

Ecological Approach to Male Diamondback Moth Response to Sex Pheromone

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Abstract

Natural populations of diamondback moth (*Plutella xylostella* L) were collected from five different vegetable fields and the response of males to a synthetic female sex pheromone was monitored using a Y-test in the laboratory throughout the year. Mass trapping of adult males using five blends of the pheromone was also conducted in the field. The antennal esterase activity of diamondback moth was monitored by the use of either 1-naphthylacetate or ¹⁴C (Z)-11-hexadecenyl acetate as substrates. In addition, zymograms of antennal esterase were studied by poly-acrylamide gel electrophoresis. Fifth instar larvae of the moth were monitored for malathion resistance. It was found that variations in the pheromone response of adult males to trinary of (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, and (Z)-11-hexadecen-1-ol, were age dependent. Assays of antennal esterase activity were positively correlated with the behavior of the male moth. Antennal esterase zymograms revealed 10 bands, each with different characteristics. The pheromone hydrolytic activity of antennal esterase of male adults had a high titer which was coincident with a high rate of malathion degradation activity in the larval stage of the moth. However, this is not interrelated with a strong pheromone response by male adults. Two cycles of male response alternated during the year. Relative humidity was possibly the major factor affecting the male response to the pheromone blends. Temperature may play only a minor role in the response in tropical Taiwan. A pheromone blend of 3:7:0.1 (Ald:OAc:OH) was recommended for use in male trapping in subtropical areas.

Introduction

Organic pesticides are invaluable in suppressing damage to agricultural products. However, the side effects of these chemicals on the environment have become a serious problem over the past decades. In addition, the rapid development of resistance, especially to insecticides, in target insect pests makes control more difficult. This has imposed pressure on scientists to find alternatives that do not depend on toxic agents. This in turn has led scientists to delve into biological and biochemical transformations within the insect system.

Sex pheromones of lepidopterous insects were introduced as biorational chemicals for pest management programs in Taiwan in the early seventies (Chow et al 1973). The female sex pheromone (FSP) of diamondback moth (DBM), *Plutella xylostella* L (Lepidoptera: Yponomeutidae), was first isolated by Chow et al (1974). Tamaki et al (1977) then identified the pheromone as a mixture of (Z)-11-hexadecenal (Z-11-16Ald) and (Z)-11-16-hexadecenyl acetate (Z-11-16:OAc). Chow et al (1977) confirmed that the best attractant ratio of the two compounds in the field was from 1:1 to 1:3 at a concentration of 10 µg in Taiwan. Chow et al (1978) announced that when methyl (Z)-11-hexadecenoate was added at low concentrations to the mixture of Z-11-16:Ald

and Z-11-16:OAc in a ratio of 1:1, a synergistic effect was displayed in field screen tests. Later Ando et al (1979) found that attractiveness in the field of the 1:1 mixture of Z-11-16:Ald and Z-11-16:OAc at 10 μg was obviously enhanced by adding 1-10% of (Z)-11-hexadecen-1-ol (Z-11-16:OH). Chisholm et al (1979) in Canada found that a combination of Z-11-16:Ald, Z-11-16:OAc, and Z-11-16:OH in a ratio of 7:3:0.1 in 100 μg achieved the optimum male catch. The catch could be enhanced by the addition of 0.02 part of Z-9-14:OAc (Chisholm et al 1983). In Taiwan, Chow et al (1983) reported that a blend of Z-11-16:Ald, Z-11-16:OAc, and Z-11-16:OH in a ratio of 5:5:0.1 was most attractive to the male adults in the field. Re-evaluation of pheromone blends for achieving high male catch revealed that there was considerable variation in responses to a range of ratios (Maa and Lin 1983). A blend of 3:7:0.1 (Ald:OAc:OH) was found to be the most efficient for mass trapping in the field in Taiwan (Maa et al 1984, 1985d). In Japan, ratios and dosages of the blend varied with the crop growing seasons. Temperature was proposed as the major factor affecting mating behavior and the catch of male adults, as in other lepidopterous insects (Roelofs 1978, Yamada and Koshihara 1980). Variations in the response of male adults to DBM pheromone observed in Taiwan, Japan, and Canada may be due to exogenous factors such as seasonal variations of environmental conditions or to endogenous factors including the genetic differences in the insect itself. This article summarizes the author's work on the variables that were possibly associated with male response to synthetic FSP of DBM in Taiwan.

Materials and Methods

Insects

DBM larvae were reared under a constant temperature of $25 \pm 1^\circ\text{C}$, and photoperiod condition of L:D, 14:10. Larvae were fed on rape seedlings (Koshihara and Yamada 1976) and adults on 20% honey-water (Chow et al 1975). Stocks of newly emerged adults within 12 h period were collected for several days. Male adults of different ages, ranging from 12 h to four days, were bioassayed simultaneously to study the age-dependent variation in pheromone response. For other experiments, male adults aged between 1.5 to 2.5 days were used (Maa et al 1983).

Chemicals

The pheromone components Z-11-16:Ald, Z-11-16:OAc and Z-11-16:OH, used for the bioassay, were synthesized and were of 98% purity as evidenced by GC tests (Lin and Chow 1983b). Blends of the three components in ratios of 1:9:0.1 to 9:1:0.1 (Ald:OAc:OH) were used in both laboratory bioassay and field tests. The doses and blends of the pheromone used depended on the purpose of the individual experiment. The isotope-labeled pheromone Z-11-16:OAc was also synthesized for enzyme assay to monitor the hydrolytic activity of male antennal esterases. All chemicals and reagents were of analytical grade or at the best grade available.

Laboratory bioassay and field test

The pheromone response of male adults to the synthetic FSP was measured according to the procedure of Chang et al (1979). The laboratory bioassay experiments included: (1) assay on age-dependent variations of male response to the pheromone, (2) assay of the optimal blend(s) of the three pheromone components, (3) assay of seasonal variation in male response to the pheromone, and (4) assay of the effect of relative

humidity (RH) and temperature on the pheromone response. For the first and the third experiments, a blend of 5:5:0.1 (Ald:OAc:OH) in 1.0 μg portions dissolved in hexane were used. For the second experiment, pheromone blends of 1:9:0.1 to 9:1:0.1 (Ald:OAc:OH) were used. The blends of the pheromone mixture which achieved a good male catch were used for field tests. For the fourth experiment, pheromone blends of 3:7:0.1, 5:5:0.1, and 7:3:0.1 (Ald:OAc:OH) were used.

The data from the first experiment were analyzed according to Mann and Whitney (1947). Data from the third and the fourth experiments were analyzed according to the equation of the least square and the best linear unbiased estimator according to the theorem of Gauss-Markov (Mendenhall 1981). The results of the third experiment were re-evaluated in the field in Hualien and Yeongjing. Blends of 3:7:0.1 to 7:3:0.1 pheromone mixture were further prepared with the addition of 200 μg butylated hydroxytoluene (BHT) for field tests according to the procedure of Lin and Chow (1983). Fifty μg of each pheromone blend with BHT was then injected into plastic microtubes each with one end sealed. The microtubes were fastened under the top of the sticky paper pheromone trap. Experiments were conducted in 100 or 64 sq m vegetable plots using 5 x 5 or 4 x 4 latin square design. The traps in the treatment plots, using a pheromone blend of 8:2:0.1 (Ald:OAc:OH), were hung around the experimental plots. Moth catches were checked every seven days continuously for two to five weeks. The traps and baits were replaced after every check. The experiments were repeated at least twice. The results of the field tests were analyzed according to Duncan's multiple range test.

Daily weather records were transcribed from the daily report of the Central Weather Bureau, Republic of China. Daily temperatures and mean RH were used as the major physical parameters to evaluate the impact of climate on the response of the male adults to the pheromone.

Enzyme preparation and enzyme assay

The appendages of the male insects were excised, homogenized, sonicated, and centrifuged (Maa et al 1985). The supernatant of the preparation was compared with the crude homogenate of the antennae to determine esterase activity. The protein contents of the two different preparations were determined according to Read and Northcote (1981). General esterase assay followed the method of Van Asperen (1962). Eserine, paraoxon, and parahydroxymercuribenzoate were used as inhibitors to characterize the antennal esterases (Bigley and Plapp 1960, Stephen and Cheldelin 1970). Pheromone esterase assay followed the method of Maa et al (1985).

Electrophoresis of antennal esterases

The supernatant of 1000g homogenate of insect antennae was examined electrophoretically according to the method of Davis (1964). Inhibitors were also used to characterize biochemically the isozyme bands of the antennal esterase according to the procedure of Maa and Terriere (1983).

Results

Age-dependent variation of pheromone response and antennal esterase activity

Figure 1 shows that the activity of the antennal esterase to naphthylacetate was at its lowest level at adult emergence. Activity increased thereafter, climbing to the highest level during the next 24 h, and staying at the same level for the next two days. On the

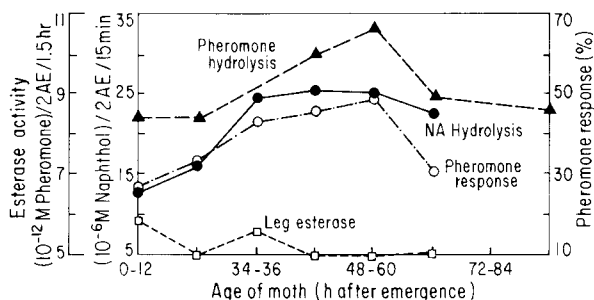


Figure 1. Variation of antennal and leg esterase activity and pheromone response of male DBM (source: Maa et al 1985a)

other hand, the activity of pheromone esterases was low when the male adults emerged. Activity increased gradually during the next 24 h, peaking at 12th hour and decreased again to the initial level later on. The fluctuations of the pheromone response of male adults and of pheromone hydrolytic activity of male antennal esterase were differentiated in parallel with each other during the ontogenesis of the male adult. The leg esterase activity did not exhibit a parallel relationship with the male pheromone response, nor did with antennal esterase activity, throughout the period of investigation. The leg esterase activity ranged from 4.3 to 5.5 μ mole naphthol produced per two leg equivalent which is about one-third to one-fifth the activity of the antennal esterase.

Comparison of enzyme activity of the five DBM populations

Activity of the general esterase of the antennae of Geoufang, Bamboo Lake, and Kaohsiung males were high, and that of Shehtzu low (Table 1). The pheromone-hydrolytic activity of the male antennal esterases were 13 pico mole/mg protein/1.5 h for Geoufang; 10 for Bamboo Lake, Nankang, and Shehtzu, and 7.6 for Kaohsiung. There is a two fold difference in antennal non-specific esterase activity between the males of Geoufang or Bamboo Lake, and those of Nankang or Shehtzu. However, pheromone hydrolytic activity of antennal esterases of the males of these four populations display similar titer. The differentiation of these two groups of enzymes indicates that these two enzyme systems might be equally associated with pheromone degradation. The high titer of aliesterase found in larvae of the two malathion-resistant strains seems incidental to the high level of antennal esterase activity. The results of enzyme inhibition studies revealed that the major components of the antennal esterase of male adults are of aliesterase type (Table 2).

Table 1. Comparison of antennal esterase activity of the male DBM

Insect population	Esterase activity		Pesticide resistance LD ₅₀ μ g malathion/larve
	$\times 10^{-6}$ M naphthol produced/mg antennal weight/10 min.	$\times 10^{-9}$ M pheromone metabolized antennal weight/1.5 h	
Geoufang	334.4 \pm 18.7 ^a	13.1 \pm 0.6 ^b	45 ^c
Bamboo Lake	268.2 \pm 11.5	10.4 \pm 0.1	—
Nankang	156.2 \pm 14.8	10.3 \pm 0.6	33
Shehtzu	139.4 \pm 17.1	10.2 \pm 0.1	84
Kaohsiung	302.1 \pm 11.6	7.6 \pm 0.1	91

^a Two assays with triplicate samples; mean \pm standard deviation; crude homogenate of eight antennae per assay; incubation at 28°C (source: Maa and Lin 1985). ^b Two assays with two to four replicates each, mean \pm standard error; crude homogenate of 100 antennae each at 30°C (Source: Maa and Lin 1985). ^c Mean of four replicates (Source: Maa et al 1985a).

Table 2. Effect of inhibitor on antennal esterase activity of the DBM

Insect population	Inhibition (%)		
	Eserine	Paraoxon	PHMB
Geoufang	84 ^a	90	74
Nangkang	74	90	77
Bamboo Lake	74	90	71
Kaohsiung	73	90	60
Shehtzu	70	90	57

^a Average of two experiment, each with three replicates. (Source: Maa et al 1985a)

Comparison of esterase activity of appendages

Table 3 shows that foreleg esterase enzyme activity is one-tenth that of antennal esterase activity when naphthylacetate is used as a substrate. Table 3 also shows that pheromone hydrolytic activity of antennal esterase is about four times as strong as that in the legs, and six times that in the wings. This result strongly suggests that the antennae, organs of pheromone reception and degradation, are appendages equipped with an efficient enzyme system to break down an adhesive molecule like Z-11-16:OAc. The cuticles of the wing and foreleg, with rather larger surface areas exposed to the atmosphere, have only limited pheromone degradative activity.

Table 3. Comparison of esterase activity of appendages of male DBM

	Esterase activity	
	$\times 10^{-6}$ M naphthol ^a produced/mg protein/30 min.	$\times 10^{-9}$ M Pheromone ^b metabolized/mg protein/1.5 h
Antennal	1417 + 46	9.14 + 0.83
Forelegs	137 + 23	2.36 + 0.01
Wings	—	1.57 + 0.08

^a Six experiments with triplicates; mean \pm standard error. Supernatant of 1,000g.

^b Two experiments with duplicates; mean \pm standard error. Crude homogenate. (Source: Maa et al 1985a)

Polymorphism of antennal esterase

Antennal esterase of male DBM was resolved into isozymes by electrophoresis (Figure 2). Eleven to twelve esterase bands, depending on the age of the adult or origin of the population of the insect, were detected in zymograms. These bands were scattered throughout two zones of the gel, a fast moving upper zone and a slightly slower moving middle one. All the isozymes migrated to the cathode end of the gel. The fast moving zone, composed of one narrow and one wide bands, was detected in all the zymograms examined. The middle zone was composed of nine to ten bands. These bands varied in number, width, and density. Two faint bands in front of the main body of the middle zone were also present in all zymograms. These two faint bands and the two fast moving bands were consistent in appearance, while the other bands varied depending on the males investigated.

The zymograms of the five insect populations could be roughly grouped into four categories according to the patterns of isozymes in the gels. The zymograms of Nangkang and Bamboo Lake strains were characterized by a single dense band in the median area; the Kaohsiung strain by two; the Shehtzu strain by three and Geoufang by four. The

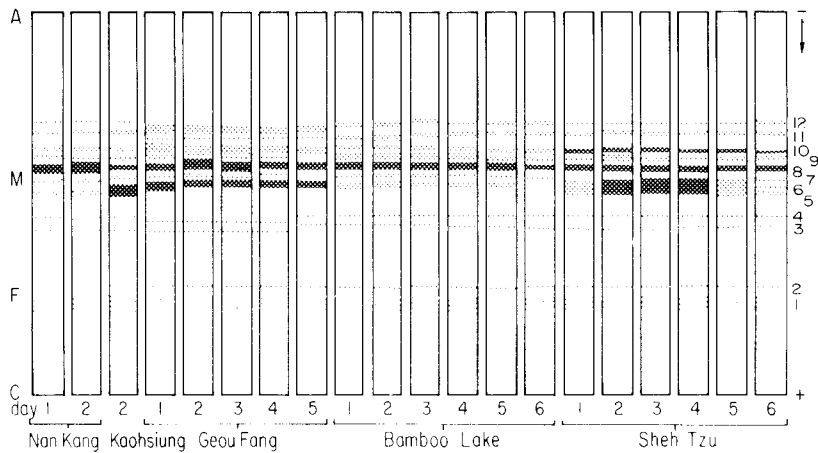


Figure 2. Zymogram patterns of antennal esterases of male DBM adults. Zymogram code: A, anode end; M, medium bands; F, fast moving bands; C, cathode end; and d, age of the moths, days (source: Maa et al 1985a)

variation of these dense bands is believed to be associated with the hydrolytic activity of antennal esterase to the acetate pheromone. The age-dependent variation of esterase isozymes shows that band No. 8 was the most dense and consistent one. This band was at its widest when the male adults were of two days old, the age when insects are most sensitive to the pheromone. On the other hand, the four-band pattern, with No. 8 the widest band, was characteristic of Geoufang male antennal esterase zymograms, which coincidentally corresponded to the high hydrolytic activity to both naphthyl acetate and acetate pheromone. These results might explain why Geoufang males were the ones with most active pheromone response to the synthetic material (Table 4). However, this case demonstrates a positive interrelation between naphthylacetate activity and pheromone hydrolytic activity of antennal esterase, and the pheromone response of males to the bait.

Table 4. Variation of pheromone response of male DBM adults of four different local populations collected in Taiwan

Insect populations ^a	U values ^b for male adults of		
	young stage (0-24 h)	mature stage (36-60 h)	aged stage (80-100 h)
BL vs KS	15	82.0	17.0
BL vs NK	3	43.5	6.0
BL vs GF	12	44.5	4.0
NK vs GF	8	34.5 ^c	4.0
KS vs GF	28	40.5	9.0
KS vs NK	8	51.0	15.5

^a BL: Bamboo Lake. KS = Kaohsiung. NK = Nankang. GF = Geoufang. ^b Mann-Whitney U-test: 15-30 males per assay. ^c Significant at $p > 0.05$. Degree of freedom: 0-24 h: KS 7; NK 2; GF 4; BL 3. 36-60 h: KS 12; NK 6; GF 6; BL 10. 80-100 h: KS 7; NK 4; GF 2, and BL 3. Ratio of Ald to OAc in lure, 5:5. (Source: Maa et al 1983)

Insecticide resistance and pheromone response

Bioassay on two insecticide-susceptible strains (Bamboo Lake and Geoufang) and one resistant strain (Kaohsiung) did not show any detectable variation of the pheromone response of male DBM adults to the 5:5:0.1 (Ald:OAc:OH) blend when the temperature was set at $25 \pm 2^\circ\text{C}$, and the RH at 80% (Table 5). It is proposed that variation of male response to the different pheromone blends observed in the field (Table 6) is unlikely to be due to genetic differences between the DBM of individual field populations. Although polymorphism of antennal esterase isozymes was observed (Figure 2), we should not exclude the potential influence of endogenous factors on the male adults. Besides, the level of stimulation by a pheromone to the male adult in the laboratory under controlled conditions does not necessarily correspond to the situation in the field. More work is needed before we can reach a final conclusion on this. The influence on male sexual behavior of other external factors, like temperature and RH, therefore needs to be clarified.

Table 5. Comparison of pheromone response of the male adults of three different malathion-resistant strains

Ratio of Ald to OAc in lure	Insect Population		
	Geoufang (GF)	Bamboo Lake (BL)	Kaohsiung (KS)
	Value of weighted mean		
5:5	7.14	5.50	7.36

Degrees of freedom: GF, 7; BL, 12; KS, 11. The difference is not statistically significant. LD₅₀ of malathion to DBM larvae in $\mu\text{g}/\text{larva}$: GF, 45; BL, 25; KS, 85 (Source: Maa and Ying 1985)

Table 6. Effect of humidity on pheromone blend preference of DBM male adults in field tests

Test No.	Ratio of Ald to OAc in lure			
	2:8 ^a	3:7	4:6	5:5
	Relative Humidity (%)			
1 ^b	74.5 ^c	80.0	86.8	86.7
2	68.1	84.9	88.7	—
3	73.0	76.7	—	—
4	67.8	81.7	—	—
Mean	71.6	82.7	86.3	86.7

^aWith addition of 0.1 part of alcohol component in the lure. ^bEach test composed of one to two field tests, Latin square design (5×5 treatments), continuously checked for two to five weeks. ^cAverage RH of 4 to 7 days interval, transcribed from report of Central Weather Bureau, Taiwan, ROC. (Source Maa and Ying 1985)

The bioassay and the field tests

Bioassay of male response to nine sex pheromone blends revealed (Table 7) that the blends with high aldehyde content attracted comparatively fewer males. The five best choices out of the nine combinations were 3:7:0.1, 4:6:0.1, 5:5:0.1, 6:4:0.1, and

7:3:0.1 (Ald:OAc:OH). For temperature and RH effect studies in the laboratory, a blend of 5:5:0.1 (Ald:OAc:OH) was used.

Table 7. Laboratory evaluation of male adults responding to nine combinations of female sex pheromone^{a,b}

Ratio of Ald to OAc	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
% males attracted	15 ^c	11 ± 6	41 ± 22	35 ± 14	30 ± 14	23 ± 16	33 ± 11	29 ± 12	27 ^c

^a Four replicates. ^b 15-30 males per assay. ^c Average of two experiments. (Source: Maa et al 1984).

Effect of temperature and RH on pheromone response

Indoor assay showed that the interaction of monthly RH and adult male orientation response to the pheromone baits was linear, with interception at RH 82.6% (Figure 3). The figure also shows that RH over the interception point of 82.6% would cause a decrease in male response. Temperature and male response also had a linear interrelationship with an interception at 18.4°C on the axis. Temperature over this critical point would have negative impact. Thus bioassay with 5:5:0.1 (Ald:OAc:OH) blend at 18.4°C and 82.6% RH would be ideal. These conditions, however, are not necessarily standard for all blends in respect of optimal male response in the laboratory. In fact, male sexual behavior interaction to three different blends (Table 8) shows that there is a linear interaction of RH and the Ald:OAc ratios of the pheromone. When RH drops below 82.8% a blend of 5:5 (Ald:OAc) is no more effective than above this level.

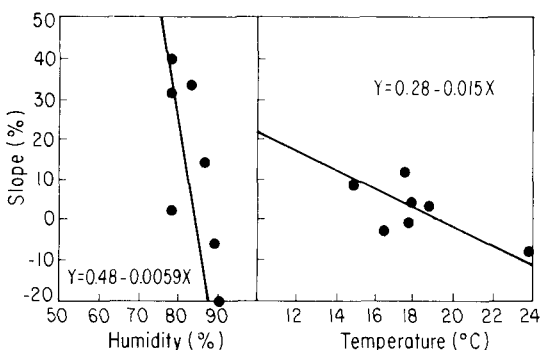


Figure 3. Influence of relative humidity and temperature on male orientation response

Table 8. Effect of relative humidity on the pheromone blend preference of male DBM in laboratory assay

Ratio of Ald to OAc in lure	Statistical factors			
	slope	confidence interval	degrees of freedom	alpha value
7:3 ^a	0.031	81.1-87.2	11	0.1
5:5	-0.055	77.9-83.9	65	0.1
3:7	-0.058	73.0-78.0	19	0.1

^a Addition of 0.1 part of alcohol component (Source: Maa and Ying 1985)

Effect of physical field conditions on male pheromone response

In order to find out how the RH and Ald:OAc ratio of different blends affect DBM male sexual behavior, these two variables were monitored in relation to the optimal male catch in field. Fifteen tests were carried out around Taiwan. Eleven tests proved valuable for analysis. The other four tests involved males showing preferences for more than one pheromone blend, and were therefore not taken into account for the analysis. As for the 11 tests, in one of them the males preferred the 5:5 blend, and in two others the 4:6 blend, when the RH was above 90%. In four cases the male adults were attracted by a 3:7 blend when the RH was around 78% to 85%. In another two cases the males were ensnared by the lure of a 2:8 blend when the RH ranged from 68% to 75%. Two other cases revealed no significant preference for any lure of of between 3:7, 4:6, 5:5, and 6:4 (Ald:OAc) blends when the RH was around 68% to 75%.

Seasonal variation of male response to the pheromone

A year-round bioassay with a synthetic sex pheromones of 5:5 blend showed seasonal variation of male response to the lure. Figure 4 shows that the males responded weakly to the synthetic pheromone during winter and summer (Maa et al 1985c), but responded strongly during spring and autumn. Generally, the insect is inactive when it is cold. The pheromone response of the males to the bait gradually increased when the daily temperature and the photoperiod increased during early spring. One out of 14, 18, and 16 bioassays performed respectively during December, January, and February, showed a strong male response to the lure. The frequency of strong response increased to eight out of 16 assays performed during March and 10 out of 23 in April. A few assays conducted during May, June, and July showed distinctly reduced effectiveness of the synthetic material. In August, the DBM were scattered and reduced in number in the vegetable fields of Shehtzu and the Taipei Basine and they were non-responsive to the pheromone (Figure 4). The males became active again in late autumn. Fourteen out of 27 assays showed that the males had a strong response to the lure in September,

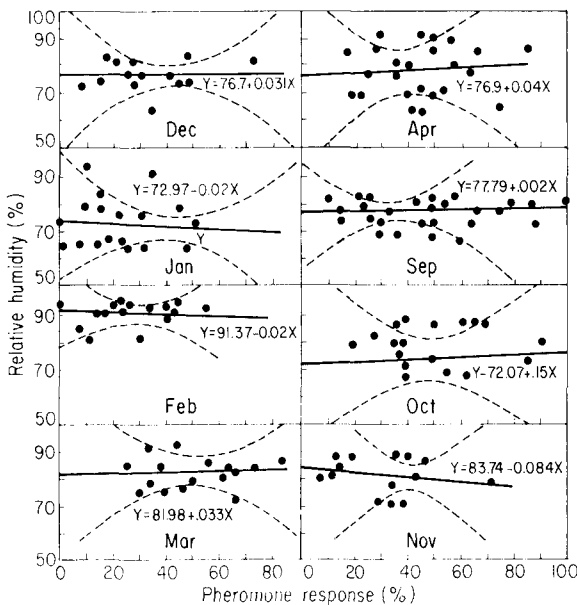


Figure 4.
The interactions of daily relative humidity and orientation response of male adults to synthetic female sex pheromone of DBM (source: Maa et al 1985b)

and nine out of 18 in October. This, however, did not last long. For an unknown reason, the synthetic sex pheromone lost its effectiveness in November.

Temperature, which is generally recognized as a major factor affecting the mating behavior of male adults of many lepidopterous species, nevertheless had a limited influence on the orientation of males to the lure (unpublished data). The pheromone response was low during December, January, and February, when the temperature was low. In September, the monthly average temperature rose to 28°C, and the insect became active. In March, the monthly average temperature declined to 15°C, but the insect was still active to the pheromone. The strong pheromone response of the male adults at either high or low temperature thresholds shows that temperature conditions in this island have a limited regulating effect on the sexual behavior of DBM males.

Discussion

Variations of male adult response to synthetic female sex pheromone of DBM have been reported in Taiwan (Chow et al 1977), Japan (Ando et al 1979), and Canada (Chisholm et al 1979). It was also noticed that the male moths responded to the lure differentially depending on not only the combinations of the trinary components of the pheromone, but also on the concentration of the pheromone used in the bait. In Japan, 100 µg of pheromone was needed for maximum catch in spring and early summer, and 1.0 mg in winter (Yamada and Koshihara 1980a). In Canada, 100 µg proved sufficient for trapping (Chisholm et al 1979). In Taiwan, 50 µg of the pheromone would do the job (Lin and Chow 1983). Koshihara and Yamada (1981) interpreted their findings on the differential response of DBM males in summer and winter to the vapor pressure of the pheromone in the specific atmosphere of these seasons. In Japan it is reported that the mating of DBM mostly depended on ambient temperature (Yamada and Koshihara 1980b).

The weather and the climate of the three countries mentioned above are different from one another. It is reasonable to postulate that these independent variables in the environment of the insect's habitat would eventually affect male sexual behavior elicited by the pheromone. However, certain questions still remained unanswered. For example, how would one explain the variation of male response to different blends of the synthetic sex pheromone observed in areas of the same geographic zone, as well as in different geographic zones? Will the variation prove to be rooted in the genetic diversity of the individual insect populations investigated, as in the case of the European cornborer (Carde et al 1981, Klun and Cooperators 1975), or will it prove to be a result of the moth perceiving the blend at a variety of different thresholds under the influence of different environmental conditions? Another intriguing question concerning the use of synthetic pheromones for mass trapping is to what extent the insecticide resistance will influence the DBM's response to synthetic sex pheromone under variety of environmental conditions since this species has developed degrees of resistance to numerous insecticides (Cheng 1981). As evidence the following facts are offered.

Age-dependent variation and antennal esterases

The profile study of male adult response to the pheromone showed that the response fluctuated in parallel with esterase activity, especially with that of pheromone-degrading esterases of the antennae, when the male adults were between 1.5 and 2.5 days old. Male pheromone response also peaked at this time. The coincidence of pheromone response and antennal pheromone esterase activity of the male DBM indicates that the antenna, as an important olfactory organ, is specific to pheromone reception and

degradation in order to promote normal transmission of the biochemical message through the peripheral nervous system. The leg and the wing, on the other hand, with low titer of esterase activity, are comparatively less specific to the pheromone stimuli. It is known that these organs in the cabbage looper (*Trichoplusia ni*) also have a considerable part to play in hydrolyzing the adhesive pheromone molecule (Ferkovich et al 1982). Nevertheless, the possibility cannot be excluded that esterases of these organs in DBM do not carry on the degradation of the pheromone molecule adhered on the cuticle of these organs.

Another aspect of antennal esterases of DBM is the heterogenous nature of these enzymes. This includes the polymorphism of isozyme patterns and the variation in general and pheromone esterase activity in particular. The activity of pheromone esterase of male adults collected in northern Taiwan is in general of similar titer. This is comparatively higher than in those collected in Kaohsiung in southern Taiwan. The general esterase activities of the male antennae, however, varied between the two regions by a factor of two. Besides, results of general esterase inhibition revealed that the isozymes of the antennae, although susceptible to paraoxon inhibition, are more or less heterogeneously responsive to other inhibitors.

From this complexity, it is difficult to clarify the interrelationship between male pheromone response, esterase activity, and/or isozyme pattern of the antennal esterases of the males investigated. It would appear that a potential diversity of genetic intrigue is possessed by males of DBM populations, although it was apparent that the pheromone response of males of malathion-resistant populations were not significantly different from that of the susceptible one.

At the same time it was found that variation in esterase isozymes of different stages of adult males of the same DBM clone is correlated with adult development. For example, two-day old males from Geoufang showed strong response to the bait. The antennal esterase activity of the males of these insects was also greater in degrading acetate pheromone, the substrate. Meanwhile, the zymogram bands of isozymes of antennal esterase of the adults of the same age were also distinctly stained. These facts indicated that zymogram studies would be useful for monitoring the sexual maturation of the male adult, although we were not able to define the so-called 'pheromone esterase' (Taylor et al 1981) in DBM. More detailed work is obviously needed for a fuller understanding of the pheromone perception in DBM.

Seasonally dependent variation and physical conditions

The seasonal variation of pheromone response of male adults to their female sex pheromone is reported for many insect species. Roelofs et al (1972) found that trans-11-14:OAc which is most effective on the male adults of European cornborer of the first brood during June and July, becomes less effective to moths of the second brood during August and September. They suggested that the cornborers present in large numbers in August employ a different communication system. Stech et al (1982) also reported that the synthetic pheromone of W-marked cutworm, *Spaelotica clandestina* (Harris), was unattractive during June and July but became attractive during August and September. Data of bioassay studies presented in previous papers (Maa et al 1985a) reveal that the male adult of DBM responds fairly well to the synthetic pheromone during spring and autumn, but becomes inactive during winter and summer. It is possible that the variation is due to a change in the intraspecies communication system. It is also possible that it could be due to the change in the physical conditions of the habitat of the insect. Results of field test and bioassay (Maa et al 1984, 1985a, 1985d) reveal that variation of male pheromone response due to the change in physical conditions is more

likely the case with DBM. This is supported by three sets of experimental data described in the following paragraphs.

Firstly, male DBM show optimal response to a pheromone blend of 5:5:0.1 (Ald:OAc:OH) under the conditions of 80% RH and 18°C temperature (Maa et al 1985c). RH over this limit has a negative impact. This indicates that a fixed pheromone blend has a limited stimulation threshold under fixed conditions. Secondly, the male preference to the pheromone shifts to the combination of 3:7:0.1 (Ald:OAc:OH) when this blend is available. This is apparent both from laboratory assays and in field tests (Maa et al 1985d). The RH for optimum response to this blend was 82% for laboratory assays and 78% to 85% for field tests. The third piece of evidence comes from the results of field tests (Maa et al 1985b). Of 15 field tests, eleven showed that male adults significantly ($P = 0.01$) preferred certain kinds of pheromone blend. The preference was mostly dependent on the RH. In general, the higher the RH the higher the aldehyde content of the pheromone needed for optimal male catch. The converse is also true. Certainly there are higher and lower threshold limits for the broad range of pheromone component ratios. This ratio range was estimated to be between 2:8:0.1 and 5:5:0.1 (Ald:OAc:OH) for DBM in the field in Taiwan. This range corresponds to an RH from 50% to 90%. In high RH conditions, pheromone blends with higher aldehyde display lower efficacy. Consequently, such a blend would lose its competitiveness to the natural blend released by the ambient population of virgin females in the field.

In Japan the pheromone with aldehyde and acetate in ratios of 1:9:0.1, 2:8:0.1, 3:7:0.1, and 4:6:0.1 lured 6, 31, 30, and 47 male adults respectively (Koshihara and Yamada 1980, Yamada and Koshihara 1980). This indicated that increasing the amount of aldehyde would initiate a positive stimulus to the male adults. On the other hand, aldehyde and acetate in ratios of 5:5:0.1, 6:4:0.1, 7:3:0.1, 8:2:0.1, and 9:1:0.1 attracted on an average, 34, 27, 23, 15, and 5 males respectively. This indicated that the male catch from the pheromone decreased with an increasing amount of aldehyde in the lure. In Canada, the male DBM was found to favor a pheromone combination of 7:3 (Ald:OAc) (Chisholm et al 1979). A combination of 9:1 (Ald:OAc) was able to lure only a few adult males. It seems that an aldehyde content over 80 to 90% of the total amount of pheromone would initiate an inhibitory effect on the sexual behavior of the male adults. It is interesting to note that a blend of high aldehyde content is needed for optimal male catch in Canada, but lower aldehyde content is needed in Taiwan.

In Taiwan, with its humid and warm subtropical weather, the male DBM adults respond to a blend of comparatively high acetate content. When the humidity is nearly 100%, a blend of 4:6:0.1 (Ald:OAc:OH) lures the majority of the males in the field. A blend of 5:5:0.1 (Ald:OAc:OH) is effective if there is no unusual change in temperature at dusk. Blends with ratios of 6:4:0.1 and 7:3:0.1 (Ald:OAc:OH) could not catch great numbers of adult males in the field. These blends are weak lures for male DBM adult in Taiwan. It is not known whether very dry and cold conditions, such as those which exist in Canada, drive the male adults to a pheromone blended with high aldehyde content. How is it possible to interpret this pheromone activity under the 'Hypothesis of Activation Threshold' suggested by Roelofs (1978), when one considers that the active space of sex pheromone of DBM in the field is only about 1.0 meter (Ishii et al 1981)? Could it possibly be another case of geographic variation with sibling species, such as the European cornborer found in North America and Europe (Klun et al 1975; Carde et al 1978)? These and other questions are worth further exploration.

Although no routine check is made on the component ratios for female-released pheromones for each season, the author suspects the possibility of some change in the pheromone secretions of the female adults.

In conclusion, it is interesting to note that the ratio of pheromone blend for optimal male catch varied in Taiwan, Japan, and Canada on a broad range from 3:7:0.1 to 7:3:0.1

(Ald:OAc:OH). The weather and the climate of these three countries are different from one another. Global cooperation on the investigation of DBM pheromone would possibly be helpful in explaining this insect's response to pheromones.

Investigations of the endogenous and exogenous variables encountered by DBM males are of utmost importance both theoretically and practically. Their importance in integrated pest control research will be evident if we consider the utilization of pheromones in the prediction of insect epidemics or in their mass trapping or mating disruption to control DBM.

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