韓應昆誌 33(2) : 74~80 (1994)
Korean J Appl. Entomol.

Studies on the Selections, the Cross-Resistance and the Esterase Activity in the Strain of German Cockroach, *Blattella germanica* L. Selected with Dichlorvos

바퀴에 대한 Dichlorvos(DDVP)의 累代淘汰, 交叉抵抗性 및 酵素活性에 관한 研究

Hyung-Rae Lee, Jeong-Wha Kim, Jong-Ryeol Bang and Kwan-Sun Choi
李炯來・金正和・方鍾烈・崔觀善

ABSTRACT The german cockroach, *Blattella germanica* L. populations were successively selected with dichlorvos for 11 generations. The resulting selected strain was investigated the resistance development. the cross resistance and the esterase activity. In the dichlorvos-selected(Rd) strain, the values of LC_{50} increased 8.58 times more compared to the susceptible (S) strain. In the dichlorvos-selected (Rd) strains, the cross-resistance to chlorpyrifos, propoxur, fenvalerate and permethrin showed 3.35, 4.09, 2.83 and 2.00 times. Esterase-activity of the Rd strain showed 1.33 times higher than that of the S strain in the filter paper test. In comparison of zymogram patterns of the esterase isozyme by thin agarose gel electrophoresis against the german cockroach, the S strain was separated by 4 bands of esterase 3, 5, 6 and 8 bands and the Rd strain was separated by 6 bands of esterase 1, 2, 4, 5, 7 and 8 bands, and the resistant mechanisms of the Rd strain were considered as the 4 bands of esterase-1.2.4 and 7 bands except the common 2 bands of esterase-6 and 8 bands.

KEY WORDS German cockroach, insecticide resistance, dichlorvos

초 록 바퀴에 대한 dichlorvos(DDVP) 약제로 11세대 누대 도태한 계통에 대한 저항성 유발정도, 교차저항성 및 효소 활성을 조사한 결과는 반수치사농도 비교시 감수성에 비하여 8.58배 증가하였고 교차저항성은 chlorpyrifos, propoxur, fenvalerate와 permethrin 약제에서 각각 3.35, 4.09, 2.83 및 2.00배의 저항성을 보였으며 효소활성은 filter paper test에서 감수성 대비 33%가 증가하였고 전기영동시험에서는 4개의 band인 esterase-1,2,4 및 7 band가 확인되었다

검색어 바퀴, 살충제 저항성, dichlorvos

The german cockroach, *Blattella germanica* L. is one of the most important pests in households and in the commercial food processing establishments It is also of medical importance because of its ability to mechanically transmit organisms causing human diseases.

The german cockroach has made a well documented history of developing resistance to various insecticides since the application of synthetic pesticide compounds (Nelson & Wood 1982). Resistances of organophosphate and carbamate compounds to the german cockroach were reported in the various spheres of science such as on resistant levels (Rust & Reierson 1978, Milio *et al.* 1987, Schal 1988, Cochran 1989), on multiple- and cross-resistances (Collins 1973, Nelson & Wood 1982, Scott *et al.* 1990, Hemingway *et al* 1993) and on toxicological characteristics including genetics (Bull *et al.* 1989, Scott *et al.* 1990, Siegfried & Scott 1990, Siegfried 1992. Hemingway *et al.* 1993, Prabhakaran & Ka-

Dept. of Agro-Biology, Coll. of Agri., Chungbuk Nat'l Univ. Cheongju, 360-763, Chungbuk porvince, Korea(충북대학교 농과대학 농생물학과)

mble 1993). Unfortunately, we could not find the experimental results of dichlorvos to the german cockroaches until now.

During the past few years, the pyrethroid compounds have assumed important role in the control of german cockroaches. Cochran (1987) reported the development of resistance to pyrethroids at six or seven generations of laboratory selection in this species. He also reported the widespread resistance to pyrethroids and emerging pictures of resistance to allethrin, permethrin, phenothrin, fenvalerate and cyhalothrin. Furthermore he has reported the experiment of selected through 17 generations for investigations of potential development on pyrethroid resistance in 1991. Its results of insecticide resistance against the german cockroach confirmed that the resistant genes were fixed by continuous selections with insecticides and affected to the other pyrethroids not selected by insecticides.

The control of german cockroach in multiple family dwellings is currently one of the toughest problems in urban pest control of Korea. In the researches on resistance of several chemicals to the german cockroach, Shim & Lee (1979), Shim *et al.* (1979) reported that the dichlorvos showed the most susceptibility with 0.31 μg/female insect (98 mg/body weight) of LD_{50} values and also reported the resistant development to the malathion and DDT. The other researchers such as Cha *et al* (1970) and Choi & Yoo (1972) reported the comparisons of toxicity with several insecticides against the several strains in Seoul and laboratory strains of the german cockroaches and its results showed the different responses of susceptibility according to the insecticides and the strains.

By this reason, we examined the resistant development, the cross-resistance and the esterase activity of the selected strain from susceptible strain for 11 generations with dichlorvos which was one of the popular pesticides for control of german cockroaches in Korea and reported its results in this paper.

MATERIALS AND METHODS

The chemicals used were supplied by the chemical manufacturers and suppliers in Korea (Table 1). The susceptible strain (S) of german cockroach which has been colonized without exposure to insecticides at the National Institute of Health (NIH) in Seoul since 1978, was used as the primary strain at the entomological laboratory of our University since in 1989 The S strain was utilized as the standard susceptible strain for determination of the resistance levels. The german cockroaches were reared at 28±℃, 50±2% RH and a photoperiod of 12:12 (L:D). The cockroach colonies were fed by Purina dog chow, sponge cake and water ad libitum and maintained in cheesecloth-covered glass container (L30 X W90 X H45 cm). The rim of this container was coated with petroleum jelly and the hiding place was created with the furrowed cardboard placed in the container.

Selections for the development of resistance were made for 11 generations on 5~6 nymphal stages of german cockroaches by exposing them to glass surfaces treated with known amounts of dichlorvos.

Table 1. Insecticides used in the present study

Insecticides	Chemical name	Purity(%)
Organophosphates		
Dichlorvos	2,2-dichlorovinyl dimethyl phosphate	95.0
Chlorpyrifos	o,o-diethyl-o-(3,5,6-trichlor-2-pyri-dyl) phosphorothioate	85.0
Carbamates		
Propoxur	2-isopropoxyphenyl-N-methyl carbamate	98.0
Pyrethroids		
Fenvalerate	(RS)-α-cyano-3-phenoxybenzyl(RS)-2-(4- chlorophenyl)-3-methyl-butyrate	96.4
Permethrin	3-(phenoxyphenyl) methyl(1RS)-cis, trans-3-(2,2-dichloroethenyl)-2-2-dimethyl cyclopropane carboxylate (approximately 60% trans, 40% cis isomers)	98.0

The measured amount of dichlorvos was dissolved in 2.5 ml of reagent acetone and was spreaded on the inner surface of 0.5 liter size glass jars by rotating jar in fume hood until the solvent was evaporated. This left a coat of insecticide on the jar surface. A thin layer of petroleum jelly was placed on the inner lip of the jars to prevent the insects from escaping. Selection pressures of dichlorvos were exposed at a rate of *ca*. 50% mortality levels in each generation. The survived nymphs for 24 h after treatment were transferred to the untreated clean containers of same size. Minimum 200 nymphs per generation were used for the selections.

Bioassays were performed as similarly to the experiment for development of resistance. However, the cockroaches were replaced with the young adult males at 6 days after emergence and the concentrations of dichlorvos were increased at least five degrees for probit analysis. Untreated control was exposure with reagent acetone alone. Ten male insects were placed in each of three glass jars with identically prepared concentration. Mortality was recorded at 24h after exposure. The data from the three replications were analyzed to determine 50% mortality. Bioassay of cross-resistance against the 11 generation strains selected by dichlorvos was performed by above mentioned method and the insecticides used in bioassay were tested chlorpyrifos, propoxur, fenvalerate and permethrin shown in Table 1.

The method used in the filter paper test for rapid detection of the esterase activity was of Pasteur & Georghiou (1981). Three kinds of stock solutions and the three kinds of working solutions named A, B, C respectively for the filter paper test were prepared as follows. The stock A solution called phosphate buffer solution (pH 6.5) was made by melting both 4.8g of Na_2HPO_4 and 9.2g of NaH_2PO_4 with 1000 ml of distilled water, the B solution called substrate solution was made by melting 1g of α-naphthyl acetate with 100 ml of reagent acetone, and the C solution called fixing solution, 10% acetic acid was used. The working A solution was made by mixing 100 ml of phosphate buffer with 100 ml of substrate solution, the B solution was made by melting 0.3g of the Fast Garnett G.B.C in 100 ml of distilled water and the 100 ml of C solution was made same as fixing solution.

The experimental procedure of filter paper test are as follows; A adult female german cockroach weighing *ca*. 98 mg was milled with 200 μl of 0.01 M sodium phosphate buffer (pH 6.8). Then coarse fragment was filtered out by gauze. And this solution was centrifuged with refregeration centrifuge (4℃ Europa 24 M) at 12,000 rpm for 15 min to get it homogenized. Then 100 μl solution of the supernatants was collected from the first solution and was centrifuged at 8,000 rpm for 10 min. Finally, the enzyme assay solution was obtained by micropipetting the supernatant part of the solution. The 2 μl enzyme assay solution was lodged in the No. 2 type of Watman filter paper. The assay paper was soaked in working solution A for substrate reaction during 90 seconds. After the excess solution on the paper surface was excluded by the absorbed papers, the assay paper was soaked in working solution B for colouration of hydrolytic substance (α-naphthyl). The coloured paper was soaked in working solution C for fixing coloured substances. The activity of esterase was measured by a digital densitometer (Shandon, CGA).

To investigate the esterase isozyme patterns, the electrophoresis was carried out using thin agarose electrophoresis method reported by Ohba & Sasaki (1968). The enzyme assay solution for electrophoresis was the same solution as above mentioned of the filter paper tests. The 2 μl of micropipetted enzyme assay solution was dropped in applicator plate and lodged in 0.8% agarose gel plate with applicator comb. The agarose gel plate was sprayed with substrate solutions which were made by mixing both 2.5 % α-naphthyl acetate and 0.5% β-naphthyl acetate with reagent acetone, and then the agarose gel plate was placed at 38℃ in incubator (Precision Incubator) for 20 min to be reacted with substrate. The staining of the agarose gel plate was performed by soaking this plate in solutions of 0.5% α-Fast Blue BB Salt with distilled water at 38℃ in incubator for 20 min,and then this plate was flooded with tap water for 24h and was dried in the oven (Precision Drying Oven) at 38℃.

RESULTS AND DISCUSSIONS

Selections for development of insecticide resistance against the 5~6th nymphal stages of german cockroach were conducted by exposing in dry film method at rate of LC_{50} with dichlorvos for 11 generations from the S strain and its results are presented in Table 2.

Development of resistance in the dichlorvos-selected up to 6th generation was not increased as much as its development from 9th to 11th generation The resistant developments in the dichlorvos-selected strain from 6th to 11th generation was increased from 1.89 to 8.58 times of the S strain. The result of this experiment could not be compared with the other researcher's because that informations regarding the dichlorvos on german cockroach were not currently available Fortunately, Bang *et al.* (1993b) reported the similar results that the chlorpyrifos which was one of the organophosphate groups showed a slight increase of the resistance on the german cockroach, 3.23 times by continuous selections with chlorpyrifos for 6 generations. Cochran (1987) reported that the development of resistance to the german cockroach which was continually selected with insecticide from the laboratory susceptible strains, was slow. However, resistant development of the field strains of german cockroach was found fast at the begining of selections. This reason can be explained as the field strain influenced by various kinds of selection agents, the past history of resistance and the number of generations during which selection has occurred. In the results of experiment by Cochran (1987, 1991), the pyrethroid-susceptible strain, anyway, developed resistance to pyrethroid insecticides in six to seven generations regardless of kinds of selection agents. The LC_{50} values and resistant ratio of 6 to 9 generations showed 61.90, 114.51 ppm and 1.89, 3.50 RR, respectively and thus, the susceptible strain of german cockroach developed resistance to the dichlorvos in 9 generations selected. The results of this experiment, in general, considering the rapidly developmental resistance with pyrethroid insecticides, were well accord with the results of reported by Cochran (1987) and Bang *et al.* (1993b).

The pattern of cross-resistance is important since it can indicate not only what materials will kill resistant insects but also how reistance may develop. In these points, cross-resistance of Rd strain were

Table 2. Resistance development of the german cockroach by continuous selection with dichlorvos

Generation	N	Slope± SE	LC_{50}± 95% FL	RR
Parent	270	3.67± 0.46	32 72± 4.12	1.00
F1	210	3.72± 0.50	32.12± 5.33	0.98
F3	180	3.74± 0 50	33 91± 5.10	1.04
F6	210	4 48± 0.64	61.90± 9 46	1.89
F9	180	3.27± 0.43	114 51± 20.39	3.50
F11	180	4.34± 0.62	280 59± 43.44	8 58

N: Total number of insects tested; RR (Resistance Ratio)· LC_{50} of test generation/LC_{50} of parental strain

Table 3. Cross-resistance with dichlorvos-selected (Rd) of the german cockroaches to insecticides

Insecticide	Susceptible (S) strain		Rd strain		RR
	LC_{50}(ppm)± 95%FL	Slope± SE	LC_{50} (ppm)± 95%FL	Slope± SE	
Chlorpyrifos	1.79± 0 28	3.87± 0.54	5 99± 0.92	4.47± 0.65	3.35
Propoxur	3.04± 0 32	2 04± 0.32	12.42± 2.23	2.52± 0 35	4.09
Fenvalerate	1.81± 0.35	2.48± 0.34	5.21± 0 78	4.77± 0.71	2.83
Permethrin	4.37± 0.75	2.74± 0.36	8.74± 1.65	2.89± 0.38	2.00

RR (Resistance Ratio): LC_{50} of Rd strain/LC_{50} of S strain invesigated. Its experimental results showed in Table 3.

Moderate or low cross-resistance of Rd strain was shown in all chemicals tested. Especially, propoxur which is belongs to the carbamate groups was obtained the highest value of cross- resistance (4.09 times) among the chlorpyrifos (3.35 times), the fenvalerate (2.83 times) and the permethrin (2.0 times) in comparison with LC_{50} values of the S strain.

Although the comparisons of cross-resistance between $R_{dichlorvos}$ (11 gen. selected) strain and $R_{chlorpyrifos}$ (6 gen. selected) strain which came from the same susceptible strain by reported Bang *et al* (1993b) were not done directly by levels of resistance, this experimental results showed same tendancy as the previous report. Although, the $R_{permethrin}$ (6 gen. selected) strain showed higher cross-resistance in fenvalerate, a pyrethroid than in the other chlorpyrifos and propoxur, and the $R_{dichlorvos}$ strain showed higher cross-resistance in fenvalerate (2.83 times) than in the permethrin (2.0 times), it, thus, appeared that the strains selected with dichlorvos and chlorpyrifos, which were belong to organophosphate groups, showed the high development of cross-resistance to the fenvalerlate. Collins (1973) reported that the cross-resistant patterns of the strain selected with diazinon for 12 generations by topical microapplication method showed the high resistance in case of the DDT (40 times), the malathion (27 times) and the pyrethrins (23 times) in comparison with LT_{50} of susceptible strain.

The field collected strain which had showed the high resistance to bendiocarb reported by Nelson & Wood (1982) showed the high cross resistance to the chlordan (8.2 times), the diazinon (3.7 times), the malathion (6.5 times) and the propoxur (13.3 times). As reported by Cochran (1987), cross resistance patterns of the strain selected with fenvalerate for 7 generations were extensively developed to the other pyrethroids, allethrin, permethrin and phenothrin which were not exposed. Cohran (1991), also, reported that the pyrethroid susceptible strain had developed resistance to pyrethroids early in the selection process and this strain ultimately had become a cross resistance to the pyrethroid chemicals which included allethrin, phenothrin, permethrin, fenvalerate, cyfluthrin and cypermethrin, and the strain selected with fenvalerate had become a cross resistance to all pyrethroid chemicals after continuous selections for 17 generations. Additionally, a further research should be continued to investigate the resistant patterns of german cockroach which are genetic, physiological, biochemical and ecological factors. Because these factors influence the onset and spread of resistance among field populations.

The comparisons of esterase-activity between S strain and Rd strain by the filter paper test is shown in Table 4 and Fig. 1.

The experimental results showed that esterase activities on the S and the Rd strains by filter paper test were 1.16 and 1.54 μm of hydrolyzed α-naphthyl acetate, respectively. It means that the 33% of hydrolytic activity of esterase enzyme with α-naphthyl acetate was increased in the Rd strains compared with in the S strain.

The mechanisms of insecticide resistance in the german cockroach to the organophosphate chlorpyrifos and the carbamate propoxur (Siegfried & Scott 1990, Siegfried 1992) were the combined effects of oxidative and hydrolytic metabolic enzymes and had increased the quantity of metabolites by hydrolytic and oxidative enzyme activity in both *in vitro* and *in vivo* tests. The esterase activity of chlorpyrifos- and permethrin-selected strains of german cockroa-

Table 4. Comparison of esterase activity in the dichlorvos-selected (Rd) and susceptible (S) female german cockroach strains by filter paper test

Strains	Replication	Esterase-α activity[a]
S	22	1.16± 0.17(1.00)[b]
Rd	22	1.54± 0.33(1 33)

[a] Esterase activity was expressed as μmole of hydrolyzed α-naphthyl acetate/98 mg/sec; [b] The parenthesis means the ratio of esterase activity between the Rd and S strains

Fig. 1. Comparisons of the esterase- actitvity in the female german cockroaches by the filter paper test.

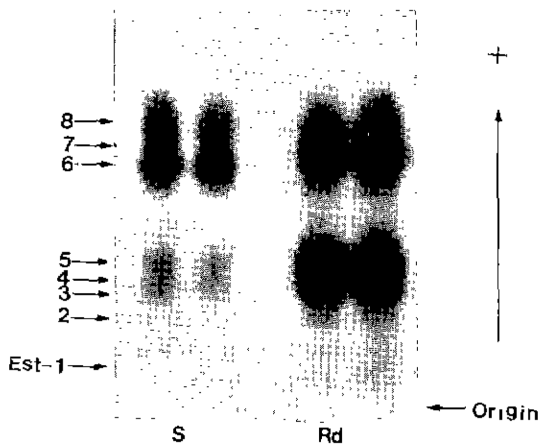

Fig. 2. Zymogram patterns of esterase isozyme of the german cockroach separated by thin agarose electrophoresis.

ches was increased to 2.65 and 1.82 times as compared with that of susecptible strain, respectively (Bang *et al.* 1993c). The resistant strains of $R_{chlorpyrifos}$ had significantly higher hydrolytic activity of esterase enzyme than the Macy susceptible strain of german cockroaches reported by Prabhkaran & Kamble (1993). The results of this experiment well correspond with aforementioned results.

The filter paper test, a rapid methods determining the insecticide resistance on organophosphate insecticides, was first reported by Pasteur & Georghiou (1981). Bang *et al.* (1993a), also, reported the possibility of resistant determination based on using chlorpyrifos-selected and permethrin-selected strains of german cockroaches. In order to be applied in the fields, this method should be developed for the preparing of assay enzyme solutions and also determining the reference which is highly and significantly correlated with the levels of insectcide resistance in the colours or densities of reaction symptoms.

The comparisons of esterase isozyme by thin agarose gel electrophoresis between the Rd and the S strains of the german cockroach were shown in the Fig. 2. The S strain was separated by 4 bands of esterase-3, 5, 6 and 8 bands and the Rd strain was separated by 6 bands of esterase-1, 2, 4, 5, 7 and 8 bands The resistant mechnisms of the Rd strain were considered the 4 bands of esterase-1, 2, 4 and 7 bands except the common 2 bands of esterase-5 and 8 bands. The strong activities at the esterase-6 band of the S strain and the esterase-4, 5, and 7 bands of the Rd strain were influenced by the resistant genetics. The patterns of esterase isozyme at the $R_{chlorpyrifos}$ and the $R_{permethrin}$ of the german cockroaches reported by Bang *et al.* (1993c) showed the strong activities at the esterase-2 and 3 bands as comparison with the S strain, and the $R_{permethrin}$ strain was separated the additional esterase-5 band. In comparisons of activity and electrophoretic of esterases in resistance and susceptible strains of german cockroach reported by Prabhakaran & Kamble (1993), the differences in staining intensities may be related to the individual isozyme activity or the genetic variations among strains. The results of this experiment showed well in accordance with the experimental results of aforementioned and provided the baseline information for futher research on the involvement of esterases in the resistance mechanisms of german cockroach

The latest date, we reported the resistant development, the cross-resistance and the esterase activity of the successively selected with chlorpyrifos and permethrin strains from the susceptible strain of german cockroaches for six generations in laboratory. The selected strains showed that the resistant ratio was increased from 2.10 to 3.23 times the cross-resistances of $R_{chlorpyrifos}$ and $R_{permethrin}$ strains were increased to 3.89 and 5.23 times for fenvalerate, respectively, and the esterase activity was increased in the selected strains in comparison of susceptible strain (Bang *et al.* 1993b, c).

In conclusions of the Rd strain, the resisrant development increased 8.58 times and the cross-resistance showed 3.35, 4.09 and 2.83 times for chlorpyrifos, propoxur and fenvalerate, respectively. The esterase-activity of the Rd strain increased 33% by the filter paper test and the zymogram patterns of esterase isozyme by the thin agarose gel electrophoresis showed more seperated bands than that of the S strain

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(Received March 25, 1994)