Insect science

Insect Science (2013) 20, 358–366, DOI 10.1111/j.1744-7917.2012.01529.x

ORIGINAL ARTICLE

Does cypermethrin affect enzyme activity, respiration rate and walking behavior of the maize weevil (*Sitophilus zeamais*)?

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Abstract Insecticides cause a range of sub-lethal effects on targeted insects, which are frequently detrimental to them. However, targeted insects are able to cope with insecticides within sub-lethal ranges, which vary with their susceptibility. Here we assessed the response of three strains of the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) to sub-lethal exposure to the pyrethoid insecticide cypermethrin. We expected enzyme induction associated with cypermethrin resistance since it would aid the resistant insects in surviving such exposure. Lower respiration rate and lower activity were also expected in insecticide-resistant insects since these traits are also likely to favor survivorship under insecticide exposure. Curiously though, cypermethrin did not affect activity of digestive and energy metabolism enzymes, and even reduced the activity of some enzymes (particularly for cellulase and cysteine-proteinase activity in this case). There was strain variation in response, which may be (partially) related to insecticide resistance in some strains. Sub-lethal exposure to cypermethrin depressed proteolytic and mainly cellulolytic activity in the exposed insects, which is likely to impair their fitness. However, such exposure did not affect respiration rate and walking behavior of the insects (except for the susceptible strain where walking activity was reduced). Walking activity varies with strain and may minimize insecticide exposure, which should be a concern, particularly if associated with (physiological) insecticide resistance.

Key words insecticides, insecticide resistance, pyrethroids, stored grain insect, sub-lethal effects

Introduction

Some xenobiotics can modulate the activity of not only detoxification enzymes in living organisms, but also of digestive and energy metabolism enzymes (Nath *et al*., 1997; Nath, 2000; Natsuhara *et al*., 2004; Philippou & Moores, 2010). Enhanced detoxification activity is frequently associated with insecticide resistance, but the activity of digestive and energy metabolism enzymes may also be important in mitigating fitness costs usually associated with insecticide resistance (Araújo *et al*., 2008a,b; Scott, 1999; Hemingway, 2000; Silva *et al*., 2010a,b). The insecticide-induction of detoxification enzymes have been well documented (Feyereisen, 1999, 2005; Ranson & Hemingway, 2005); however, the potential insecticide modulation of digestive and energy metabolism enzymes has been an object of little attention, especially considering the possibility of strain variation (Ahmed *et al*., 1998; Wilkins *et al*., 1999).

Insect homeostasis is maintained through proper diet and subsequent digestion and mobilization of carbohydrates, lipids and proteins, in addition to gas exchange.

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Insecticide exposure may impose changes to allow insect survival leading to altered patterns of digestion, energy metabolism and respiration rate (Orr & Downer, 1982; Hostetler *et al*., 1994; Alaoui *et al*., 1994, 1997; Nath *et al*., 1997; Harak *et al*., 1999; Nath, 2000, 2003; Ge *et al*., 2011; Guedes *et al*., 2006; Oliveira *et al*., 2007; Araújo *et al*., 2008,b; Silva *et al*., 2010a,b). Variation in such traits were observed among the key pest of stored maize in the neotropical region – the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Guedes *et al*., 2006; Oliveira *et al*., 2007; Araújo *et al*., 2008a,b; Silva *et al*., 2010a,b; Lopes *et al*., 2010), but their possible induction in different strains of this species has not yet been investigated and is the objective of the present study.

Shifts in metabolism, particularly energy investment in defense mechanisms against xenobiotics, are important to allow survival of exposed insects. However, such metabolic changes in the absence of the stressor agent are unnecessary and thus suggestive that they may be induced by stressor exposure. This was the hypothesis tested in the present study, where three strains of the maize weevil with different pyrethroid (insecticide) susceptibilities were exposed to sub-lethal concentrations of cypermethrin. The activity of digestive and energy metabolism enzymes was determined in cypermethrin-exposed and unexposed insects of each strain, which were also subject to determinations of respiration rate and walking activity. We expected enzyme induction and higher respiration rate in cypermethrin-exposed insects, particularly in the more resistant strains. However, lower walking behavior was expected in the more susceptible strains, particularly when exposed to cypermethrin as a means to minimize insecticide exposure as earlier predicted by Georghiou (1972).

Materials and methods

Insects and reagents

Three strains of *S. zeamais* were used, all of them derived from colonies maintained in the laboratory since the 1990s. One colony is the standard susceptible colony frequently used in insecticide resistance studies (coded as "SL" due to its original place of collection: Sete Lagoas county), while the other colonies were derived from pyrethroid-resistant colonies (coded as "JF" and "JA", after Juiz de Fora and Jacarezinho counties, their original sites of collection), both exhibiting resistance to DDT and pyrethroid insecticides, mainly due to reduced target-site sensitivity (Guedes *et al*., 1995; Ribeiro *et al.*, 2003; Araújo *et al*., 2011). The strains were maintained in whole maize grains free of insecticides under controlled temperatures (25 ± 2°C), relative humidity (RH: 70% ± 5%) and photoperiod (12 : 12 h L : D).

Benzamidine, N-α-benzoyl-l-Arg-p-nitroanilide (L-BApNA), $CaCl_2$, KCl, carboxymethyl cellulose, 3,5-dinitrosalicyclic acid (DNS), sodium acetate, trehalose, Tris-HCl and N-α-p-tosyl-l-Arg methyl ester (L-TAME) were all were purchased from Sigma-Aldrich Química Brasil (São Paulo, Brazil). In contrast, acetone was obtained from Cromato Produtos Químicos Ltda (Diadema, São Paulo, Brazil), and technical grade permethrin (92.2% pure) was provided by Syngenta (São Paulo, Brazil).

Insecticide bioassays and exposure to cypermethrin

Insecticide concentration-mortality bioassays were carried out using 20 mL glass scintillation vials and 48-h exposure (Fragoso *et al*., 2003; Ribeiro *et al.*, 2003). Briefly, each replicate encompassed a 20 mL glass vial coated inside with dried insecticide residues (applied at the rate of 0.4 mL/vial) to which 20 non-sexed adult insects (2 weeks old) were transferred. At least four replicates were used in the bioassays following a completely randomized experimental design and a minimum of seven concentrations were used to establish the concentration-mortality curves for each strain (0.0014, 0.0140, 0.14, 0.28, 0.56, 1.20, 2.40, 4.80, 9.60 μg/cm^2 for the SL strain; 0.0140, 0.14, 1.40, 2.80, 5.60, 11.20, 22.40 μg/cm^2 for the JA strain; 0.14, 1.40, 2.80, 5.60, 11.20, 22.40, 44.80, 89.60 μg/cm^2 for the JF strain). The concentrations were established based on preliminary tests using a series of 10-fold dilutions and establishing the concentration range leading to 10%–90% mortality (LC_{10}) for each strain, after which a 2-fold dilution series was used within this range. Control vials were treated with acetone only. Insects were considered dead if unable to walk when prodded with a fine hair brush. The same 20-mL vials were also used to expose adult weevils (2 weeks old) of the three strains to cypermethrin, but only for 24 h at a concentration equivalent to the LC_{10} for the SL strain obtained in the concentration-mortality bioassay previously described (LC_{10} = 1.29 μg/cm^2; such reduced exposure prevented insect mortality in our bioassays securing a sub-lethal exposure for the subsequent bioassays). Exposed and control insects were kept under the same conditions in growth chambers (25 ± 2°C, 70% ± 5% RH, and 12 : 12 h L : D) and used for enzyme colorimetric assays, respirometry and behavioral assays as described below.

Preparation of enzyme extracts

Three batches of 150 and 250 unsexed adult insects of each strain and treatment group were used as enzyme

sources for the determination of trehalase and cellulase activity after their immersion in 1.5% KCl and subsequent homogenization in 3 mL of 0.1 mol/L Tris-HCl buffer (pH 8.0). The crude (whole body) 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 40 and 100 adult insects (unsexed) were used for the determination of cysteine- and serine-proteinase activity (amidolytic and esterolytic), respectively, while batches of 20 insects were used for amylase and lipase determinations in homogenates with 5 mL buffer.

Protein concentration and enzyme assays

Protein concentration was determined following Warburg and Christian (1941). Trehalase activity was determined based on Dahlqvist (1968) and using trehalose as the substrate (50 mmol/L trehalose), also complemented by the reducing sugar method (Miller, 1959). Cellulase activity was determined by methods described by Lee *et al*. (2004), based on the method of reducing sugars (Miller, 1959). The reaction substrate was prepared by dissolving 1.0% (w/v) carboxymethyl cellulose in 50 mmol/L sodium acetate buffer (pH 6.0). The reaction mixture contained 1.0 mL substrate and 1.0 mL enzyme extract, which was incubated at 37°C for 30 min. The reaction was stopped by adding DNS (1 mL of a 0.044 mol/L solution), which reduced the glucose released in the reaction. The absorbance readings were carried out at 540 nm.

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 modified by Caraway (1959). The soluble starch shows a blue color in the presence of iodine, and the starch hydrolysis by amylase progressively eliminates the blue color. The absorbance is read at 660 nm. Lipase activity was determined using the K025 enzymatic kit, also from BIOCLIN, following methods adapted from Cherry and Crandall (1932). This method is based on the activity of lipases over a glycerol ester, releasing a chromogenic compound quantified at 410 nm. Activity values for amylase and lipase were expressed as amylase units (AU) and international units (IU), respectively. AU refers to the amount of amylase that hydrolyzes 10 mg starch in 30 min at 37°C, while the IU of lipase activity refers to the amount of lipase that releases 1 μmol of fatty acid per minute.

The activity of serine-proteinases was assessed using two substrates: L-BApNA as a substrate for determination of amidolytic activity, and L-TAME as a substrate for determination of esterolytic activity. The amidolytic activity was determined using the methods of Erlanger *et al*. (1961) using 60 mmol/L L-BApNA in 0.1 mol/L Tris–HCl buffer (pH 8.2) containing 20 mmol/L $CaCl_2$. The reaction mixture encompassed 5 mL substrate and 0.6 mL enzyme extract, which was incubated at 25°C for 2.5 min. The absorbance reading was carried out at 410 nm and the extinction coefficient 8800 mol/L/cm was used to calculate the enzyme activity. The esterolytic activity was determined following Hummel (1959) using 0.1 mmol/L L-TAME in the same buffer system used for amidolytic activity. The reaction mixture encompassed 1 mL substrate and 250 μL enzyme extract, which was incubated at 25°C for 2.5 min. The absorbance reading was carried out at 247 nm and the extinction coefficient, 540 mol/L/cm, was used to calculate enzyme activity. Cysteine-proteinase activity was assessed using L-BApNA as substrate as previously described, but adding 0.1 mL of 10 mmol/L benzamidine, a serine-proteinase inhibitor, in the reaction mixture.

Respirometry

Four flasks containing 20 insects each were used in respirometry determinations for each strain and treatment in a completely closed system (Guedes *et al*., 2006; Oliveira *et al*., 2007). Consumption of O_2 was measured in a O_2 Analizer (TR3, Sable Systems International, Las Vegas, NV, USA) using methods described by Guedes *et al*. (2006). The measurements were obtained by injecting atmospheric air into the flasks, which then directed it to an infrared reader connected to the system. Respiration values were presented as μL of O_2/h/insect (O_2 consumed per hour). Body mass was determined for insects of each strain, after their removal from the respirometer flasks, using an analytical balance (Sartorius BP 210D, Goettingen, Germany).

Behavioral assays

Adult insects (2 weeks old) were exposed to cypermethrin as previously described. After this exposure, the insects were individually placed in the test arenas and their walking behavior was recorded for 10 min. The test arenas were Petri dishes (9.0 cm diameter) with filter paper (Whatman no. 1) lined on the bottom and with inner walls coated with Teflon® PTFE (DuPont, Wilmington, DE, USA) to prevent the insects from escaping. Arenas with individual insects were used for each treatment group

Table 1 Relative toxicity of cypermethrin to three strains of *Sitophilus zeamais*, including a standard susceptible strain (SL).

Strain	No. insects	Slope ± SEM	LC_{50} (95% CI) ($\mu g/cm^2$)	Resistance ratio	χ^2	df	*P*
SL	860	1.46 ± 0.10	1.29 (1.03 – 1.66)	–	4.37	7	0.74
JA	720	0.56 ± 0.03	4.28 (2.58 – 7.01)	3.3	6.54	6	0.37
JF	800	0.74 ± 0.04	15.87 (10.44 – 24.23)	12.3	5.76	6	0.46

(exposed and control). The movement of each insect within the arena was recorded for 10 min and digitally transferred to a computer using an automated video tracking system equipped with a charge-coupled device (CCD) camera (ViewPoint Life Sciences Inc., Montreal, Canada). The parameters recorded were walked distance (cm), velocity (cm/s), and time spent walking (s). Behavioral tests were carried out between 9:00 to 17:00 hours in a room with artificial, incandescent light and average temperature of 25 ± 3°C. The experiments were set using a completely randomized design and following a factorial scheme (3 strains × 2 treatments) with 20 replicates, each one consisting of a single insect. Before initiating the video tracking in each trial the insects were allowed to recognize the arena for 1 min.

Statistical analyses

Concentration-response bioassays with cypermethrin were analyzed using probit analysis (PROC PROBIT: SAS Institute, 2008). The overall results for enzyme activity were subjected to a two-way (strain × exposure) multivariate analysis of variance (PROC GLM with MANOVA statement) followed by two-way univariate analysis of variance (ANOVA) for each enzyme (PROC GLM) and Fisher's least significant difference (LSD) test ($P < 0.05$) if appropriate. A similar procedure was used for the behavioral changes under cypermethrin exposure. Body mass and respiration rates for the insect strains were analyzed using analysis of variance and Fisher's LSD test ($P < 0.05$) (PROG GLM: SAS Institute, 2008).

Results

Concentration-mortality bioassays

The concentration-mortality results for cypermethrin were properly described by the probit model (goodness-of-fit exhibiting low χ^2-values [<6.5] and high *P*-values [>0.37]), which was therefore suitable to estimate the intended toxicological parameters (Table 1). The resistance ratios were estimated relative to the LC_{50} for the standard susceptible population (SL) and ranged from 3.3-fold (JA strain) to 12.3-fold (JF strain) (Table 1).

Enzyme activity

The overall specific activity of the enzymes studied varied significantly among the strains of *S. zeamais* (Wilks' Lambda < 0.001, $F_{14,12} = 52.32$, $P < 0.000\,1$) and was affected by exposure to the insecticide (Wilks' Lambda = 0.016, $F_{7,16} = 53.26$, $P < 0.000\,1$). Also, the interaction strain × exposure was significant (Wilks' Lambda = 0.013, $F_{14,12} = 0.013$, $P = 0.001\,2$) indicating that the effect of cypermethrin on the catalytic activity of the enzymes was dependent upon the strain of *S. zeamais*.

The results of the two-way univariate ANOVA of the activity of each enzyme are shown on Table 2. The interaction strain × exposure was only significant ($P < 0.05$) for amylase, trehalase and cellulase activity, indicating diverse insecticide effect with the insect strain. Cysteine-proteinase activity varied among strains and insecticide exposure, but their interaction was not significant. The activity of lipase and serino-proteinases (amydolytic) were affected by strain, but not by insecticide exposure. There was no significant variation in the esterolytic activity of serino-proteinases.

Table 3 shows the specific activity of carbohydrate- and lipid-metabolizing enzymes. Overall, cypermethrin exposure reduced the activity of these enzymes, except lipase (and trehalase in the SL strain), which maintained the same level of activity. Without cypermethrin exposure, amylase activity was higher in the JF strain. In contrast, cypermethrin exposure reduced the amylase activity in all strains and particularly in the JF strain (ca. 4-fold decrease). After cypermethrin exposure, amylase activity was similar between the two resistant strains, which differed from the significantly smaller activity of the susceptible strain. Levels of lipase and trehalase activity were constitutively higher in the resistant strains, which remained after insecticide exposure despite a smaller decrease in trehalase activity in these strains.

Table 2 Results of the (univariate) analysis of variance showing the main effects of strain of *Sitophilus zeamais* and cypermethrin exposure, as well as their interaction, in the specific activity of seven metabolic enzymes.

Source	df	Amylase		Lipase		Trehalase		Cellulase		Cysteine-protease		Serine-proteases			
												Amydolytic		Esterolytic	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Strain (A)	2	90.48	<0.001	10.49	0.002	59.10	<0.001	22.89	<0.001	161.28	<0.001	16.09	<0.001	1.39	0.28
Exposure (B)	1	79.79	<0.001	0.18	0.681	6.09	0.030	145.04	<0.001	189.09	<0.001	0.02	0.88	1.37	0.26
Interaction (AB)	2	44.57	<0.001	0.56	0.588	5.73	0.018	15.65	<0.001	2.17	0.157	1.80	0.21	0.33	0.72
Error	12														

Regardless of the insect strain, cypermethrin exposure significantly reduced cellulase and cysteine-proteinase activity (Table 4). Constitutively, celullase and serine-proteinase activities were higher in the JF strain, while cysteine-proteinase activity was higher in the JA strain. Such strain differences were maintained with cypermethrin exposure.

Respirometry assays

No significant difference was detected in O_2 consumption among strains of *S. zeamais* ($F_{2,17} = 1.36$, $P = 0.28$), between insecticidal exposure ($F_{1,17} = 2.37$, $P = 0.14$), and interaction between these two variables ($F_{2,17} = 2.32$, $P = 0.13$) (average O_2 consumption = 1.74 ± 0.09 μL/h/insect). In addition, no significant difference in body mass was observed (average adult weight = 2.96 ± 0.07 mg; $P > 0.05$).

Behavioral assays

MANOVA indicated that the overall walking behavior parameters varied among strains (Wilks' Lambda = 0.81, $F_{6,180} = 3.44$, $P = 0.003$) and between insecticidal treatments (Wilks' Lambda = 0.91, $F_{3,90} = 3$, $P = 0.03$). However, there was no effect of the interaction strain × treatment (Wilks' Lambda = 0.97, $F_{6,180} = 0.39$, $P = 0.88$).

Univariate ANOVA indicated significant differences among strains for distance walked ($F_{6,180} = 7.63$, $P = 0.001$), walking velocity ($F_{6,180} = 6.93$, $P = 0.002$) and resting time ($F_{6,180} = 7.06$, $P = 0.001$). The insecticide-resistant strains are more active than the susceptible strain regardless of insecticide exposure (Fig. 1). Cypermethrin exposure did not affect distance walked and walking velocity, but increased the resting time ($F_{6,180} = 6.35$, $P = 0.013$) (Fig. 2).

Table 3 Effect of cypermethrin exposure on the specific activity of carbohydrate- and lipid-metabolizing enzymes in three strains of the maize weevil S*itophilus zeamais*.

Enzyme	Strain	Specific activity		% Control[†]
		Control	Cypermethrin	
Amylase	SL	0.51 ± 0.14 Ca[‡]	0.32 ± 0.03 Ba	63
(AU/mg protein)	JA	2.22 ± 0.22 Ba	1.67 ± 0.06 Aa	75
	JF	4.82 ± 0.35 Aa	1.27 ± 0.05 Ab	26
Lipase	SL	898 ± 79 Ba	1214 ± 245 Ba	135
(μmol/min/mg protein)	JA	2148 ± 307 Aa	2009 ± 129 Aa	94
	JF	1820 ± 364 Aa	1875 ± 190 Aa	103
Trehalase	SL	13.53 ± 0.55 Ca	14.54 ± 0.41 Ba	107
(ηmol/min/mg protein)	JA	21.58 ± 1.38 Ba	20.18 ± 1.12 Aa	94
	JF	25.80 ± 0.68 Aa	20.85 ± 1.38 Ab	81

Values are means ± SEM of nine determinations in three independent (biological) replicates.

[†](Activity in exposed insects /activity in non-exposed [control] insects) × 100.

[‡]Values followed by the same uppercase letter in columns and lowercase letter in rows are not significantly different ($P < 0.05$) by Fisher's protected least significant difference test.

Table 4 Effect of cypermethrin exposure on the specific activity of cellulase and proteinases in three strains of the maize weevil S*itophilus zeamais*.

Enzyme	Strain	Specific activity (ηmol/min/mg protein)		% Control†
		Control	Cypermethrin	
Cellulase	SL	10.40 ± 0.23 Ca‡	3.47 ± 0.06 Ab	33
	JA	15.11 ± 2.04 Ba	5.10 ± 0.27 Ab	34
	JF	25.66 ± 0.83 Aa	5.19 ± 0.39 Ab	20
Cysteine-proteinase	SL	9.80 ± 0.55 Ba	6.32 ± 0.16 Cb	64
	JA	14.94 ± 0.89 Aa	9.95 ± 0.36 Ab	67
	JF	7.93 ± 0.51 Ca	3.95 ± 0.27 Bb	50
Serine-proteinase (amidolytic)	SL	0.192 ± 0.043 Ba	0.163 ± 0.018 Ba	85
	JA	0.162 ± 0.010 Ba	0.152 ± 0.013 Ba	94
	JF	0.235 ± 0.019 Aa	0.211 ± 0.005 Aa	90
Serine-proteinase (estereolytic)	SL	33.81 ± 1.53 Aa	32.89 ± 7.70 Aa	97
	JA	35.33 ± 1.28 Aa	29.04 ± 6.37 Aa	82
	JF	47.71 ± 3.25 Aa	37.00 ± 6.84 Aa	78

Values are means ± SEM of nine determinations in three independent (biological) replicates.

†(Activity in exposed insects /activity in non-exposed [control] insects) × 100.

‡Values followed by the same uppercase letter in columns and lowercase letter in rows are not significantly different ($P < 0.05$) by Fisher's protected least significant difference test.

Discussion

The working hypothesis of this study was that metabolic changes take place when insects are exposed to sub-lethal concentrations of insecticide to maximize their survival. Such changes are likely to vary among insect strains, particularly when they differ in insecticide susceptibility, and metabolic changes may reflect changes in respiration rate and walking activity. Curiously, no enzyme-induction was observed in the strains of *S. zeamais* studied when activity of digestive and energy metabolism enzymes was quantified with and without cypermethrin exposure at sub-lethal concentrations. In truth, depression of enzyme activity was the outcome observed particularly for cellulase and cysteine-proteinase, besides amylase and trehalase for the JF strain only, while the other enzymes were not affected by cypermethrin exposure. This trend in depression of enzyme activity prevailed regardless of the level of susceptibility to cypermethrin, although the level of enzyme activity varied with insect strain.

The JA and JF strains exhibit resistance to cypermethrin, but the resistance levels were only low and moderate respectively, in contrast with the original strains from which they were derived (Guedes *et al*., 1994, 1995; Fragoso *et al*., 2003; Ribeiro *et al*., 2003; Corrêa *et al*., 2011). Arbitrary divergence from the original (parental) colonies certainly occurred during the establishment and maintenance of the derived colonies used in our study, which has already been demonstrated to occur (Fricke & Arnqvist, 2004). Regardless of that, cypermethrin resistance was detectable in levels high enough for the intended study and variation of enzyme activity among strains was significant for all digestive and energy metabolism enzymes, except esterolytic activity of serine-proteinases, as expected based on previous studies with *S. zeamais* (Araújo *et al*., 2008a,b; Lopes *et al*., 2010; Silva *et al*., 2010a,b).

Cypermethrin exposure also affected expression of some enzymes, but not others, and the most cypermethrin-resistant strain (i.e., JF) was subjected to the strongest depression of enzyme activity with insecticide exposure. This result was unexpected because we believed that a more cypermethrin-resistant strain would be able to better cope with sub-lethal exposure of cypermethrin. However, this cypermethrin-resistant strain was more active than the other strains, regardless of insecticide exposure and without changes in its respiration rate. Walking activity may favor insect survival and even more so in insecticide-resistant strains favoring their escape from the contaminated area, which is a trait independent from physiological resistance to insecticides, unlike earlier predictions by Georghiou (1972), but later revised and subsequently confirmed in experimental studies (Lockwood *et al*., 1984; Guedes *et al*., 2009; Braga *et al*., 2011; Corrêa *et al*., 2011). In addition, the JF strain is likely to exhibit fitness cost associated with insecticide resistance, as reported in

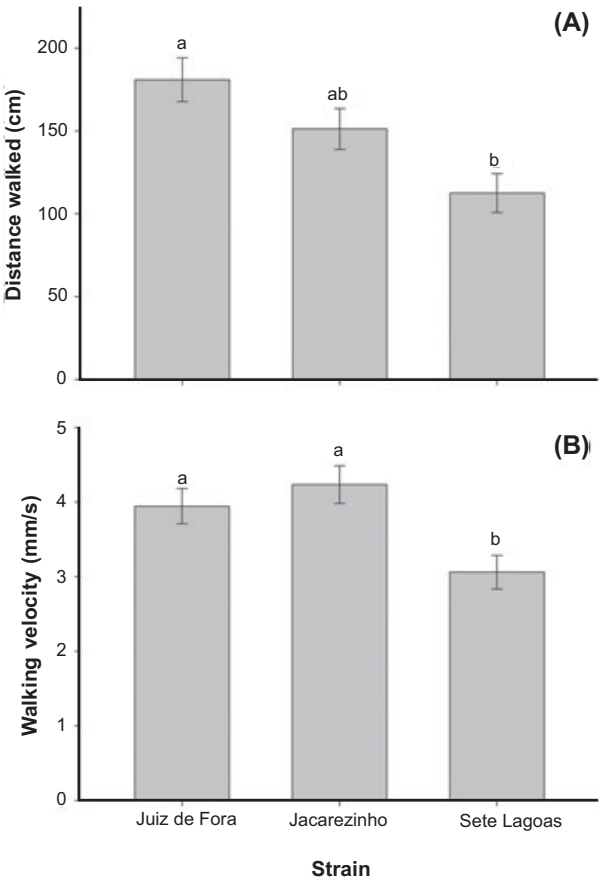

Fig. 1 Distance walked (A) and walking velocity (B) (± SE) of insects from three strains of *Sitophilus zeamais.* The results of insects exposed and not exposed to cypermethrin were pooled together since they were not significant (Fisher's *F*-test; $P > 0.05$). Bars followed by the same letter are not significantly different by Fisher's protected least significant difference test ($P > 0.05$).

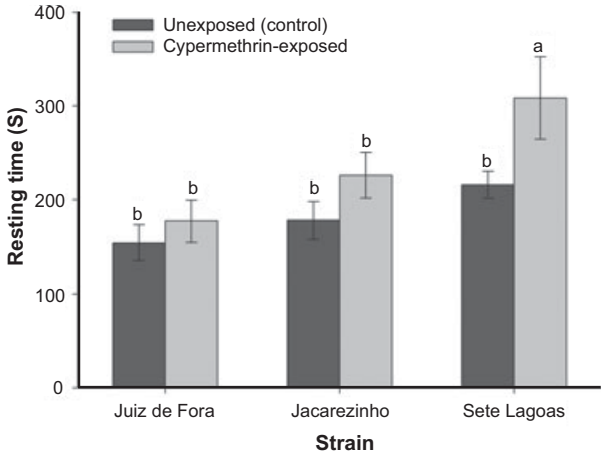

Fig. 2 Resting time of insects from three strains of *Sitophilus zeamais* exposed and not exposed to cypermethrin. Bars followed by the same letter are not significantly different by Fisher's protected least significant difference test ($P > 0.05$).

its original (parental) strain, which may account for the depression in activity of some of its metabolic enzymes favoring insecticide detoxification enzymes in a physiological trade-off (Guedes *et al*., 2006; Fragoso *et al*., 2005, 2007; Oliveira *et al*., 2007; Ribeiro *et al*., 2007). Therefore, insecticide survival in the case of this JF strain may be maximized, not only by costly insecticide-resistant mechanisms, but also by its higher activity favoring its escape from cypermethrin-treated surface.

In summary, cypermethrin-exposure affected only some metabolic enzymes (particularly celullase and cysteine-proteinase) decreasing their activity, which may impair insect fitness. However, such exposure did not affect respiration rate and walking behavior of the insects (except for the susceptible strain where walking activity was reduced). Walking activity varies with strain and may minimize insecticide exposure, which should be a concern, especially if associated with (physiological) insecticide resistance.

Acknowledgments

Appreciation is expressed to the Minas Gerais State Foundation for Research Aid (FAPEMIG), the CAPES Foundation (Brazilian Ministry of Education), and the National Council of Scientific and Technological Development (CNPq; Ministry of Science and Technology) for the financial support provided.

Disclosure

The authors declare that they have no conflict of interests.

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Accepted March 15, 2012