Bull. Inst. Zool., Academia Sinica **24**(2): 165-176 (1985)

ESTERASE OF DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA* L.).
II. THE ANTENNAL CARBOXYLESTERASE OF ADULT MALE WITH REFERENCE ON MALE RESPONSE TO SYNTHETIC FEMALE SEX PHEROMONE

WILLIAM CAN-JEN MAA AND YUI-MEEI LIN

Institute of Zoology, Academia Sinica
Taipei, Taiwan 115, Republic of china

(Received April 2, 1985)

William Can-Jen Maa and Yui-Meei Lin (1985) Esterase of diamondback moth (*Plutella xylostella* L.). II. The antennal carboxylesterase of adult male with reference on male response to synthetic female sex pheromone. *Bull. Inst. Zool., Academia Sinica* **24**(2): 165-176. Natural populations of diamondback moth, *Plutella xylostella* L., collected from different vegetable fields were investigated. The response of male adults to the female pheromone was revealed by a *Y*-test apparatus in laboratory. The antennal esterase activity of the moth was monitored by either 1-naphthylacetate or C^{14} (Z)-11-hexadecenyl acetate. In addition, the zymogram of antennal esterase was also studied by poly-acrylamide gel electrophoresis. We found that variations of pheromone response of the adult male to trinary of (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate and (Z)-11-hexadecen-1-ol, was either age-dependent or population-dependent. Although, the population-dependent variation of male pheromone-response is statistically insignificant. Age-dependent variation of antennal esterase activity was positively correlated with the behavior of the male moth. Zymogram of antennal esterases also varied in their apperance. Certain bands of the esterase isozyme seems to be associated with the response of male adults to the pheromone. However, from the data on hand it is difficult to draw a clear cut between the pheromone-response of the male moth, the enzymatic hydrolysis of antennal esterases to the acetate pheromone and NA, and the zymogram pattern of the esterases. No evidence currently to show that the variation of esterases were interrelated with the malathion resistance of the insects from different populations investigated.

Diamondback moth (DBM), *Plutella xylostella* L., is one of the most notorious insect pests of cruciferous vegetable in Taiwan. The rapid development of resistance of this insect to various pesticides were noticed early in the sixties (Tao, 1973). Strategic adjustment on pest management of this pest besides chemical application was tried out, but with little success (Chiang and Maa, 1970; Chiang 1972). Meanwhile, female sex pheromone of lepidopterous insects were introduced into this country for pest management program (Chow *et al.*, 1972). The female sex pheromone (FSP) of DBM was the first one being synthesized and tried out for male mass trapping in field. The attractant as a mixture of (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate in a ratio of 5:5 was found with optimal catch in vegetable field of Young-Ming park, Taipei (Chew *et al.*, 1974,

1. Paper no. 261 of the Journal Series of the Institnte of Zoology, Academia Sinica.

Chew *et al.*, 1977). This tactic was then experimentally applied to mass trapping of the moth in northern and central Taiwan. The result is somehow ambigious (Lee *et al.*, 1982), Contemporarily, preliminary experiment of male response to the synthetic female sex pheromone of the DBM was varied according to the age of the insect as well as the individual insect population investigated (Maa *et al.*, 1983). Variation of male response to the pheromone was also noticed in the field (Maa and Lin, 1983; Maa *et al.*, 1984). Age-dependent variation of pheromone response of insect was believed to be due to the differentiation of the enzyme systems in the olfactory receptors on antennae. Ferkovich *et al*, (1982) found that in cabbage looper the quantitative as well as quantitative changes of the pheromone degradative enzyme(s) in antennae may accompany with variation of male adult response to the FSP during the adult maturation.

Very little of pheromone reception mechanism of male adult of diamond-back moth was known, although results of indoor and outdoor experiments reflected that antennal esterase activity, pheromone-response, and insect maturation were associated with male sex behaviour of the insect (Maa *et al*, 1983). Lack of adequate appreciation of individual variability or local population variability sometimes strongly affected the effective development of control program employing synthetic insect pheromone.

The weather and the climate of northern and southern Taiwan are somehow different. We suspected the independent variables, around which the insect habitated, would eventually affect the male sex behavior elicited by the FSP.

In other words, would this variation be a result of a moth percepting the blend under the variability of the thresholds with different environmental stimuli? Another important intriguing question about using the synthetic FSP for mass trapping is whether this species, which is known to be with various degree of resistance to numberous kinds of insecticides, is such as to present indiscriminate response to the pheromone. To answer these questions, pheromone reception of DBM male adults associated with pheromone degradation enzymes of antennae were, investigated in the following aspects:

1. What is the isozyme categories of the antennal esterases?
2. How is the non-specific esterases and the pheromone esterases associated with the response of the adult to the FSP?
3. Will the variation of male pheromone response associated with the insecticide resistance?
4. Were, if there is any, the esterase of male antennae varied with genetic intrinsic of the insects from different populations.
5. How are the epicuticular tissues of the legs and the wing associated with the pheromone degradation.

MATERIALS AND METHODS

Insects

Diamondback moth, *Plutella xylostella* L., collected from five sites in northern Taiwan and one site in southern Taiwan, were reared in the insectarium. Larvae were reared under constants temperature, 25±1°C, and light L:D, 14-10, conditions according to Maa and Chuag (1983). The larvae were fed on seedlings of rapeseed, with four changes of the food source during the growth (Koshihara & Yamada, 1976), and adults on 20% honey water (Chow *et al.*, 1975). Sheh-Tzu (ST) DBMs were mostly heavily selected by pesticide application under practical program supported by the local government of Taipei City. Nan-Kang (NK) is a suburban area of Taipei City. Geou-Fang (GF) is a montain village, northwest to Taipei, with no commercial pesticides of any kind are used during the passed five years. Manhand-pick is the only method for pest control. Bamboo-Lake (BL) is another montain village of Taipei metro-

politan. Kao-Shoung (KS) DBMs were collected from a seed company. I-Lan (IL) is a farmer county northeast to Taipei. Stocks of newly emerged adults were synchronized within 12 hr intevals for four days. Male adults of 1-day to 4-day old, were bioassayed to pheromone response in Y-shaped apparatus, and enzyme assayed for esterase activity. The appendages of the tested adult males were excised and kept frozen at −20°C. There would be used as enzyme source for the assay.

Chemicals

All chemicals and reagents were of analytical grade or the best trade available. Chemicals for electrophoresis were from Bio-Rad Lab., or Sigma Chemical Co. Materials for Bray's solution was from New England Nuclear Co. The three pheromone components used for the bioassay were synthesized in this laboratory. The purity of the pheromone was of 98% by GC test. A lend of (Z)-11-hexadecenyl acetate, (Z)-11-hexadecenal, and (Z)-11-hexadecen-1-ol in a ratio of 1.0:1.0:0.02, was mixed. One μg of this mixed pheromone was dissolved in *n*-hexane in a small polyethylene tube for bioassay. The isotope-labeled pheromone, (Z)-11-hexadecenyl acetate which was used as a substrate of antennal esterase, was also synthesized in this laboratory and purified by TLC according to Lin and Chow (1983).

Bioassay of male response to pheromone

Pheromone response of male adults to female sex pheromone was measured according to Chang *et al.* (1979). The age-dependent variation of male response to the lure were monitored and data were analyzed statistically according to Mann and Whitney (1953). The air flow rate, in our case, was adjusted to 60 ml per minute at room temperature. (Maa *et al.*, 1984). The tested male adult was counted as with strong (pheromone) response when 50 percent or more of the tested insects moved into the lure. The weak responser was the one with less than 49 percent of the tested individuals approaching the bait.

Enzyme preparation

Antennae, wings, or legs were excised from adult males of different ages (day) and homogenized in 0.1 M phosphate buffer (pH 7.5). The homogenate was sonicated for 1 minute. After sonication the homogenate was centrifuged at 1,000 g for 10 minutes at 4°C. Enzymes of the crude homogenate was used freshly.

Protein assay

The supernatant or the crude homogenate was assayed for protein content, with bovine gamma-globulin as standard, according to Read and Northcoto (1981).

General esterase assay

The enzyme sources of antennae, legs, and wings were analysed using naphthyl acetate (NA) as substrate to determine the activiity of general esterase according to Van Asperen (1962).

Effect of inhibitors to antennal esterases

Eserine, parahydroxymercurybenzoate (PHMB), 1×10^{-4} M, and paraxon, 1×10^{-8} M, were used as inhibitors to characterize the general esterase according to the methods of Bigley and Plapp (1960) and Stephen and Cheldelin (1970). Pre-incubation of the enzyme solution with inhibitors was carried out in dark at room temperature (Maa and Terriere, 1983) before the enzyme reaction was initiated.

Pheromone esterase assay

Radioactive (Z)-11-hexadecenyl acetate was incubated with crude homogenate of the insect appendages in a shaking water bath at 30±1°C for 1.5 hours. A total of 0.25 ml pheromone solution contained 34,000 cpm (about 16 pico mole of labeled pheromone) was coated on polyethylene beads and was blowed gently with nitrogen to dryness. Fifty pairs of antennae, legs and wings were used for each assay. Antennal homogenate boiled in a hot water bath for 15 min. was used as control solution. Incubation was followed by

three extraction of the pheromone component with 0.25 ml hexane each. The solvent phase and the aqueous phase of the mixture was separated by centrifugation, 1,000 g for 15 minutes. Aliquots of the solvent or aqueous phase were drawn with a syringe and injected into vails with Bray solution, counted in a scientillation counter. Measurements of substrate disappearance of the acetate component was counted as hydrolytic activity of antennal esterases.

Electrophoresis of antennal esterases

The supernatant of 1,000 g homogenate of insect appendages were examined electrophoretically according to the method of Davis (1964). All procedures, except preincubation, staining and destaining, were conducted at 4°C. After the run the gels were separatively preincubated with inhibitors for 25 minutes. These inhibited the choline, aliphatic, or aryl esterase activities of the antennae. The gels were then stained for 25 minutes with 0.1% 1-naphthylacetate in 10% aqueous acetone, washed with tape water, developed in 0.1% of diazo-dye (fast blue RR) in 0.1 M phosphate buffer, pH 7.0 at room temperature. Upon development of deep brown colored esterase bands in eight minutes, the gels were washed with three changes of distilled water and stored in 10% acetic acid.

RESULTS

Age-dependent variation of pheromone response

Male adults of different ages responsed differentially to the lure as show in Fig. 1. The young adult moth responsed mostly weakly to the bait. The response increased gradually to a higher level at 1.5-2.0 day after eclosion, and to its maximum at 2.5 day, and dropped to the initial level thereafter. The pheromone response of the 2.5-day-old moth is about 1.8 fold higher than that of the 3.0-day-old male adults.

Age-dependent variation of antennal esterases

The non-specific esterase activity of antennae of the male adults was found to fluctuate not quite in parallel with the pheromone response of the male insects. Fig. 1 shows that the activity of the antennal esterase to both substrates; naphthylacetate and acetate pheromone, were at the lowest level during the day of emergence. The activity of the non-specific esterase increased thereafter, climbed to the highest level during the next 24 hr, stayed at the same level for the next two days. On the other hand, the activity of pheromone esterase was low when the male adults emerged. The activity increased gradually during the next 24 hr interval, peaked the next 12 hr, and decreased to a low level as the initial later on. The fluctuation of pheromone response of male adults and pheromone hydrolytic activity of the male antennae were possibly differentiated in parallel with each other during the ontogenesis of the adult stage. The leg esterase activity does not reflect a parallel relation with the the male pheromone response, nor does it with the antennal esterase activity throughout all period of the examination. Comparatively, the leg esterase activity; ranged from 4.3 to 5.5 μm naphthol produced per two leg equivalent is about one-third to one-fifth as that of the antennal esterases.

Comparison of enzyme activity of the antennal esterases

In this study we try to compare the esterase activity of antennae of male adults collected from different areas. Table 1 shows that the activity of non-specific esterase of the antennae ranged 334 n mole naphthol produced per mg protein for GF; 268 for BL; 156 for NK; 139 for ST and 302 for FS. The pheromone-hydrolytic activities of the crude preparation of male antennal esterases were ranked 13 picol mole per mg protein per 1.5 hr for GF; 10 for BL, NK, TS; and 7.6 for FS. These results revealed that the activity of non-specific and pheromone esterases were not quite corresponding with one another. The Table 1 also shows that there is about two fold difference in antennal non-specific

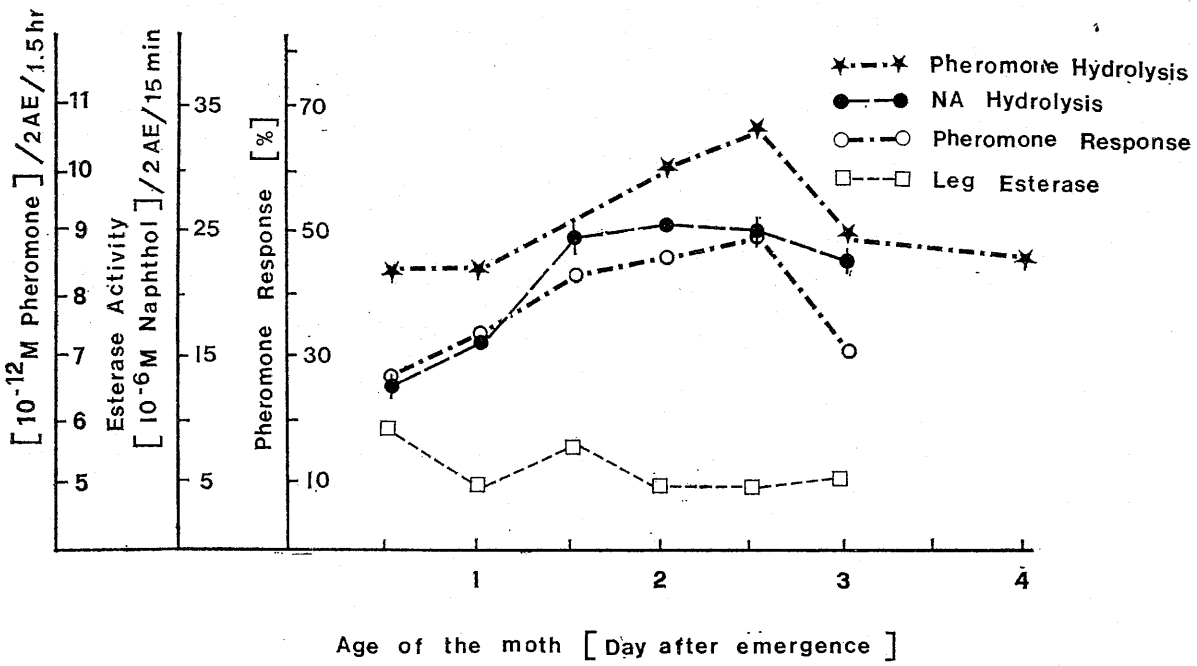


Fig. 1. Variation of antennal and leg esterase activity and pheromone response of male diamondback moth. Triplicate for enzyme assay., four to six replicates for pheromone assay.

esterase activity between the males of GF and/or BL, and these of NK and/or ST. However, pheromone hydrolytic activity of antennal esterases of the males of these four populations are with similar titer.

The inhibition study revealed that these esterases are all susceptible to paraoxon, indicating that the majority of the esterase components of the male antennae- are aliesterase in nature (Table 2). The antennal esterase of GF male are most sensitive to the inhibitors. The males of NK and BL were about equally sensitive to inhibition. Antennal esterases of ST males are less sensitive to the inhibitors. The variation of enzyme inhibition revealed that the genetic diversity of esterases component of the male antennae is possibly present in the insect populations.

TABLE 1
Comparison of antennal esterase activity of male diamond-back moth

Insect populn	Enzyme activity	
	10^{-6} M NA/mg prot/10 min.	10^{-9} M AOC/mg prot/1.5 hr.
GF	334.4±18.7[a]	13.1±0.6[b]
BL	268.2±11.5	10.4±0.1
NK	156.2±14.8	10.3±0.6
ST	139.4±17.1	10.2±0.1
KH	302.1±11.6	7.6±0.1

a) Triplicate with m±S.D., crude homogenate of 8 antennae incubating in 28°C.
b) Two experiments with two to four duplicates, m±S.D., crude homogenate of 100 antennae per each assay in 30°C.

TABLE 2
Effect of inhibitor to antennal esterase activity of the diamond-back moth

Insect populn	Percentage of inhibition		
	Eserine	Paraoxon	Phmb
GF	84	>90	74
NK	74	>90	77
BL	74	>90	71
KS	73	>90	60
ST	70	>90	57

Average of two experiments with triplicate.

Comparison of esterase activity of appendages of male adults

Table 3 shows that foreleg esterase has about one tenth of enzyme activity as that of antennae when naphthylacetate was used as substrate. This result strongly evidenced that antennae, in terms of hydrolytic activity to exogeneous ester, is the most efficient appendage to breakdown the adhesive molecule with ester group. In the case of acetate pheromone, we find a similar answer as to naphthylacetate. The table also shows that pheromone hydrolytic activity of antennal esterase is about 4 fold as strong as those of legs, and 6 fold as those of wings. This revealed that forelegs and wings had very limited capability to degradate the adhensive pheromone around the surface of the appendages other than antennae, although the wing and the fore leg had a rather large area that exposed to the atomosphere.

TABLE 3
Comparison of esterase activity of appendage of male DBM

Appendage	Esterase activity	
	10^{-6} M Naphthol[a] per mg. prot. per 30 min.	10^{-9} M Pheromone metabol[b]. per mg. prot. per 90 min.
Antenna	1417±46	9.14±0.83
Foreleg	137±23	2.36±0.01
Wing	—	1.57±0.08

a. Six experiments with triplicate: M±S.E., 1,000 g. supernatant.
b. Two experiments with duplicate: M±S.E., curde homogenate.

Variation of antennal esterases

Supernatants of 1,000 g of 1-6 day old male adult antennae of five DBM populations were resolved into its component esterases by electrophoresis. The results are illustrated schematically in Fig. 2.

Eleven to twelve isozymes, depending on the age or the population of the insect, were detected during the period of examination. These bands are scattered in two zones of the gel: fast move and middle move zone. Two bands, a narrow band and a wide band, are present in the fast moving zone of all the zymograms examined. A middle zone is composed of nine to ten bands, varied in number, density, and width. All of the isozymes included fast moving and middle moving bands migrating to the cathode end of the gel. There are two faint middle bands in front of the main body of the middle zone are also present in all the zymograms studied. These two bands and the two fast bands are consistant in appearance, while the other bands varied depending upon the age of the male adult, or upon the population investigated. These four bands are usually difficult to detect. The rest of isozymes of the antennal esterse are easily stained.

In the case of ST, the maximum number of isozymes occurred on day 1 and day 6, when twelve clearly defined bands were detected. There are only six bands detectable during day 2-4. It is possible that the corresponding bands: 5, 6, and 7 were fused together into one thick and wide band when the protein density of these isozymes increased, and separation technique of this method fail to satisfy the resolution. Band 8 was found in all zymograms of the gels. The density and band-width of this band is at its obvious condition when the insect is 2-3 day old. Band 10 is comparatively dense in staining color than the corresping band of other insect population. Band 11 and 12 are similar to that of the BL, but was much thinner than those of GF ones. Zymograms of BL antennal esterase are composed of 11 bands with one of the middle band absent from the position of band 5, 6, and 7 of that of ST. It has a dense 8th band and some other less dense bands. The isozyme pattern of this insect, in fact, represented the esterase pattern of most insect populations we collected from the island (8 localities). The ST insects also possessed similar pattern to those of BL. Nevertheless, there are minor variations of each band of the isozymes found in various

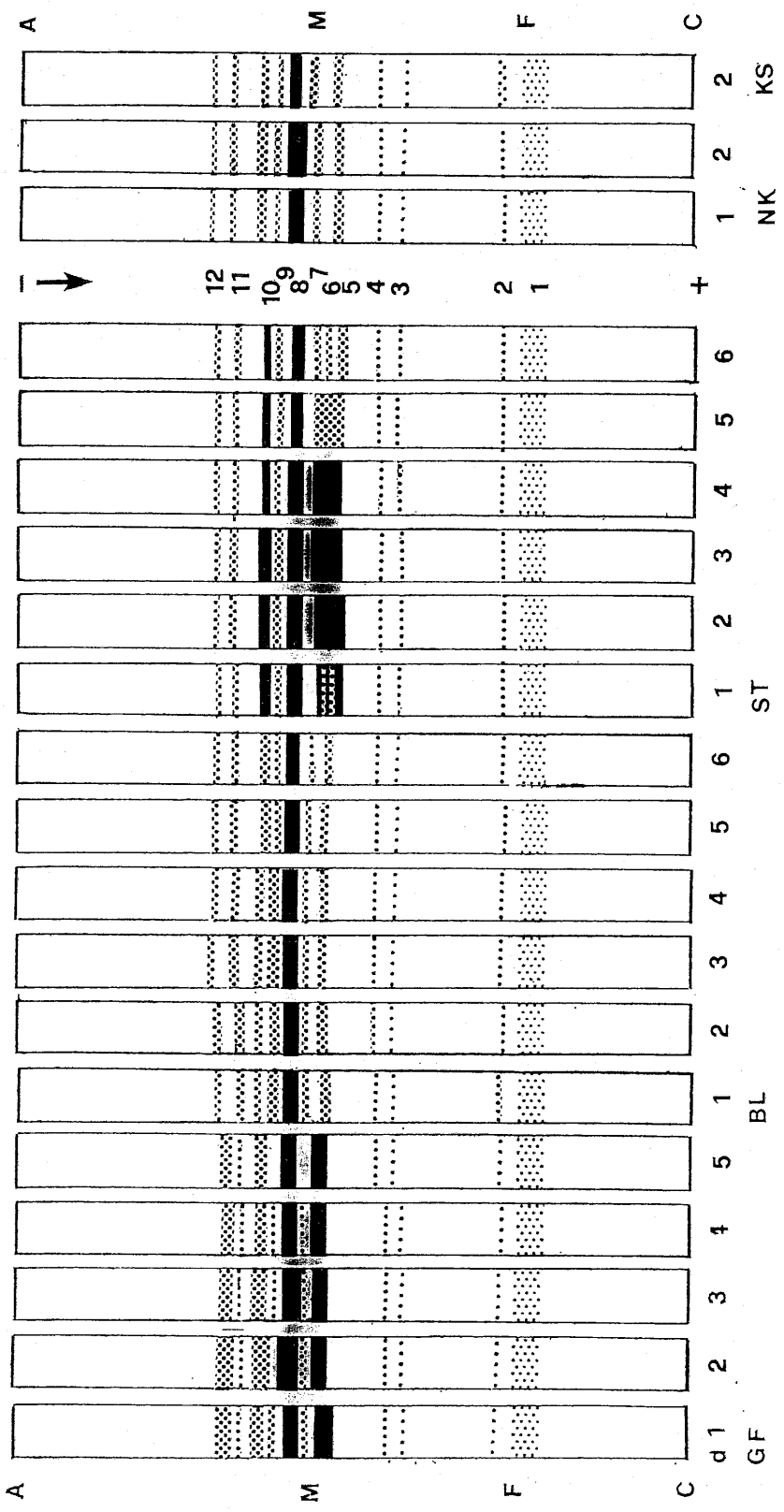
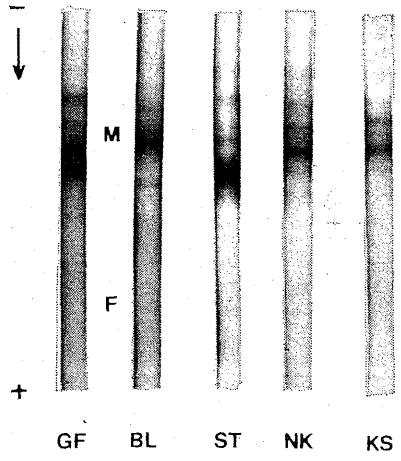


Fig. 2. Zymogram patterns of antennal esterase of male diamond back moths.
Zymogram code:
A, anode end; M, medium bands; F, fast moving bands;
C, cathode end; d, age of the moth, day.

insect populations. Isozyme zymogram of GF males in the most peculiar one among all the other groups of diamond-back moth collected for this study. In the case of BL and ST, bands 9, 10, 11, and 12 are narrow bands in appearance. In the case of GF, both band 10 and 12 are thick with strong staining, even when the male adults aged while all the isozyme decreased in staining density. This "four-dense-band" pattern was possibly related with the high enzyme activity to naphthyl acetate and the acetate pheromone tested in this study. It is possible that these dense bands are associated with the strong response of the male adult to the female sex pheromone.



ZYMOGRAMS OF ANTENNAL ESTERASES

Fig. 3. Photographs adults of zymograms of antermal erterares from male of different DBM popalation.

Male response to synthetic female sex pheromone

Indoor investigation on male adult responsed to female sex pheromone was carried out by *Y*-test. A combination of pheromone mixture with equal amount of acetate and alcohol component in 1.0 μg was used for the bioassay.

Fig. 4 shows that GF male showed the strongest response to this pheromone combination. It is estimated that about 48.7% of the insect had a strong response to the lure, and 51.3% of the insect show weak response. The male from NK ranked second with 43.4% of strong response. Male of KS population and BL followed with 36.0% and 30.2% respectively. ST males were the ones with lowest response activity to the pheromone bait.

For further investigation on male sexual response to pheromone, the male of GF population, a pesticide-susceptible population, and of KS, a pesticide-resistant population, were bioassayed under RH 75–85% and temperature, 25°C. Table 4 shows that the Weighting means of these two grous of DBM varied in the pheromone response. However, the difference between these two populations in response was statistically insignificant.

TABLE 4

Comparison of pheromone response of male adults of three malathion susceptible strins of diamondback moth

Insect population	Weighting mean	Repeats	F	$F_{0.05}$	LD_{50}
GF	7.14	7	3.92	19	45.39
IL	5.50	12	n. s.		24.95
KS	7.36	11			84.72

Fifteen to thirty 1.5 to 2.5 day adult males per assay.
Conditions controlled under 75–85% relative humidity and 25°C at dark.
LD_{50} of 4th instar larvae; from Maa and Guh (unpublished data).
F; observed value., $F_{0.05}$; theoretical value at p=0.1.
n. s.=Not significant.

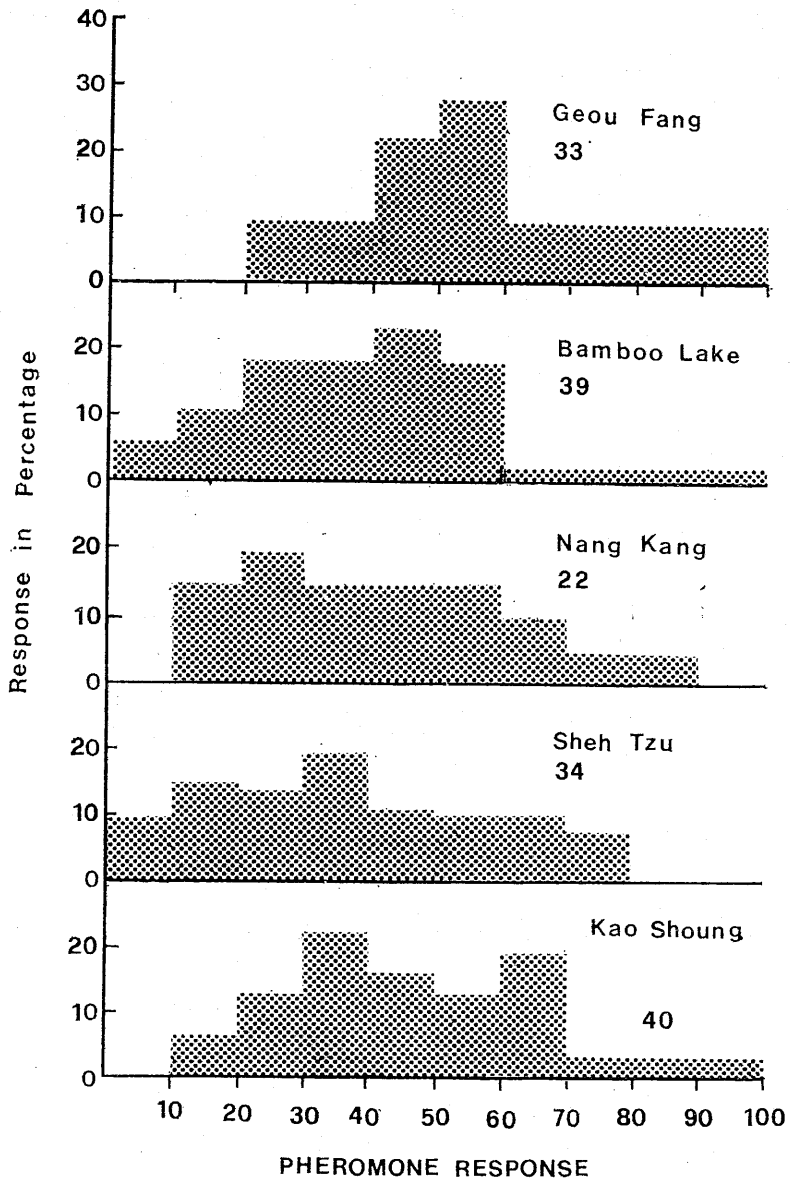


Fig. 4. Variation of male response to the synthetic female sex pheromone on five local DBM populations of Taiwan., 33, 39, 22, 34 and 40; numbee of replications of bioassay.

DISCUSSION

The unsaturated long-chain alkyl acetate, acted as one of the pheromone multicomponent was to be the most common compound in the lepidopterous insect. In case of DBM, we found that a high content of acetate pheromone was needed for optimal male catch in field. The ratio of the three components of the female sex pheromone was 7:3:0.1 of (Z)-11-hexadecenyl acetate, (Z)-11-hexadecenal, and (Z)-11-hexadecend (Maa *et al.*, 1984). Enzymatic degradation of acetate by antennal esterase or antennal hydrolase was found in other insect. In cabbage looper, *Trichoplusia ni*, the acetate pheromone was found enzymatically and nonenzymatically hydrolyzed to its alcohol (Ferkovich *et al.* ,1973).

Schneider (1970) has suggested that enzymes capable of degrading pheromone may play a double role, in olfaction on antennae, and on the bodies and legs of the insect to prevent a buildup of pheromone molecules in those regions. In case of DBM we found that the antennal esterase activity was much higher than the leg or wing esterase activity, indicating that antennal esterase might well be associated with the major catabolism of the pheromone molecule. It also suggested that the leg and wing are possibly not subjected to the pheromone stimulation. Nevertheless, we shall not exclude the possibility that the esterase of these appendages would carry out the cleavage of the pheromone molecules adhered to the epicuticule of these appendages. Evidence also showed that the activities of leg and wing esterase were not corresponding to the sexual maturation of the adult male. On the other hand, the enzymatic activity of male pheromone esterase was fluctuated in parallel with the male response to the FSP. The non-specific esterase activity was, however, not quite corresponding to the male response. In sense of substrate overlapping of different groups of esterase, it is difficult to discriminate the enzymatic activity of pheromone esterase from that of non-specific esterase. Our result, nevertheless, confirmed that the hydrolytic activity of antennal esterase was associated with the male sexual maturation and the sexual behaviour of the male adult. This was already reproted in cabbage looper (Taylor *et al.*, 1981).

The second finding of this study was the population-dependent variation of antennal esterase of the male DBM. The esterase was characterized as of B-type in majority. However, the components of the esterase varied depending on BDM population. They varied at least in two aspects including sensitivity to inhibitors and variation in zymograms of the esterase isozymes. For the first, the antennal esterase of DBM of ST and KS, the two well known pesticicide resistant group, showed less susceptibility to both eserine and PHMB. This hinted that antennal esterase of these DBM were more likely to be aliesterase in nature. A comparatively high hydrolytic activity of these antennal esterase to acetate pheromone was expected for these BDM. However, the fact revealed that the antennal esterase of the adult male of these two populations were with lowest titer to hydrolyze NA and pheromone. The strong resistance of these DBM to organophosphate pesticides, most of ester in nature, was possibly associated with enzyme systems other than esterase. Since mechanism of pesticide resistance of DBM was not fully understood currently, it is difficult to clarify whether the variation of male pheromone response was to be associated with the insecticide resistance.

Variation on isozyme is obvious in antennal esterase. The esterase zymogram of GF was found with 4 strong stainned bands of 9, 10, 11 and 12. These bands of antennal esterase isozymes from male of all the counter populations were faint in staining. On the other hand, the zymogram of TS males had denser color on band 5, 6, and 7 which were different from those of GF and the other populations either. The antennal esterase of KS males revealed another trend on its low activity to acetate pheromone, less dense bands, but with high enzyme titer to NA.

From these complex mentioned above, it is difficult to draw a clear line to clarify the interrelation between male pheromone response, esterase activity, and/or isozyme pattern of the antennal esterase of the male adult. These evidences, nevertheless, hinted that it is likely to have genetic diversity on DBM populations of Taiwan. Although, with respect to pheromone response, the male adults of pesticide-resistant population was statistically not discriminated from those of susceptible ones.

Acknowledgements: This research was supported by National Science Council, ROC, with grant no. NSC 72-0201-B001a-35.

REFERENCES

ASPERN, VAN K. (1962) A study of housefly esterases by means of a sensitive colorimetric method. *J. Insect Physiol.* **8**: 401-416.

BIGLEY, W. S. and F. W. JR. PLAPP (1960) Cholinesterase and aliesterase activity in organophorus-susceptible and resistant house flies. *Ann Ent. Soc. Am.* **53**: 360-364.

CHENG, E. Y. (1981) Insecticide resistance study in *Plutella xylostella* L. II. Ageneral survey (1980-81). *J. Agric. Res. China* **30**: 285-293.

CHIANG, J. J. H. (1972) Diamondback moth (*Plutella xylostella* L.) control and eradiation through sexual sterility induced by gamma radiation. *Bull. Soc. Entomol.*, Natl. Chung Hsing Univ. **9**: 31-42.

CHIANG, J. J. H. and C. J. W. MAA (1970) Gamma radiation sterilization on diamondback moth as a control measurement (in Chinese). on *Symposium on Control of Vegetable Pests.* Sept. 28-29, 1970. Taipei, Taiwan, ROC. (Abstract).

CHANG, F., KOU, R. and Y. S. CHOW (1979) The effect of prececene I and II on sex attractancy in the diamondback moth, *Plutella xylostella* L. *Procd. Natl. Sci. Council* **3**(1): 67-76.

CHOW, Y. S., F. M. LU., C. T. PENG and P. C. CHENG (1972) Isolation of lipids and sex pheromone from hard ticks. *Bull. Inst. Zool., Academia Sinica.* **11**(1): 1-8.

CHOW, Y. S., S. C. CHIN and C. C. CHIEN (1974) Demonstration of a sex pheromone of diamondback moth (Lepidoptera: Plutellidae). *Ann. Entomol. Soc. Amer.* **67**: 510-512.

CHOW, Y. S. and Y. M. LIN (1983) Pheromone in Taiwan. *Monogr. Inst. Botany., Academia Sinica* **5**: 135-140.

CHOW, Y. S., Y. M. LIN and C. L. HSU (1977) Sex pheromone of the diamondback moth (Lepidoptera: Plutellidae). *Bull. Inst. Zool., Academia Sinica.* **16**(2): 99-105.

DAVIS, B. J. (1964) Disc electrophoresis II. Method and application to human serum proteins. *Ann. B. T. Acad. Sci.* **121**: 404-427.

FERKOVICH, S. M., OLIVER, J. E. and C. DILLARD (1982) Pheromone hydrolysis by cuticular and interior esterases of the antennae legs, and wings of the cabbage looper moth, *Trichoplusia ni* (Hübner). *J. Ecol. Chem.* **8**: 859-866.

KOSHIHARA, T. and H. YAMADA (1976) A simple mass-rearing technique of the diamondback moth, *Plutella xylostella* (L.), on germinating rape seeds. *Jap. J. appl. Ent. Zool.* **20**: 110-114.

LEE, L. H. (1982) Unpublished data from annunal report of Sex Pheromone Control of Vegetable Pest of Taiwan.

LIN, Y. M. and Y. S. CHOW (1983) Field evaluation and stability studies of the synthetic pheromone of the diamondback moth, *Plutella xylostella. Monogr. Inst. Botany., Academia Sinica* **5**: 147-157.

MAA, C. J. W. and M. L. CHUANG (1983) Esterase of diamondback moth (*Plutella xylostella* L.) I. enzymatic properties of larval esterases. *Bull. Inst. Zool., Academia Sinica.* **22**(2): 123-131.

MAA, C. J. W. and Y. M. LIN (1983) Reevaluation of male catch efficience of synthetic female sex pheromone of diamondback moth, *Plutella xylostella* (L.) in northern Taiwan. *Plant Prot. Bull.* **25**(4): 293. (Abstract).

MAA, C. J. W., Y. M. LIN and Y. S. CHOW (1983) Variation of pheromone response and antennal esterase activity of the male diamondback moth (*Plutella xylostella* L.) of Taiwan. *Monogr. Inst. Botany., Academia Sinica.* **5**: 157-164.

MAA. C. J. W., Y. M. LIN and Y. S. CHOW (1984) Population variation of male response to female sex pheromone of *Plutella xylostella* L. in northern Taiwan. *Plant Prot. Bull.* **26**(3): 249-255.

MAA. C. J. W. and L. C. TERRIERE (1983) Age-dependent variation in enzymatic and electrophoretic properties of house fly (*M. domestica*) carboxylesterases. *Comp. Biochem. Physio.* **74**C: 461-467.

MANN, H. B. and D. R. WHITNEY (1947) "on a test of whether one of two random variables is stochastically larger than the other" *Ann. Math. Stat.* **18**: 50-60.

READ, S. M. and D. H. NORTHCOTE (1981) Minimization of variation in the response to different proteins of the coomassie blue G dye-binding assay for protein. *Analyst. Biochem.* **116**: 53-64.

ROELOFS, W. L. and L. B. BJOSTAD (1984) Biochemical intermediates and their relationship to sex pheromone components. XVII International Congress of Entomology. Abstract Volume p. 464. August 20-26. 1984. Hamburg, Federal Republic of Germany. (Abstract).

STEPHEN, W. S. and I. H. CHELDELIN (1970) Characterization of soluble esterases from the thoratic muscle of american cockroach, *Periplantela americana. Biochem. Biophys. Acta* **201**: 109-118.

TAO, C. C. (1973) A review of vegetable insect pests in Taiwan during seven years. (in Chinese). *Sci Agric.* **21**: 230-240.

TAYLOR, T. R., S. M. FERKOVICH and F. VAN ESSEN (1981) Increase of pheromone catabolism by antennal esterases after eclosion of the cabbage looper moth. *Experientia* **37**: 729-731.

YAMADA, H. and T. KOSHIHARA (1981) Interspecific attractiveness of female sex pheromone components of the diamondback moth, *Plutella xylostella* (L.), and their analogue to the stone leek miner, *Acrolepiopsis sapporensis* Matsumara. *Kontyu, Tokyo.* **48**(1): 104-110.

小菜蛾 (*Plutella xylostella* L.) 之酯酶

Ⅱ 雄成蟲觸角酯酶之特性

馬堪津　林玉美

五個不同地區蔬菜田中所採集到的各小菜蛾族羣，分別在室內飼養。取其雄成蟲在室內以Y-型嗅覺器作雌性費洛蒙的誘雄試驗。以萘酸乙酯及碳十四同位素標示的順-11-十六乙酸烯酯爲受質。來測定雄蛾觸角酯酶之活性。同時；利用聚丙醯胺膠粒泳動法分離雄蟲觸角酯酶作其類酶之比較研究。

結果發現，雄性小菜蛾對雌性費洛蒙之反應，因雄蟲本身蟲齡，或因不同族羣而有別。雖然因族羣之不同而生之差異在統計上是屬不明顯者。

另一方面，雄成蟲因年齡之不同，其觸角酯酶之活性之變異却與該蟲齡之雄成蟲所表現出之性反應有正的相關性。

觸角酯酶中某些類酯之活性，還可能與雄蟲對雌性費洛蒙之反應有關。就手頭現有資料而言，仍然很難在雄蟲性費洛蒙之反應上，觸角酯酶水解費洛蒙之活性上，及酯酶之類酶差異上找出一個明顯的區分點來。目前的證據也無法證實小菜蛾是否抗馬拉松與酯酶活性大小有任何關連。