Comunicação Científica

Acetylcholinesterase Inhibition in Organophosphate-Resistant *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

Raul N. C. Guedes[1] and Barry A. Dover[2]

[1]Departamento de Biologia Animal, Universidade Federal de Viçosa, 36571.000, Viçosa, MG.
[2]Department of Entomology, Kansas State University, Manhattan, KS, 66502, U.S.A.

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Inibição de Acetilcolinesterase em *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) Resistente a Organofosforados

RESUMO - Populações de *Rhyzopertha dominica* (F.) resistente e susceptível a inseticidas fosforados foram investigadas para verificar a ocorrência de acetilcolinesterase alterada. Malaoxon, a diferentes concentrações, foi utilizado como inibidor em ensaios colorimétricos "*in vitro*". Populações resistentes provenientes dos municípios de Patrocínio (Minas Gerais, Brasil) e Riley (Kansas, E.U.A.) apresentaram, respectivamente, 9,3- e 12,3- vezes menor sensibilidade ao malaoxon que a população susceptível de laboratório. Acetilcolinesterase de insetos resistentes foi inibida mais lentamente que a enzima de insetos susceptíveis com razão de constante bimolecular (k_i) diferindo em 5,5- e 12,8- vezes para o malaoxon. Esses resultados confirmaram estudos prévios indicando o envolvimento de acetilcolinesterase alterada como mecanismo de resistência a inseticidas organofosforados em populações brasileiras e norte-americanas de *R. dominica*.

PALAVRAS-CHAVE: Insecta, praga de grãos armazenados, malatiom, insensibilidade de acetilcolinesterase, resistência a inseticidas.

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a notorious pest of stored cereals that has shown insecticide resistance worldwide (Champ & Dyte 1976, Badmin 1990, Guedes 1990, Subramanyam & Hagstrum 1996). Organophosphates are the main insecticides used against this insect, but resistance to them have been reported in Brazil and in the United States (Guedes *et al.* 1996). We recently examined the biochemical mechanisms of resistance to organophosphates in Brazilian and U.S. populations of lesser grain borer and no evidence was found suggesting the involvement of detoxification enzymes in the resistance, but indication of existence of target site insensitivity was obtained (Guedes *et al.* unpublished). We investigated the insensitivity of acetylcholinesterase (AChE) to malaoxon (the active metabolite of malathion) in two populations field-collected from Patrocínio (State of Minas Gerais, Brazil) and Riley (State of Kansas, U.S.A.). A standard susceptible population from the Laboratory of Stored

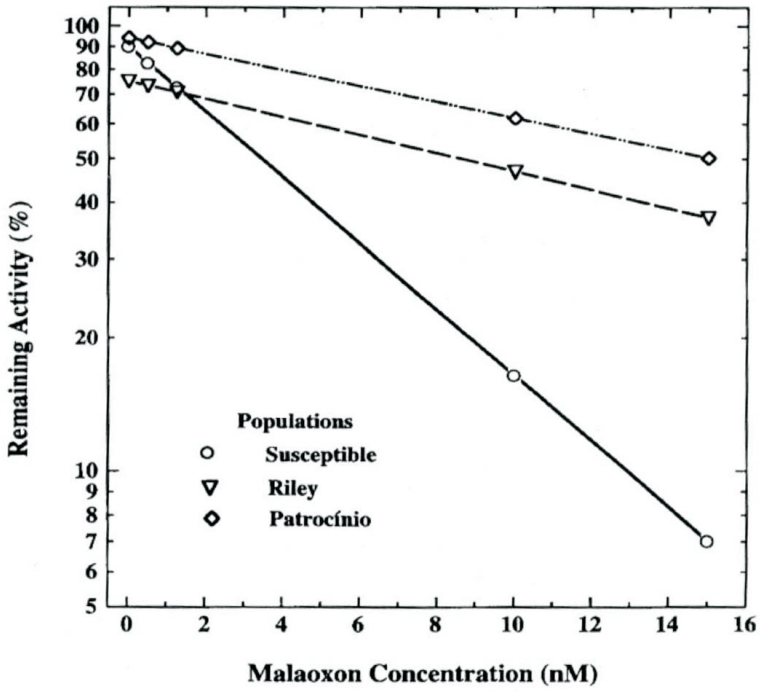

Figure 1. Acetylcholinesterase inhibition by malaoxon in *Rhyzopertha dominica* susceptible (Y = 1.95 - 7.38x107 X, p < 0.01; R2 = 0.98) and resistant, Riley (Y = 1.87 - 2.05x107 X, p < 0.01; R2 0.95) and Patrocínio (Y = 1.97 - 1.81x107 X, p < 0.01; R2), to insecticides.

Product Insects, Department of Entomology, Kansas State University, was also used.

Technical grade malaoxon was supplied by Cheminova Agro (Lemvig, Denmark) and the other chemicals were purchased from Sigma Chemical Co. AChE activity was measured using acetylthiocholine iodide (ATChI) as substrate and detecting the released thiol colorimetrically at 412 nm by its reaction with 5,5'-dithiobis(2-nitrobenzene) (DTNB). AChE activity was assayed following Ellman *et al*. (1961) modified by Karunaratne & Plapp (1993) using homogenates of 10 adult insects in 2 ml 0.05 M phosphate buffer (pH 7.5) and three replicates for each concentration and insect population. Curves of AChE

Table 1. Inhibitory power of acetylcholinesterase from susceptible and resistant populations of *Rhyzopertha dominica*.

Population	I_{50} (M)	R/S (I_{50})	k_i (M-1 min-1)	S/R (k_i)
Susceptible	5.3×10^{-8}	-	15.4×10^{8}	-
Riley	65.2×10^{-8}	12.3	1.2×10^{8}	12.8
Patrocínio	49.1×10^{-8}	9.3	2.8×10^{8}	5.5

inhibition by malaoxon in resistant and susceptible *R. dominica* were estimated by the procedure PROC REG from SAS (SAS Institute 1987 - Fig. 1). Malaoxon concentrations required to inhibit 50% of the AChE activity (I_{50}) for each population were calculated using the procedures PROC PROBIT and PROC REG from SAS and bimolecular rate constants (k_i) were calculated as described by Aldrich (1950) and Main (1964).

Both resistant populations showed less sensitivity to inhibition by malaoxon than the susceptible strain (Fig. 1). Based on I_{50} values, the Brazilian and the U.S. populations of *R. dominica* showed respectively 9.3- and 12.3-times less sensitivity to the inhibitor than the susceptible population (Table 1). In addition, AChE from the resistant populations was 5.5 and 12.8-times (for Patrocínio and Riley populations, respectively) more slowly inhibited by malaoxon than the enzyme from the susceptible population based on values of bimolecular rate constants (k_i) (Table 1). These results provide further support for the hypothesis of a major involvement of altered AChE in *R. dominica* resistance to organophosphates in Brazil and the U.S.

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