サウジアラビア産ネッタイイエカにおける各種ピレスロイド剤の化学構造と抵抗性レベルの相関

誌名	日本農薬学会誌
ISSN	03851559
著者	葛西, 真二 正野, 俊夫 Weerasinghe, I.S.
巻/号	26巻2号
掲載ページ	p. 158-161
発行年月	2001年5月

J. Pesticide Sci. **26**, 158–161 (2001)

Note

Correlation of Pyrethroid Structure and Resistance Level in *Culex quinquefasciatus* Say from Saudi Arabia

Indira S. WEERASINGHE,[#] Shinji KASAI*,[##] and Toshio SHONO[###]

Laboratory of Applied Zoology, Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

(Received June 5, 2000; Accepted December 18, 2000)

Key words: cross-resistance, pyrethroid, *Culex quinquefasciatus*, mosquito, *kdr*, cytochrome P450 monooxygenase.

INTRODUCTION

Pyrethroids are one of the most widely used insecticides in the world. When photo-stable pyrethroids are introduced as agricultural insecticides, they are promising compounds because of their broad and potent insecticidal activity, and their low toxicity to mammals. However, extensive use of pyrethroids results in the development of resistance in many agriculturally and medically important pests.[1-4] Early pyrethroids can not be used near aquatic environments because of their high fish toxicity. However, some pyrethroids with low fish toxicity have been developed for control of pests in rice paddy fields.[5,6] A major concern is the development of resistance in non-target insects such as mosquitoes, including vectors of tropical diseases, through the use of these insecticides.[7]

A pyrethroid resistant strain of *Culex quinquefasciatus* Say (JPal-per strain), originally collected from Saudi Arabia, showed high resistance (1546-fold) to permethrin.[8] Previously, we reported that the major mechanism of permethrin resistance in this strain is enhanced permethrin detoxification via cytochrome P450(s) in addition to a *kdr* (knock down resistance)-type nerve insensitivity.[9] We also estimated the resistance levels of JPal-per strain against three organophosphates (fenitrothion, profenofos and parathion), a carbamate (carbaryl) and an insect growth regulator, pyriproxyfen. The resistance ratios to these insecticides were relatively low and ranged between 1.8 to 6.4-fold.[9] However, the spectrum of resistance against pyrethroid insecticides in JPal-per strain is still unknown. In this study we investigated the toxicity of 13 pyrethroid insecticides against the JPal-per strain and discuss the correlation between their chemical structures and resistance levels.

* To whom correspondence should be addressed.
[#] Present address: Department of Entomology, Medical Research Institute, Colombo 08, Sri Lanka
[##] Present address: Department of Medical Entomology, National Institute of Infectious Diseases, 1 23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan
[###] Present address: Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853-0901, USA

MATERIALS AND METHODS

1. *Insects*

The pyrethroid resistant strain of *Culex quinquefasciatus* (JPal-per) obtained from Dr. J. Hemingway (Univ. of Wales Cardiff, UK) was collected from Palestine Street in the University district of Jeddah, Saudi Arabia and selected with permethrin for 20 consecutive generations at a mortality level of 60–75%.[8] The susceptible strain was obtained from National Institute of Infectious Diseases collected from Chichijima, Ogasawara Island, Japan in 1968 and cultured without exposure to insecticides. Larvae were fed ground rat pellets. Adults were maintained on 10% sucrose and females were given blood meals from mice. Both strains were reared at 27±1°C and a photo period of 16 : 8 (L : D) hr.

2. *Chemicals*

The following insecticides were used: bifenthrin (90.4%; FMC), deltamethrin (99.9%; AgrEvo), etofenprox (96.0%; Mitsui Toatsu Chemicals, Inc.), and insecticides obtained from Sumitomo Chemical Co., Ltd. including allethrin (91.1%), cyfluthrin (88.4%), cypermethrin (94.5%), cyphenothrin (94.3%), fenvalerate (94.9%), furamethrin (88.0%), permethrin (91.2%), phenothrin (94.0%), resmethrin (94.1%) and tetramethrin (94.3%).

3. *Larval Bioassay*

Larval bioassays were carried out using standard bioassay techniques for mosquito larvae.[10] Twenty to thirty early fourth-instar larvae were exposed to different concentrations of pyrethroids in 50 ml of distilled water. The treated individuals were kept at 27±1°C and the mortality was assessed 24 hr after exposure. Ethanol was used as a carrier of the insecticides and control animals were dosed with ethanol only. The alcohol concentration never exceeded 1% of the total volume (sublethal concentration). At least 3 replicates were run for each insecticide concentration. LC_{50} values for each insecticide were calculated using log-probit mortality regression analysis.[11] The resistance ratio for each insecticide was calculated by dividing the LC_{50} values of the resistant strain by that of the susceptible strain.

RESULTS AND DISCUSSION

The toxicity of 13 pyrethroids to susceptible and resistant JPal-per strains is listed in Table 1. We observed that the JPal-per strain showed cross-resistance to all pyrethroids tested. The resistance ratios, however, ranged from 5.6 to

Table 1 Toxicities of pyrethroids with and without α-cyano groups on the susceptible and resistant JPal-per strains of *Culex quinquefasciatus* larvae.

Insecticide	Susceptible				JPal-per				RR[b]
	n[a]	Slope±SE	LC_{50}(μg/ml)	95% CL	n[a]	Slope±SE	LC_{50}(μg/ml)	95% CL	
Without α-cyano group									
Etofenprox	439	6.7±0.40	0.017	0.016–0.018	284	4.4±0.41	70.8	64.3–76.8	4160
Permethrin	287	5.3±0.02	0.0040	0.0030–0.0040	290	2.6±0.33	10.0	8.4–12	2500
Phenothrin	416	5.6±0.37	0.016	0.015–0.017	257	2.8±0.33	38.8	33.6–44.7	2430
Resmethrin[c]	590	7.4±0.64	0.044	0.042–0.046	500	4.2±0.83	57.5	46.9–69.6	1310
Furamethrin[c]	386	7.9±1.00	0.0024	0.0023–0.0026	325	4.1±1.20	0.15	0.12–0.18	63
Bifenthrin[c]	592	3.8±0.29	0.0050	0.0047–0.0057	266	3.3±0.41	0.18	0.16–0.21	36
Allethrin[c]	323	10.4±1.17	0.10	0.096–0.11	318	8.6±2.04	0.86	0.72–1.05	8.6
Tetramethrin[c]	342	5.6±0.61	0.13	0.10–0.15	379	11.4±1.46	0.73	0.70–0.77	5.6
With α-cyano group									
Cyfluthrin[c]	595	3.5±0.23	0.00085	0.00078–0.00093	542	3.8±0.35	0.050	0.046–0.054	59
Deltamethrin	755	4.7±0.41	0.00041	0.00039–0.00044	521	3.4±0.28	0.023	0.021–0.026	56
Cypermethrin	561	4.3±0.47	0.0021	0.0019–0.0024	286	3.3±0.27	0.099	0.088–0.11	47
Cyphenothrin	239	3.8±0.48	0.011	0.010–0.013	406	0.94±0.10	0.45	0.34–0.68	41
Fenvalerate	749	5.0±0.29	0.038	0.036–0.041	286	1.1±0.16	1.5	1.1–2.4	39

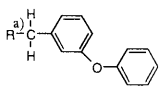
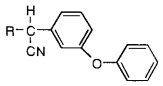
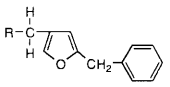
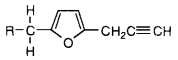
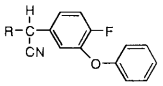
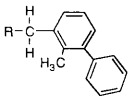
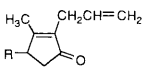
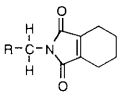
[a]Total number of larvae used. [b]Resistance ratio = LC_{50}(JPal-per strain)/LC_{50}(Susceptible strain). [c]Pyrethroid which does not have a 3-phenoxybenzyl moiety.

4160-fold. Pyrethroids can be classified into two types according to the absence (type I) or presence (type II) of an α-cyano group in the alcohol moiety.[12] JPal-per strain showed higher resistance to permethrin, etofenprox, phenothrin and resmethrin which belong to type I pyrethroids. Furthermore, three of these insecticides (permethrin, etofenprox, phenothrin) have a 3-phenoxybenzyl moiety in their chemical structures (Group I, Table 2). Etofenprox is unique because it contains an ether bond instead of the more common ester bond between the acid- and alcohol-moieties found in pyrethrins and most synthetic pyrethroids. The ether bond of etofenprox, however, does not reduce but rather increase the high level of resistance found in other pyrethroids having a phenoxybenzyl group such as permethrin and phenothrin. On the other hand, JPal-per strain showed only moderate resistance to pyrethroids that have a 3-phenoxybenzyl moiety with an α-cyano group (Group II, Table 2). The resistance ratios of JPal-per strain against these 4 compounds were between 39- to 56-fold. The only difference between permethrin and cypermethrin or phenothrin and cyphenothrin is the absence or presence of an α-cyano group, suggesting that the α-cyano group affects resistance mechanism(s) of the JPal-per strain. In contrast, the JPal-per strain showed relatively low levels of resistance to tetramethrin and allethrin (the resistance ratios were 5.6- and 8.6-fold, respectively), which have neither a 3-phenoxybenzyl moiety nor an α-cyano group (Table 2). The LD_{50} values to these two compounds were larger than most pyrethroids having a 3-phenoxybenzyl moiety with an α-cyano group (Table 1).

There appear to be at least two major mechanisms in pyrethroid resistance. One is decreased sensitivity of target site (*i.e.* sodium channel) against pyrethroids. The *kdr*-type mechanism (low sensitivity to DDT and pyrethroids in the voltage-dependent sodium channel) is a common mechanism for resistance to pyrethroids and DDT in many insects.[13] JPal-per strain also showed high resistance (300-fold) to DDT.[9] The resistance ratio to DDT did not decrease under the treatment of metabolic enzyme inhibitors such as piperonyl butoxide (PBO), 2-propynyl 2,3,6-trichlorophenyl ether (both oxidase inhibitors) and DMC (1,1-bis (*p*-chlorophenyl) ethanol, DDT-dehydrochlorinase inhibitor) suggesting the possible involvement of a *kdr*-like factor in this strain.[9] However, insects with a *kdr* factor generally show cross resistance to both type I and II pyrethroids.[1,14,15] The JPal-per strain may have different nerve insensitivity to each pyrethroid (*i.e.* particularly insensitive to the pyrethroids that have a 3-phenoxybenzyl moiety without an α-cyano group (Group I, Table 2)) as previously proposed by Chandre *et al.*[16] In fact, recent results of an electrophysiological study showed that the nerve sensitivity of adult JPal-per strain was 1/10 of the susceptible strain to a non-cyanopyrethroid, permethrin (Umeda, personal communications) while no difference in susceptibility was observed to lambda-cyhalothrin (with α-cyano 3-phenoxybenzyl moiety).[8] A similar phenomenon was reported from a resistant strain of *Spodoptera littoralis* which showed decreased sensitivity of the central nerveous system to permethrin but not to cypermethrin.[17]

Another mechanism of pyrethroid resistance is detoxification by metabolic enzymes. Previously, we investigated the synergistic effects of oxidase inhibitors on the toxicity of permethrin and *in vitro* metabolism of [^{14}C]-permethrin, and concluded that cytochrome P450 monooxygenases are one of the major mechanisms responsible for permethrin resistance in the JPal-per strain. In this metabolism study, we observed that monooxygenases hydroxylated

Table 2 Comparison of alcohol moiety of pyrethroids and resistance ratios

Group	Alcohol moiety	Compound	Resistance ratio
Group I (phenoxybenzyl without α-cyano)		Etofenprox	4160
		Permethrin	2500
		Phenothrin	2430
Group II (phenoxybenzyl with α-cyano)		Deltamethrin	56
		Cypermethrin	47
		Cyphenothrin	41
		Fenvalerate	39
Group III (Without phenoxybenzyl)		Resmethrin	1310
		Furamethrin	63
		Cyfluthrin	59
		Bifenthrin	36
		Allethrin	8.6
		Tetramethrin	5.6

[a]R = Acid moiety of each pyrethroid.

the phenoxybenzyl moiety of permethrin.[9]

Cytochrome P450s play a very important role in resistance to various insecticides in many pest insects.[18,19] In the pyrethroid resistant strain of the house fly (LPR), enhanced levels of pyrethroid detoxification via a cytochrome P450 (CYP6D1) are related to resistance.[20,21] Curiously, the LPR strain shows extremely high resistance to pyrethroids with or without an α-cyano group though the presence of phenoxybenzyl moiety is always associated with high levels (>900-fold) of resistance.[22] This difference may come from a large variation in substrate specificity of different P450s which is one of the remarkable features of the monooxygenases. For example, cytochrome P450 1A1 (CYP1A1) can metabolize more than 20 substrates while CYP7A1 has only one known substrate.[23] In the JPal-per strain, the P450 that is involved in resistance may have higher substrate specificity than CYP6D1 (*i.e.*, the isoform that hydroxylates only the 3-phenoxybenzyl moiety (without an α-cyano group) may cause the resistance). Recently, we cloned and determined the nucleotide sequence of cytochrome P450 cDNAs (*CYP6E1* and *CYP6F1*) from JPal-per strain in addition to four other partial cDNAs.[24,25] *CYP6F1* was observed to be over-expressed in the JPal-per strain relative to the susceptible strain suggesting the possible involvement of this isoform in insecticide resistance.[25] Heterologous expression and characterization of these isoforms individually will bring us closer to understanding the resistance mechanisms of the JPal-per strain.

Sheppard (1995) selected horn flies (*Haematobia irritans*) with a cyanopyrethroid, cyhalothrin. The resultant strain showed high resistance to all cyanopyrethroids tested (671- to 12,831-fold) as compared to non-cyano group pesticides (30- to 69-fold). By means of a PBO synergism study, it was concluded that enhanced activity of oxidative enzyme(s) is the major mechanism of cyanopyrethroid resistance in this strain.[26] It seems that oxidative enzyme(s) recognizes the α-cyano group in the substrates, and that different types of cytochrome P450(s) can be selected depending on the presence or absence of an α-cyano group in the pyrethroid insecticide used for the selections.

ACKNOWLEDGMENTS

We thank Dr. J. Hemingway, Cardiff University, UK, for supplying the resistant JPal-per strain, and Dr. T. Tomita and C. Shudou, National Institute of Infectious Diseases, Japan, for supplying the susceptible strain. We are grateful to Dr. D. Taylor for his critical reading of the manuscript and Dr. J. G. Scott and Dr. P. J. Korytko, Cornell University, USA, for their helpful suggestions. This work was supported in part by a Grant-in-Aid for Developmental Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (07406003 and 07556014) and by the University of Tsukuba Research Projects.

REFERENCES

1) M. Liu, Y. Tzeng & C. Sun : *J. Econ. Entomol.* **74**, 393 (1981)
2) J. G. Scott & G. P. Georghiou : *J. Econ. Entomol.* **78**, 316 (1985)
3) D. J. Pree, D. E. Archibald, K. W. Ker & K. J. Cole : *J. Econ. Entomol.* **83**, 2159 (1990)
4) D. C. Sheppard & J. A. Joyce : *J. Econ. Entomol.* **85**, 1587 (1992)
5) K. Nakatani, T. Inoue, S. Numata, K. Oda, T. Udagawa & M. Gohbara : 5th Int. Congr. Pestic. Chem., Kyoto, 1a-9 (1982)
6) T. Ohtsuka : *J. Pesticide Sci.* **18**, S145 (1993) (in Japanese)
7) G. P. Georghiou : "Pesticide Resistance in Arthropods," ed. by R. T. Roush & B. E. Tabshnik, Chapman and Hall, New York and London, pp. 183-202, 1990
8) A. M. Amin & J. Hemingway : *Bull. Ent. Res.* **79**, 361 (1989)
9) S. Kasai, I. S. Weerasinghe & T. Shono : *Arch. Insect Biochem. Physiol.* **37**, 47 (1998)
10) WHO : Unpublished document WHO/VBC/81, 807, World Health Organization, Geneva (1981)
11) D. J. Finney : "Probit Analysis," 3rd ed., Cambridge University Press, Cambridge, 1971
12) D. W. Gammon, M. A. Brown & J. E. Casida : *Pestic. Biochem. Physiol.* **15**, 181 (1981)
13) T. Shono : *J. Pesticide Sci.* **10**, 141 (1985)
14) D. H. DeVries & G. P. Georghiou : *Experientia* **36**, 226 (1980)
15) T. M. Priester & G. P. Georghiou : *Pestic. Sci.* **11**, 617 (1980)
16) F. Chandre, F. Darriet, M. Darder, A. Cuany, J. M. C. Doannio, N. Pasteur & P. Guillet : *Med. Vet. Entomol.* **12**, 359 (1998)
17) D. W. Gammon : *Pestic. Biochem. Physiol.* **13**, 53 (1980)
18) C. E. Wilkinson : "Pest Resistance to Pesticides," ed. by G. P. Georghiou & T. Saito, Plenum Press, New York, pp. 175-228, 1983
19) J. G. Scott : *Insect Biochem. Mol. Biol.* **29**, 757 (1999)
20) G. D. Wheelock & J. G. Scott : *J. Exp. Zool.* **264**, 153 (1991)
21) T. Tomita, N. Liu, F. Smith, P. Sridhar & J. G. Scott : *Insect Mol. Biol.* **4**, 135 (1995)
22) J. G. Scott & G. P. Georghiou : *Pestic. Sci.* **17**, 195 (1986)
23) S. Rendic & F. J. Di Carlo : *Drug Metab. Rev.* **29**, 413 (1997)
24) S. Kasai, T. Shono & M. Yamakawa : *Insect Mol. Biol.* **7**, 185 (1998)
25) S. Kasai, I. S. Weerasinghe, T. Shono & M. Yamakawa : *Insect Biochem. Mol. Biol.* **30**, 163 (2000)
26) D. C. Sheppard : *J. Econ. Entomol.* **88**, 1531 (1995)

要　　約

サウジアラビア産ネッタイイエカにおける各種ピレスロイド剤の化学構造と抵抗性レベルの相関

Indira S. Weerasinghe, 葛西真治, 正野俊夫

サウジアラビア産のパーメスリン抵抗性ネッタイイエカ(JPal-per 系統)について各種ピレスロイド剤に対する抵抗性レベルを調べた. JPal-per 系統は実験に用いた 13 種のピレスロイド剤すべてに対し抵抗性を示したが, そのレベルには 5.6～4160 倍という開きが認められた. 抵抗性レベルはピレスロイド剤のアルコール部位の構造に依存する傾向が認められ, 3-フェノキシベンジル基をもつピレスロイド剤では, α 位にシアノ基をもたない化合物に対しては 2000 倍以上の高い抵抗性を示したが, α-シアノ-3-フェノキシベンジル基をもつ化合物に対しては 39～56 倍の抵抗性しか示さなかった. 3-フェノキシベンジル基の α 位にシアノ基が存在するか否かが本系統の抵抗性機構に大きな影響を及ぼしていることが示され, この抵抗性レベルの違いがピレスロイド剤の作用点であるナトリウムチャネルの薬剤感受性の差違, もしくは解毒酵素チトクロム P450 の基質特異性の差違に起因している可能性について考察した.