中華農業研究 (Jour. Agric. Res. China) 39(3) : 208～220 (1990)

Insecticide Resistance Study in *Plutella xylostella* L.

X. The IGR-resistance and the possible management strategy[1]

Cheng, E. Y., C. H. Kao and C. S. Chiu[2]

Abstract : The benzoylphenyl urea-type insect growth regulators (IGRs) have caused the newest type of resistance in diamondback moth (DBM, *Plutella xylostella*). The possible mechanism, the declining rate, the genetics and the possible management strategy of this resistance have been investigated. The results indicated that DBM can develop IGR-resistance up to thousand folds in less than one and half years or equal to 30 generations in field, and this resistance is attributed to the metabolic degradation by a new mixed function oxidase (mfo) in DBM induced from the intensive IGR sprays. The resistance can only be maintained by continuous IGR selection, or it will decline when the selection pressure was lift. The decline of IGR-resistance can be accelerated by crossing the susceptible strain with the resistant population, and by such a simple manipulation, greater reduction in resistance was accomplished than the addition of synergist, piperonyl butoxide. Genetically, the IGR-resistance is not sex-linked and is highly recessive, and has not been confirmed as the monofactorial control. When the susceptible gene for IGR was re-introduced into the resistant population, this genetical dominance can self-propagate as the generations proceed. In a few generations, the whole population will restore the high sensitivity to IGRs, which was similar to the susceptible strain. By establishing sanctuary in the field to harbor a portion of DBM population free from the IGR selection, the dominant susceptible gene or genes will be preserved in the field DBM to delay or prevent the development of IGR-resistance. This strategy can be utilized in intensively cultured vegetable areas where small areas are kept free from IGRS to serve as the sanctuary for IGR-susceptible DBM.

Key words : *Plutella xylostella*, insecticide resistance, teflubenzuron, chlorfluazuron

Introduction

Ever since the benzoylphenyl urea (BPU)-type insect growth regulators (IGRs) were introduced to Taiwan in 1987 for the diamondback moth (DBM, *Plutella xylostella*) control, the possibility of developing IGR-resistance has become of great concern because sporadical alerts on the IGR-resistance in DBM had been reported previously in

1. Contribution No. 1526 from Taiwan Agricultural Research Institute. The study is partially supported by research grant from National Science Council (NSC 78-0409-B055-09) .

2. Senior entomologist, assistant entomologist and research assistant, respectively, Department of Applied Zoology, TARI, Wufeng, Taichung, 41301, Taiwan Republic of China.

Thailand (Cheng, 1988; Vattanatangum, 1988). The DBM had been shown to possess a natural mfo metabolic activity toward diflubenzuron and triflumuron (Cheng et al., 1988), and a mild teflubenzuron resistance was induced in DBM by the laboratory selection (Perng et al., 1989). The mfo degradation serves as the resistance mechanism for IGRs in the houseflies and DBM (Pimprikar and Georghiou, 1979; Lin et al., 1989). Within two years of marketing of IGRs in Taiwan, the IGR-resistance appeared in DBM and reached more than thousand folds. During the same span, TARI had been continually monitoring the field DBMs for the IGR-resistance. Several characters on the IGR-resistance in DBM were studied which included the possible mechanism, the natural declining rate of resistance, and the IGR-resistance in genetics. Hopefully, a better understanding of IGR-resistance in DBM and the possible management strategy can be elucidated

Materials and methods :

Insect materials

The susceptible IL(TC)-strain (Cheng et al., 1988) was used and the generations being used during this study were 58 to 78, a span of more than 20 generations. No changes in the IGRs sensitivity were observed.

IGR-resistant DBMs were collected either from different locations or from the same site at different times, and were used to (1) identify the IGR-resistance and (2) select the high IGR-resistant DBM for particular study. The resistant strains included:

Lu-chu (LC) strain (collected several times during 1988).

Juang-wei (JW) strain (Sept. 1989 from I-lan county).

Hsi-hu (HH) strain (Oct. 1989 from Changhua county).

San-chung (SC) strain [Oct. 1989 from Taipei county and the collecting site was close to the BC-strain appeared in previous DBM resistance study (Cheng et al., 1988)].

Insecticides

All insecticides used were commercially formulated and they were :

Teflubenzuron, 5% EC

Teflubenzuron, 13.5% FP

Chlorfluazuron, 5% EC

Fenvalerate, 10% EC

For the test, high concentrations of insecticide stocks were first made, and a series dilutions according to experimental design were followed. Usually, 5 to 7 dosages between LC_{10} and LC_{90} were chosen.

Synergist

Piperonyl butoxide (pb) was obtained from Tokyo Kasai, and was first formulated into 50% emulsion stock, and then mixed with target insecticide in 5 to 1 ratio (active ingredient) for testing.

Testing procedures

Third instar larvae of DBM were subjected to various insecticide susceptibility tests by using the Potter Spray Tower for spraying treatment. For every test, one side of a

cabbage leaflet ($5\times5cm^2$) was sprayed with 1ml of insecticide solution first, and then 20 DBM larvae were loaded on the other side of the leaf and sprayed again by another 1ml insecticide solution. A minimum total of 60 larvae in three replicas were treated per dosage. The post-treatment holding temperature and humidity were controlled at $25\pm1°C$ and $85\pm10\%$, respectively. The mortality was counted every 24 hours until pupation. The final mortality, usually at 120 hours after treatment, was recorded for probit analysis.

Testing items

1. The development of IGR-resistance in the field DBM population : Ever since IGRs were introduced for DBM control, the DBM in Lu-chu area has been periodically sampled for IGR-sensitivity test to examine the resistance development. Later, IGR-sensitivity test was also conducted on DBMs sampled from other locations in the insecticide resistance monitoring program.

2. The synergistic effect of pb: IGR-resistant DBMs from 5 locations around Taiwan were used to test whether the synergistic effect of pb is universal and to probe if there is any resistance mechanism other than the oxidative metabolic degradation.

3. The decline of IGR-resistance after release from the selection pressure : To understand the possible changes of IGR-resistance after the stopage of IGR use by farmers. The sensitivities of LC-strain to three commercial IGR products were determined on every three generations for 17 generations.

4. Genetic tests by crossing the IGR-resistant and the susceptible strains for the following purposes :

a. Whether the IGR-resistance is sex-linked

b. Whether the genetic nature of IGR-resistance is dominant or recessive

c. Whether the IGR-resistance is controlled by a single gene.

5. Laboratory testing on the reduction of IGR-resistance through the genetic manipulation.

Results

1. Development of IGR-resistance in the field DBM populations

In Taiwan, the original sensitivities (LC_{50}) of field DBMs to chlorfluazuron and teflubenzuron had never exceeded 10ppm in early 1987, however, the DBM resistance to IGRs had developed within a short period in the LC-areas (Fig. 1). The susceptibility to chlorfluazuron had changed from 3.8ppm in March to 924ppm in December, 1988, an increase of 243 folds in resistance ratio. By the end of 1989, DBMs collected from four other locations showed varied chlorfluazuron resistance as the LC_{50} ranged from 61.0 to 1685.7ppm, while the sensitivities to teflubenzuron in five DBM populations ranged from 100.5 to 72,628.6ppm (Table 1). The above results indicated clearly that the field DBM populations in Taiwan had developed IGR-resistance.

2. The synergistic effect of pb

The IGR-resistance observed in Taiwan DBM populations was greatly affected by the action of piperonyl butoxide. The synergistic ratio (S. R.) were 7.9 to 10.4 for

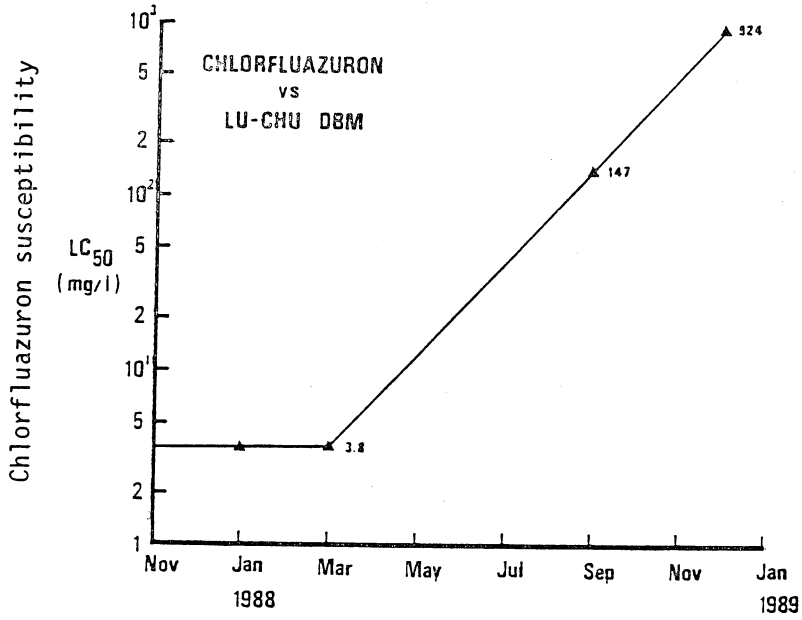


Figure 1. The chlorfluazuron susceptibility changes in Lu-chu DBM from Jan. to Dec, 1988.

Table 1. The synergistic effect of piperonyl butoxide to IGRs and synthetic pyrethroids against field resistant *Plutella xylostella**

Insecticide treatments	LC_{50} in ppm				
	HH(F_2)	KW(F_3)	SC(F_4)	IL(F_3)	LC(F_2)
IGRs					
Teflubenzuron alone	209.4	100.5	4,613.3	4,534,3	72,628.6
Teflubenzuron +5pb	26.4	—	444.8	475.2	—
Synergistic ratio	(7.9)	—	(10.4)	(9.5)	—
Chlorfluazuron alone	162.0	61.0	701.3	1,685.7	924.2
Chlorfluazuron+5pb	61.8	23.5	251.0	103.6	113.4
Synergistic ratio	(2.6)	(2.6)	(2.8)	(16.3)	(8.2)
Synthetic pyrethroid					
Fenvalerate alone	5,790.0	—	—	5,409.9	—
Fenvalerate+5pb	522.2	—	—	268.4	—
Synergistic ratio	(11.1)	—	—	(20.2)	—

* Collection dates : HH (Oct. 6th, 1989); KW (Sept. 23rd, 1989); SC (Oct. 21st, 1989); IL (Sept. 23rd, 1989); LC (Dec., 1988)

teflubenzuron in three tested DBM populations, while the S. R. ranged from 2.6 to 16.3 for chlorfluazuron in five IGR-resistant DBMs (Table 1). The action of pb was also examined by testing fenvalerate resistance on two resistant populations. The synergistic effect of pb was proven positive and universal in DBM's resistance to currently commercially available IGRs in Taiwan.

3. The declining of IGR-resistance after releasing from the selection pressure

The DBM collected from LC-area in December, 1988 carried a high IGR-resistance, and the resistance magnitude were 7,621 and 243 folds for teflubenzuron and chlorfluazuron, respectively. In a period of 17 generations, the resistance declined to only 6.5 and 3.1 folds for teflubenzuron and chlorfluazuron, respectively (Fig. 2). The resistance decline for the EC formulation of teflubenzuron showed a similar trend.

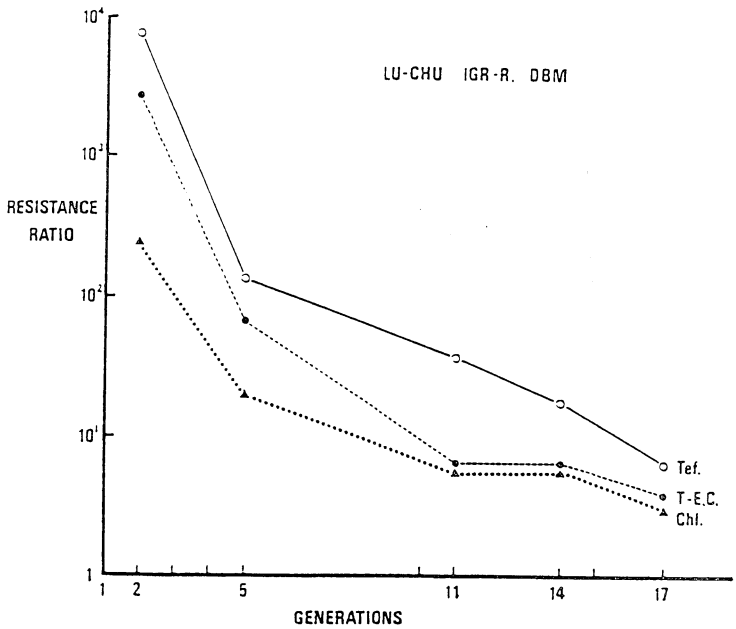


Figure 2. The decline of IGR-resistance of Lu-chu DBM in laboratory after releasing from the selection pressure.
Tef. : teflubenzuron, flowable powder
T-EC : teflubenzuron, emulsifiable concentrate
Chl. : chlorfluazuron, emulsifiable concentrate

4. Genetic tests of crossing the susceptible and two IGR-resistant strains

a. The reciprocal cross results of the susceptible and IGR-resistant DBM strains were presented in Table 2. In both tests, significant decrease in resistance was the only common conclusion, and the evidence of sex-linked (Russell, 1986) relation in IGR-resistance could not be found.

b. When the IGR-susceptibilities of the F_1-generation were compared to the probit lines of their parental strains (Figs. 3 and 4), significant increase in susceptibilities were found for both chlorfluazuron and teflubenzuron. If both compounds confronted with a common resistance, the genetic behavior of IGR-resistance would be considered as highly recessive.

Table 2. The reciprocal cross of the susceptible DBM and two IGR-resistant DBM strains for testing the possibility of sex linkage in IGR-resistance

Treatments	LC_{50} in ppm			
	S*	S♂×R♀	S♀×R♂	R**
1st test				
Teflubenzuron	9.2	150.1	44.0	4,934.0
Resistance ratio	(1.0)	(16.3)	(4.8)	(536.3)
Chlorfluazuron	5.3	98.7	43.7	1,210.9
Resistance ratio	(1.0)	(18.6)	(8.2)	(228.5)
2nd test				
Teflubenzuron	9.2	22.1	35.7	209.4
Resistance ratio	(1.0)	(2.4)	(3.9)	(22.8)
Chlorfluazuron	5.3	36.3	96.9	162.0
Resistance ratio	(1.0)	(6.8)	(18.2)	(25.7)

* S : susceptible IL-strain (collected in 1983).

** R : resistant SC-strain for the first test (F_3).
resistant HH-strain for the second test.

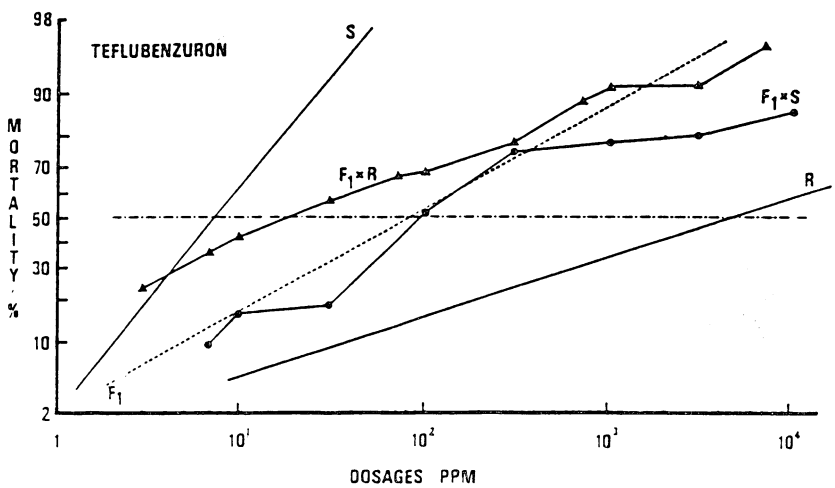


Figure 3. The teflubenzuron sensitivities of DBM in the susceptible (S), the resistant (R), the cross (F_1) and two backcross (F_1×R, F_1×S) strains.

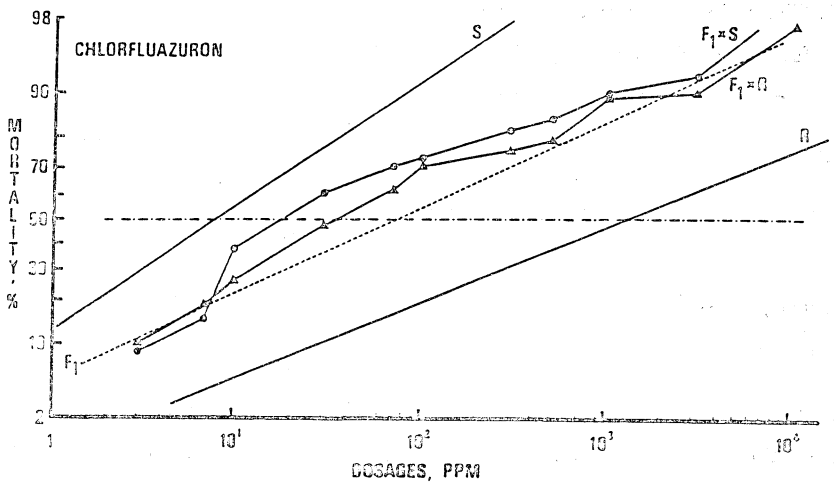


Figure 4. The chlorfluazuron sensitivities of DBM in the susceptible (S), the resistant (R), the cross (F_1) and two backcross ($F_1 \times R$, $F_1 \times S$) strains.

c. To test the monofactorial control of IGR-resistance, the IGR-susceptibilities were determined on the backcross of F_1 to the susceptible and resistant strains separately (Figs. 3 and 4). The results did not fit the suggested pattern of single gene control. The F_2 generation did not provided evidence to support the single gene control hypothesis either (Tsukamoto, 1983) (Figs. 5 and 6). The only conclusion could be made in these tests was that the IGR-resistance drastically reduced in all test crosses and continually declined as the generation proceeded. One problem related to this genetic test is that the degree of genetical homogeneity of the resistant strain. A better or clearer result would be obtained when a strain with only the homozygous IGR-resistance was used.

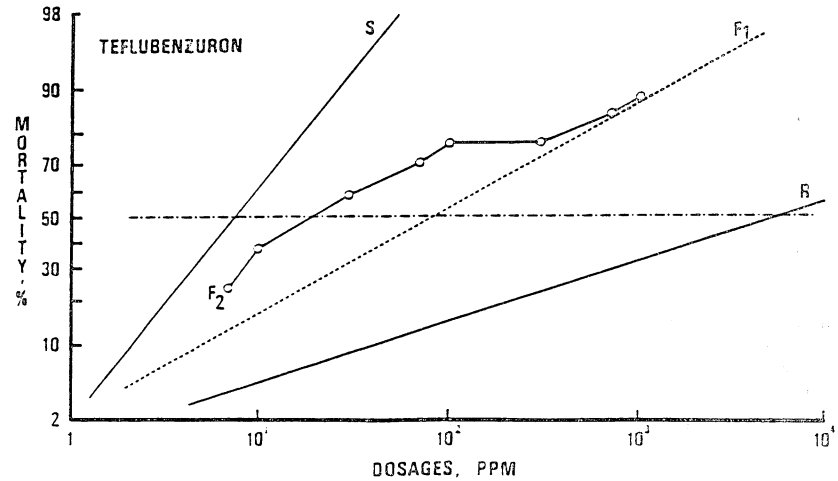


Figure 5. The self-cross (F_2) compared to its parental F_1, S and R-strains in their teflubenzuron mortality distribution pattern.

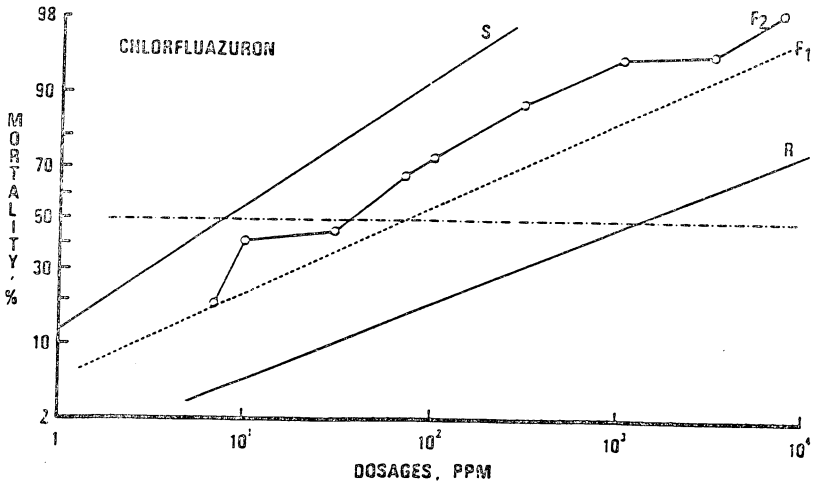


Figure 6. The self-cross (F_2) compared to its parental F_1, S and R-strains in their chlorfluazuron mortality distribution pattern.

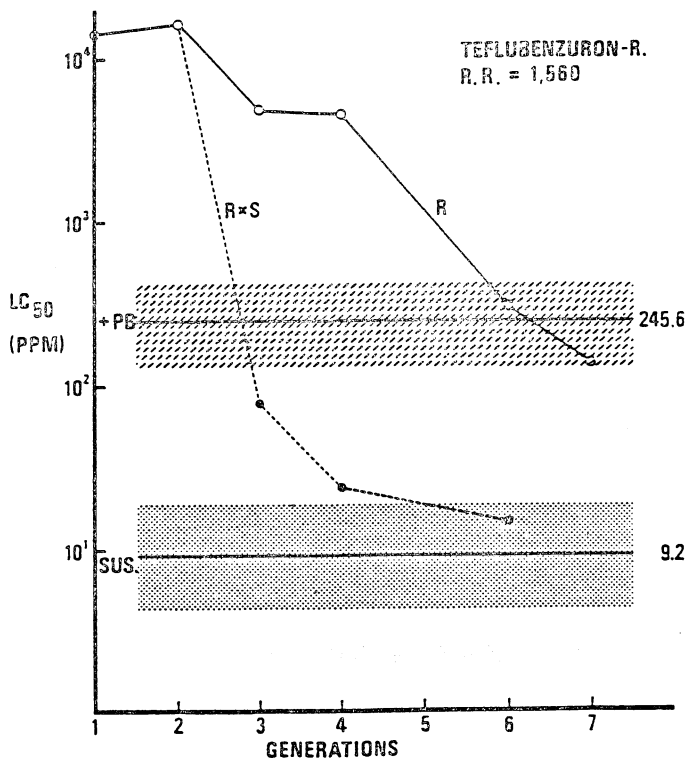


Figure 7. The teflubenzuron resistance decline (°)/accelated by crossing with the susceptible DBM (•)

LC50 (averaged from three tests of F1 to F5 of SC-strain with pb added) with range from 444.8 to 128.4 ppm

LC50 (averaged from 7 determinations of F59 to F77 of susceptible IL-strain) with range from 19.55 to 4.47 ppm

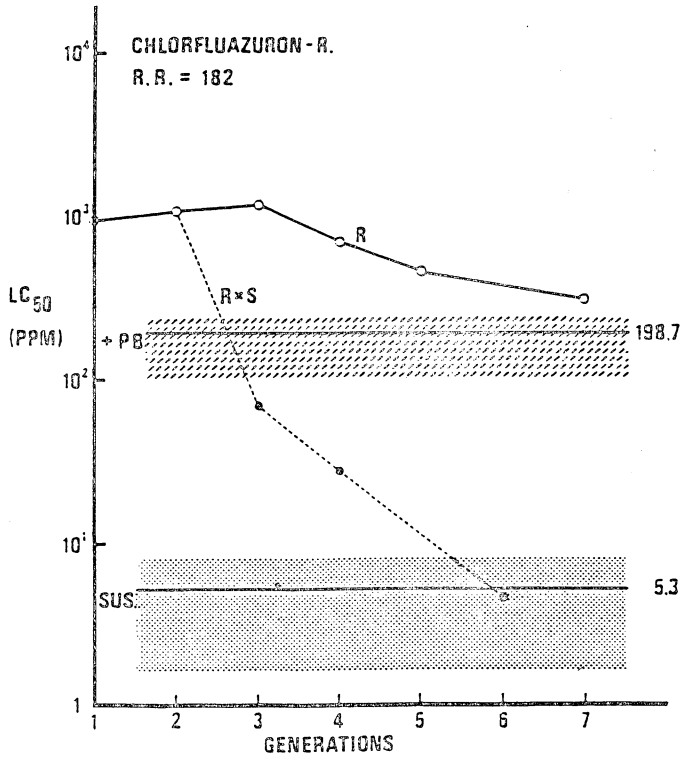


Figure 8. The chlorfluazuron resistance decline (°)/accelerated by crossing with the susceptible DBM (•)

LC50 (averaged from three tests of F1 to F5 of SC-strain with pb added) with range from 251.0 to 100.7 ppm

LC50 (averaged from 7 determinations of F59 to F77 of susceptible IL-strain) with range from 8.32 ppm to 1.68 ppm

5. Laboratory test for the possible management strategy of IGR-resistance

Through the genetic manipulation, we had observed the IGR-resistance of DBM could be greatly reduced by one cross with the susceptible strain. This genetic manipula tion already caused greater reduction in the IGR-resistance than the addition of pb. The sensitivity of teflubenzuron and chlorfluazuron with the addition of pb were determined in three tests from F_1 to F_5, and the values averaged at 245.6 and 198.7 ppm, respectively, and the ranges were 444.8 to 128.4ppm and 251.0 to 100.7 ppm in both cases.

The after-effect of this single cross on the teflubenzuron and chlorfluazuron-resistance (Figs. 7 and 8) was examined in the following generations and compared to the resistant DBM without such manipulation. In three more generations, the IGR-resistance in the offsprings of R×S population reduced to almost zero and the sensitivities to both teflu-benzuron and chlorfluazuron were restored and equal to that of the susceptible strain, while the F_5-F_7 of the resistant strain was still 78 to 20 folds resistant to teflubenzuron and chlorfluazuron, respectively. The sensitivities to both teflubezuron and chlorfluazuron

in the susceptible strain were determined 7 times during F_{59}-F_{77}, and the average LC_{50} were 9.2 and 5.3ppm, respectively. The ranges of these determinations were indicated in Figures 7 and 8. The fact proved that by utilizing the recessive characters of IGR-resistance for genetic dilution, the decline of IGR-resistance can be speeded up.

Conclusions and discussions

Based on results of the field monitoring and the laboratory tests, several conclusions had been drawn on the characters of BTU-type IGR-resistance in DBM :

(1) Under intensive IGR spray without any alternation with other insecticides, the DBM can develop high resistance to IGRs rapidly. The span usually was less than one and half years or equal to 30 generations in the field, and the resistance level could reach more than thousand folds comparing to the original effective dosage. Eventually, the DBM simply became unaffected at any dosage.

(2) The metabolic degradation of IGRs in DBM is mainly attributed to the oxidation by mfo. The IGR-resistance in DBMs collected in Taiwan could be reduced by pb. The substrate affinity of this new mfo, which degraded teflubenzuron and chlorfluazuron, is independent from previously found mfos that degraded other pesticides such as synthetic pyrethroids.

(3) It has not been found that the IGR-resistance with any sex-lined character genetically.

(4) Genetically, the IGR-resistance is recessive or highly recessive. Whenever a resistant individual hybred with a susceptible counterpart, great reduction in IGR-resistance occurred.

(5) Once the susceptible factor, genetically dominant, has been re-introduced into a resistant DBM population, this susceptible factor will propagate naturally as the generation proceeds, and the whole population will re-establish its high IGR sensitivity promptly.

Chou and Cheng (1983) first reported that DBM can develop a new and highly specific factor to detoxify a particular insecticide as the carbofuran-resistant DBM was proven to act independently from methomyl and carbaryl. Thereafter, the specificity of mfo's detoxiacation in DBM had been recognized, and at least three lines of mfo which were separately responsible for the detoxication of synthetic pyrethroids, carbofuran and other carbamates were confirmed (Cheng et al., 1986). Before IGRs were commercialized in Taiwan, four IGRs had been studied in their mfo degradation in relation to effectiveness of the susceptible DBM. Indications of mfo degradation for the two non-effective IGRs did exist in DBM, while the naturally inherited mfo in the susceptible DBM could not utilize teflubenzuron and chlorfluazuron as their enzymatic substrate (Cheng et al., 1988). It had been proven that the degradation of diflubenzuron is mostly contributed to mfo in the housefly (Pimprikar and Georghiou, 1979). In the induced teflubenzuron-resistant DBM, the mfo was confirmed to be the major resistance mechanism (Perng et al., 1988; Lin et al., 1989). The significant role of this resistance mechanism for IGR was again confirmed in the general survey of IGR-resistant DBMs in Taiwan in this report (Table 1).

All those facts indicated DBM had a very efficient, active and inducible mfo system (or complex). Once the molecular structure of xenobiotic chemical was fit to be the substrate for one of the many hidden mfos, this sub-line of mfo will respond quickly to the selection pressure and a new type of resistance appeared. The problem is that when mfo become the responsible resistance mechanism, it would respond indefinitely or without any limitation in resistance amplitude, and rendered the control chemical useless at any dosage. Carbaryl, methomyl, carbofuran, synthetic pyrethroids, teflubenzuron and chlorfluazuron all encountered the same fate. The mfo system of DBM is very diversified, and it responded to the selection pressure of other experimental insecticide as well. For example, by selecting DBM with SN72129 (Cheng et al., 1987), a mfo that could metabolize SN72129 appeared.

When the resistance is genetically recessive, the selection pressure is needed to maintain the resistance in the population. By crossing the resistant strain with the susceptible strain, the immediate reduction of resistance in their offsprings can be obtained. This phenomenon had been demonstrated very well in the synthetic pyrethroids and many other insecticides (Liu et al., 1984; Cheng et al., 1988). By genetic manipulation, a recessive type of resistance is manageable if it does not intangle with the cross resistance from other insecticide.

The BPU-type IGR-resistance in DBM is recessive, selective and independent from other insecticide resistance. Under these conditions, the IGR-resistance of DBM in the field may become manageable if the sanctuary could be provided in field to harbor a portion of DBM population free from the IGR-selection, for example, mosaic sprays of IGR and *Bacillus thuringiensis* (BT) in alternate row or rows. Genetic dilution would occur when the survivors of mosaic spray mated. The environment will favor the prevention or delay the IGR-resistance naturally. The proposed sanctuary in a mosaic spray should be designed in a way to provide satisfactory mixing environment

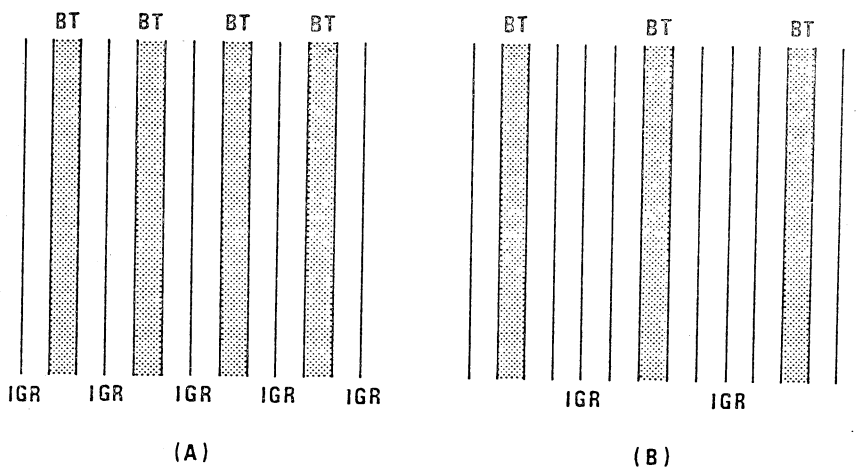


Figure 9. Proposed field mosaic sprays of IGR and BT. The survived DBM population in BT plots served as the susceptible strain to cross those DBM selected from IGR spray, and to delay or prevent IGR-resistance.

for DBM adults, which in turn may depend on factor like rate of dispersal of DBM adult (Fig. 9). The applicability of this strategy in what kind of field design will be the subject in our future study. Theoretically, the mosaic sprays of IGRs and BT is desirable for having minimum possibility in producing cross resistance and both insecticides favor the survival of natural enemies. Prosperous natural enemies, sanctuaries for the dominate susceptible gene, and two insecticides with independent modes of action and free from cross resistance should be considered as the most favorable combination in an integrated pest management (IPM) scheme.

If the IGR-resistance still slowly developed in IGRs and BT mosaic spray areas, insecticides such as mevinphos that cause only short-lived resistance in DBM (Cheng et al., 1985) can substitute as the control measure for a period of time to relieve the IGR-selection. The thorough study is definitely essential to construct a rational IPM implementation program to counter the resistance problem in DBM.

References

1. Cheng, E. Y. 1988. Problems of control of insecticide-resistant *Plutella xylostella*. Pestic. Sci. 23: 177-188.
2. Cheng, E. Y., T. M. Chou, and C. H. Kao. 1985. Insecticide resistance study in *Plutella xylostella* L. VI. An experimental analysis of organophosphorus and synthetic pyrethroid resistances. J. Agric. Res. China 34 : 96-104.
3. Cheng, E. Y., C. H. Kao, and D. F. Lin. 1987. The induction and the mechanism of SN72129 resistance in the diamondback moth, *Plutella xylostella* L. J. Agric. Res. China 36 : 228-236.
4. Cheng, E. Y., C. H. Kao, D. F. Lin, and T. C. Tsai. 1986. Insecticide resistance study in *Plutella xylostella* L. VIII. The specificity of oxidative detoxication mechanism in larval stage. J. Agric. Res. China 35 : 375-386.
5. Cheng, E. Y., D. F. Lin, T. C. Tsai, and C. H. Kao. 1988. Insecticide resistance study in *Plutella xylostella* L. IX. The selective metabolism of insecticides. J. Agric. Res. China 37 : 328-339.
6. Chou, T. M., and E. Y. Cheng. 1983. Insecticide resistance study in *Plutella xylostella*. III. The insecticide susceptibilities and resistance response of a native susceptible strain. J. Agric. Res. China 32 : 146-154.
7. Lin, J. G., C. F. Hung, and C. N. Sun. 1989. Teflubenzuron resistance and microsomal monooxygenases in larvae of the diamondback moth. Pestic. Biochem. Physiol. 35 : 20-25.
8. Liu, M. Y., J. S. Chen, and C. N. Sun. 1984. Synergism of pyrethroids by several compounds in larvae of the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 77 : 851-853.
9. Perng, F. S., M. C. Yao, C. F. Hung and C. N. Sun. 1988. Teflubenzuron resistance in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 81 : 1277-1282.
10. Pimprikar, G. D., and G. P. Georghiou. 1979. Mechanisms of resistance to diflubenzuron in the house fly, *Musca domestica* (L.). Pestic. Biochem. Physiol. 12 : 10-22.
11. Russell, P. J. 1986. Genetics. pp. 70-90. Little, Brown and Company, Boston.
12. Tsukamoto, M. 1983. Methods of genetics analysis of insecticide resistance. in "Pest Resistance to Pesticides", G. P. Georghiou and T. Saito (eds.), pp. 71-98. Plenum Press , New York and London.

13. Vattanatangum, A. 1988. Recent problems on chemical control of Thailand agricultural insect pests related to insecticide resistance of diamondback moth and other major species, in "Proceedings of Report Meeting of the Joint Research Project: Insect Toxicological Studies on Resistance and Integrated Control of the Diamondback Moth" pp. 8—13. Bangkok.

小菜蛾抗藥性之研究

十、昆蟲生長調節劑抗性特質及防治策略之探討[1]

鄭 允 高靜華 邱紀松[2]

摘 要

本省自1987年起，大量使用新登記之昆蟲生長調節劑（Insect Growth Regulator, IGR）防治小菜蛾以來，雖在田間僅使用一年半(相當於30個小菜蛾世代)，即已遭逢高達千倍之抗藥性。針對此一問題進行的研究得知，其抗藥性之機制爲一新誘發之氧化代謝酶，添加協力劑piperonyl butoxide（pb），可使此抗性之幅度下降。利用遺傳雜交試驗顯示，此一 IGR 之抗性無「性連遺傳」關係，且係隱性基因，利用雜交、回交及自交分析，皆未能得到與單基因控制理論相符合之結果，但此抗性主要爲隱性基因控制之事實已可確認。敏感品系在遺傳上具有顯性之優勢，將抗性族羣與感性族羣進行一次雜交即可造成 IGR 抗性之大幅下降，其幅度甚至超過添加協力劑之效果。雜交後再任其自交3—4代，全族羣對 IGR 之感度即已恢復，而與感性品系相當。引入感性品系對該抗性族羣之抗性下降幅度及速度均有促進之作用，此一特質可在未來之 IGR 抗性管理策略上加以應用，如保留部份間雜蔬菜區僅使用蘇力菌防治，以便在田間保護部份小菜蛾族羣不受 IGR 選汰，藉以保存在遺傳上占優勢之 IGR 敏感因子，可以自然雜交之方式延遲或阻止 IGR 抗藥性之發生。

關鍵詞：小菜蛾、抗藥性、昆蟲生長調節劑。

---

1. 臺灣省農業試驗所 研究報告第 1526 號。本計畫承國科會部份補助（編號 NSC78-0409-B055-09），簡淑貝、盧燕鈴、林愉珊協助工作，謹此一併致謝。

2. 本所應用動物系研究員、助理研究員及助理。臺灣省 臺中縣 霧峰鄉。