J.Pest Cont. & Environ. Sci. 9 (2): 1-14 (2001).

Investigation of the insecticide resistance mechanisms of the termite, *Coptotermes formosanus* (Shiraki), and the sweet potato weevil, *Cylas formicarius elegantulus* (Summer).

By

Helmy Anber*, Jun Zhu And Shinkichi Tawata****

***Department of Plant Protection, Faculty of Agriculture, University of Tanta, Tanta, Egypt.**

**** Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa 903-0129, Japan.**

Received17/10/2000 , Accepted 6/12/2000.

ABSTRACT

Toxicity of fenobucarb, chlorpyrifos, fenitrothion and dichlorvos insecticides against adult stages of the termites and the sweet potato weevils was estimated. LC_{50} values were 1.2, 0.224, 0.368 and 1.12 ng/mg body weight respectively for the termites, and 1350, 17.2, 18.8 and 660 ng/mg body weight for the sweet potato weevils. All these compounds were much more active against termites than the sweet potato weevils. Chlorpyrifos was the most active insecticide against both insects followed by the two other organophosphorus compounds (fenitrothion and dichlorvos). The carbamate fenobucarb was the least effective.

The biochemical resistance mechanisms were studied by using the crude homogenates of the resistant individuals of both insect adult stages, which had survived through the treatments by the LC_{50} values of the insecticides mentioned above. The *in vitro* studies resulted in insecticide degradation products. Insect mechanisms of resistance were investigated

by calibrating each insecticide and its degradation products on TLC and by gas chromatography. A clear correlation between the two analytical methods showed that the resistance mechanism in the termites was mainly due to esterase hydrolysis of all insecticides used. The results for the sweet potato weevils were not conclusive. Therefore the resistance mechanism may be due to other detoxification enzymes included in the insect supernatant, such as oxidases or glutathione S-transferase.

Key words: Fenobucarb, chlorpyrifos, fenitrothion, dichlorvos, toxicity, resistance mechanisms, the termite and the sweet potato weevil.

INTRODUCTION

Smith, in 1941, was the first one to realize that insecticide resistance is due to interaction of genetic variability and selection with insecticides. Later on, development of insect resistance to insecticides was intensively studied and numerous review articles were published. The studies were carried out to obtain a better understanding of the genetic bases and biochemical changes in resistant insects (Oppenoorth 1985).

The biochemical mechanisms of insecticide resistance have been attributed mainly to: (1) changed sites of action conferred by reduced neuronal sensitivity of altered AChE, the target enzyme of organophosphorus and carbamate insecticides or reduced neuroreceptors and ion channels, as a toxic action of DDT and pyrethroids compounds, which is a factor conferring resistance by a rapid paralytic (kdr) as a lethal action of these compounds. (2) increased detoxification by three groups of enzymes; esterases, oxidases and glutathione S-transferases, which hydrolyze all classes of insecticides except the chlorinated cyclodienes. Esterase enzymes were responsible for resistance found in insect homogenates. Hydrolysis of ester bonds by esterase play a significant role in the metabolism of organophosphorus, carbamates and pyrethroids, but carbamate hydrolysis plays only a minor role in insects.

Several reviews deal with the role of esterases in the metabolism of insecticides (Dauterman 1985 and Oppenoorth 1985). Glutathione S-transferases are also groups of detoxification enzymes but their action is mainly restricted to detoxification of organophosphorus, lindan and DDT. Their role in insect resistance was reviewed by Dauterman 1983, Oppenoorth 1985 and later on by Carlini *et al* 1995. These enzymes catalyse a first step in the detoxification e.g. by transferring methyl or p-nitrophenyl group of methyl parathion to the sulfur atom of reduced glutathione. Glutathione S-transferases are found in the insect supernatant of homogenates at low speed centrifugation. Esterase's and glutathione S-transferase's enzyme activities are mainly higher in resistant insects than in susceptible ones. In several cases of resistant insects, one or more mechanisms can cause the insecticide resistance (Scharf *et al*, 1998 and 1999). Microsomal oxidation enzymes differ from the two other mechanisms discussed, since these enzymes are found in the endoplasmatic reticulum, and can be obtained only by high centrifugation at approximately 100,000 rpm. Therefore, the detoxification enzymes which degraded insecticides used in this study, belong to the esterases included in the whole termite homogenates used. They hydrolyze these insecticides to two products, as was clear from calibrating each insecticide hydrolysis product on TLC and by gas chromatography.

MATERIALS AND METHODS

Chemicals: Insecticides used were obtained from Ryukyusankei Co. Ltd (Okinawa, Japan), and each had purity of more than 97%. They are widely used in agriculture for the control of various insects. Figure 1 shows the chemical structures of these insecticides and their expected hydrolysis products for the water and organic phases.

Experimental Insects: The adult stages of termites used in the experiments were collected from infested trees on the mountains of the University of the Ryukyus, Agricultural Faculty's farm in Okinawa. The sweet potato weevil adult stages used were a field strain collected from Okinawa island and reared in the laboratory on sweet potato roots at room temperature.

Susceptibility test: Toxicity of four insecticides was tested against adult stages of termites and the sweet potato weevil by using residue method of insecticide solutions in acetone. Test insects were placed in petri dishes for 48 hr in which 200 µl of each concentration were applied. LC_{50} values were statistically calculated according to Finney's probit analysis in 1972.

Calibration of the metabolites or degradation products of insecticides used. The calibration and analytical methods were used according to Tokieda *et al* 1997, with only some modification made. Fifteen of the adult stages of termites and sweet potato weevils, which survived after 24 hr of treating them by LC_{50} value of each insecticide, were used. Adult individual termites weighed an average of 37.5 mg and sweet potato weevils 60 mg. Surviving insects were homogenized in 200 µl of ice sodium phosphate buffer 0.1 M pH 7.0 (consisting of 297 ml of 0.1 M NaOH and 500 ml of 0.1 M KH_2PO_4 diluted with distilled water to 1000 ml) and prepared with buffer solution to 1 ml final volume in a 10 ml glass tube. 10 µl of each insecticide (final concentration 10^{-2} µg) were added to the crude homogenate of the insect (which included the soluble and insoluble degradation enzymes). Homogenates and insecticides incubated for 30 min at 37° C in a water bath while shaking. Following the incubation time, 5 ml of 0.5 N NaOH solution were added to stop the enzyme's reactions. The aqueous solution was shaken with 30 ml of mixed solvent of methanol and acetone (1+2 v/v) for 10 min in a 50 ml vessel. Then the vessel contents were filtered through a sodium sulfate anhydrous layer (3 cm thickness) in filter paper wattman No 2 and

organic phase of acetone and methanol mixed solvent were collected in a new vessel. Four different volumes of methanol and acetone mixtures were used to extract insecticide hydrolysis products of water (mobile) phase filtered through the sodium sulfate anhydrous layer.

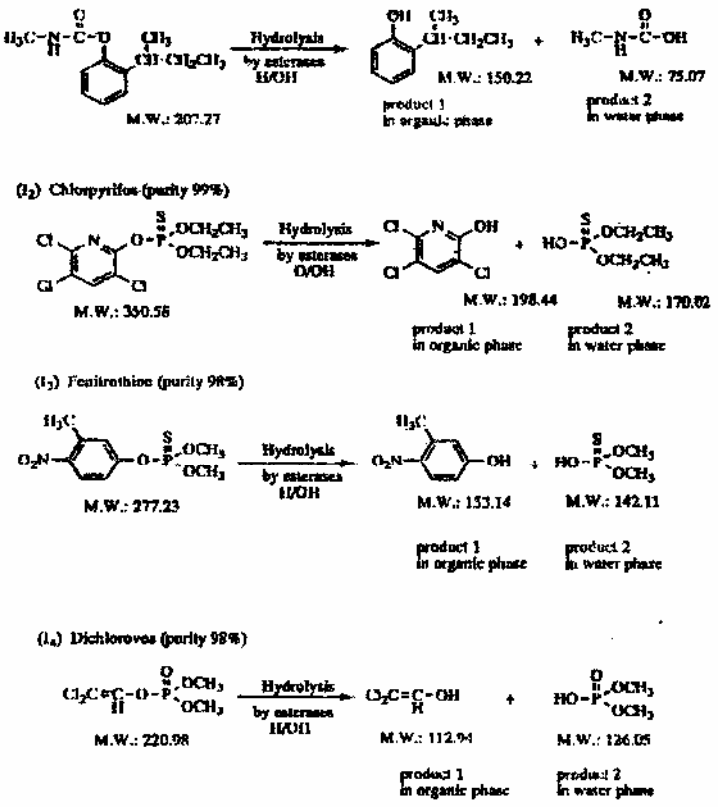

Fig (1): Fenobucarb, chlorpyrifos, fenitrothion and dichlorvos and their expected degradation products by hydrolsis enzymes.

The uneffected insecticide or metabolites and degradation products were extracted in a mixed solvent, including organic and water phase combined and filtered through a column of silica gel 60 GC 10 cm

length and 2 cm inner diameter. The extracts of the insecticide were unaffected and the metabolites or the degradation products were taken out of the silica gel column with 100 ml acetone. Each sample obtained was concentrated to 10 ml at 40°C in a water bath. Controls of each insecticide (A) and each insect homogenate (B) were studied as described above.

Analytical Methods:

(a). Thin Layer Chromatography (TLC). Precoated silica gel 60 F254 chromatoplates (20 × 20 cm, 0.25 mm thickness, E. Merck, F.R.G) and chloroform-acetone (1:1 v/v) solvent system were used. Insecticides used, their degradation products and insect homogenate spots on TLC were detected under UV light (Topcor FI-31, Tokyo Kogakukikai Kiki, Japan).

(b). Gas chromatography (GC). Analysis of the parent compounds of the termite and sweet potato weevil insect homogenates, insecticides and their degradation products was carried out in gas chromatography, GC-14A-Shimaddzu system, equipped with a flame thermionic detector under the following conditions: column, G-100 (1 μ, 1.2 mm i. d × 40 m) injector and detector temp., 160°C; column oven temp., 140°C ; carrier gas (He) flow rate, 10 ml/min; gas pressure for H_2 and air, 0.5 and 0.6 kg/cm^2. 1.5 μl of sample solutions in acetone were injected into the machine. Insecticides and their degradation products were characterized by TLC spots as well as by GC calibration curves.

RESULTS AND DISCUSSION

Insecticidal activities of fenobucarb, chloropyrifos, fenitrothion and dichlorvos against two field strains of termites and sweet potato weevil were assayed. As shown in Table 1 termites were much more susceptible to all insecticides than the sweet potato weevil. Ratios of LC_{50}'s values for these insecticides with the sweet potato weevil to that with the

termites were 1125, 77, 51 and 589-fold, respectively. That is possibly because the termites lived inside of tree trunks where little or no insecticide residues reached them. The differences in susceptibility between the population individuals may be due only to their genetic variability. However, the sweet potato weevil is one of the most destructive pests for the sweet potato plant (*Ipmoea batatas*) all over the world and as such, comes into constant contact with insecticides. Larval and adult stages feed on shoots and roots. In the present study chlorpyrifos was the most active compound showing lethal activity on the two strains. Jackson *et al.*, in 1998 also studied the insecticidal activities of a series of amides and esters of benzofuran 2-carboxylic acid against adult stages of the sweet potato weevil, which were more active compared with the dimethoate insecticide activity.

Table (1): Toxicity of some insecticides against the adult stages of termite (T*) and sweet potato weevil(SPW).**

Insecticide	LC_{50} at 48 h (ng/mg body weight)		Confidence limits (ng/mg body weight)		Ratio of LC_{50}'s of SPW**/LC_{50}'s of Termites
	T*	SPW**	T*	SPW**	
(I_1) Fenobucarb	1.2	1350	0.32-8.8	990-2350	1125-Fold
(I_2) Chloropyrifos	0.224	17.2	0.064-1.520	7-64	77-Fold
(I_3) Fenitrothion	0.368	18.8	0.056-7.2	7-90	51-Fold
(I_4) Dichlorvos	1.12	660	0.04-240	280-2800	589-Fold

Two analytical methods have been used to study the biochemical mechanisms of those insecticides mentioned above. The homogenates of resistant insects, which survived after treating them with the LC_{50} values of insecticides, were used. The insecticide unaffected, and its metabolites or degradation products were calibrated using two methods. First the presence of the degradation products of the insecticides was studied on silica gel TLC and the biochemical mechanisms of resistance were investigated. In Table 2, columns one and two show the R_f values for the insecticides used and the termite homogenates respectively. Column three shows the R_f values for the termite homogenates when incubated with the insecticides. Results confirmed the presence of two hydrolyzing products for all insecticides used. This identification was also clear from the results obtained from gas chromatography analysis, as shown in Figures 2 and 3. These two patterns of the hydrolysis products on TLC were similar to the products that were eluted through the GC calibration curves in different retention times. Both of the results obtained by the two analytical methods showed that enzymes belonging to esterases hydrolyzed ester bonds of all the compounds in resistant individual termites. Konno, in 1996, found that the carboxylesterase activity of the rice stem borer, *Chilo suppressalis* (Walker), was responsible for resistance to fenitrothion, one of the four insecticides we used. Chlorpyrifos and two other organophosphates were used by Baskaran *et al* 1999 as termiticals. They investigated the degradation behaviour of these compounds on soil. Chlorpyrifos was the most unstable one and was degraded to primary metabolie (TCP) 3,5,6-trichloro-2-pridinal by microorganism degradation enzymes.

Table (2): Thin layer chromatographic R_f values of insecticides used (column 1), termite homogenate (T) (column 2) and insecticides products hydrolyzed by termites degradation enzymes (column 3).

Column1		Column 2		Column 3				
Insecticides	R_f	Spots of (T) homogenate	R_f	Spots	With I_1 R_f	With I_2 R_f	With I_3 R_f	With I_4 R_f
(I_1) Fenobucarb	0.51	S_1	0.02	S_1	0.03	0.02	0.06	0.06
(I_2) Chloropyrifos	0.75	S_2	0.37	S_2	0.16*	0.19*	0.22*	0.17*
(I_3) Fenitrothion	0.79	S_3	0.45	S_3	0.38	0.30	0.38	0.34
(I_4) Dichlorvos	0.49	S_4	0.61	S_4	0.52	0.41	0.46	0.47
				S_5	0.64	0.53	0.59	0.59
				S_6	0.76**	0.72**	0.71**	0.65**

*Product 1, **product 2.

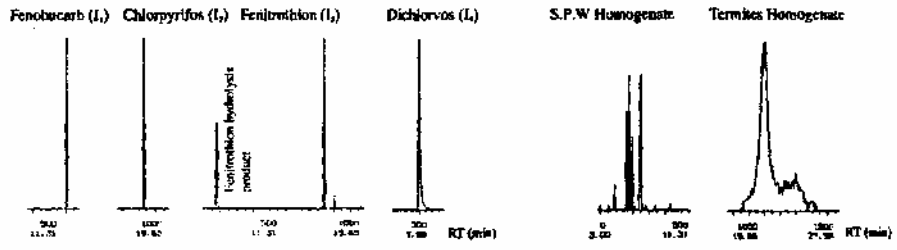

Fig (2): Gas chromatography chromatograms of insecticides used in amounts of 1.5 ng (in 1.5 ml acetone) as shown in A and insect homogenates as shown in B.

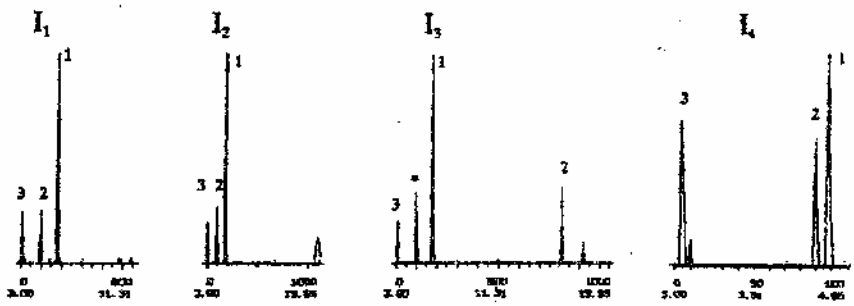

Fig (3): Gas chromatography chromatograms of insecticides used and their hydrolysis products degraded by termite enzymes compared with A and B. (1 is recovered insecticide, 2 and 3 are degradation products, and * is fenitrothion hydrolysis product with parent insecticide).

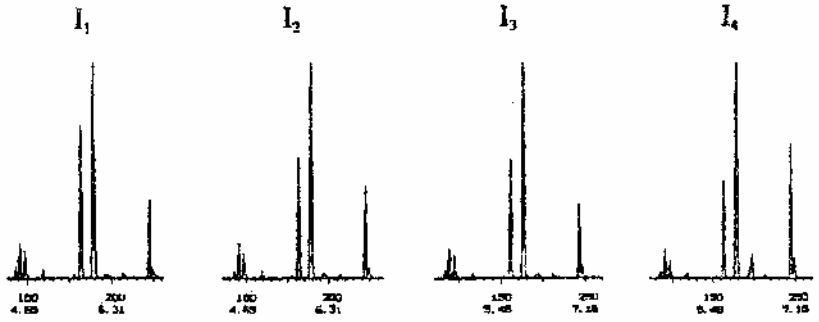

Fig (4): Gas chromatography chromatograms of the four insecticides incubated with the SPW homogenates showed no differences between them and the parent compound of the insect homogenate.

The most interesting finding in our study is that termites hydrolyse ester bonds of the carbamate compound (fenobucarb), as well as organophosphorus ones by esterases, which are known to play only a minor role in insects. On the other hand, there were no clear results observed from either of the two analytical methods conducted on the

homogenates of the sweet potato weevil resistant individuals. Figure 4 shows the results of the gas chromatography chromatograms calibration curves. In addition, no spots ran over and separated on silica gel plates with acetone-chloroform (1:1 v/v) or methanol-acetone (1:1 v/v) solvent systems.

Therefore, new experiments are still to be developed for using the supernatant of the sweet potato weevil homogenate on low and high speed centrifugations, in order to explore the group of detoxification enzymes, such as glutathion S-transferase or microsomal oxidation enzymes, which could be responsible for resistance, or otherwise may degrade insecticides.

REFERENCES

Baskaran S., R. Kookana, R. Naidu, Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates, *Pestic. Sci.*, 55, 1222-1228 (1999).

Carlini E.J., B.A. Mc Pheron, C.M. Felland, and L.A.Hull, Biochemical mechanisms of azinphosmethyl resistance in the tufted apple bud moth *Platynota idaeusalis*, *Pestic. Biochem. Physiol.* 51, 38-47 (1995).

Dauterman W.C., Insect metabolism, extramicrosomal, in "Comprehensive Insect Physiology, Biochemistry and Pharmacology" (G.A Kerkut and L.I. Giilbert, Eds.), Pergamon Press, New York, Vol. 12, pp. 713-730 (1985).

Dauterman W.C., Role of hydrolases and glutathions S-transferases in insecticide resistance, in "Pest Resistance to Pesticides, Challenges and Prospect" (G.P. Georghiou and T. Satio, Eds), Plenum press, New York, Vol. 12, pp. 229-247 (1983).

Finney D. J., Probit analysis, a statistical treatment of the sigmoid response curve. Cambridge, pp. 318-333 (1972).

Jackson Y. A., M.F. Williams, L.A.D. Williams, K. Morgan and F.A. Redway, Insecticidal properties of benzofuran-2-carboxylic acid derivatives, *Pestic. Sci.*, 53, 241-244 (1998).

Konno Y., Carboxylesterase of the rice stem borer, *Chilo supperessalis*, Walker (Lepidoptera: Pyralidae), responsible for fenitrothion resistance as a sequestering protein, *J. Pesticide Sci.* 21, 425-429 (1996).

Oppenoorth F. J., Biochemistry and genetics of insecticide resistance, in "Comprehensive Insect Physiology, Biochemistry and Pharmacology" (G. A. Kerkut and L.I. Gilbert, Eds.), Pergamon Press, New York, Vol. 12, pp. 731-773 (1985).

Scharf M.E., J. Neal and G. Bennett, Changes of insecticide resistance levels and detoxication enzymes following insecticide selection in the German cockroach, *Blattela germanica* (L.), *Pestic. Biochem. Physiol.* 59, 67-79 (1998).

Scharf M.E., L. Meinke, R.Wright, L. Chandler, and Siegfried, Metabolism of carbaryl by insecticide resistant and –susceptible western corn rootworm populations (Coleptera: Chrysomelidae), *Pestic. Biochem. Physiol.* 63, 85-96 (1999).

Smith F.F., Radical segregation in insect-population and its signification in applied entomology, *J. Econ. Entomol.*, 34, 1 (1941).

Tokieda, M., M. Ozawa, S. Kobayashi, and T Gomyo, Method of determination of total residues of the insecticide acetamiprid and

its metabolites in crops by gas chromatography, *J. Pesticide Sci.*, 22, 77-83 (1997).

الملخص العربى

توضيح ميكانيكيات مقاومة المبيدات فى حشرة النمل الابيض و خنفساء البطاطا

حلمى عنبر* ، جون زو، شنكيشى تاواتا****

*قسم وقاية النبات – كلية الزراعة بطنطا – جامعة طنطا – طنطا – جمهورية مصر العربية

** قسم العلوم البيولوجية و التكنولوجية – كلية الزراعة – جامعة ريوكيوس – أوكيناوة – اليابان

لقد تم دراسة سمية كلا من مبيدات الفينوبيوكرب ، الكلوربيروفوس ، الفينوتروثايون و الداى كلوروفوس على الحشرات الكاملة للنمل الأبيض و خنفساء البطاطا . و لقد كانت قيم الـ LC_{50} لهذه المبيدات ١,٢، ٠,٢٢٤ ، ٠,٣٦٨ ، ١٠١٢ نانو جرام / مليجرام من وزن جسم الحشرة على التوالي للنمل الأبيض و كانت ١٣٥٠ ، ١٧,٢ ، ١٨,٨ ، ٦٦٠ نانو جرام / مليجرام لحشرة خنفساء البطاطا. و هذه النتائج توضح أن هذه المبيدات كانت اكثر سمية على النمل الأبيض عن خنفساء البطاطا و أن مبيد الكلوروبيروفوس كان أعلى المبيدات سمية و مبيد الفينوبيوكرب كان أقلهم على كلا الحشرتين تأثيرا.

تم استخدام المخلوط المتجانس من جسم الحشرة في دراسة ميكانيكيات المقاومة في الأفراد المقاومة من طور الحشرة الكاملة لكلا من النمل الأبيض و خنفساء البطاطا و التي مازالت حية بعد معاملتهم بقيم الـ LC_{50} للمبيدات السابقة . ولقد أوضحت هذه الدراسات عن طريق تقدير نواتج تحطم هذه المبيدات بالأنزيمات بواسطة أستخدم طريقة TLC و التحليل الكروماتوجرافي الغازي ميكانيكية المقاومة لهذه المبيدات في كلا الحشرتين , و قد أوضحت النتائج أن ميكانيكية المقاومة لهذه المبيدات في النمل الأبيض ترجع أساسا الى أنزيمات الاستريز التي تحلل هذه المبيدات . أما في خنفساء البطاطا فلم تكن النتائج واضحة و لذلك ربما تكون ميكانيكية المقاومة فيها ترجع إلى وجود أنزيمات أخرى في المحلول الرائق للمخلوط المتجانس من الحشرة بعد الطرد المركزي مثل أنزيمات الأكسدة أو الجلوتاثيون ترنس فيريز.