Bull. Inst. Zool., Academia Sinica **24**(1): 75-84 (1985)

TEMPERATURE AND HUMIDITY EFFECT TO SEX BEHAVIORS OF THE MALE ADULTS ELICITED BY THE SYNTHETIC SEX PHEROMONE OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.)[1]

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(Received September 27, 1984)

William Can-Jen Maa, Yuh-Meei Lin and Yui-Jang Ying (1985) Temperature and humidity effect to sex behaviors of the male adults elicited by the synthetic female sex pheromone of the diamondback moth, *Plutella xylostella* (L.). *Bull. Inst. Zool., Academia Sinica* **24**(1): 75-84. The male diamondback moth, *Plutella xylostella* (L.), was bioassayed to the synthetic female sex pheromone: (Z)-11-hexadecenyl acetate, (Z)-11-hexadecenal, and (Z)-11-hexadecenl-ol in a ratio of 5:5:0.1 by a Y-test apparatus. The assay was carried on throughout the whole year to explore the influence of the climate factors to the male response elicited by the synthetic female sex pheromone. The optimal relative humidity for the male response to the pheromone was estimated at around 75-85%. Over this range would cause a decreasing pheromone response of the male adults. Daily average temperature also affected the male behaviors to the bait with comparative less importance. The optimal temperature was estimated at 18.6°C. The male response to the bait fluctuated seasonally through the year. The response was comparatively strong during Spring and Fall and was weak during the other seasons. The climate variables that affected the male response was discussed. Besides, seasonal variation of male response to the pheromone was also discussed.

Willmer (1982) indicated that climate plays a critical role in the life of terrestrial insects. He mentioned that climate conditions affected the geographical and ecological distributions, the site and timing of activities of an insect. Reports of geographical diversity of male response to the female sex pheromone (FSP) of diamondback moth, *Plutella xylostella* (L.) were found in Japan (Koshihara *et al.*, 1978; Yamada and Koshihara, 1980), Taiwan (Chow *et al.*, 1977), and Canada (Chisholm *et al.*, 1979). In Japan, it is reported that the blend of optimal male catch of the moth was a mixture of (Z)-11-hexadecenyl acetate, (Z)-11-hexadecenal, and (Z)-11-hexadecen-1-ol in a ratio of 5:5:0.1 was the best combination for male catch in the field In Canada, combination of 3 acetates with 7 aldehydes for the optimum male catch was reported. In Taiwan, population variation of male response to synthetic FSP of this insect species was recently reported (Maa *et al.*, 1984). The optimal blends of the trinary mixture of the female pheromone for male catch were varied from 5:5:0.1, 4:6:0.1 to 3:7:0.1 (aldehyde:acetate:alcohol) It is proposed that either the environmental factors of the insect habitat, or the genetic intrigue of the insect would impose influences onto the male sex behavior to the synthetic FSP (Klun and cooperators, 1975; Carde *et al.*,

1. Paper No. 258 of the Journal Series of the Institute of Zoology, Academia Sinica.

1978). The results of male catch, reported in Japan, with different ratio or dosage of the above mentioned compounds, somehow varied with the growing seasons of the year. They explained that the variation of pheromones used for optimal male catch was mainly because of the change of evaporation rate of the compounds under different weather conditions. Nevertheless, temperature was acclaimed to be a major factor that affected mating and pheromone-responding behavior of male adults to the female sex pheromone in Japan (Yamada and Koshihara, 1980).

In this study, we are trying to explore whether the two major climate factors: relative humidity (RH) and temperature would affect the sex behavior of the male adults to the adults to the synthetic FSP. Since seasonally dependent variation of male response to the synthetic FSP of *Spaelotics clandestina* (Harris) was reported (Stecch *et al.*, 1982), it is our hope to see whether this phenomenan will occurred in the diamondback moth. Therefore, a close look on the seasonal fluctuation of male response to the pheromone bait was also under investigation. In addition, the results of laboratory assay were also compared with those found in the field.

MATERIALS AND METHODS

Insects

Diamondback moth, *Plutella xylostella* (L.), used for bioassay (DBM) were reared under constant temperature of 25±1°C, and a light-dark cycle of 14-10. Larvae were fed on seedlings of rapeseeds according to Koshihara and Yamada (1976). Stocks of newly emerged adults from the insectarium were synchronized within 12-h intervals according to Maa *et al.*, (1983).

Chemicals

The pheromone components of 98% purity by GC test were synthesized in this laboratory and used for the bioassay. The components included (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate (Z)-11-hexadecen-1-ol. A combination of 5:5:0.1 (aldehyde:acetate:alcohol), in 1.0 μg was injected into a microplastic tube. Hexane was used as solvent. The tube was blowed with an electric fan for 1 h to evaporate the extra hexane before use.

Bioassay and Climate Factors

Newly emerged male adults were collected in a large glass jar and fed with honey water. Males of 1.5-2.5 day-old were used for assay. Around 15-30 males were used for each test. One hundred and eighty-three assays were done, with about 23 assays, in average, for each month. Percentage of male adults resonse to the lure was counted according to Chang *et al.*, (1979). The males that moved forward over the arm chambers were considered of being with positive orientation response to the lure. Those that set around, by the end of the assay, within 1.5 cm at the bait, were considered of being with positive landing response.

The conditions of the daily climate were transcribed from the daily report of the Center Weather Bureau (CWB), Republic of China. We used mean of daily temperature and mean of RH as major parameters to evaluate the impose of climate factors to the sex response of the male adults to the pheromone bait.

Data Analysis

Two variables: RH and temperature recorded by CWB, were separatively plotted against the percentage of male response to the lure. This is to test the linear interrelation between each of the two climate factors and the male response of male adults to the synthetic FSP. The linear regressions of RH and male response of each assay of the day were analyzed statistically following the equation of Least Square (Mendenhall *et al.*, 1981). The data, of the eight months, were in further analyzed to test the Best Linear Unbaised Estimater (BLUE) according to Gaurs-Markov Theroms (Mendenhall *et al.*, 1981). Similar treatment was done onto another variable: temperature.

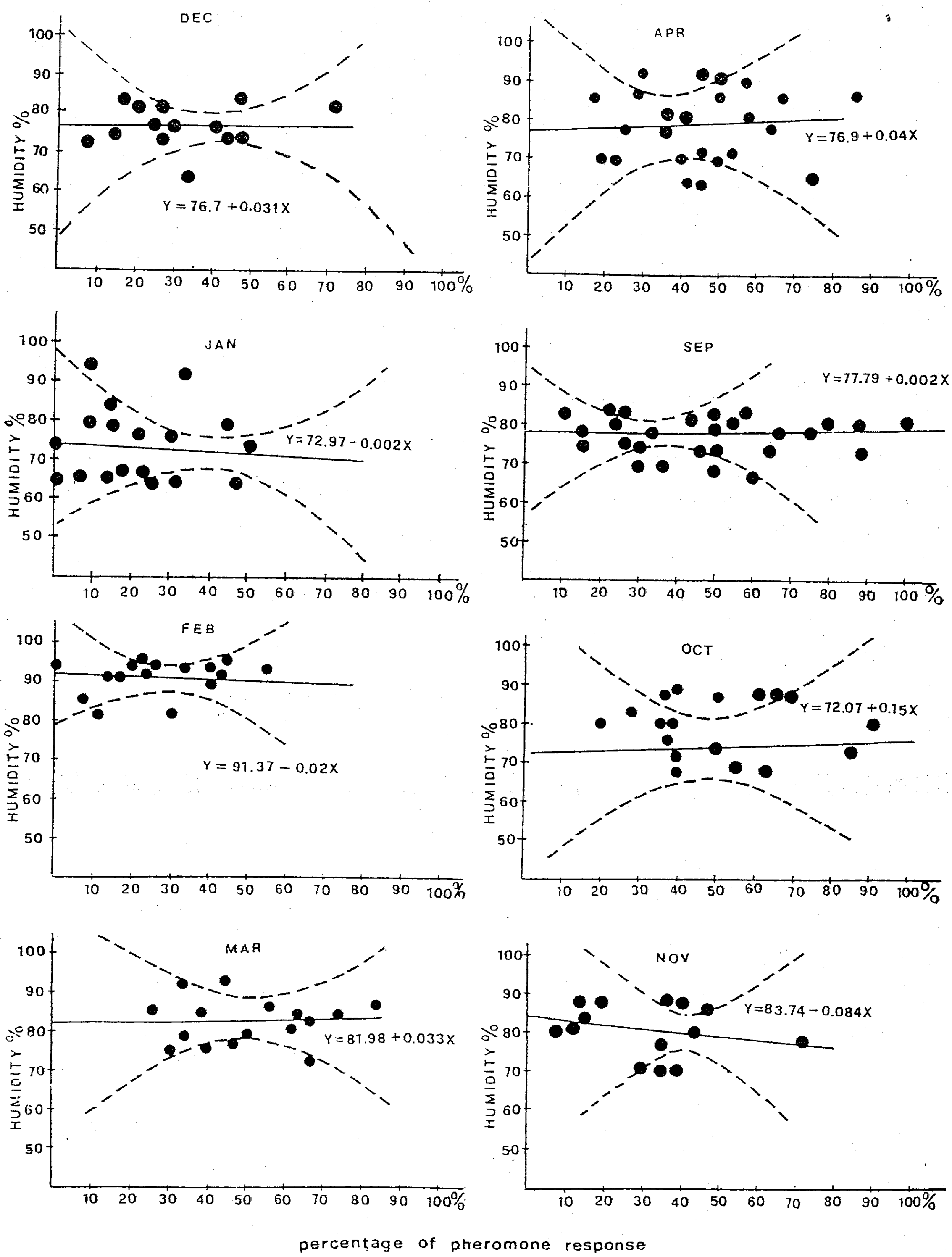

Fig. 1. The interactions of daily RH of each month and orientation response of male adults to synthetic FSP of diamondback moth. Calculation following least Square Method with $p=0.1$ level.

Bioassay

The whole bioassay was started from October, 1982 and was completed in April, 1983. The duration of bioassay covered ten months. This period of time included three seasons namely; Winter, Spring and Fall. We did a few assay during May and June. No assay was done during July and August since it became dry and warm and is not suitable for the growth of both the host plants and the insect.

Relative Humidity

The percentage of the male response to the female sex pheromone in each day of the month was plotted against the RH of that day. The relation between these two factors were analysed and the results were pictured. Fig. 1 also shows that in four cases: March, April, September and October the male adults are being with strong orientation response to the pheromone while the RH are under 77% to 82% except October. In cases of November, December, January and February the orientation response of male adults to the lure are weak when the RH were higher than 82%. There is a strong pheromone response of the male to the low RH in October, and a weak response to the proper RH in December. In view of the whole year assay, we find that there are more than one fluctuation of male response to the pheromone. The first wave of the strong-response of male adults to the pheromone was found during the early Spring. The second wave came during September. These strong response of the male adults last for more than two months each season. When it came to November the response decreased. The regression interrelation between the RH and the pheromone response of the adults, showing in the results of the bioassay of each of the eight months, were further plotted to extimate the BLUE. Fig. 2 shows that the linear regression of RH and the pheromone response of males was significant at $p=0.1$. The critical RH for the response is set around 82% (the interception). In fact, a RH value around 79-85% are all

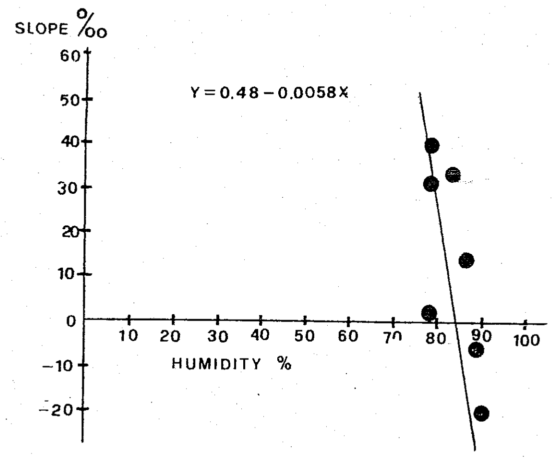

Fig. 2. The interactions of monthly average RH of the year and orientation response of male adults to the pheromone bait of the diamondback moth. Calculation following the equation of Best Linear Unbasied Estimation with $p=0.1$ level; interception at RH 82.6%.

accepted for the optimal male orientation response. A RH over this point would cause a decrease of male response. It is postulated that RH of the environment played an important role in the regulation of the male response to the female sex pheromone.

Temperature Effect

Temperature, which is generally recognized as a major factor affecting the mating behavior of male adults of many lepidopterous species, nevertheless, shows with less influence to the orientation response of the male moths to the pheromone lure of the DBM in Taiwan. Fig. 3 shows that the mean of temperature of each month was 17.7°C for December and January, 16.2°C for February, 15°C for March, 18.5°C for April, 28°C for September, 17.4°C for October, and 23.9°C for November. We found that the pheromone response of the male adults were low during December, January, and February when the temperature was low. The response increased, althrough average monthly temperature of March was even

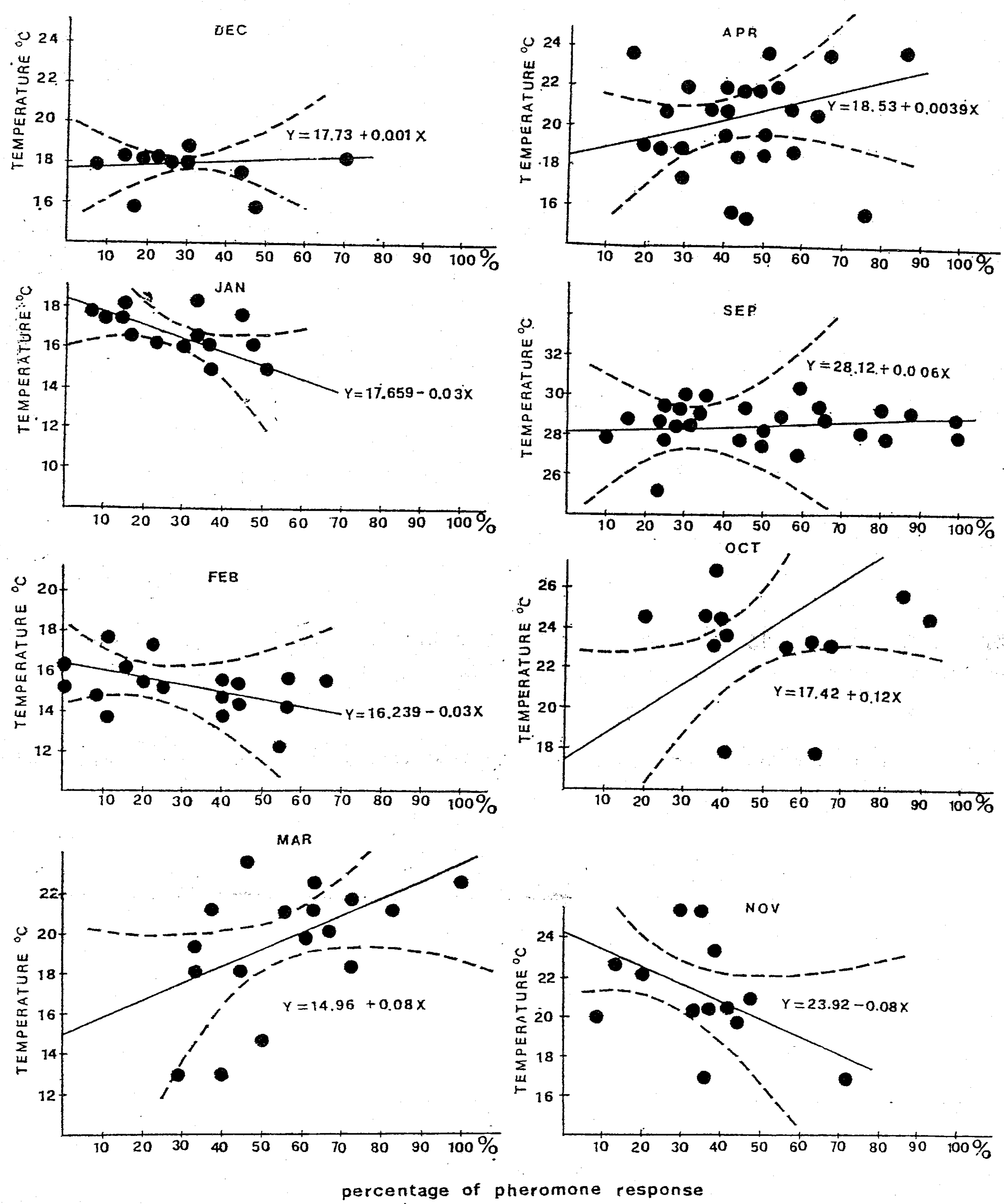

Fig. 3. The interactions of daily temperature of each month and orientation response of male adults to synthetic FSP of diamondback moth. Calculation following the Least Square Method with $p=0.1$ level.

lower than that of other months. This evidently revealed that the sex behavior of the male adults to the pheromone was not affected by the low temperature. In April the temperature increased with an increase of response. In September, monthly temperature arose to 28°C while the insect was still active to the pheromone bait. In the following two months the response dropped to low level again. The strong pheromone response of male adults to the synthetic FSP found in either a high or a low temperature condition, somehow, reflected that temperature had limited regulating effect to the sex behavir of the adults males of diamondback moth. Nevertheless, the regression interrelation between the temperature and the pheromone response of the males in BLUE analysis revealed that the circumstance temperature over 18.6°C would cause a decrease pheromone response of the male to the lure (See Fig. 4). The regression linearity is significant at $p=0.1$ level.

According to this study, it is believed that a condition of 75-85% RH with average

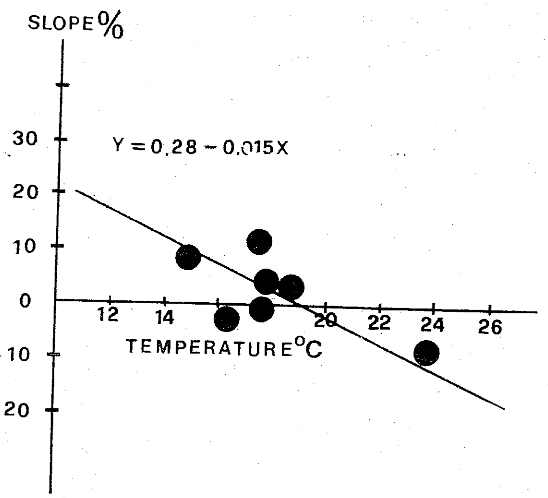

Fig. 4. The interaction of monthly average temperature of the year and orientation response of male adults to the pheromone bait of the diamondback moth. Calculation following the equation of Best Linear Unbasied Estimation with $p=0.1$ level; interception at 18.6°C.

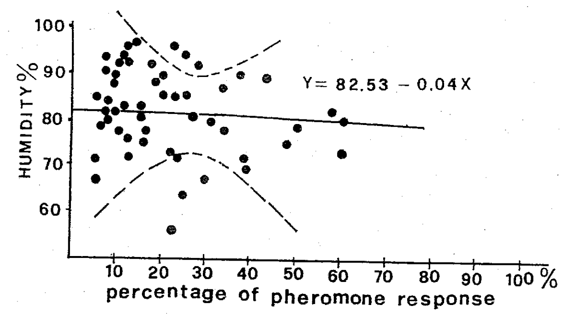

Fig. 5. The interaction of daily RH and landing response of male adults to the pheromone bait of diamondback moth. Calculation following the Least Square Method with $p=0.1$ level.

temperature of 16.5-18.5°C would be a satisfied conditions for the *Y*-test in the laboratory.

Relative Humidity to Landing Behavior

Sixty-five out of 183 assays were found with positive landing response of the male adults to the synthetic FSP. All these data of landing response of male were plotted against the RH according to the Least Square Equation. The result is with linear regression significant at $p=0.1$. The mean RH for landing behavior was estimated: $y=82.53-0.04x$. This leaded to a RH even lower than that of orientation response of male adults. It is possible that a critical humidity of the atomsphere is required for the landing behavior of male. Temperature, on the other hand showed less effect to the landing response of the insect (see Fig. 5a and 5b).

Seasonal Variation of Male Response to Pheromone

Fig. 1 shows that the male response to the synthetic FSP is low during the Winter: moths; namely November, December and January. We found one case each in November and December experiments with strong response and none in January experiments. It is possibly related with the low temperature and short photoperiod of daytime during those months. The pheromone response of the male adults to the bait gradually increased when the daily temperature and the photoperiod of daytime increased during early Spring. As it shows that 4 out of 18 cases of tested males showing strong response to the lure during February. The strong response increased to 10 out of 17 in March, and dropped to 11 out of 25 in April. Althrough, cruciferous vegetables are still cropped in Taipei Basine during May, the habitated diamondback moth population, somehow, drastically dropped to its mimimum before the end of May. Following the cultivation-rotation, no more cruciferous vegetables are available as hostplants for the DBM in the fields of Taipei Basine. When the hot Summer with elongated photoperiod finally dominated the land the dismissed. The farmers recropped the cruciferous vegetable during Septembber. The pheromone response of male adults realived and reached to another peak of the sensitive response cycle. It showd that 13 out of 27 cases of tested males with strong response to the lure during September, and 6 out of 13 during October. It dropped once more when the cold weather arrived this land.

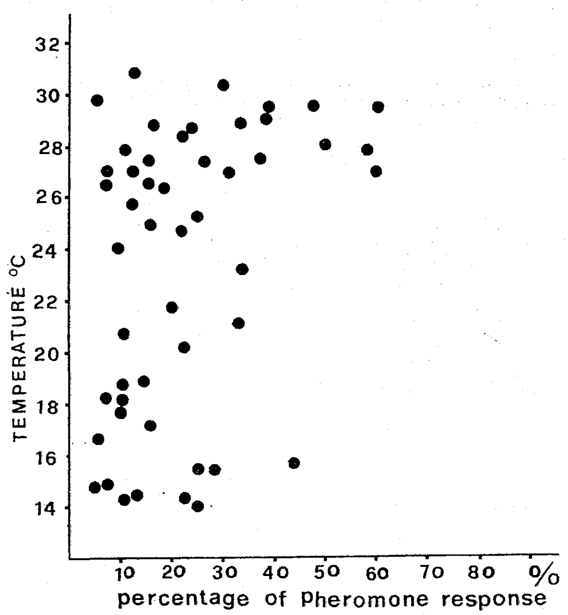

Fig. 6. The interaction of daily temperature and landing response of male adults to the pheromone bait of diamondback moth. A random type of interreaction.

DISCUSSION

We use a pheromone blend of 5 parts of each of (Z)-11-hexadecenyl acetate and (Z)-11-hexadecenal in addition of 0.1 part of (Z)-11-hexadecen-1-ol in 1.0 μg to assay the male response to the synthetic FSP of the DBM. The pheromone blend is with optimum male catch of the moth reported in Japan and Taiwan (Chow *et al.*, 1977; Koshihara *et al.*, 1978). Our study on Y-test in the laboratory revealed that there are several factors possibly associated with the status of male response to the synthetic FSP of diamondback moth. These factors involved mainly: the diversity of genetic intrigue of the insect populations, seasonally dependent variation, influence of the physical conditions.

The Seasonary Dependent Variation

This variation was first reported in W-marked cutworm, *Spaelotics clandestina* (Harris) by Stech *et al.*, (1982). He found that the synthetic material was unattractive during June and July but became attractive during August and September. Our data revealed that the male adults of diamondback moth responsed to the lure in an order of low-high-low level of attractance in a sequence of Winter-Spring-Summer on the first cycle and Summer-Fall-Winter on the second cycle of the year. Unlike the W-marked cutworm, our pest has two cycles of the male response to the lure in the lāborātory. These periods of sensitive stage of the moth lasted for more than four months approximately. Since the weather conditions of Winter and Summer in Taiwan are not proper for the reproductive activity of this insect pest, it is expectable that sex behavior of both sexes of this insect pest would mimimize during these seasons. It is also expectable that an active reproduction behavior of diamondback moth would extend in field during Spring and Fall. We are not sure whether the experimental coincidence of strong pheromone response of adults to the lure during these two seasons would justify what is really happened in the field. The results of *Y*-test carried out in this laboratory, nevertheless, may reflect that this seasonally dependent variation of pheromone response of male adults to the lure, in a sense of mating behavior, was possibly an important content with the diamondback moth for self regulation of nature population in the field. The evaluation of this point is still under investigation.

Physical Conditions

Mating behavior of the male adults of most lepidopterous insect species to either virgin female or synthetic FSP was found temperature-dependent (Baker and Roelofs, 1981; Kanno, 1980; Miyahara, 1981; Kanno and Sato, 1980; Yamada, 1979). Kanno and Sato (1979) indicated that in rice stem borer moth, *Chilo sappressalis* Walker, the percentage of mating was scarcely affected by humidity variation at 20°C. Coincidently, the highest incidence of female calling occurred at temperature near 20°C. They concluded that the relative humidity had little effect on mating percentage at 20°C. The sexual behavior of both male and female adults are therefore cases of temperature-predominated activity.

On the other hand, the european corn borer, *Ostrinia nubialis* Hubner, showed another trend in sexual behavior which was affected absolutely by the free water of the air (DeRozari *et al.*, 1977). It is reported that the presence of the free water is only a condition required for the initiation of sexual activity. Whether this potential is expressed depends on the availability of both sexes combined with temperature, RH and illumination. Obviously, either temperature or RH may play some kinds of roles on stimulating the sexual activity of both male and female adults of a specific insect species. Whether the stimulation is triggled by either factors is depending on how the adults were constrained by the physical parameters. For example, male rice stem borer responds positively to the pheromone when the temperature is around 15–25°C while the RH shows little effect to the insect. As the temperature increased the RH became a factor that will affect the status of the male sexual behavior. In case of diamondback moth of Taiwan, a subtropical island with mild temperature of 15–32°C through all the year, RH will naturely take its course to monopolize its influence to the male sexual behavior. The effect of temperature will then be with less significance. We would expect that the male adults became sensitive to the pheromone on the impact of the RH both in laboratory and in field, when it is a time to reproduce and grow. We would also expect that the RH would have less effect to the sexual behavior of the male adults to the bait when the conditions is not suitable for the insect during the hot Summer and the cold Winter. The pheromone-sensitive cycle of male response to the synthetic FSP of diamondback moth during Spring and Fall might conserve a channel, seasonally, for population-self-regulation mechanism of the insect.

The optimum temperature for the male response was estimated experimentally 16.5–18.4°C in our laboratory. Kao and Cheng (1984) could not detect the difference of the respiration rate of the two groups of male adults which were loaded separatively in conditions of 18°C and 24°C. They acclaimed that the optimum temperature condition for the activity of male adults laided on 18°C. Their results coincidently confirmed what we found of the optimum temperature for male response to the synthetic FSP in the laboratory. Our data further shows that temperature over 18.6°C will cause deterrent effect to the pheromone response of the male adults to the lure.

The optimum RH was 82.6% for the orientation response and 82.5% for the landing response. The extremely high RH would constrain the attractance of the pheromone. Evidences found in field (data not published) also confirmed it. The low RH in the laboratory, with a pheromone blend of 5 aldehyde and 5 acetate, was found with fair male response. The low RH in the field would have a fair male catch only when a pheromone blend of low aldehyde content and high acetate content was used. These evidences may reflect that there is some other mechanisms involved with the releasing of the pheromone blend by the female adults, or with the acceptance of pheromones by the male adults. It is known that the antennae of DBM adults are equipped with pit-peg sensilla (Chow *et al.*, 1984) which were suggested to be hygroreceptor (Schneider, 1964). However, we do not know how will, in aspect of humidity detection, the DBM females make favorable adjustment of their behavior to fit the physical conditions of the environment that changed from time to time. It seems that use of a pheromone to imitate the signal molecule(s) released by the alive female adults of an insect is rather a comprehensive process than a single reaction model of physical conditions, pheromone release of female and pheromone acceptance of male. Further investigation is neened before practical methodology of mass trapping and sexual disruption is ever established.

Acknowledgements: We thank financial support of Council for Agriculture Planning Development, with grnts no. 73 Agri. Constr.-4.1-Prodt-153(2) and 72 Agri. Constr.-4.1-Prodt-83. Thanks also extent to Dr. Y. S. Chow, for his encouragement during this study. For the use of weather reports of Center Weather Bureau, Taipei, Taiwan, ROC, we gave our great appreciation.

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溫度及濕度對合成之雌性蒙洛蒙誘發雄性小菜蛾性反應之影響

馬堪津　林玉美　尹玉珍

以小葉蛾雌性費洛蒙的三種成分；(順)-11-十六烯醛，乙酸(順)-11-十六烯酯和(順)-11-十六烯醇之 5：5：0.1 之配方經一年間之室內Y型嗅覺試驗，以日爲計量單位去分析每個月中不同溫、濕度狀況下雄蛾對合成的雌性費洛蒙反應之結果來測定溫度、濕度對其反應之影響。結果顯示雄蛾的趨向反應 (Orientation response) 在相對濕度 75～85%之區間有較佳之結果，高於此範圍則反應趨弱。結果也顯示，在 79～82%相對濕度下，着陸反應 (Landing response) 最佳。性反應最佳之溫度區間爲 16.5～18.5°C，高於此上限，反應漸減。然而，在適宜之濕度區間時，溫度對雄蛾性反應之影響不大。全年試驗結果顯示出小葉蛾雄蟲對合成之雌性資洛蒙之反應有季節性之差異。在春，秋季月份時，試驗結果遠較多，夏季月份者爲佳。文中探討了氣溫、濕度及季節之變化與誘發雄蛾性反應之影響。