Ajin Rattanapan 2007: Biochemical and Molecular Detection of Cypermethrin and Rotenone Resistance in the Tropical Armyworm, *Spodoptera litura* (Fabricius). Doctor of Philosophy (Bioscience), Major Field Bioscience, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Suraphon Visetson, Ph.D. 127 pages.

This dissertation was carried out to investigate resistance of the tropical armyworm, *Spodoptera litura* (Fabricius), against 3 insecticides, i.e., cypermethrin, standard rotenone and derris crude extract, and their effects on this insect's detoxification enzyme systems over 10 generations. Ethanolic derris extract gave high yields both as crude extract and in rotenone content, *ca*. 48.60 and 17.91%w/w, respectively. The toxicity of the 3 insecticides estimated by the dipping method showed that cypermethrin gave the best control of 3rd instar larvae at LC_{50}=9.93 (r^2=0.98) after 72 hours exposure. *In vitro* study of carboxylesterase, acetylcholinesterase (AChE) and glutathione-s-transferase (GSTs) enzymes revealed that the pH most suitable for homogenization and incubation was pH 10. After 24 hours exposure to the three insecticides, carboxylesterase was found to be the most important enzyme for insecticide detoxification. When larval instars were examined, cypermethrin and derris extracts seemed to express enhanced enzyme activity in all instars while rotenone seemed to inhibit these three enzymes' activities in some larval instars.

The toxicological response after sequential treatment with these insecticides through 10 generations was examined and it was found that cypermethrin gave the highest levels of toxicity and resistance development. The cypermethrin treatment gave a resistance ratio of 104.58 fold for the F_{10} generation, which was much higher than those for the other 2 treatments (standard rotenone, RR = 5.79; derris crude extract, RR = 3.14). Through 10 generations, carboxylesterase showed the greatest increase in activity and all enzymes were characterized as enhancing activity. Although standard rotenone and derris crude extract resulted in enhanced enzyme activities, the increased activities were slight when compared with those resulting from cypermethrin treatment. SDS-PAGE showed bands for carboxylesterase, two subunits of AChE, and GSTs at 60, 24 and 48, and 24 kDa, respectively, in every generation. RT-PCR characterized a part of the gene associated with expression of the carboxylesterase enzyme in the F_{10} generation of each insecticide-treated population as a single band of 553 bp with 99% identity of nucleic acid and amino acid sequences to a *S. litura* partial sequence in GenBank (accession number DQ445461). Five positions, T^{30}, C^{76}, A^{102}, G^{111} and C^{381}, of partial sequences were found different from those in the GenBank database. Four of these differences (C^{76}, A^{102}, G^{111} and C^{381}) belonged to the cypermethrin population while another one (T^{30}) belonged to every population of this study. In addition, the deduced amino acid of cypermethrin also gave a single substitution from E^{37} to D^{37} which meant that Aspartate (Asp) substituted Glutamate (Glu). The results indicated that cypermethrin gave much faster resistance than did rotenone, which was caused by enhancement of detoxification mechanisms. Therefore, rotenone is an alternative safe choice to control *S. litura*. These results provide evidence for novel gene mutations that may lead to changes in the carboxylesterase enzyme mechanism in *S. litura*.

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