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Enzyme activity of the energy-metabolism of pyrethroid-resistant and -susceptible populations of the maize weevil (*Sitophilus zeamais*)

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Abstract

Populations of *Sitophilus zeamais* of distinct susceptibility to insecticides show differences in the accumulation and mobilization of energy reserves, what may allow the production of their defensive tools against such compounds without impairing their reproductive performance. Enzymatic assays with energy-metabolism enzymes, including those involved in sugar hydrolysis, were therefore carried out to test this hypothesis. Activity levels of trehalase, glycogen phosphorilase, lipase, glycosidase and amylase were determined in the Jacarezinho and Juiz de Fora resistant populations, and in the Sete Lagoas susceptible population. Respirometry bioassays were also carried out for these maize weevil populations. According to the results obtained, the Jacarezinho population showed respiration rate significantly higher than the other two populations, which were similar. No significant differences in glycogen phosphorilase and glycosidase were observed among the maize weevil populations. Among the enzymes studied, trehalase and lipase showed significant difference among the three populations with higher activity in the Juiz de Fora population. The results obtained in the assays with amylase also indicate significant difference in activity among the populations, with higher activity in the Jacarezinho population. The inverse activity trends of lipases and amylases in both resistant populations, one showing fitness disadvantage without insecticide exposure and the other not showing it, may underlay the mitigation of insecticide resistance physiological costs observed in the Jacarezinho population.

Key words: Insecticide resistance, amylase, lipase, trehalase, fitness cost.

Introduction

Insecticide resistance evolution is usually associated with adaptative costs in the absence of insecticides (Coustau et al., 2000). The result of such costs is the impairment of the reproductive performance of the resistant individuals due to resource allocation from a basic physiological process to the protection against insecticides favoring their survival at the expense of the reproduction (Coustau et al., 2000; Foster et al., 2000). Adaptative costs associated with insecticide resistance were reported in a Brazilian population of the maize weevil,

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Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), but other resistant population of the same species does not exhibit costs of insecticide resistance (Fragoso et al., 2005; Guedes et al., 2006). This finding allows the investigation of the underlying costs of insecticide resistance and their mitigation mechanisms, what has yet to be done.

The O_2 consumption or the CO_2 production represents the summation of the energy requirements for the organism physiological processes (Clarke, 1993; Marais and Chow, 2003). Variations in respiration rate may therefore assist in detecting stress and stress response with potential for detecting costs associated with insecticide resistance, while modifications in fat body morphology indicate the availability and mobilization of energy reserves for the individual maintenance leading to its survival when exposed to toxic compounds (Guedes et al., 2006). These patterns and tools of investigation were developed in studies with populations of maize weevils resistant to pyrethroids by Guedes et al. (2006), where the resistant population showing mitigation of fitness costs associated with insecticide resistance exhibited higher respiration rate and body mass than the susceptible and another resistant population. The higher respiration rate in this case seems to favor a more efficient accumulation of energy reserves and their consequent mobilization allowing the maintenance of the resistance mechanism without compromising reproductive performance.

The objective of the present study was to test the hypothesis that differences in mobilization of energy reserves do exist between insecticide-resistant populations of the maize weevil, what may account for the mitigation of the costs associated with insecticide resistance in one of the strains. Enzymes assays were therefore carried out for enzymes involved in sugar and lipid digestion (amylase and lipase) and energy metabolism (glycogen phosphorliase, glycosidae and trehalase). The levels of enzyme activity thus obtained were confronted with results of concentration-mortality bioassays and respiration rate.

Material and methods

Insects and chemicals

Three populations of *S. zeamais* were used in the present investigation. The standard susceptible population was collected in Sete Lagoas County and provided by Embrapa Milho e Sorgo (Sete Lagoas, state of Minas Gerais, Brazil). The resistant populations are pyrethroid-resistant, one collected in Jacarezinho County (state of Paraná, Brazil) by the late 1980's and the other collected in Juiz de Fora County (state of Minas Gerais, Brazil) in 1999. All three populations were maintained in whole maize grains free of insecticides under controlled temperature (25 ± 2 ^{o}C), relative humidity (70 ± 5 %) and photoperiod (LD 12:12 h). All reagents were purchased from Sigma-Aldrich Química Brasil (São Paulo, Brazil), except acetone, which was obtained from Cromato Prod. Quim (Diadema, São Paulo, Brazil), and technical grade deltamethrin, which was provided by Bayer CropScience (Paulínia, São Paulo, Brazil).

Insecticide and respirometry bioassays

Insecticide bioassays were carried out as described elsewhere using 20 ml glass scintillation vials (Fragoso et al., 2003; Ribeiro et al., 2003). Production of CO_2 was measured in a CO_2 Analiser (TR2, Sable Systems International, Las Vegas, NV, USA) using methods described by Guedes et al. (2006). Respiration values were not normalized by body mass, in accordance with recommendations by Packard and Boardman (1999).

Preparation of enzyme extracts

Three batches of 300 non-sexed adult insects of each population were used as enzyme source for determination of glycosidase and trehalase activity after their immersion in 1.5 % KCl and subsequent homogenization in 6.0 ml 0.1 M Tris-HCl buffer (pH 8.0). The crude homogenate was filtered through glass-wool and centrifuged at

10,000 g_{max} for 15 min. The pellet was discarded and aliquots of the supernatant were taken for determination of protein content and enzyme activity. Three batches of 100 adult insects (non-sexed) were used for determination of glycogen phosphorilase activity using the same amount of buffer, while batches of 20 insect were used for amylase and lipase determinations in homogenates with 5 ml buffer.

Protein determination and enzyme assays

Protein concentration was determined following Warburg and Christian (1941). Glycosidase activity was determined as described by Hill and Orchard (2005) complemented by the method of the reducing sugar (DNS) of Miller. Trehalase activity was determined following Dahlqvist (1968), also complemented by the reducing sugar method, while glycogen phosphorilase was determined by methods of Tolman and Steele (1980). Amylase activity was determined with the K003 enzymatic kit from BIOCLIN (QUIBASA – Química Básica Ltda., Belo Horizonte, Minas Gerais, Brazil) by incubating the samples with starch following the method as modified by Caraway (1959). Lipase activity was determined using the K025 enzymatic kit, also from BIOCLIN, following methods adapted from Cherry and Crandall (1932). The kinetic parameters were determined for trehalase using increasing substrate concentrations (trehalose concentration from 0.31 to 25 mM) and fitting the results into a non-linear regression (Michaelis-Menten equation).

Statistical analysis

Concentration-response bioassays with deltamethrin were subjected to probit analysis (PROC PROBIT; SAS Institute, 1997). Respiration rates for the insect population were subjected to analysis of variance and Fisher's LSD test ($p < 0.05$) (PROG GLM; SAS Institute, 1997). The levels of enzyme activity were also subjected to analysis of variance and Fisher's LSD test ($p < 0.05$), if appropriate. Non-linear regression (Michaelis-Menten equation) was carried out to estimate the kinetic parameters (K_m and V_{max}) using the curve-fitting procedure of SigmaPlot (SPSS, 2000).

Results

Insecticide resistance and respiration rate

The results of the ÷² tests (÷² and p values) used to measure how well the data of each concentration-mortality curve fit the assumption of the probit model indicate that it was suitable for the intended estimates (low ÷²-values and $p > 0.05$). The LC_{50}'s (95% FL) were 0.62 (0.50-0.75) ì g a.i./cm², 177.44 (27.38-451.88) ì g a.i./cm² and 75.12 (1.85-317.23) ì g a.i./cm² for the susceptible population from Sete Lagoas and the resistant populations from Juiz de Fora e Jacarezinho respectively, which showed resistance ratios of 286.19-fold and 121.16-fold compared with the susceptible population at the LC_{50}. Respiration rate was also significantly different among the populations of maize weevil ($F_{2,6} = 23.05$, $p = 0.003$) with the Jacarezinho resistant population showing significantly higher respiration rate than both the susceptible and the insecticide-resistant population from Juiz de Fora (Table 1).

Enzyme activity

Among the enzymes involved in mobilization of carbohydrate (glycogen phosphorilase and trehalase) and hydrolysis sugars from the metabolism (glycosidase), only trehalase activity was significantly different among the maize weevil populations ($F_{2,6} = 72.39$, $p < 0.0001$), with the insects from Juiz de Fora showing the higher specific activity than the remaining populations, which resembled one another (Table 1). Activity of glycogen phosphorilase ($F_{2,6} = 0.25$, $p = 0.79$; 1,569.63 ± 104.36 nmol/min/mg protein) and glycosidase ($F_{2,6} = 1.43$, $p = 0.31$; 12.31 ± 0.79 nmol/min/mg protein) were similar in all three maize weevil populations. Activity

levels of amylase and lipase were also significantly different among maize weevil populations ($F_{2,6}$ = 20.19, p = 0.002 for amylase; and $F_{2,6}$ = 22.80, p = 0.002 for lipase) with Jacarezinho showing the highest levels of amylase activity and Juiz de Fora showing the highest levels of lipase activity (Table 1).

Table 1. Respiration rate and specific activity of amylase, lipase and trehalase (± SEM) in one insecticide-susceptible and two insecticide-resistant populations of the maize weevil *Sitophilus zeamais*.

Populations	Respiration rate (ì mol/h/insect)	Enzyme activity		
		Trehalase (nmol/min/mg protein)	Amylase (x 10^{-3} AU/ mg protein)	Lipase (IU/mg protein)
Susceptible (Sete Lagoas)	51.38 ± 1.82 b	33.39 ± 0.65 b	4.68 ± 0.28 b	12.95 ± 1.62 b
Resistant (Juiz de Fora)	52.98 ± 2.79 b	41.55 ± 0.51 a	1.81 ± 0.17 b	113.42 ± 21.43 a
Resistant (Jacarezinho)	68.83 ± 7.75 a	33.29 ± 0.50 b	10.92 ± 1.78 a	7.70 ± 2.22 b

Means followed by the same letter in a column do not differ significantly by Fisher's LSD test ($p < 0.05$).

Maize weevil trehalase follows the Michaelis-Menten kinetics within the range of substrate concentrations used (Figure 1). By taking the reciprocal of both sides of the Michaelis-Menten equation (hyperbolic model), the disadvantages of non-linear kinetic analysis are avoided converting it into the Lineweaver-Burk relationship, which is linear. The kinetic parameters K_m and V_{max} were therefore estimated for the three maize weevil populations as follows: K_m = 0.16 ± 0.04 mM and V_{max} = 46.10 ± 1.44 for the susceptible population (Sete Lagoas); K_m = 0.28 ± 0.08 mM and V_{max} = 66.14 ± 3.06 for the resistant population from Juiz de Fora; and K_m = 0.29 ± 0.08 mM and V_{max} = 51.08 ± 2.33 for the resistant population from Jacarezinho. Amylase and lipase also followed the Michaelis-Menten kinetics (Figure 2), but the estimation of the kinetic parameters was not possible for the enzymes due to the use of enzymatic kits.

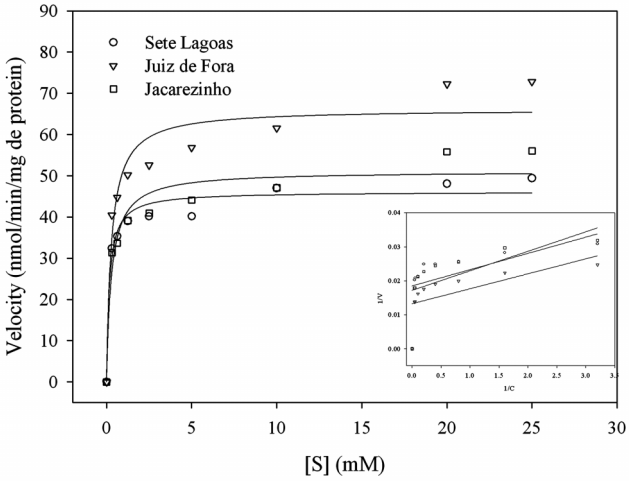

Figure 1. Michaelis-Menten plot of trehalase activity from three populations of the maize weevil *Sitophilus zeamais*, one insecticide-susceptible (Sete Lagoas) and two insecticide-resistant (Juiz de Fora and Jacarezinho) populations, using trehalose as substrate. Insert: Lineweaver-Burk plot (double reciprocal).

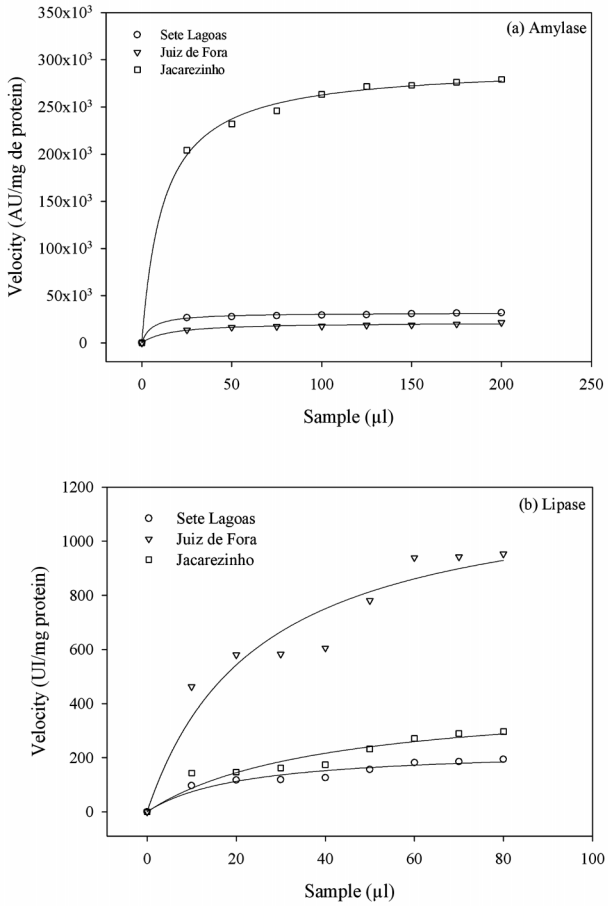

Figure 2. Michaelis-Menten plot of specific activity of amylase (a) and lipase (b) with increasing volumes of sample extract from three populations of maize weevil *Sitophilus zeamais*, one insecticide-susceptible (Sete Lagoas) and two insecticide-resistant (Juiz de Fora and Jacarezinho) populations.

Discussion

The levels of deltamethrin resistance and the respiration rates observed in the present study reinforce those obtained by Guedes et al. (2006), who hypothesized that the higher respiration rate, body mass and energy reserves from an insecticide-resistant population may mitigate the fitness cost usually associated with insecticide resistance. Such mitigation allows the maintenance of insecticide resistance mechanisms without impairing other physiological processes, such as reproduction. The study here reported aimed to further test this hypothesis and to provide insights on the potential mechanisms underlying the mitigation of insecticide resistance costs.

Differences in activity among enzymes from energy metabolism were expected between insecticide-susceptible and -resistant populations and also between resistant populations showing or not fitness costs associated with insecticide resistance. Indeed such differences were observed in the present study. Activity of trehalase, besides amylase and lipase, were always higher in one of the insecticide-resistant populations suggesting higher energy storage and eventual mobilization compared with the susceptible population. The resistant population from Juiz de Fora exhibits significantly higher activity of trehalase and lipase than the resistant population from Jacarezinho, which exhibits higher amylase activity than Juiz de Fora. The differences were particularly high for amylase and lipase.

Amylase cleaves starch and related polysaccharides allowing their eventual storage and use as energy source, which become substrate of activity of another group of carbohydratases (e.g., glycosidase and trehalase) that hydrolyze oligosaccharides and disaccharides (Chown and Nicolson, 2004). Trehalase, which is widespread in insects, hydrolyses trehalose into glucose; lipases are involved in lipid digestion. Lipid digestion was therefore more efficient in the resistant population from Juiz de Fora, while starch digestion was more efficient in the resistant population from Jacarezinho. In any case, the higher activity of these hydrolytic enzymes would favor energy storage in insects from these populations and are likely to favor their higher body mass. In fact, body mass is higher in the insecticide-resistant populations and even higher for the resistant population from Jacarezinho compared with Juiz de Fora (Guedes et al., 2006). Higher amylase activity seems therefore a particularly efficient tactic to better explore this insect food source (i.e., maize grains, which are a rich source of starch) leading to more efficient energy storage and higher body mass. Lipid digestion is poorly understood in insects (Arrese et al., 2001), and does not seem to be as important as starch digestion in maize weevil populations

regarding the mitigation of insecticide resistance costs, since the resistant population from Juiz de Fora, with high lipase activity, shows fitness disadvantaged in the absence of insecticide (Fragoso et al., 2005).

Trehalase activity was significantly higher in the resistant population from Juiz de Fora, followed by the resistant population from Jacarezinho suggesting that energy mobilization is also greater in the insecticide resistant populations. The K_M values for trehalase from the resistant populations indicate lower affinity for the substrate (i.e., trehalase) than the enzyme from the insecticide-susceptible population. The higher trehalase activity then observed in the resistant populations is probably due to the higher levels of these enzymes in the resistant insects. Since trehalase activity was particularly higher in the Juiz de Fora population, sugar mobilization is probably higher in insects from this population preventing energy storage at the levels observed for the Jacarezinho population (Guedes et al., 2006). The other enzymes involved in energy mobilization and studied here (i.e., glycogen phosphorilases and glycosidase) were similar in all three populations, therefore trehalase is probably the main responsible for the higher energy mobilization in the resistant populations required for maintaining active insecticide resistance mechanisms in the resistant insects. Extensive selection with insecticide however favors selection of modifier genes that mitigate such cost. The mitigation of the cost of insecticide resistance may relax the need of this high energy mobilization and favors energy storage instead aiming the maintenance of both – insecticide resistance mechanisms and basic physiological processes without significant trade-off between them, as observed for the resistant population from Jacarezinho, but not for Juiz de Fora.

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