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The Induction and the Mechanism of SN72129 Resistance in the Diamondback Moth, *Plutella xylostella* (L.)

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Abstract: SN72129 resistance has been induced in the diamondback moth, *Plutella xylostella* (L.), by consecutive LC_{75} selection in the laboratory. The Taiwan native susceptible strain developed 12 fold resistance after 14-18 generations of selection. No significant resistance had built up in the first 8 generations, then the resistance doubled every 4 to 5 generations.

The SN72129-resistant diamondback moth was tested with organophosphorus, synthetic pyrethroids, carbamate and tertiary amine insecticides. No significant cross resistance was found, and only minor cross effects were observed for phenthoate, carbofuran, and deltamethrin.

In the resistance mechanism investigation, the esterases and hydrolases were excluded from the study because the SN72129 molecule was not subjected to the action of those enzymes. The mixed function oxidases are most likely to be the enzymes that are responsible for the detoxication of SN72129. Topical application of SN72129/synergist/acetone combinations on the resistant third instar diamondback moth larvae produced synergistic ratios of 1.87 and 2.65 for piperonyl butoxide and 4-chloro-1-naphthyl propargyl ether. The test by adding the synergists into the regular SN72129 spray had better results. The resistance ratio of 11.70 to SN72129 was lowered to 8.22, 5.26, and 2.45 by MGK264, piperonyl butoxide, and 4-chloro-1-naphthyl propargyl ether respectively. Obviously, 4-chloro-1-naphthyl propargyl ether is the strongest synergist among three synergists for SN72129. By increasing the piperonyl butoxide to 10 : 1 and 20 : 1 in the mixture, the SN72129 resistance can be lowered further.

The above evidence indicates that the diamondback moth has the ability to develop a unique SN72129 resistance after a latent selection period, and the mixed function oxidase is the main factor in detoxifying the SN72129 in the resistant diamondback moth larvae.

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Introduction

The experimental thiazole insecticide, SN72129, has been proven of its effectiveness on the diamondback moth, *Plutella xylostella* (L.). The SN72129 demonstrates itself a rather unique insecticidal action from other traditional insecticides and encounters with no cross resistance from all existing resistances[4]. However, it is questionable that how the diamondback moth (DBM) react under the longterm exposure of SN72129. Can the DBM generate resistance toward the SN72129 ? If this is possible, then how fast the resistance will appear and to what extend ? Furthermore, what is the possible mechanism for the new resistance, and will other insecticides be affected by its cross effect ? For better understanding of the above questions, we have conducted the SN72129 resistance selection, and once a SN72129-resistant DBM strain is obtained, many aspects of this new resistance can be investigated in details.

Materials and methods

Chemical: The SN72129 was supplied by the Schering AG as 50% w/w wettable powder. Pure SN72129 was isolated from the wettable powder and used in the topical test. Piperonyl butoxide (PB) was purchased from the Tokyo Kasie; MGK264 was a gift from Cheng-hong Chemical Co. Ltd ; 4-chloro-1-naphthyl propargyl ether (PE) was obtained from the Union Carbide. Commercially formulated mevinphos, phenthoate, profenofos, prothiophos, carbofuran, cartap, deltamethrin, and permethrin were used for the cross resistance test.

Insects: The I-lan and Lu-chu strains of DBM were subjected to the SN72129 selections. Both strains were similar in their original SN72129 susceptibility except that the former is more susceptible to other insecticides than the latter[1]. The SN72129-selected I-lan strain and the parental I-lan strain are then used in the synergistic effect and cross effect tests as the resistant and susceptible strains respectively.

Experimental procedures : The late 3rd instar DBM larvae were treated with SN72129 and the survived individuals were collected for propagating the next generation. The selection pressure was set at 75% and the required LC_{75} dosage was re-determined in every succeeding generation on 100-200 early emerged DBM larvae of that particular generation. The change of selection pressure (LC_{75} in ppm) from generation to generation was counted as the induction rate for resistance. The actual resistance to SN72129 was carefully determined until significant change was noticed, i.e., 14 generations for the I-lan strain and 12 for the Lu-chu strain.

The synergistic effects of PB, PE, and MGK264 were tested by either adding the synergists into the regular SN72129 spray or incorporating the synergists into the acetone solution of purified SN72129. The regular spray was carried out by a Burkard spray tower, whereas the topical application was performed by using the microapplicator.

The mortality of all treatments were counted 24 hours after the treatment; the post treatment holding temperature and relative humidity were controlled at 25 ± 1°C and 85 ± 10% respectively.

Results

1. Induction of SN72129 resistance

The SN72129 resistance had been successfully induced in both the I-lan and Lu-chu strains of DBM by consecutive LC_{75} selections. The SN72129 resistance induction in the I-lan strain is illustrated in Figure 1 by graphing the needed changes of LC_{75} for

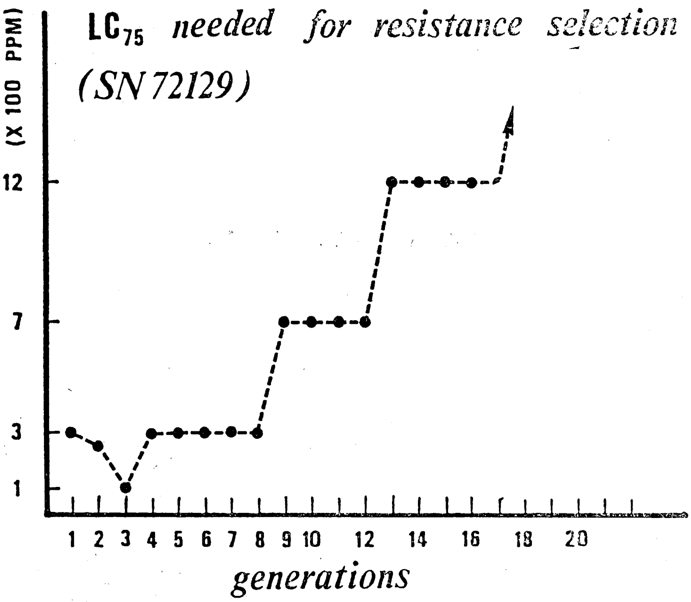

Fig. 1. The dosage increase curve of SN72129 resistance selection in the I-lan strain diamond-back moth, selection pressure=75% mortality.

succeeding selections. After 14 generations of selections, the I-lan strain gained 11.7 fold resistance. The build-up of this SN72129 insensitivity is rather non-significant in the first 8 generations, and the resistance then appeared almost doubled every 4-5 generations. Meanwhile, the Lu-chu strain developed 6 fold resistance after 12 selections. The resulted LC_{50} were 521.9 and 635ppm for the I-lan and Lu-chu strains respectively, and the comparison of their probit lines are shown in Figure 2(a) and 2(b). Continual increase of resistance is possible upon more selections.

2. Synergistic effects of MFO inhibitors

In order to counter the SN72129 resistance and probe the possible role of MFO in regard to this newly developed resistance, we have tested the synergistic effects of PB, PE, and MGK264. The first part of the test was carried out topically with the SN72129/synergist combination in acetone solution. The resistance ratio was merely 2.15 (Table 1) in contrast to 11.70 in the regular spray test (Table 2). By the help of synergists, the resistant DBM had recovered their susceptibility to SN72129 and the synergistic ratios were 1.87 and 2.65 for PB and PE respectively (Table 1). The differences of both the

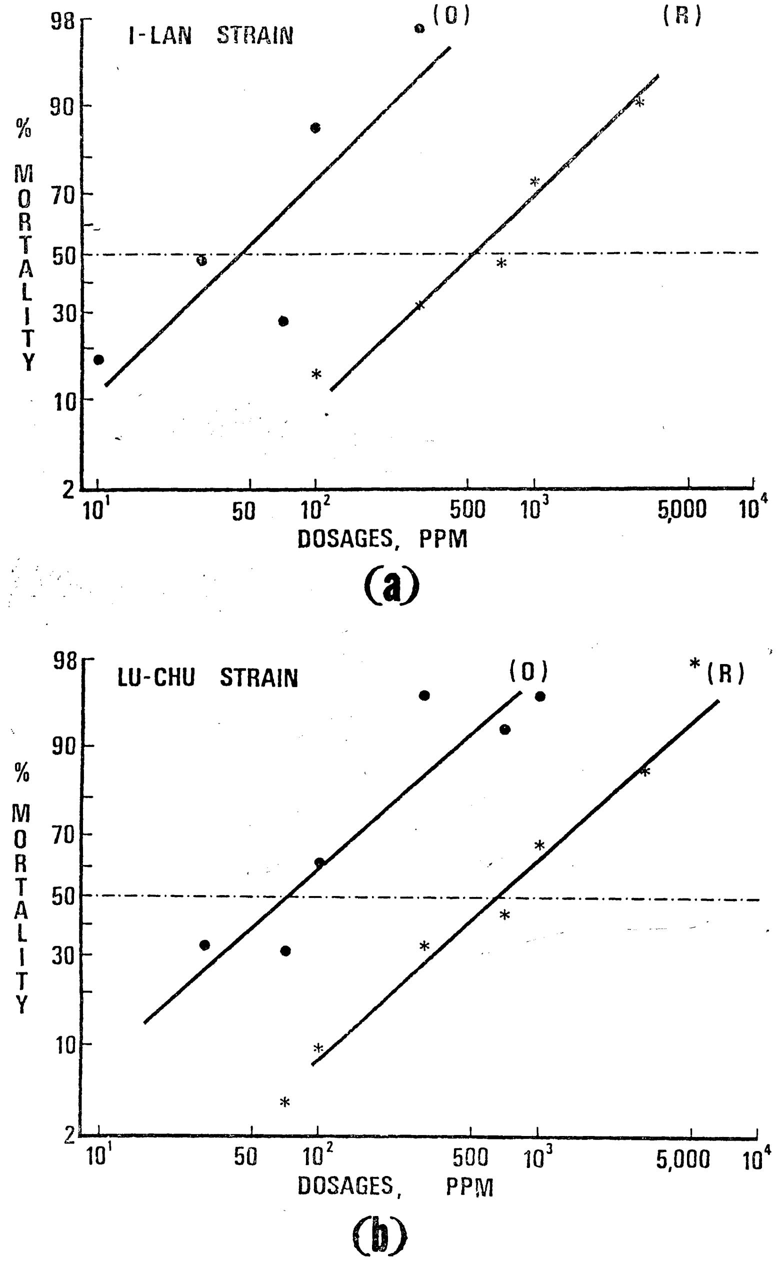

Fig. 2. The induction of SN72129 resistance of I-lan and Lu-chu strains of DBM
(O) : original strain, (R) : resistant strain.

resistance ratio and synergistic ratio were too little to demonstrate the significance of possible MFO metabolism of SN72129.

The second test was carried out by incorporating the synergists into the regular SN72129 spray and the results are shown in Table 2. For the SN72129-selected strain, the resistance ratio stood at 11.70 and could be lowered to 5.26, 8.22, and 2.45 by the synergistic action of PB, MGK264, and PE respectively. The synergistic ratios

Table 1. The synergistic effects of piperonyl butoxide (PB) and 4-chloro-1-naphthyl propargyl ether (PE) on the susceptible and resistant diamondback moths, determined by topical application method.

Treatment	LD_{50}* in ng/(Slope)/(Synergistic ratio)		R. R. **
	Susceptible DBM	Resistant DBM	
SN72129 only	13.3ng/(0.78)/(--)	28.5ng/(0.59)/(--)	2.15
PB+SN72129(5 : 1)	10.8ng/(0.79)/(1.23)	15.3ng/(0.70)/(1.87)	1.42
PE+SN72129(5 : 1)	11.8ng/(0.65)/(1.13)	10.8ng/(0.57)/(2.65)	0.92

*LD_{50} in ng a.i. of SN72129 in acetone/larva **R. R. : resistance ratio

Table 2. The synergistic effect of MGK264, PB, and PE on the susceptible and resistant diamondback moth, determined by spray tower method.

Treatment	LC_{50}* in ppm/(Slope)/(Synergistic ratio)		R. R. **
	Susceptible DBM	Resistant DBM	
SN72129 only	44.6ppm/(0.79)/(--)	521.9ppm/(0.90)/(--)	11.70
PB+SN72129 (5 : 1)	46.1ppm/(1.45)/(0.97)	242.5ppm/(1.02)/(2.15)	5.26
(10 : 1)	Not tested	95.9ppm/(1.45)/(5.44)	—
(20 : 1)	Not tested	49.9ppm/(1.06)/(10.5)	—
MGK+SN72129(5 : 1)	34.8ppm/(0.88)/(1.28)	285.9ppm/(1.39)/(1.83)	8.22
PE+SN72129(5 : 1)	20.5ppm/(1.09)/(2.17)	50.3ppm/(2.11)/(10.4)	2.45

*LC_{50} in ppm a.i. of SN72129 as wettable powder in water. **R. R. : resistance ratio

were 2.15, 1.83, and 10.4 for PB, MGK264, and PE respectively. By increasing the ratio of PB to SN72129 to 10 : 1 and 20 : 1, the SN72129 resistance can be lowered. The results clearly demonstrated that the MFO is actively involved in the SN72129 metabolism in resistant DBM.

3. Cross resistance to other insecticides

The susceptibilities of the SN72129-resistant DBM and the parental I-lan strain of DBM to 4 organophosphorus compounds, 2 synthetic pyrethroids, one carbamate, and one tertiary amine insecticide are compared in Table 3. In general, there is no significant cross effect among the SN72129 and other insecticides. Only slight reduction of the susceptibilities to phenthoate, carbofuran, and deltamethrin were noticed. Nevertheless, the susceptibilities of SN72129-resistant DBM toward mevinphos, profenofos, prothiophos, cartap, and permethrin remained unchanged.

4. Possible entry route of SN72129 in the DBM

When applying the SN72129 on the DBM, we had noticed that topical application

Table 3. Test for cross resistance of SN72129-resistant diamondback moth to other insecticides.

Insecticide	LC_{50} in ppm/(Slope)		R. R.
	Susceptible DBM	Resistant DBM	
SN72129	44.6ppm/(0.79)	521.9ppm/ (0.90)	11.7
Mevinphos	91 ppm/ (1.03)	161 ppm/ (1.01)	1.8
Phenthoate	284ppm/(0.76)	879 ppm/ (1.05)	3.1
Profenofos	367ppm/(0.94)	474 ppm/ (0.96)	1.3
Prothiophos	831ppm/(0.79)	1407ppm/ (0.78)	1.7
Carbofuran	150ppm/(0.57)	343 ppm/ (0.49)	2.3
Cartap	431ppm/(0.71)	551 ppm/ (0.51)	1.3
Deltamethrin	26.2ppm/(0.62)	62.4ppm/ (0.59)	2.4
Permethrin	98.0ppm/(1.18)	77.7 ppm/ (0.97)	0.8

did not give distinct lethal action as compare to the regular spray method. For this reason, a more detailed investigation concentrated on how the DBM was intoxicated by the SN72129 was conducted. Four different treatments were compared after we had discovered that the cabbage leaf sprayed with 1ml of 300ppm SN72129 was enough to kill the DBM (Table 4), and they were (1) SN72129 sprayed on leaf only, (2)

Table 4. The mortality of DBM by SN72129 via different intoxication treatments.

Sprayed object	Conc. (ppm)	Mortality (%)	Number of DBM larvae tested
First test			
Leaf only	300	95	20
	100	61	18
	70	32	19
	0	0	20
Second test			
Leaf only	300	70	43
Insects only	300	8	53
Petri dish only	300	44	36
Leaf and insects	300	72	25
	0	0	12

sprayed on the DBM larvae only, (3) sprayed on petri dish, and (4) sprayed on leaf with the DBM larvae on it. In treatment (1) and (3), the DBM larvae were transferred onto the leaf or petri dish later for the intoxication test. The results in Table 4 indicate that the DBM larvae were not killed by contact of SN72129, but rather by feeding of SN72129 on the vegetable leaves. Although the DBM larvae crawling on the pre-treated petri dish resulted in some lethal effect, it was suspected that feeding of fine SN72129 particles had caused the death of insects. Therefore, the DBM larvae were tested again by sealing their mouth parts with wax to prevent the ingestion of SN72129. The data in Table 5 had confirmed our speculation. The DBM larvae with their mouth parts sealed showed no mortality at all, whereas the DBM crawling on the SN72129-treated petri dish without sealing their mouth parts had the same mortality as those fed on the SN72129 sprayed leaves.

Table 5. Comparison of DBM mortality by different SN72129 treatments on vegetable leaf or crawling surface.

Sprayed object	Conc. (ppm)	Mortality (%)	Number of DBM larvae tested
Leaf only	10,000	100.0	39
Petri dish only			
DBM mouth parts sealed	10,000	8.3	36
DBM mouth parts not sealed	10,000	100.0	31
No insecticide spray			
DBM mouth parts sealed	0	9.1	11
DBM mouth parts not sealed	0	0.0	20

Discussion

The successful induction of SN72129 resistance in the DBM has once again demonstrated that the DBM have the ability to adapt themselves to the presence of xenochemical pressure. Unlike the resistance induction for other insecticides [2,3,5], the SN72129 resistance did not appear in the beginning of the selection. The resistance appeared only after 8 generations of selection. The MFO of the DBM did not metabolize the SN72129 very well and it took a long time to activate proper MFO for the above purpose. Whether the LC_{75} had provided enough pressure in selection is a question remain to be answered. The Lu-chu strain of DBM did not show faster or higher resistance coincident with the previous finding that no cross resistance was found from other insecticides [4].

Several synergists were successful in countering the SN72129 resistance hence, indicates the MFO metabolism is the main mechanism. The finding fits with the conclusion of von Keyserlingk that the selectivity of SN72129 to different insects is related to the species-specific MFO activities [6]. In the DBM, the common mixing ratio of 5 : 1 of synergist: insecticide was not enough for PB, and had to be raised to 10 : 1 or 20 : 1 for effective synergistic action. The digestive absorption might be the reason for the failure of PB at 5 : 1 ratio. The intoxication route test revealed that the

DBM had to ingest the SN72129 for the lethal effect, whereas the synergist test also indicated that feeding method resulted in more pronounced mortality than that of topical application. So far, it is not clear whether the metabolism of SN72129 occurs in the epithelium of digestive tract or in the haemolymph, but the subject is rather interesting for further study.

Since the independence of the lethal action of SN72129 and the resistance from other insecticides had been confirmed, the thiazoles can be used in alternating with other insecticides to relieve the resistance problem in the future.

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SN72129 汰選小菜蛾抗性及其抗性機制之研究[1]

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摘 要

以試驗代號為 SN72129 的 Thiazoles 類殺蟲劑於室內連續汰選小菜蛾，發現本省的感性小菜蛾在汰選14代之後，可得到12倍的抗藥性。此抗藥性在汰選初期發生極緩慢，第8代抗性開始上升，平均每4—5代提高約一倍。以有機磷、合成除蟲菊精、氨基甲酸鹽及有機氯等殺蟲劑測試抗SN72-129 小菜蛾對常用藥劑的感度，結果僅賽達松、加保扶及第滅寧有輕微之交互抗性產生。添加協力劑於SN72129測試其對小菜蛾之效果時發現，小菜蛾三齡幼蟲行表面施藥時，添加 PB及 PE分別有1.87及 2.65 倍的協力作用；若以噴施葉面餵食的方式，則 PB、PE 及 MGK 264 分別有 5.26、2.45及8.22倍的協力效果，顯見多功能氧化酵素 (mixedfunction oxidases) 為小菜蛾抗 SN72129 的重要因子。

以上各項結果顯示，長期使用 SN72129 後，小菜蛾會產生抗藥性，而此抗性小菜蛾體內對 SN72129 發生之解毒作用，係誘發出可解毒的多功能氧化酵素，此一新誘發之氧化酵素並不對其他殺蟲劑發生交互抗性。

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