Review

Pyrethroid Resistance : Importance of the *kdr*-Type Mechanism*

Toshio SHONO**

Department of Medical Zoology, School of Medicine, University of Occupational and Environmental Health, Yahatanishi-ku, Kitakyushu 807, Japan

(Received August 31, 1984)

Syntheses of photo-stable pyrethroids, such as permethrin, cypermethrin, deltamethrin and fenvalerate, have led to possible agricultural use of pyrethroid class insecticides. Pyrethroid insecticides have very high toxicity to insects and very low toxicity to mammals; there is no problem of environmental contamination because pyrethroids easily decompose in the environment. Due to these favorable characteristics, pyrethroids are expected to be widely used in the areas of both agriculture and public health in the next decade. Development of resistance in pest insects is the only obstacle to the future success of these ideal insecticides. In this review, information regarding the development of pyrethroid resistance is summarized, and the importance of nerve insensitivity to pyrethroids in resistant insects is pointed out. Nerve insensitivity as a mechanism of pyrethroid resistance is controlled in houseflies by a gene termed *kdr*, and this resistance is therefore called the *kdr*-type. The *kdr*-type resistant insects show cross resistance to all kind of pyrethroids, so far synthesized, thus making the ideal insecticides, pyrethroids, impotent.

INTRODUCTION

After the structures of pyrethrins, the insecticidal components of pyrethrum flowers, were determined, an effort was made to synthesize more potent analogues. This effort led to the discovery of so-called first generation synthetic pyrethroids, *i.e.*, allethrin, tetramethrin, furamethrin, resmethrin, phenothrin, *etc.* Lack of photostability in these compounds, however, made their use on agricultural crops impractical and their use was limited to the

control of sanitary and household pests. Such lack of photostability was overcome when permethrin and fenvalerate were synthesized by Elliot¹⁾ and by Ohno *et al.*,²⁾ respectively. These were subsequently called second generation synthetic pyrethroids and, together with cypermethrin and deltamethrin, are now widely employed in agriculture. Several other pyrethroids will be put into practical use in the near future, and a great effort is now being made to develop others.

Pyrethroids in general have very high toxicity to insects compared to other insecticides, and this fact enables minimization of the amount used per given area. There is also little fear of environmental contamination because most of them readily decompose in the environment. These facts plus very low mammalian toxicity make pyrethroids an ideal agricultural insecticide, though they have high toxicity to fish and natural enemies of pest insects. It is, therefore, expected that pyrethroids will be one of the world's most popular insecticides in the latter half of the 1980's.

* This review was supported by Grant-in-Aid for Scientific Research No. 58560053, from the Ministry of Education, Science and Culture of Japan.

A part of this paper was presented at the International Symposium of Pesticides and the Environment in Lima, Peru, in April, 1984, organized by the National Institute for Evaluation of Natural Resources of Peru.

** Present address: Department of Medical Entomology, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan

Insecticide resistance, however, casts a shadow over the seemingly bright future of pyrethroids. An increase in their use might cause the appearance of pests which are resistant to these compounds. There are many questions yet to be answered regarding this development of resistance such as the species of insects in which it appears, the rate of development, cross resistance pattern, efficiency of synergists, whether a mixture of compounds delays resistance development, or for what period of time each pyrethroid can effectively be employed. In this article I will briefly review the information accumulated thus far regarding the resistance problem of pyrethroids and will try to depict what can be anticipated in the future.

It has quite often been found that when organochlorine insecticides such as DDT, or organophosphates or carbamates were put into wide use, within several years insects became resistant to them and eventually they were no longer effective as pest control agents. With pyrethroids, however, few cases of resistance were seen although several of them, *i.e.*, natural pyrethrins or allethrin were used for long periods. It was thus assumed by some that pyrethroids have a character that naturally restrains the development of resistance. Others argued that the lack of resistance was merely because of the limited use of pyrethroids in both the amount and the frequency of application and that if such factors were increased, development of resistance would be inevitable.

HOUSEFLIES IN DENMARK

Keiding^{3,4)} provided an answer to this question of whether insect populations are capable of developing resistance to pyrethroids when exposed to intensive control pressure. He carried out a large scale control experiment on houseflies in 23 farms in Denmark. Pyrethroids such as natural pyrethrins, bioresmethrin and tetramethrin or those substances plus a synergist, piperonyl butoxide, were applied as aerial sprays twice a week from May to October, 1973, in buildings housing livestock. Housefly susceptibility was monitored by topical application before, during and after the control program, and moderate to

high levels of resistance were found in most of the farms investigated after only 4 months. As this experiment distinctly revealed, pyrethroids are not free from encountering resistance development when they are intensively used.

PYRETHROID RESISTANCE IN THE DIAMONDBACK MOTH— AN EXAMPLE FROM TAIWAN

Recently several cases of pyrethroid resistance were found in the diamondback moth (*Plutella xylostella*) in Taiwan where the pyrethroids fenvalerate, permethrin, cypermethrin and deltamethrin had been used for its control on cruciferous crops since 1976. Liu *et al.*⁵⁾ sampled diamondback moths from ten locations, of which one for permethrin, 7 for cypermethrin, 5 for deltamethrin and 7 for fenvalerate showed a resistance factor of more than 10. The highest resistance factor was observed in the Ban-Chau strain, where the factor was 14 for permethrin, 87 for cypermethrin, 67 for deltamethrin, and as high as 207 for fenvalerate. Similar results were obtained by Cheng⁶⁾ who examined diamondback moth populations from 20 locations during the period December, 1980 to April, 1981 in the western half of Taiwan. He found 14 permethrin resistant populations with resistance factors of more than 10, and 5 populations with resistance factors of more than 30. For fenvalerate, out of 20 populations examined, 17 and 10 populations showed resistance factors of more than 10 and 30, respectively. These two reports clearly indicate that pyrethroid resistance developed in diamondback moth populations in Taiwan within 5 years of the first exposure to the compounds. This rapid development of resistance was probably influenced by the fact that in Taiwan the moths have more than a dozen generations a year and that frequent applications of pyrethroids had been made since vegetable crops were involved.

PYRETHROID RESISTANCE IN OTHER INSECTS

Aside from the housefly and the diamondback moth few cases of pyrethroid resistance are known. This paucity is probably because pyrethroid use has not been intensive enough

to cause a higher concentration of resistant genes in populations. The housefly,⁷⁾ the louse,⁸⁾ the German cockroach,⁹⁾ and a tick¹⁰⁾ showed resistance to natural pyrethrins, while resistance to synthetic pyrethroids was found in a mosquito, *Aedes aegypti*,¹¹⁾ and a tick.¹²⁾ On the other hand, as an example of cross resistance between organophosphates and pyrethroids, an aphid, *Myzus persicae*, resistant to dimethoate was highly resistant to permethrin, cypermethrin, deltamethrin and fenvalerate.¹³⁾

One species of insect worth mentioning here is a pest of cotton in the U.S.A., the tobacco budworm, *Heliothis virescens*, because this species is now being subjected to the most intensive pyrethroid application ever attempted. Development of resistance in this species is attracting major attention from those interested in pyrethroids around the world. In Texas, the center of the cotton belt, reduction in sensitivity has not yet been observed to any appreciable degree¹⁴⁾ although some sensitivity reduction^{15,16)} and development of resistance¹⁷⁾ have been found in the same insect in the Imperial Valley in California.

Selection under laboratory conditions, however, led to a resistance factor of 4000 after 16 generations when a mosquito *Culex quinquefasciatus* was subjected to selection pressure with *d-trans*-permethrin.¹⁸⁾ Another selection for 60 generations with bioresmethrin resulted in a resistance factor of 90 in the housefly.¹⁹⁾ The Egyptian cotton leaf worm (*Spodoptera littoralis*), the most critical cotton pest in Egypt, showed 32-fold resistance to fenvalerate after laboratory selection for 23 generations.²⁰⁾

MACHANISMS AND INHERITANCE OF PYRETHROID RESISTANCE IN THE HOUSEFLY

The housefly is probably one of the most thoroughly investigated insects in terms of resistance mechanisms and their inheritance. It is well known that pyrethroid resistant insects also show DDT resistance,^{8,10-12,21)} and the housefly is no exception.^{7,22)} There are three major genes confirming DDT resistance in the housefly. Two of them are involved with the metabolism of DDT: *Deh* on the second chromosome and *DDT-md* on the fifth chromo-

some. The other gene is a recessive *kdr*, which is not concerned with metabolism, on the third chromosome. This *kdr* gene is thought to lower the sensitivity of the nerve toward DDT.²³⁾ Flies that have homozygous *kdr* genes also show resistance to pyrethroids²⁴⁾ and electrophysiological experiments have revealed that their nerve sensitivity to pyrethroids is lower than that of the normal fly.^{25,26)} This lowered nerve sensitivity was also found in bioresmethrin selected houseflies.¹⁹⁾

Enhanced activity of detoxifying enzymes plays a major role in resistance against organochlorines, organophosphates and carbamates. Although such metabolic genes, e.g., *py-ses* and *py-ex*, played important roles in resistance toward natural pyrethrins or the first generation synthetic pyrethroids,²⁴⁾ metabolic degradation was not involved in highly resistant houseflies as a mechanism of resistance toward the second generation synthetic pyrethroids.²⁷⁾ This may seem strange since the second generation synthetic pyrethroids can also be broken down by detoxifying enzymes such as oxidases or hydrolases.^{28,29)} It is, however, reported that moderately resistant houseflies (5 to 13-fold resistant to several pyrethroids), which lack the *kdr* gene, more rapidly metabolize permethrin than do susceptible flies.³⁰⁾ This seems to indicate that the contribution of metabolism to the mechanism of pyrethroid resistance in houseflies is much smaller than nerve insensitivity. On the other hand, in *Spodoptera littoralis* larvae,³¹⁾ the esterases are thought to contribute to pyrethroid resistance. Reduced penetration also works as supplementary mechanism in pyrethroid resistance.^{19,24)}

In summary, we may say that the *kdr* gene plays a principal role in the resistance to second generation synthetic pyrethroids in houseflies. Another gene called *super-kdr* that is allele to *kdr* is also known to exist and flies that possess this *super-kdr* as homozygous show even higher resistance to DDT or pyrethroids; for example, *super-kdr* alone shows 600-fold resistance to deltamethrin.³²⁾

KDR-TYPE RESISTANCE

The pyrethroid resistance due to lowered nerve sensitivity is called *kdr*-type resistance, and has several characteristics:

Table 1 Insect and acarina species which have an insensitive nerve as a mechanism of pyrethroid resistance.

Identified by electrophysiological method
<i>Anopheles stephensi</i> ²¹⁾
<i>Blattella germanica</i> ³⁶⁾
<i>Musca domestica</i> ^{19, 25, 26)}
<i>Spodoptera littoralis</i> ³⁷⁾
<i>Boophilus microplus</i> ³⁸⁾
Suggested by synergist use or metabolism study
<i>Aedes aegypti</i> ^{39, 40)}
<i>Culex quinquefasciatus</i> ¹⁸⁾
<i>Plutella xylostella</i> ⁵⁾
<i>Spodoptera exigua</i> ⁴¹⁾
<i>Amblyseius fallacis</i> ⁴²⁾

1. The *kdr* gene causes lower sensitivity toward DDT and pyrethroids in nerves.

2. It confirms resistance to all pyrethroids so far known.

3. Even alone it can give high resistance, especially when the allele *super-kdr* gene is involved.

4. The gene is recessive.

The most remarkable of the above points is the second, that the gene confirms resistance to all pyrethroids. According to the absence or presence of the cyano group in the alcohol moiety, pyrethroid insecticides are classified as type I or type II. Each of these two types has a different neurophysiological mode of action and different target site (reviewed by Casida *et al.*³³⁾). The *kdr*-type houseflies, however, showed cross resistance to all kinds of both types I and II pyrethroids.^{32, 34)}

Similar cases of cross resistance were also observed in a mosquito, *Aedes aegypti*³⁵⁾ and in the diamondback moth.⁵⁾ Table 1 lists some pyrethroid resistant insects and acarina in which the *kdr*-type resistance mechanism has been confirmed or is presumed. Electrophysiological techniques were employed to verify the presence of such a mechanism in five species of arthropods. Such presence was also suggested from the results of experiments where several synergists were used, or metabolism was studied.

As previously stated, not many cases of pyrethroid resistance have been detected; only about twenty species of arthropods have be-

come resistant. It is thus worthy of note that, among those twenty, ten cases of *kdr*-type resistance were found. This fact indicates the common occurrence of the *kdr* gene in arthropod populations, giving rise to *kdr*-type pyrethroid resistance when those organisms are exposed to selection pressure with these substances. If this is indeed the case, we are justified in fearing that use of one pyrethroid insecticide and the subsequent development of resistance (possibly *kdr*-type), might lead to the ineffectiveness of this group of insecticides since such resistant organisms will be resistant to all pyrethroid insecticides.

CONCLUDING REMARKS

A pessimistic scheme may be drawn of pyrethroid resistance and its implications as follows;

The observations on the diamondback moth in Taiwan suggest that such a consequence is not unrealistic. In addition, since the *kdr* gene is one of the major genes responsible for DDT resistance, the resistance after years of heavy use of DDT may have brought about today's higher *kdr* gene frequency in pest populations, even though the use of this chemical was abandoned a decade ago. It was suggested, for example, that the rapid development of resistance to pyrethroids in Danish houseflies was due to the use of DDT in the 1940's. We may not have to be so pessimistic, however, because metabolic degradation also acts as a DDT resistance mechanism, so that DDT resistance is not necessarily accompanied by high *kdr* gene frequency.

The *kdr*-type resistance is also of extreme importance in terms of the development of new pyrethroids. Nowadays at least seven to eight years are usually required to develop a new insecticide. If the *kdr*-type resistance becomes common among pests from the use of already marketed pyrethroids, the above time period could conceivably render the newly

developed compounds ineffective before they appear on the market. Therefore, the issue of the *kdr*-type resistance mechanism must be kept in mind when new pyrethroids are to be developed.

Does the *kdr* mechanism confirm resistance to any possible structure of pyrethroids? Is there any possibility that we can develop pyrethroids which kill pests having the *kdr* mechanism? Answering these questions will require a knowledge of pyrethroid action at the molecular level and of the actual mechanisms of *kdr*-type resistance, neither of which, unfortunately, is available at this stage. Several findings such as that Ca-ATPase inhibition by DDT is lower in cockroaches with *kdr*-type resistance,⁴³⁾ or that phospholipids from the nerves of houseflies with such resistance have different characteristics from those of normal flies,⁴⁴⁾ or that there is a reduced number of pyrethroid receptors in *kdr* flies,⁴⁵⁾ or that there is reduced pyrethroid sensitivity of sodium channels in the *kdr* insect nerve⁴⁶⁾ may serve as clues to elucidate the mechanisms of *kdr*-type resistance. At present, however, the only strategy we are able to employ in the development of pyrethroids effective toward *kdr* seems to be trial-and-error with various compounds. Nevertheless, thus far we have always been able to come up with new compounds to suppress resistant pests. Therefore, we need not abandon hope of developing new pyrethroids that will overcome the *kdr*-type resistance, and these we might call the third generation synthetic pyrethroids.

Although the importance of *kdr*-type resistance in pest control by pyrethroids is rather emphasized in this article, the appearance of pyrethroid resistant insects which primarily depend upon another mechanism such as metabolism is quite possible, though only a few cases have been reported to date. The resistance caused by enhanced metabolism can be avoided by using synergists with pyrethroids or by modification of the chemical structures of pyrethroids. Such avoidance, however, is not possible in the *kdr*-type resistant insects.

REFERENCES

- 1) M. Elliot, A. W. Fahnham, N. F. Janes, P. H. Needham & D. A. Pulman: *Nature* **244**, 456 (1973)
- 2) N. Ohno, K. Fujimoto, Y. Okuno, T. Mizutani, M. Hirano, N. Itaya, T. Honda & H. Yoshioka: *Pestic. Sci.* **7**, 241 (1976)
- 3) J. Keiding: *Pestic. Sci.* **7**, 283 (1976)
- 4) J. Keiding: "Pesticide Management and Insecticide Resistance," ed. by D. L. Watson & A. W. A. Brown, Academic Press, New York, pp. 261-302, 1977
- 5) M. Y. Liu, Y. J. Tzeng & C. N. Sun: *J. Econ. Entomol.* **74**, 393 (1981)
- 6) E. Y. Cheng: *J. Agric. Res. China* **30**, 285 (1981)
- 7) B. C. Fine: *Nature* **191**, 884 (1961)
- 8) M. M. Cole & P. H. Clark: *J. Econ. Entomol.* **54**, 649 (1961)
- 9) D. G. Cochran: *J. Econ. Entomol.* **66**, 27 (1973)
- 10) G. P. Whitehead: *Nature* **184**, 378 (1959)
- 11) C. Prasittisuk & J. R. Busvine: *Pestic. Sci.* **8**, 527 (1977)
- 12) J. Nolan, W. J. Roulston & R. H. Wharton: *Pestic. Sci.* **8**, 484 (1977)
- 13) D. Otto: *Arch. Phytopathol. Pflanzenschutz, Berlin* **16**, 283 (1980)
- 14) F. W. Plapp, Jr.: *J. Econ. Entomol.* **74**, 207 (1981)
- 15) T. C. Sparks: *Bull. Entomol. Soc. Am.* **27**, 186 (1981)
- 16) P. H. Twine & H. T. Reynolds: *J. Econ. Entomol.* **73**, 239 (1980)
- 17) J. L. Martinez-Carrillo & H. T. Reynolds: *J. Econ. Entomol.* **76**, 983 (1983)
- 18) T. M. Priestler & G. P. Georgiou: *J. Econ. Entomol.* **71**, 197 (1978)
- 19) D. H. DeVries & G. P. Georgiou: *Pestic. Biochem. Physiol.* **15**, 234 (1981)
- 20) M. R. Riskallah, S. F. Abd-Elghafar, M. R. Abo-Elghafar & M. E. Nassar: *Pestic. Sci.* **14**, 508 (1983)
- 21) S. M. Omer, G. P. Georgiou & S. N. Irving: *Mosq. News* **40**, 200 (1980)
- 22) J. R. Busvine: *Nature* **171**, 118 (1953)
- 23) M. Tsukamoto, T. Narahashi & T. Yamasaki: *Botyu-Kagaku* **30**, 128 (1965)
- 24) A. W. Farnham: *Pestic. Sci.* **4**, 513 (1973)
- 25) T. A. Miller, J. M. Kennedy & C. Collins: *Pestic. Biochem. Physiol.* **12**, 224 (1979)
- 26) M. P. Osborne & R. J. Hart: *Pestic. Sci.* **10**, 407 (1979)
- 27) D. H. DeVries & G. P. Georgiou: *Pestic. Biochem. Physiol.* **15**, 242 (1981)
- 28) T. Shono & J. E. Casida: *J. Pesticide Sci.* **3**, 165 (1978)
- 29) T. Shono, K. Ohsawa & J. E. Casida: *J. Agric. Food Chem.* **27**, 316 (1979)
- 30) R. A. Nicholson & R. M. Sawicki: *Pestic. Sci.*

- 13, 357 (1982)
- 31) M. R. Riskallah: *Pestic. Biochem. Physiol.* **19**, 184 (1983)
- 32) R. M. Sawicki: *Nature* **275**, 443 (1978)
- 33) J. E. Casida, D. W. Gammon, A. H. Glickman & L. J. Lawrence: *Ann. Rev. Pharmacol. Toxicol.* **23**, 413 (1983)
- 34) D. H. DeVries & G. P. Georgiou: *Experientia* **36**, 226 (1980)
- 35) T. M. Priester & G. P. Georgiou: *Pestic. Sci.* **11**, 617 (1980)
- 36) J. G. Scott & F. Matsumura: *Pestic. Biochem. Physiol.* **16**, 21 (1981)
- 37) D. W. Gammon: *Pestic. Biochem. Physiol.* **13**, 53 (1980)
- 38) R. A. Nichololson, A. E. Chalmers, R. J. Hart, R. G. Wilson: "Insect Neurobiology and Pesticide Action (Neurotox 79)," Society of Chemical Industry, London, pp. 289-295, 1980
- 39) C. J. Brealey, P. L. Crampton, P. R. Chadwick & F. E. Rickett: *Pestic. Sci.* **15**, 121 (1984)
- 40) P. R. Chadwick, R. Slatter & M. J. Bowron: *Pestic. Sci.* **15**, 112 (1984)
- 41) S. L. Fullbroock & J. S. Holden: "Insect Neurobiology and Pesticide Action (Neurotox 79)," Society of Chemical Industry, London, pp. 281-287, 1980
- 42) J. G. Scott, B. A. Croft & S. W. Wagner: *J. Econ. Entomol.* **76**, 6 (1983)
- 43) S. M. Ghiasuddin, A. A. Kadous & F. Matsumura: *Comp. Biochem. Physiol.* **68C**, 15 (1981)
- 44) C. Chialiang & A. L. Devonshire: *Pestic. Sci.* **13**, 156 (1982)
- 45) C. P. Chang & F. W. Plapp, Jr.: *Pestic. Biochem. Physiol.* **20**, 86 (1983)
- 46) V. L. Salgado, S. N. Irving & T. A. Miller: *Pestic. Biochem. Physiol.* **20**, 100 (1983)

要 約

ピレスロイド剤抵抗性: *kdr* 型抵抗性機構の重要性

正野俊夫

フェンバレート, パーマスリン, サイパーメスリン, デルタメスリンのような, 光分解に対して安定なピレスロイド系殺虫剤の開発は, ピレスロイド剤の農業用殺虫剤としての利用に道を開いた. ピレスロイド剤は昆虫に対しては強い毒性を示すが, 哺乳類に対しては低毒性である. また, 環境中では速やかに分解されるので, 環境汚染のおそれもまったくない. これらの利点によって, ピレスロイド剤は, 今後, 農業用, 防疫用殺虫剤の中心的な地位を占めるものと考えられている. この理想的殺虫剤の将来に障害となるのは抵抗性の発達である. この総説では, 各種の昆虫におけるピレスロイド剤に対する抵抗性の発達を概説するとともに, 抵抗性の機構として, 神経の低感受性 (*kdr* 型) 抵抗性が重要であることを指摘し, この機構をもつ抵抗性害虫が蔓延した場合, すべてのピレスロイドの使用が困難になることを警告した.