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Improvement of rapid and sensitive enzyme assays for monitoring pyrethroid resistance in mosquitoes.

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Mosquitoes have developed resistance to numerous organophosphorous and more recently to pyrethroid insecticides in California and elsewhere globally. In the case of pyrethroid resistant mosquitoes, enhanced esterase and P450 monooxygenase(P450) activity represent the two major enzyme mediated mechanisms for resistance, but the existing diagnostic assays for detection of these esterases and P450s lack specificity, are of low sensitivity and are time consuming. Therefore, there is an urgent need for development of rapid and sensitive techniques for monitoring and studying enzyme mediated pyrethroid resistant mechanism in mosquitoes.

We have found a new class of fluorescent reporters that offer advantages over the commonly used reporters. These materials give a larger Stokes' shift, hight molar absorptivity, a greater red shift, lower fluorescent background of the substrates, and greater chemical stability than the commonly used fluorescent reporters. Using these reporters, we have been designed and synthesized esterase and P450 fluorescent substrates bearing α-cyanoester or α-cyanoether. With esterases the improved fluorescent properties, lower fluorescent background and greater chemical stability combine to give over a 500-fold increase in sensitivity. Especially we could measure the pyrethroid specific esterase activity in single mosquito with pyrethroid mimic substrates. In the case of P450 substrates, the O-dealkylation rate of the substrates with mammal microsomes is faster than that of commercial substrate. However, although these new substrates were more sensitivity than commercial substrates, considerable improvements in the sensitivity of the substrates for measuring P450s

activity in single mosquito need to be made.