Female Sex Pheromone of the Diamondback Moth, *plutella xylostella*

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The diamondback moth, *Plutella xylostella* Linné (Lepidoptera; Plutellidae), is a notorious pest of the cruciferous vegetables in many parts of the world. In Japan, the diamondback moth was not important as a pest before around 1965. However, the species, thereafter, more increased its populations and became the most serious pest of the crucifers such as cabbage, daikon and Chinese cabbage throughout the country. Particularly on cabbage grown extensively not only in the autumn like daikon and Chinese cabbage but also in the spring and early summer, in which the species occurs usually more abundantly, the diamondback moth has become most important. The diamondback moth larvae infest the young cabbage plants and retard their vegetal growth. The larvae also infest the outer layers of the cabbage heads later and consequently the cabbages are rendered appreciably unfit for consumption.

The diamondback moth in the central and southern parts of Japan appears all the year round and proceeds approximately 10 to 12 generations continually a year. On the other hand, in the northern part of Japan, the species commonly appears except in the winter and has several generations a year. The control measures against the diamondback moth are now needed usually on cabbage and sometimes on daikon and Chinese cabbage in Japan. For controlling the species, various kinds of and large amounts of insecticides have been applied. Dichlorvos was first used almost exclusively. Recently, acephate, prothiophos, cartap and others have come to be used. However, the control with chemical agents has become distinctly difficult in the past few years at various parts of the country. It is presumed that the diamondback moth has revealed resistance to some of the aforementioned insecticides. For controlling the diamondback moth, therefore, it is required to deal with more efficiently and rationally.

This paper reports on the femals sex pheromone of diamondback moth and its researches in Japan.

Identification of sex pheromone

A series of the researches were conducted from 1975 to 1976. Approximately 32,000 virgin female moths in total were used during the procedures for identification of the female sex pheromone in diamondback moth. The virgin female moths used were produced mostly by the mass rearing technique of the diamondback moth larvae using germinating rape seeds (Koshihara & Yamada, 1975). Methylene chloride extract of whole bodies of virgin female moths and of folded filter papers, in which active substances from the virgin females confined in small containers was absorbed, was prepared for isolation of the female sex pheromone.

Isolation and identification of the female sex pheromone were carried out at the National Institute of Agricultural Sciences. The female sex pheromone of diamondback moth was identified to consist of two components, (*Z*)-11-hexadecenyl acetate (*Z*11-16: OAc) and (*Z*)-11-hexadecenal (*Z*11-16: Ald) (Tamaki et al., 1977). The total amount of *Z*11-16: OAc and *Z*11-16: Ald isolated from 21,000 virgin female moths was about 5 μg. The ratio of *Z*11-16: OAc and *Z*11-16: Ald was estimated to be 4 to 6.

Biological activity of synthetic female sex pheromone components, *Z*11-16: OAc and *Z*11-

16: Ald, was first examined by electroantennogram (EAG) assay and behavioral response bioassay using the male moths in the laboratory. Attractive activity of the female sex pheromone components to the male moths was also examined by field trapping tests in the cabbage field. Male attraction was distinctly shown when binary mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5: 5 or 4: 6 at 0.1 or 1 μg was loaded on a cotton wick of sticky trap placed at the cabbage fields.

Incidentally, *Z*11-16: OAc and *Z*11-16: Ald have been known as female sex pheromone components of some lepidopterous insects. *Z*11-16: OAc has been reported as one of the female sex pheromone components of the cabbage armyworm moth, *Mamestra brassicae*, Linné, a most notorious pest of various vegetables and crops in the palearctic region of the world, including Japan (Hirai et al., 1978). *Z*11-16: Ald is described as one of the female sex pheromone components of the rice stem borer moth, *Chilo suppressalis* Walker, the most notorious pest of rice in Japan as well as Southeast Asia (Nesbitt et al., 1975; Ohta et al., 1976).

Attractive activity of synthetic sex pheromone

A series of the researches on attractive activity of the synthetic female sex pheromone components were extensively conducted at the Vegetable and Ornamental Crops Research Station since 1977. Field attractiveness of mixtures of *Z*11-16: OAc and *Z*11-16: Ald to the male moths was examined in detail at the cabbage fields using cotton wicks partially and rubber septa as the pheromone dispensers. The pheromone dispensers were baited to sticky traps. The traps were placed among the cabbage plants at 0.2 m in height and apart some 5 m each other.

First, field attractive activities of binary mixtures of *Z*11-16: OAc and *Z*11-16: Ald of various ratios and of various amounts were preliminarily evaluated using cotton wick as the pheromone dispenser. The results obtained here were summarized as follows (Koshihara et al., 1978). Attractive activity of *Z*11-16: OAc or *Z*11-16: Ald alone can not be appreciated. The binary mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5: 5 or 4: 6 at the total amont of 1 μg showed the most strong attractive activity equivalent to that of five live virgin females. The optimal ratio shown here seemed to be generally coincided with the ratio of two components found in virgin female moths by Tamaki et al. (1977). It was also found that attractive activity of the binary mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5: 5 at the total amount of 1 μg was equivalent to that of five female moths at mean temperatures of about 10 and 15°C in the spring and the autumn. However, attractive activity of the binary mixture was considerably inferior to the females at mean temperatures of about 20°C in the early summer. These results seemed to suggest that the female sex pheromone of diamondback moth was not composed solely of the two components but other unknown ones play an important role in the male attraction as minor components.

Accordingly, the following researches were carried on in order to acquire information on enhancing attractiveness of the binary mixture of *Z*11-16: OAc and *Z*11-16: Ald at high temperatures in the summer (Ando et al., 1979). EAG activities of many analogues of the female sex pheromone components, *Z*11-16: OAc and *Z*11-16: Ald, were first measured. EAG profiles of the antennal responses in male diamondback moth to a series of 16 carbon monoene acetates, aldehydes and alcohols were measured. The male antenna responded most strongly to *Z*11-16: OAc, and *Z*11-16: Ald, while their geometrical isomers, (*E*)-compounds, and double bond positional isomers, (*Z*)-10 or (*Z*)-12 compound, elicited the medium EAG responses. These results were in good agreement with the EAG profiles obtained so far in other species. On the other hand, although it has been known that change of functionality of sex pheromone component into others usually results in remarkable decrease of the EAG activity, (*Z*)-11-hexadecenol (*Z*11-16: OH), the parent alcohol of the sex pheromone acetate, still showed noticeably strong EAG activity. These data

Table 1. Attraction of male moths to (*Z*)-11-hexadecenyl acetate and (*Z*)-11-hexadecenal in combination with the third compounds

Third compound	No. of males attracted by two traps			
	19 to 21	22 to 24	25 to 28 July	Mean
(*Z*)-11-hexadecenol	95	35	28	51.0 *a*
(*E*)-11-hexadecenal	86	22	18	42.0 *a*
(*Z*)-12-hexadecenal	63	23	14	30.0 *ab*
(*E*)-11-hexadecenyl acetate	57	16	8	27.0 *ab*
(*E*)-11-hexadecenol	39	7	12	19.3 *bc*
(*Z*)-10-hexadecenal	37	9	10	18.7 *bc*
(*Z*)-10-hexadecenyl acetate	33	15	7	18.3 *bc*
(*E*)-12-hexadecenal	22	12	13	15.7 *bc*
(*Z*)-9-hexadecenal	23	5	7	11.7 *bc*
None	23	8	0	10.3 *bc*
Hexane control	2	2	0	1.3 *c*

Numbers followed by the same letter do not differ significantly ($P > 0.05$).

suggested that *Z*11-16: OH might be the unidentified sex pheromone component and may have synergistic effect on the field attractiveness of the *Z*11-16: OAc and *Z*11-16: Ald mixture.

The field trapping test was second conducted for evaluation of the attractiveness of binary mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5: 5 at the total amount of 10 μg alone and in combination with each 0.1 μg of their analogous compounds which possessed the strong EAG activity. The mixed chemicals were loaded on rubber septa. The results of this field trapping test are shown in Table 1. Since mean temperature was about 25°C during the experimental period of July, mid-summer, the binary mixture of the sex pheromone acetate and aldehyde alone was less attractive than at lower temperatures and the data coincided with the previous ones at high temperatures of the early summer (Koshihara et al., 1978). The mixture of two pheromone components and additional *Z*11-16: OH still showed strong attractiveness and it was confirmed that this alcohol had strong synergistic activity on the attraction of the diamondback moth. Geometrical isomers of the pheromone components and (*Z*)-12-hexadecenal also possessed the synergistic activities distinctly. These results suggest that the diamondback moth utilizes not only the two identified pheromone components, *Z*11-16 OAc and *Z*11-16: Ald, but also one or some of the following; the parent alcohol (*Z*11-16: OH), the geometrical isomers (*E*11-16: OAc and *E*11-16: Ald) and the double bond positional isomer (*Z*11-16: Ald).

Subsequently, the details of synergistic activity of *Z*11-16: OH on the field attractiveness of mixture of *Z*11-16: OAc and *Z*11-16: Ald were further examined. Then, field attractiveness of the mixture of *Z*11-16: OAc and *Z*11-16: Ald in combination with additional *Z*11-16: OH to the male moths was fully evaluated (Koshihara & Yamada, 1980). Results obtained in a series of the tests were summarized as follows. The optimal ratio of the binary mixture of *Z*11-16: OAc and *Z*11-16: Ald for the male attraction was estimated 5: 5, though the optimal ratio fluctuated something around the ratio of 5: 5 in each of the repeated tests. The mixture of *Z*11-16: OAc and *Z*11-16: Ald showed strong attractive activity to the males in the ratio of 5: 5 at 0.01 to 0.1 mg levels of rubber septa in the spring and summer except mid-summer and at 0.1 to 1 mg levels in the winter, respectively (Fig. 1). As is aforementioned, the diamondback moth appears throughout the year in the central and southern parts of Japan. Accordingly, the fact that the optimal dose of the 5: 5 mixture of *Z*11-16: OAc and *Z*11-16: Ald for male attraction changed by seasons was considered to be noticeably important. It was presumed that the optimal dose for male attraction needed more amounts

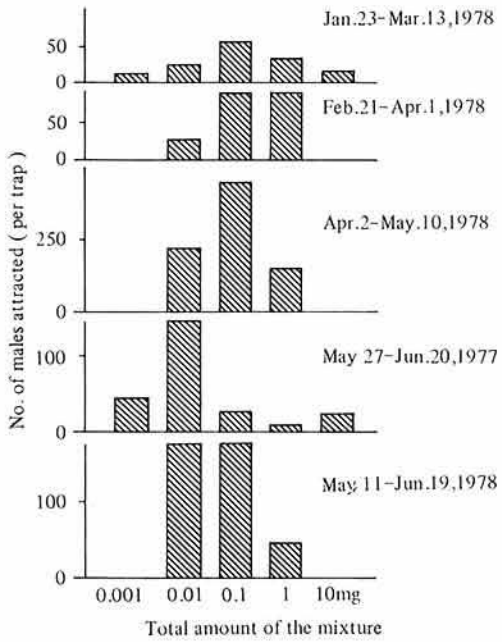

Fig. 1. Attraction of male moths to the binary mixtures of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecenyl acetate in the ratio of 5:5 in different amounts.

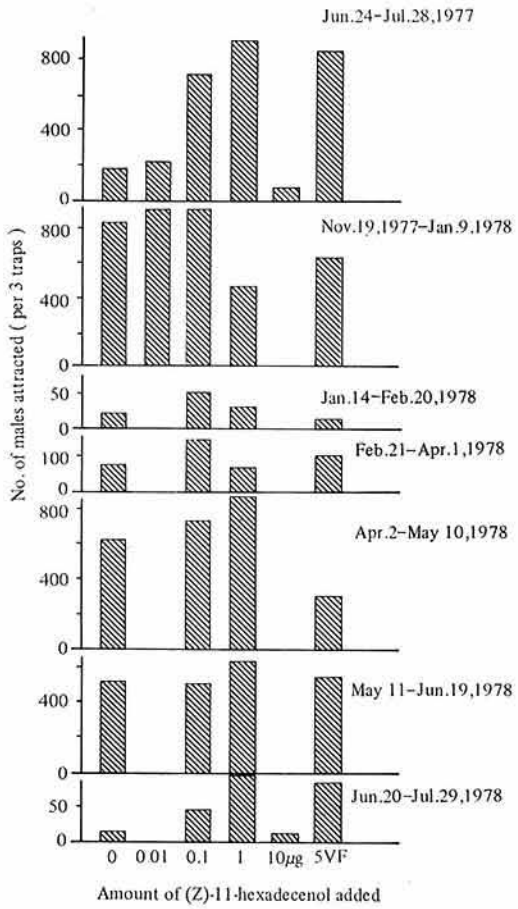

Fig. 2. Seasonal changes in male attraction to the 5:5 mixtures of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecenyl acetate, at 0.01 mg level, to which (*Z*)-11-hexadecenol was added in different ratios. 5VF means 5 virgin females aged 3 and/or 4 days.

in the winter since the pheromone chemicals of rubber septa were slowly evaporated at low temperature. Field attractiveness of the 5:5 mixture of *Z*11-16: OAc and *Z*11-16: Ald at 0.01 mg level in the mid-summer was obviously enhanced by adding 1 to 10% of *Z*11-16: OH. Attractiveness of the mixture of *Z*11-16: OAc and *Z*11-16: Ald in the spring was apperciably enhanced by adding 1 to 10% of *Z*11-16: OH The attractiveness in the winter was only poorly enhanced by addition of 1% of *Z*11-16: OH (Fig. 2). Namely, it was clarified that the mixture of *Z*11-16: OAc and *Z*11-16: Ald with additional *Z*11-16: OH has stronger attractive power than that of the binary mixture alone at high temperatures in the summer. No attractive activity of *Z*11-16: OH alone was recognized. Interestingly, adding 100% amount of *Z*11-16: OH to the mixture of *Z*11-16: OAc and *Z*11-16: Ald reduced almost all the activity.

At the conclusion of a series of the researches, the field attractiveness of mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5:5 at 0.01 and 0.1 mg levels of rubber septa with additional 1% of *Z*11-16: OH was furthermore examined continually all the year round. As shown in Fig. 3 and Table 2, it was judged that the mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5.5 at 0.1 mg level with additional 1% of *Z*11-16: OH showed fairly stronger activity compared with that of the 0.01 mg mixture and of five live virgin females nearly in all seasons. Attractive activity remained strong at least for some 40 days even in the summer. It has been shown that attractive activity of the trap baited with live virgin female moths increases most when five females per trap are baited and its activity is about 8 times

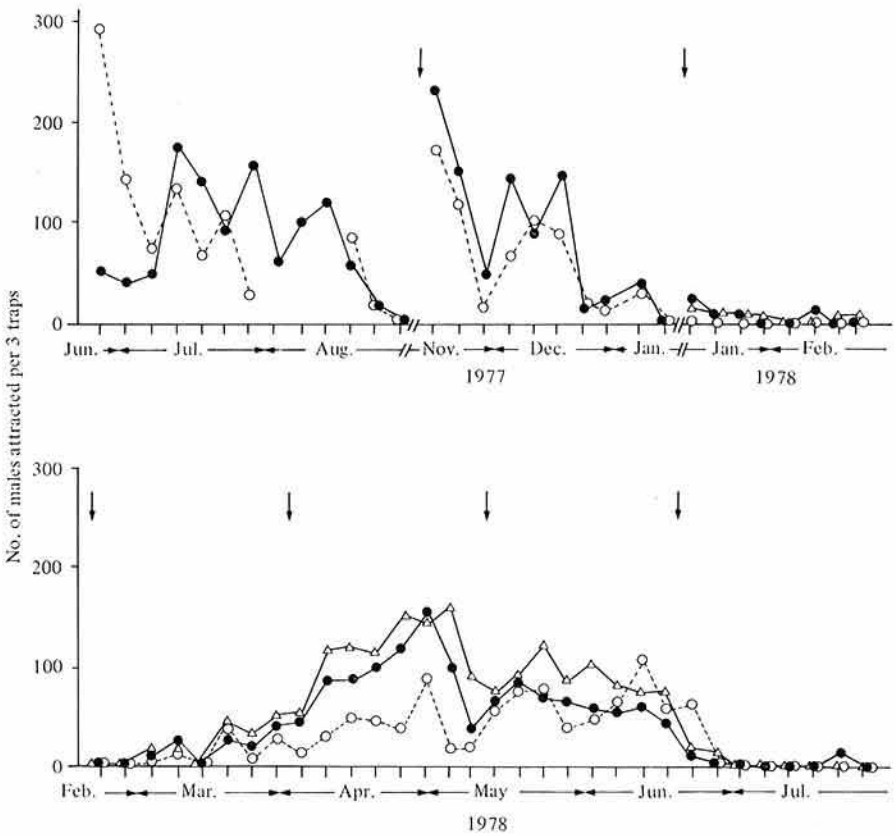

Fig. 3. Seasonal variations in attraction of male moths to the mixtures of (*Z*)-11-hexadecenal, (*Z*)-11-hexadecenyl acetate and (*Z*)-11-hexadecenol.

Note ● and △: 5:5 mixture of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecenyl acetate at 0.01 and 0.1 mg levels, respectively, to which 1% of (*Z*)-11-hexadecenol was added.
○: 5 virgin females aged 3 and/or 4 days.
↓: represents the date on which rubber septa loaded with the synthetic compounds were renewed.
Values indicated are the total number for each 5 day period with a few exceptions.

stronger than that of the trap baited with a single female (unpublished data). Therefore, the mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5: 5 at 0.1 mg level of rubber septa with additional 1% of *Z*11-16: OH was regarded to be sufficient as attractant for trapping the diamondback moths.

Practicability of pheromone trap in surveying the diamondback moth

Synthetic sex pheromone of the diamondback moth is primarily expected to be used as an attractant of the trap for surveying the population densities. Feasibility of surveying the diamondback moth populations using the binary mixture of synthetic *Z*11-16: OAc and *Z*11-16: Ald alone was suggested (Chow et al., 1977; Chisholm et al., 1979).

It was previously clarified that seasonal changes in the diamondback moth catches by the trap baited with five live virgin females well related with that in the populations at the cabbage fields (Yamada & Koshihara, 1975). Accordingly, as shown in Fig. 3, the trap baited with the mixture of *Z*11-16: OAc and *Z*11-16: Ald in the ratio of 5: 5 at 0.1 mg level with additional 1% of *Z*11-16: OH loaded on

Table 2. Attraction of male moths to the mixtures of (*Z*)-11-hexadecenal, (*Z*)-11-hexadecenyl acetate and (*Z*)-11-hexadecenol

Date	Number of male moths attracted[a]		
	Pheromone sources		
	I[b]	II[b]	5VF
Jun. 24 '77—Aug. 27 '77	543	—	773
Nov. 19 '77—Jan. 9 '77	890	—	631
Jan. 14—Feb. 20 '78	48	42	27
Feb. 21—Apr. 1	148	169	100
Apr. 2—May 10	737	959	305
May 11—Jun. 19	507	730	539
Jun. 20—Jul. 29	45	43	85
Total	1,485	1,943	1,056

[a] Values indicated are the total numbers of males (per 3 traps) attracted during each test period.

[b] Each trap was baited with mixtures of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecenyl acetate in the ratio of 5:5 at 0.01 mg (I) and 0.1 mg (II) levels, to which 1% of (*Z*)-11-hexadecenol was added.

rubber septa was expected to be used efficiently for surveying population densities of the diamondback moths infesting in the cabbage fields. The data that seasonal appearances of the diamondback moth larvae at the fields in which cole crops are grown was reflected in the males catch of trap baited with the 5:5 mixtur of *Z*11-16: OAc and *Z*11-16: Ald at 0.1 mg level of rubber septa with additional 1% of *Z*11-16 OH were quite recently shown (Nakagome & Kato, 1980). It is concluded that the trap baited with the mixture of *Z*11-16: OAc, *Z*11-16: Ald and *Z*11-16: OH in the ratio of 5:5:0.1 at 0.1 mg level loaded on rubber septa can be used effectively for surveying the diamondback moth appearances.

Conclusion

In a series of these researches, the female sex pheromone of diamondback moth has been identified and the mixture of synthetic female sex pheromone components, *Z*11-16: OAc and *Z*11-16: Ald, and parent alcohol of the former one, *Z*11-16: OH, in the ratio of 5:5:0.1 at 0.1 mg loaded on rubber septa has proved to be available in the trapping systems for surveying the population densities. The research activities should be next concentrated on use of the sex pheromone for direct control of the diamondback moth. The researches on the atmospheric permeation with sufficient synthetic sex pheromone compounds for disrupting male moths orientation to females are now being carried on.

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(Receiving for publication, October 7, 1980)