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TEMPERATURE AND OTHER EXTRANEOUS FACTORS AFFECTING MALATHION SUSCEPTIBILITY OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* L.[1,3]

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Maa, C.J. William and S.H. Guh (1988) Temperature and other extraneous factors affecting malathion susceptibility of diamondback moth, *Plutella xylostella* L. *Bull. Inst. Zool. Academia Sinica* **27**(4): 265-274. Extraneous factors that effected malathion susceptibility of diamondback moth, *Plutella xylostella* L., was investigated in this laboratory through 1984 to 1988. The result of dose-response experiment shows that two moth populations collected from Lu-Chu and Sheh-Tzu are resistant to malathion. Temperature is considered as an important factor to influence the susceptibility of Lu-chu larvae to malathion. The larva treated with malathion under a ambient condition would have a low LD_{50} of 30 μg per larva in summer and a high LD_{50} of 140 μg per larva in winter. The result of this study shows that ratios of the high LD_{50} to low LD_{50} obtained during each winter and summer of the passed four years are 2.7 for 1984, 6.9 for 1985, 3.3 for 1986 and 5.0 for 1987. In case the larva was reared in 25±1°C and tested in an optimum temperature, 20±1.0°C, variation of the eight LD_{50}s botained from the eight dosage-mortality tests and done in January through September, 1988 was around 10 percent. Average of the LD_{50} of the eight tests was 178 μg malathion per larva. A resistant strain, ST-AO, and a susceptible strain, ST-SE, both selected by means of isofemale-pairing method, showed a 29-fold difference in malathion tolerance when the test was worked out in November. The difference would be five folds when the test was done in February. Change of these resistant ratios between November test and February test was mainly due to temperature variation of the season in a year. Humidity and diet showed little affect to the outcome of the LD_{50}-test. Drenching the larva before topical treatment would, however, cause the larva being more sensitive to malathion toxicity and hence would increase the mortality of the test insect. Food deprival showed significant influence of the larval susceptibility to malathion. The resistance of DBM to malathion would increase under malathion selection. Under a malathion-free condition the resistance would be stable and last for, at least, five years in laboratory after a slowly initial drop of the resistant level.

Key words: *Plutella xylostella, Extraneous factors, Malathion, Susceptibility.*

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The rapid development of resistance of the diamondback moth to various kinds of insecticides was noticed in the sixties in Taiwan (Tao, 1973). Numerous reports on insecticide resistance of diamondback moth larva have been published since then (Sun *et al.*, 1986; Cheng, 1986). Research into the cause of resistance of diamondback moth to insecticides was also reported in other asian countries (Yamada, 1977; Ho, 1965). Decrease in insecticide resistance in the diamondback moth larva on release from selection pressure have also been studied (Noppun *et al.*, 1984; Cheng and Chou, 1985; Sun *et al.*, 1986). The gaining and the loss of insecticide resistance of diamondback moth larvae in most studied cases was derived from the measurement of the median lethal dose (LD_{50}), for the calculation of toxicity of the insecticide used for the bioassay. Trevan (1927) and his contemporaries indicated that the LD_{50} of a drug is not a fixed value, but that it depend on many extraneous factors. They were particularly aware of seasonal variations. Zbinden and Flury-Roversi (1981) indicated that many factors including ambient temperature, diet, food deprivation, method of administration and humidity of the air would affect the result of a dosage-mortality experiment. For example, in Japan it is reported that the seasonal changes of the resistance level of the diamondback moth larva to various organophosphorous was with a ten times of difference between a diamondback moth population collected in the field during September, 1984 and another moth population collected in the same field during June, 1984 (Hama, 1986). Hama also showed that cyanofenphos-resistance of the diamondback moth larva of June 1984 population was counted as one one hundredth as that of the moth larva population collected in October, 1982.

The author was told that the diamondback moth larva in Taiwan which was resistant to insecticide would become less resistant during summer, and the susceptibility of the moth larva to insecticide would also become unstable. The resistance would be recovered during winter and the coming spring. These informations revealed that either temperature or the seasonal change during the year would influence the result of dosage-response experiment. In this study we intended to explore whether the seasonal variation of LD_{50} was associated with adverse effect of the increasing sensitivity of the DBM larva to malathion due to a increasing temperature during summer time.

Other extraneous factors including diet, food deprival, treatment time in a day, humidity or drenching which are considered to be important to influence the outcome of LD_{50}-tests were also investigated in this study. By the way, we also like to know what would be happen when the DBM larva were selected with malathion, and how stable would be the resistant level of the DBM to malathion, and how long would the resistance last under a malathion-free condition.

MATERIALS AND METHODS

Insects and Chemicals

Diamondback moth (DBM), *Plutella xylostella* L., collected from vegetable farms of Lu-Chu, Kao-Hsiung County and Sheh-Tzu, Taipei City in 1982, were reared in insectarium according to Koshihara and Yamada (1976). The fourth instar larva of the moth was used as test organism. The DBM larva of Lu-Chu population was selected with 132 μg malathion per larva for two generations during November, 1984 and with 176 μg malathion per larva for additional two generations during December, the same year. Two other DBM populations collected in I-Lan County and Geou-Fang of Taipei County respectivelly were also reared in the insectarium accordingly. These four DBM populations were compared with one another for their tolerance to malathion treatment. The result of the preliminary test showed that

the DBM of Lu-Chu population was the best choice for this study since individual DBM larva of this population showed variable tolerance to malathion treatment, and this population was with a high LD_{50} to malathion. Administration of malathion to the larva was followed the method of topical treatment (Anon. 1969).

All chemicals and solvents are of analytical grade or the reagent grade. Malathion (0, 0-dimethyl-S-(1, 2-dicarboethoxyethyl) phosphorodithioate), 99% purity, was purchased from Chem Service, Co., USA.

Toxicity Test

Batches of thirty to sixty larvae were treated topically with malathion in a series of concentrations, 16.5, 33.0, 66.0, 132.0, 200.0 and 264.0 μg/0.25 μl acetone. After treatment, each batch of the larva was confined within a petridish, 6.0 cm in diameter and 1.5 cm in height, with a cover of 7.0 cm in diameter, and kept at 24.0±1.5°C for 24 hrs. The mortality of the treated larva was counted every one hour for the first six hours and every two to three hours for the additional 18 hours. Each test was repeated twice. Dosage-mortality curves were calculated using probit analysis method as proposed by Finney (1971).

The study on the effect of temperature on LD_{50}-tests was gone with that of seasonal variation on LD_{50}-tests. These tests were carried out either under ambient condition or under controlled condition at 20.0±1.0°C. The test under ambient condition was carried on from 1984 to 1987. The test under controlled condition was done during 1988. Study on the effect of other four factors on the outcome of LD_{50}-tests were also conducted under 20.0±1.0°C. The stability of malathion resistance of the Lu-Chu DBM, under malathion-free condition, was also investigated.

RESULTS AND DISCUSSION

It is known that within one population of laboratory organisms the numerical value of the lethal dosage determined experimentally was influenced by a large number of factors. The data of dosag-response-experiments carried out in this laboratory during the passed years revealed how the extraneous factors would affect the result of LD_{50}-tests in diamondback moth larva.

Temperature and Seasonal Difference

Table 1 shows the result of a general survey on malathion toxicity to four populations of DBM. The values of LD_{50} of the Geou-Fang and Bamboo-Lake populations were rather low than those of Lu-Chu and Sheh-Tzu populations. The table also shows that Lu-Chu DBM not only was a resistant population but also was homogenous in nature. Therefore, Lu-Chu population was chosen for this study. This population was then selected with malation for four generations. The selection led the offspring of the selected population to be more tolerant to malathion treatment. In fact, the LD_{50} of the selected population to malathion was

TABLE 1
Toxicity of malathion to the larva of diamondback moth

Strain	LD_{50} (μg/larva)	95% fiducial limit	Slope
Geoufang	45.39	37.49- 51.95	2.71
Bamboo Lake	66.07	52.48- 83.18	1.89
Shehtzu	91.20	63.20-131.83	1.29
Luchu	84.72	70.72-101.86	2.07

The bioassay was done in August, 1982. Fourty to sixty larvae was used for each of malathion dose. Five doses were used with a blank as control.

TABLE 2
Temperature-dependent variation in percentage of mortality of diamondback moth larvae of same batch to malathion

Temperature (°C)	Percentage of mortality 24 hr after treatment (M±S. D.)	No. of test	Percentage of Mortality at emergency (M±S. D.)
16	20.5±6.4(1)	5	64.3±12.5(2)
20	17.0±4.2	5	50.8±8.7
24	30.1±8.2	5	72.3±10.1
28	37.3±11.9	5	71.7±9.2
32	47.5±8.7	5	91.8±6.2

(1) Lu-Chu DBM was used for the bioassay; 30 larvae per each bioassay; 176 μg malathion per larva.
(2) Data were not corrected with blanks.

increased with the increased selection dose. Similar phenomenan were found in the case of mevinphos and other synthetic insecticides (Cheng, 1988; Chen and Sun, 1986, Sun *et al.*, 1978).

Table 2 shows that a linear relationship was found between temperature and toxicity as measured by the LD_{50}-test. The linear relationship was, however, within a limited temperature range from 20°C to 32°C or higher temperature. A reverse relationship was found within 20°C to 15°C. It shows a slow response to malathion treatment when the temperature was around 20°C. The time for 50 percent mortality of the treated larva would decrease with increase of temperature. The difference of the mortality of larvae treated under low and high temperature was around three-fold favaring to survival of the larva at low temperature condition. It seems that a temperature of 20°C was possibly an optimum temperature for DBM larva to tolerate the malathion toxicity. Similar result was found in Table 3 and 4. Table 3 shows that larvae treated with malathion under the optimum temperature of 18°C to 20°C died slowly. On the other hand, those treated under 14°C to 16°C died fast, and the difference is significant. The optimal temperature of 20°C was therefore as a standard condition for all the LD_{50}-test in this study.

TABLE 3
Temperature-dependent variation in LT_{50} of diamondback moth of different batch to 176 μg malathion

Date (Month/day)	Repeat (N)	LT_{50}; hr. (M±S. D.)	Temperature (°C)
1/2	2	8.8± 0.1	16.1
1/4	4	4.4± 1.7	14.6
1/6	2	7.2± 0.4	13.7
1/7	4	14.1± 6.4	15.4
1/9	2	7.2± 1.6	16.2
1/10	3	6.5± 5.6	18.5
1/12	2	33.5±10.2	20.5
1/13	4	21.2±13.1	17.6
1/14	4	17.4± 4.1	19.1
1/15	4	30.5±12.1	18.8
1/16	5	26.4±13.2	18.9

Lu Chu DBM was used for the bioassay; 25 larvae per each bioassay.

Variations of the LD_{50} that were appearently related to the season during which the test were pereformed were noted already by many investigators. Taiwan is a subtropical island. In most cases it is cold with low temperature down to 10°C during winter and is warm up to 36°C during summer. The DBM larva reared under a ambient condition was, therefore, showing different response to malathion during different season. In general, as the temperature is high, so is the mortality of the larva that treated with malathion.

TABLE 4
Temperature affecting the susceptibility of DBM to malathion during January to September

Testing month	1987(1)		1988(1)	
	LD_{50} (μg/larva) M±S.E.	Slope Mean	LD_{50} (μg/larva) M±S.E.	Slope Mean
Jan.	84.1±23.6	1.46	193.4±12.5	0.97
Feb.	92.5±13.4	1.97	189.5± 5.6	1.09
March	93.7±16.3	1.97	161.7±22.8	1.32
April	73.8±19.8	1.87	205.4±18.3(2)	1.23
May	31.9± 7.2	1.50	—	—
June	—	—	173.8±31.4	1.60
July	28.6± 8.3	1.48	157.3±17.6	1.60
August	31.6±11.2	1.40	157.6±19.7	1.77
Sept.	22.6±10.4	1.35	186.4±15.6	2.14

(1) The test of 1987 was performed under ambient temperature, while the test of 1988 was under controlled temperature of 20±10°C. Each test was repeated twice.
(2) The Test was performed during joint week between April and May.

Both Table 4 and Fig. 1 shows that the value of LD_{50} of the larva was high when it is cold and is low when it is warm. The high LD_{50} count was found in the case of December 1984, January 1985, February 1986 and April 1987. The low LD_{50} cases were found in October 1984, July 1985, July 1986 and July 1987. On an average, the LD_{50} obtained in cold day was three times higher than that obtained in warm day. The maximum difference is about seven-fold between the low and the high LD_{50} in 1985. The minimum is 2.7-fold in 1984. It is five-fold in difference in the case of 1986 and 3.3-fold in 1987.

The result of the LD_{50}-test which was done moonthly, under ambient condition during 1987 was tabulated in Table 4. The table shows that the value of LD_{50} of the dosage-mortality test of DBM larva to malathion is around 80 μg per larva for cases of January, February, March and April. The value drops down to 30 μg per larva for cases of May, July, August and September. The difference between the high and the low LD_{50} is about three-fold. The larva that was reared in a controlled condition and was treated with malathion and kept in 20°C± 1°C would have values of LD_{50} with little variation. Data on the left side of Table 4 shows that in the case of 1988 the LD_{50} of the larva to malathion was ranged from 157 to 205 μg per larva. The average of the LD_{50} of eight tests was 178.1 μg per larva. The difference between the LD_{50} of January test and that of August test was not significant. These two experimental results listed in Table 4 reveal that change of the temperature during a year would, somehow, impose certain influence on the dosage-response of the larvae to malathion treatment. It is expectable that similar phenomenon would be found in DBM larva with other kind of organophosphorous compounds.

Another interesting aspect of temperature-dependent variation of LD_{50} is that the outcome of dosage-mortality test of different DBM strains or populations would have the resistant ratio changed due to change of season in a year. For instance, three strains of DBM selected from Sheh-Tzu population shows different susceptibility to malathion. The value of LD_{50} of Sheh-Tzu (ST)-AB, ST-AE and ST-AO is 6.2, 4.7 and 136.3 μg per larva respectivelly when the test was carried on in a ambient condition during

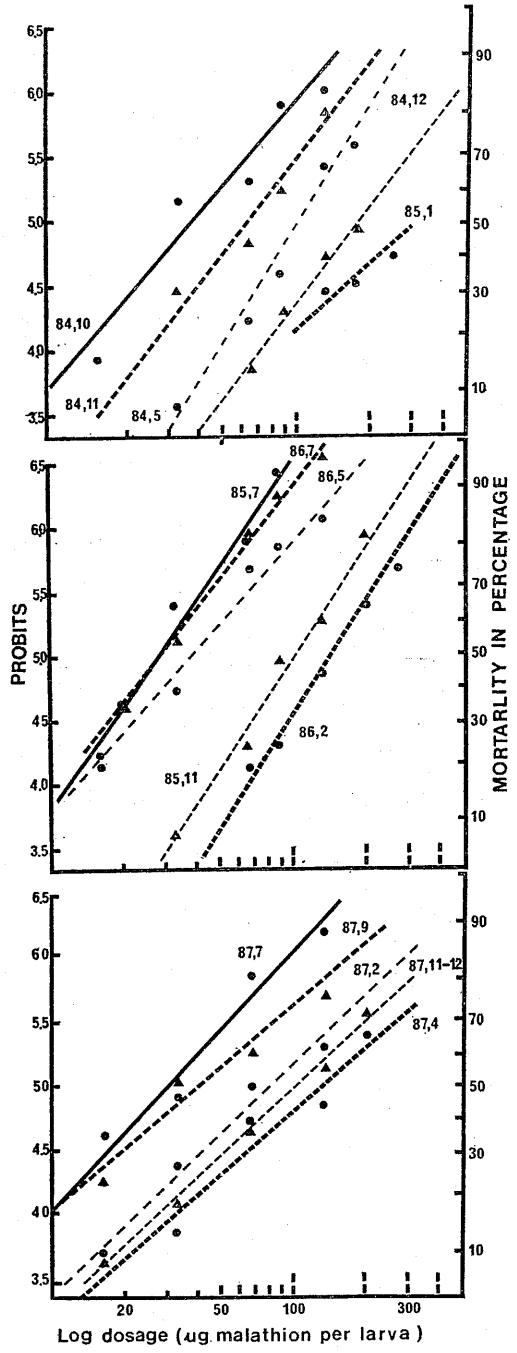

Fig. 1. The regression lines of the lethal-dosage-response of Lu-Chu diamondback moth larvae to malathion, a four years survey to show the seasonal fluctuation and the stability of the larvae to malathion, Each regression line represented average of two tests.

summer, 1985. The difference between the LD_{50} of the resistant and the susceptible strain (ST-AO to ST-AE) is 29-fold. The value of LD_{50} of the dosage-mortality test would change to 54.9, 86.8 and 412.4 for ST-AB, ST-AE and ST-AO when the test was carried out in cold weather during February. In this case, the ratio of resistant level between the most resistant strain, ST-AO, and the susceptible strain, ST-AE, would be around five to one. It is interested to note that the ratio of the resistant level between ST-AO and ST-AE would be one to 29 during October-November and would be one to five during February. These results justified that seasonal variation of tolerance of DBM larva to malathion, mentioned in Hama's work (1986), was possibly merely due to temperature change rather than the seasonal variation in a year.

TABLE 5
Strain-dependent variation on susceptibility of the Sheh-Tzu larvae to malathion treatment

	LD_{50} (μg/larva)		Slope	
	75/10	76/2	75/10	76/2
ST-AE(1)	4.7	86.8	0.67	1.94
ST-AO(1)	136.3	412.4(2)	2.13	2.62

(1) Susceptible strains were treated with dosages with a range of 0.1 μg to 8.0 μg per larva. Resistant strains: 8 μg to 176 μg per larva. One hundred or more larvae were used for a single dose.
(2) Values were obtained by extrapolation method

Similar results found in other insect species were summarized by Champ and Dyte (1976) and Brown and Pal (1971).

Banki (1978) indicated that in most of the cases it is more important to maintain the temperature at a constant level than at the optimum level. Optimum temperature can be different according to the various indicator species, they think that the maintenance of a temperature of 25±1°C over

the whole year in all the localities is expedient, adequate and sufficient for most of the indicator organisms and tests. However, Lee (1986) found that the DBM would increase the population in field when it is around 20 to 24°C. Maa and Lin (1985) also found that the male adult of DBM showed strongly sexual response to virgin female calling under a temperature range of 18 to 20°C either in field test or in laboratory test. In this study we found that 20°C is also the optimal temperature for DBM to tolerate malathion toxicity.

Humidity, Drenching and Other Factors

The result of the LD_{50}-test of malation to the DBM larva carried out in this laboratory showed that the DBM larva is less sensitive to changes in relative humidity than to those in temperature, nevertheless its control cannot be neglected. Under otherwise identical conditions, the relative humidity, as is well known, depends on the temperature (data not shown). Drenching was reported as a desperate factor to DBM population in the field (Talekar *et al.*, 1986; Lee, 1986). The drenched larva died with a low LD_{50} in the laboratory (see Table 6). Food deprival is also a desperate factor to the moth larva. Table 6 shows that the larva which was confined in petri-dish without food for 12 hours would have a LD_{50} down to 68.4 μg per larva under 20°C. This value of LD_{50} is lower than that with food supply. Diet or food source would also influence the outcome of the LD_{50}-test on the DBM larva. Table 6 also shows that the larva fed with chinese cabbage seedling had a low value of LD_{50}. The larva fed with rape seedlings or with mustard seedlings showed higher tolerance to malathion. A slight difference had been detected in the susceptibility of the DBM larva to malathion depending on the time of day at which the test is made. The tolerance was found to be maximum at night, when the mortality was low, and minimum at morning, when the mortality was high (data not shown). However, no daily rhythm could be detected in the susceptibility of the larva to malathion.

TABLE 6
Effect of diet, food deprival and drencching to mortality of diamondback moth larvae to malathion treatment

Treatment	LD_{50} (μg/larva)	Slope
Starved for 12 hours	68.4± 4.5	1.77
Starved for 6 hours	113.6±23.3	2.14
Not starved	144.3±19.7	1.77
Cabbage seedlings	122.3±32.6	1.43
Rape seedlings	153.1±27.6	2.27
Mustard seedlings	144.3±19.7	1.77
Drenched larvae	118.3±21.5	1.31
Not drenched	132.7±13.5	2.36

Lu-Chu larvae were used for the test; two repeats with 30 larvae each.
Five doses were used, done in 20±1°C condition.

Variation in Malathion Resistance and Resistance Stability

Variability in insecticide response is a characteristic of the DBM and the insecticide. Cheng (1986) indicated that the laboratory simulation experiment for organophosphorus resistance has successfully converted susceptible DBM to strains with strong resemblance to the field strains in term of resistance. In our laboratory we obtained both resistant and susceptible strain from the Sheh-Tzu population. We had difficult to get even moderate resistant strain DBM from Geou-Fang popualtion. Our study on esterase isozyme in PAGE gel of larval homogenate revealed that different Sheh-Tzu strain DBM hold their own esterase isozyme pattern that was absent from the DBM larva of Geou-Fang population (data not shown).

It is known that the resistance level of most insect would vary and, in most time, decrease under the pesticide-free condition. Susceptibility of DBM larva to pesticides was reported increased several folds after

having reared for many generations (Sun *et tl.*, 1978; Lee and Lee, 1979). Hama (1983), however, indicated that a Japanese strain (Miinohara) did not show any sighificant loss of resistance (except to a few insecticides) after laboratory rearing for more than 15 generation. A similar phenomenon was also found by Noppun *et al.*, (1984). In our laboratory we reared Lu-Chu DBM for almost five years. The resistant level of this DBM population varied accordingly with the hot and cold season every year. In general, the resistance of this population was considered to be stable. This is supported by numerous measurement of resistance level showing initial decrease after removal of selection pressure, followed by a long-term and stable resistance to malathion. Besides, the Lu-Chu DBM, in most time, is homogenate in dosage-response. The experiment of LD_{50}-test carried out during 1988 in this laboratory revealed that it is important to maintain the temperature at a constant level, below 25°C, for rearing the insect and at a optimal level, around 20°C, for performing the test. Besides, monitoring test at a temperature around 20°C would narrow down the difference of resistance levels between resistant and susceptible strain. Our data also showed that at a high temperature condition, the DBM larva of both resistant and susceptible strain showed a high mortality, with same scale, to malathion. Similar result was also found in other insect species (Pradhan and Govindan, 1954).

Monitoring the resistance of the DBM to insecticide either in field or in laboratory undern a optimum condition is important. We believe that under this condition the scale of resistant levels of resistant and susceptible populations of the DBM could be accurately measured at the very early stage of the control failure.

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溫度及其它處理狀況對小菜蛾感藥性之影響

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在不同環境狀況下以馬拉松來處理小菜蛾 *Plutella xylostella* L. 四齡幼蟲，其結果將因各環境因素對小菜蛾本身影響之大小致使小菜蛾對馬拉松反應出强弱有別之抗藥程度。本實驗室在過去五年裏，以六種影響因子進行了一系列的馬拉松之毒效試驗，結果發現：社子及路竹之小菜蛾爲高抗性之族羣，其中社子 AO 爲抗性品系、社子 AE 則爲感性品系，兩者對馬拉松之感受性可因溫度之不同而呈現二十九至五倍之差異。溫度與季節變遷對小菜蛾之感藥性有極大的影響。飼育在自然環境下之路竹小菜蛾，每至夏天其半致死劑量卽下降爲約參拾微克/每隻幼蟲，而在冬季或冷天，其半致死劑量則提昇爲百數拾微克。四年寒暑期間，差異分別是：2.7 倍、3.3 倍、5.0 倍及 6.9 倍。在定溫環境 (25±1°C) 飼育及做施藥處理 (20±1°C) 所得之結果，其寒暑間之差異則很小，最高與最低兩者間之比值爲 1.30 比 1.00。在同一時間不同溫度下，以同一劑量來處理幼蟲，發現在攝氏二十度左右時，小菜蛾具最强的抗藥性，溫度高於或低於此者，則感性增加。其它外在因素，如：濕度、光、處理時間，對小菜蛾幼蟲之感藥程度無顯著之影響。浸濕處理小菜蛾幼蟲，將致使幼蟲對馬拉松產生敏感，而顯著地降低其半數致死劑量，幼蟲對藥劑之反應亦因不同之飼料及饑餓處理而有所影響。