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Target site and metabolic insecticide resistance monitoring in *Musca domestica*

INTRODUCTION

The housefly *Musca domestica* L. (Diptera, Muscidae) is a noxious and serious public health pest. The large use of many different insecticide classes has selected insecticide resistant populations.
This study describes in two different Hungarian housefly populations: a) a new PASA method to detect *kdr* (knockdown resistance) L1014F and L1014H and *s-kdr* (M918T) ; b) biochemical assays to measure total activity of oxidases and GSTs; c) inhibition assays to investigate the potential inhibitory efficacy of piperonyl butoxide (PBO) and other synergistic compounds, under development in the framework of the EU Project "ECOSYN".

MATERIAL AND METHODS

SNPs linked with insecticide resistance were detected with PASA-PCR using genomic DNA as in Mazzoni *et al.* (2015).
Total oxidases activity and inhibition rate of synergists was determined measuring the rate of oxidation of 7-EC (7-ethoxycoumarin). The oxidation is accompanied by an increase in fluorescence at 465 nm (390 nm excitation).
Total GSTs (Glutathione S-Transferase) activity and inhibition effects were evaluated measuring at 340 nm the increase of absorbance produced by tbe conjugation rate of CDNB (1-chloro-2,4-dinitrobenzene) with reduced glutathione.
The inhibitory effects of the same set of inhibitors was measured for oxidases and GSTs.

RESULTS

Genotyping

L1014F and L1014H in the voltage sodium channel gene were found in both populations but the allele frequency is different. The M918T was not found in these populations (**Figure 1**).

Biochemical analysis

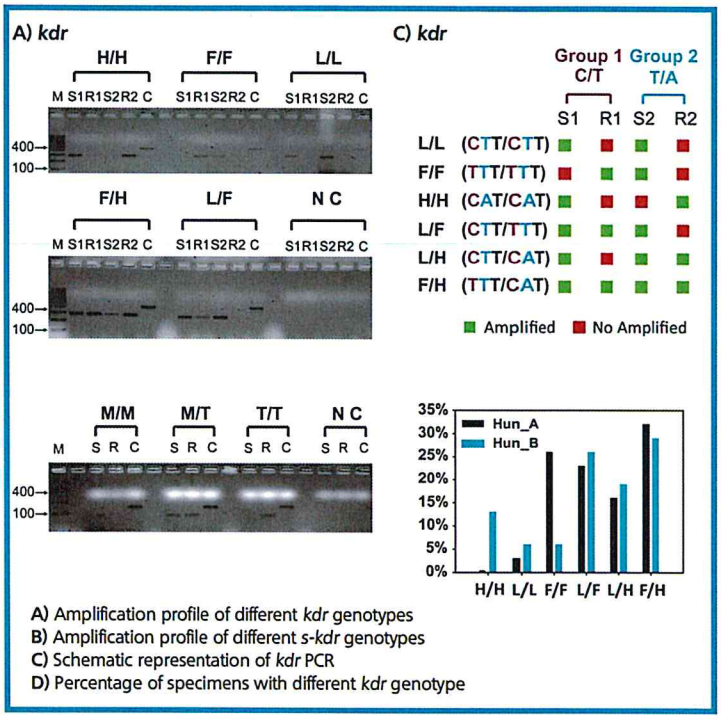
The Hungarian populations A and B showed similar oxidase and GST activities. These activities were always higher than those of the susceptible reference strain S-WHO (**Figure 2**).

Inhibition assays

PBO and other six compounds were assayed. These molecules produced various inhibition level of oxidases and generally a low inhibition of GSTs (**Figure 3**).

DISCUSSION

Target-site and metabolic resistance can be responsible of insecticide treatment failures. The development of rapid biochemical and molecular biology tests is important to identify the presence of metabolic or target-site mechanisms in unknown populations to avoid ineffective insecticide applications. Morever the study of inhibition efficacy of new potential synergists can be useful to overcome metabolic resistance and to reduce insecticide application rates.



A) Amplification profile of different *kdr* genotypes
B) Amplification profile of different *s-kdr* genotypes
C) Schematic representation of *kdr* PCR
D) Percentage of specimens with different *kdr* genotype

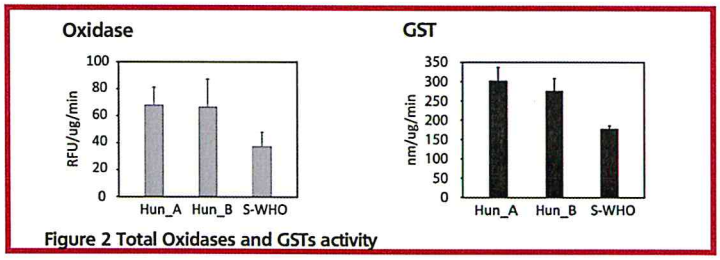


Figure 2 Total Oxidases and GSTs activity

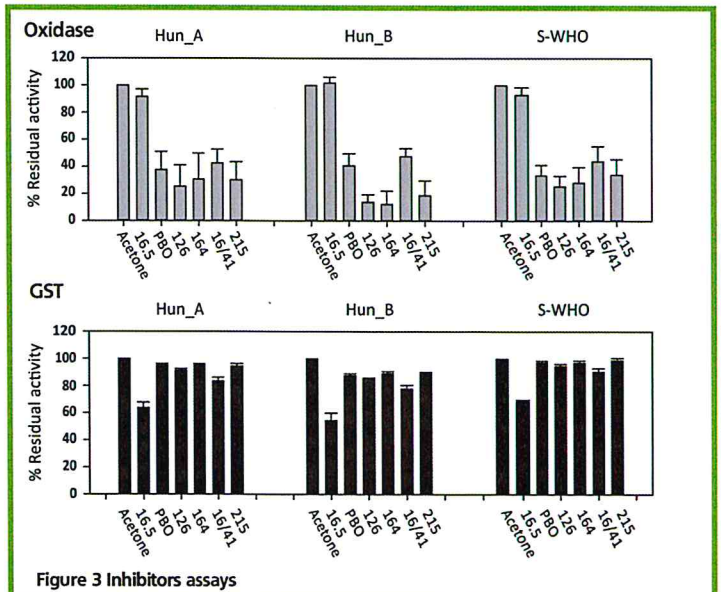


Figure 3 Inhibitors assays

ECOSYN Ecofriendly synergists for insecticide formulations
This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 605740