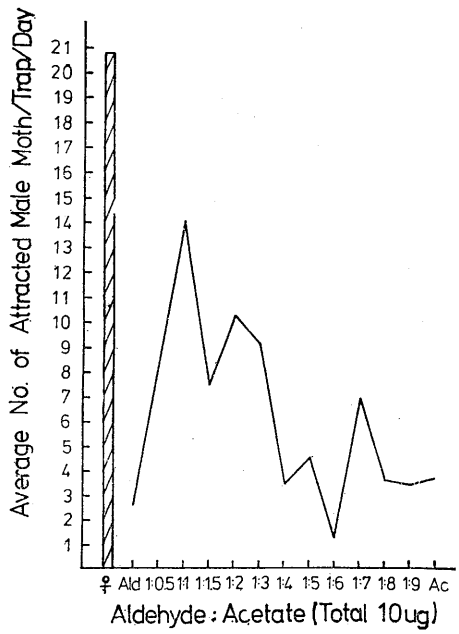

Fig. 5. Capture of male moths of the diamondback moth by 4 virgin females and field traps baited with different ratio mixtures of Z-11-hexadecenyl acetate and Z-11-hexadecenal at Young-Ming Park, Grass Mountain, Taipei, during 1977.
The figures are average of 40 replicates.

1:1 pheromone mixture will even catch more males than the virgin females. But as a whole, newly emerged females still caught more males than the 1:1 pheromone mixture (Fig. 5). If we compare Fig. 5 with Table 2, the 1:1 pheromone mixture is even better than the natural pheromone fraction (60 F. E.) in their attraction ability test in field. So, it is proposed as the sampling tools in surveys of population or the mass trapping agent for controlling of the diamondback moth.

Acknowledgements: We thank Dr. Tamaki for providing synthetic Z-11-hexdecenyl acetate as a standard to assure the purity of our synthetic pheromones for field test, Dr. C. J. persoons, Central Lab. TNO Delft for providing Z-12-pentadecenyl acetate and to Mrs. S. C. Chiu and C. C. Chien, Dept. of Applied Zoology Taiwan Agric. Res. Inst. Taipei for their assistance in field-trapping trials and cultivation of the insects.

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小菜蛾性費洛蒙之研究

周延鑫 林玉美 許秋玲

小菜蛾 *Plutella xylostella* (L.) 性費洛蒙確實是 Z-11-hexadecenyl acetate 與 Z-11-hexadecenal 自 **1：1** 到 **3：1** 的混合物。小菜蛾性費洛蒙類似物 Z-9-tetradecenyl acetate 雖然在室內用滴管法可以引起部份的雄蛾的性反應，但是在田間並不能誘蟲成功。後來再用氣相色層分析儀做生物檢定時，發現眞正的費洛蒙比 Z-9-tetradecenyl acetate 的分子量要大些，所以確定性費洛蒙應該有碳 16～18 個的骨架才對，但是 Z-9-hexadecenyl acetate 沒有反應，所以其他化合物 Z-9-tetradecenyl propionate、Z-9-tetradecenyl butyrate、Z-9-hexadecenyl propionate 及 Z-9, E-12-tetradecadienyl propionate 一一加以合成，結果只有 Z-9-hexadecenyl propionate 在室內有反應，但在田間仍然未有誘蟲的反應，所以可能是 double bond 的位置不對，故我們又合成了 Z-7-hexadecenyl acetate、Z-7-hexadecenyl propionate、Z-12-pentadecenyl acetate 及 Z-11-hexadecenyl acetate，結果只有 Z-11-hexadecenyl acetate有室內反應，但在田間仍然無誘蟲效果。最近日本報導：日本的小菜蛾性費洛蒙是Z-11-hexadecenal 及 Z-11-hexadecenyl acetate 1：1 到 1：1.5 的混合物，我們在田間仿做試驗誘蟲後，亦證明此二化合物可在臺灣誘蟲，但其所誘平均蟲數仍不如活的雌蟲，原因仍在研究中。又因其在田間已可誘蟲，故此 1:1 的混合物已可推廣使用。