Physiological Entomology (2006) **31**, 30–38 DOI: 10.1111/j.1365-3032.2005.00479.x

Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*

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Abstract. A common assumption in models of insecticide resistance evolution is the association between resistance and fitness costs in the absence of insecticides. There is empirical evidence of such associations, but their physiological basis (and mitigation) is little investigated. Pyrethroid-resistant populations of the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) offer this opportunity. Pyrethroid resistance in this species was initially observed in five Brazilian states by 1995, but the phenomenon apparently decreased and did not spread to other regions, probably due to the occurrence of a fitness disadvantage in resistant individuals in the absence of insecticides. The present investigation aims to verify whether differences in respiration rate and fat body morphology are related to differences in rate of development in Brazilian populations of *S. zeamais* resistant to insecticides, and thereby provide evidence for the existence (or not) of a physiological fitness cost acting against insecticide resistance in maize weevils. This may occur due to a possible energy trade-off between insecticide resistance and other physiological processes associated with development and reproduction. To achieve this, studies of the rate of development, respiration and fat body cytomorphology are carried out in one insecticide-susceptible (from Sete Lagoas) and two resistant populations (from Jacarezinho and Juiz de Fora) of *S. zeamais*. The resistant population from Jacarezinho shows that higher body mass is associated with higher energy reserves (larger trophocyte area) for development and reproduction, as well as for insecticide resistance. However, the resistant population from Juiz de Fora does not appear to have large enough energy allocation for insecticide-resistance expression and development and/or reproductive performance, suggesting a trade-off between resistance and other life history traits.

Key words. Adaptative cost, adult respiration rate, body mass, carbon dioxide production, grain beetles, pyrethroid resistance, rate of development, trophocyte area.

Introduction

Insecticide resistance studies are not only of practical importance in pest management programmes, but also are important as models of evolution of newly adapted phenotypes and their associated physiological (and genetic) changes (Coustau *et al.*, 2000; Raymond *et al.*, 2001). The major genes responsible for the individual adaptation to a new environment (e.g. insecticide-treated grains in the case of weevils) are usually associated with an adaptative cost because they may be at a disadvantage in the previous environment where independent selection pressures shaped the prevailing phenotypes (Coustau *et al.*, 2000; Berticat *et al.*, 2002). This rationale is based on the general view that a resource allocation takes place, affecting metabolic or developmental processes, and decreasing reproductive potential (Berticat *et al.*, 2002).

Selection for insecticide resistance is generally followed by pleiotropic effects, which may place the resistant insects

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at a disadvantage when insecticide use is discontinued, relaxed or altered (Coustau *et al.*, 2000; Arnaud & Haubruge, 2002; Foster *et al.*, 2003). This may lead to a decline in the frequency of resistant individuals over time in the absence, reduction or alteration of insecticide selection. However, there are cases where there are no apparent disadvantages, or rather fitness advantages, observed in insecticide-resistant individuals of some insect species, such as the red flour beetle *Tribolium castaneum* (Beeman & Nanis, 1986; Haubruge & Arnaud, 2001) and the mosquito *Culex pipiens* (Raymond *et al.*, 2001). Allelic replacement (by a less costly allele) and selection of modifier genes can also ameliorate the cost of insecticide resistance (Coustau *et al.*, 2000; Raymond *et al.*, 2001; Berticat *et al.*, 2002). Curiously, the basis of insecticide resistance costs and its mitigation is little investigated.

Among the four basic molecular mechanisms of insecticide resistance (constitutive overproduction, constitutive underproduction, target modification and inducible change in gene regulation), only in the last case is there no general expectation of physiological cost of resistance (Taylor & Feyereisen, 1996; Coustau *et al.*, 2000). These modifications leading to insecticide resistance usually induce deleterious effects by disrupting the pathways of their targets (or receptors) (Coustau *et al.*, 2000). As a result, a higher metabolic rate may be necessary for the resistant individuals to maintain the resistance mechanisms unbalancing their gas exchange. High body mass and differences in fat body morphology in insecticide-resistant populations are suggestive of enough energy reserves being available for the maintenance of the resistant phenotype without compromising potentially conflicting processes and mitigating the cost of insecticide resistance. If no suitable increment of energy metabolism takes place, energy reallocation may divert energy from other physiological processes involved with insect development, maintenance and reproduction (Hostetler *et al.*, 1994; Chown & Gaston, 1999; Harak *et al.*, 1999). The insecticide itself may also interfere with the storage of carbohydrates and proteins in the fat body and their mobilization in insects (Orr & Downer, 1982; Alaoui *et al.*, 1994, 1997; Nath *et al.*, 1997; Nath, 2000, 2002). However, these studies have yet to be comparatively carried out in insecticide-resistant and -susceptible populations.

Despite oxygen uptake representing the summation of the energy requirements for the insect physiological processes (Clarke, 1993), there are few studies that use respiration rate (measured as O_2 uptake or CO_2 release) to demonstrate the adaptability of insect populations to different environmental conditions through their basic physiological processes (Marais & Chown, 1993). Variations in insect respiration rate contribute to the detection of possible fitness costs associated with the insecticide resistance in insecticide-free environments, whereas modifications in fat body morphology suggest the availability and mobilization of energy reserves for organism maintenance and survival during exposure to toxic compounds.

Pyrethroid resistance in Brazilian populations of the maize weevil *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) was initially observed in five national States (Guedes *et al.*, 1995). Target site modification is the prevailing insecticide resistance mechanism in these insect populations with secondary involvement of enhanced detoxification by glutathione S-transferases and phosphotriesterases (Fragoso *et al.*, 2003; Ribeiro *et al.*, 2003). Nonetheless, retrogression in the spread of the phenomenon appears to have occurred (Fragoso *et al.*, 2003; Ribeiro *et al.*, 2003). The likely occurrence of a fitness disadvantage associated with insecticide resistance in insecticide-free environments may have prevented its spread (Muggleton, 1983; Roush & McKenzie, 1987; Coustau *et al.*, 2000).

Fitness disadvantages associated with target site resistance to pyrethroids and enhanced metabolic detoxification of different insecticide groups have been reported in resistant populations of some insect species (Boivin *et al.*, 2001; Foster *et al.*, 2003). This may also occur in the maize weevil, but whether or not higher energy reserves together with their mobilization in resistant populations ameliorates insecticide resistance costs remains to be studied. Therefore, the objective of the present investigation is to assess differences in fitness, respiration rate and fat body morphology among insecticide-resistant and -susceptible populations of the maize weevil, and how they are inter-related, thus providing evidence of the existence or not of physiological cost associated with this phenomenon in this insect-pest species, how this cost is translated into fitness disadvantage and if it may be somehow mitigated.

Materials and methods

Insect populations and chemicals

Three populations of *S. zeamais* were used in this study, each of them established from over 500 field-collected individuals, as detailed elsewhere (Guedes *et al.*, 1995; Fragoso *et al.*, 2003; Ribeiro *et al.*, 2003). A standard susceptible population was obtained from the National Research Center of Maize and Sorghum (CNPMS/EMBRAPA, Sete Lagoas, State of Minas Gerais, Brazil), where it has been maintained for over 15 years without insecticide exposure and its susceptibility to pyrethroids and organosphosphates being known (Guedes *et al.*, 1994, 1995; Ribeiro *et al.*, 2003). This population was originally collected from heavily infested maize by mid 1980s in Sete Lagoas County (State of Minas Gerais, Brazil).

The other two populations are highly resistant to pyrethroids (over 500-fold resistant) based on insecticide-resistance bioassays routinely carried out before and during the present study. One of them, field-collected in Juiz de Fora County (State of Minas Gerais, Brazil) in early 1999, is also resistant to the organophosphate insecticide malathion (Fragoso *et al.*, 2003) and exhibits poor demographic performance compared with susceptible populations of the same species, as recently reported (Fragoso *et al.*, 2005). The second resistant population shows high resistance to dicholorodiphenyltrichloroethane (DDT) and pyrethroids and was originally collected in a seed-storage facility in Jacarezinho County (State of Paraná, Brazil) in the late 1980s. The population from Jacarezinho showed a demographic performance resembling those of susceptible populations (Fragoso *et al*., 2005).

Both resistant populations showed enhanced detoxification by glutathione S-transferases and phosphotriesterases (Fragoso *et al*., 2003; Ribeiro *et al*., 2003), but these mechanisms appear to be of secondary importance and were lost in both populations after less than 2 years of their detection. The main DDT and pyrehtroid resistance mechanism in both populations appears to be target site insensitivity (Guedes *et al*., 1994, 1995; Ribeiro *et al*., 2003). All populations were reared on whole maize grain free of insecticides and maintained under controlled conditions (25 ± 2 °C; 70 ± 5% relative humidity and an LD 12 : 12 h photoperiod). All reagents were purchased from Sigma-Aldrich Química Brazil (São Paulo, SP, Brazil).

Daily emergence

Two hundred and fifty grams of whole maize were weighed into 1.5-L glass jars. Fifty unsexed adults of *S. zeamais* were released in each jar and removed 15 days later using methods developed by Trematerra *et al*. (1996) and adapted by Fragoso *et al*. (2005). Three replicates were used for each population. Progeny production was assessed every other day and the daily adult emergence data were recorded and normalized using the methods of Trematerra *et al*. (1996). The data set was subjected to nonlinear regression analyses using the curve-fitting procedures of SigmaPlot 2000 (SPSS, 2000).

Respirometry and body mass

Carbon dioxide production was measured in a CO_2 Analiser (TR2, Sable Systems International, Las Vegas, NV, U.S.A) using methods adapted from Daniel & Smith (1994) and Guedes *et al*. (2003) for a differential respirometer. A series of 25-mL flasks was used, each flask containing 20 insects of the same sex and population in a completely closed system. The adults were sexed using rostrum differences in shape and texture (Reddy, 1951; Tolpo & Morrison, 1965). Five replicates were used for each population and sex and CO_2 production was measured in each flask. Flasks were connected to the system for 3 h before measuring the CO_2 produced by the insects. The measurements were obtained by injecting CO_2-free air into the flasks for 2 min at a 600 mL min^{-1} flow. This air current directed the CO_2 to an infrared reader connected to the system allowing the prompt quantification of μmol CO_2 produced per hour. After this measurement, insects were removed from the flasks and weighed on an analytical balance (Sartorius BP 210D, Germany). Respiration values were not normalized by body mass, in accordance with recommendations by Packard & Boardman (1999) and Haynes (2001).

Cytomorphology of fat body

Twenty adult insects from each population and sex were starved for 48 h to ensure similar gut content for the intended study. During this period, adult insects up to 2 days old were maintained in 20-mL glass vials because females of this species do not mate before the third day after emergence (Walgenbach & Burkholder, 1987). After the starvation period, 15 insects of each population and sex were decapitated and their abdomens transferred to 4% para-formaldehyde in 0.1 M sodium phosphate buffer (pH 7.2). Dehydration on a crescent series of ethanol (70, 80 and 90%) was carried out and the abdomens were subsequently embedded in JB-4 historesin. Tissue sections (7 μm thick) of the abdomens were cut on a RM2255 microtome (Leica Microsystems, Germany) and stained with eosin and haematoxylin (Pearse, 1953). The trophocyte (i.e. fat body cell) area measurements were obtained using the software Image-Pro Plus, version 4.0 (Media Cybernetics, Silver Spring, MD, U.S.A).

Specific tests to recognize reaction with total proteins and neutral carbohydrates were also carried out for the different populations of *S. zeamais*. The histological sections were subjected to reaction with mercury-bromophenol (1% $HgCl_2$, 0.05% bromophenol blue, 2% acetic acid) when testing for total protein and to reaction with 4% periodic acid (periodic acid–Schiff; PAS) when testing for neutral carbohydrate, as previously described by Pearse (1953), which were adapted for historesin.

Statistical analysis

The data on CO_2 production, insect body mass and area of trophocytes were subjected to two-way analysis of variance (two sexes × three populations) followed by Tukey's multiple range test ($P < 0.05$), whenever appropriate. Each mean for the area of trophocytes was calculated based on the measurement of the individual area of 25 cells and were transformed to $\log_{10}x$ to stabilize variance and to satisfy normality assumptions. Correlation analyses between CO_2 production (nmol CO_2 $insect^{-1}$ h^{-1}), body mass (mg) and area of trophocytes (μm^2) were also carried out.

Results

Daily emergence

The data sets for each insect population were normalized and the time of the first adult emergence was used as the starting point because the relative emergence curves for the populations are more important than the overall emergence. This transformation is simple and increases the

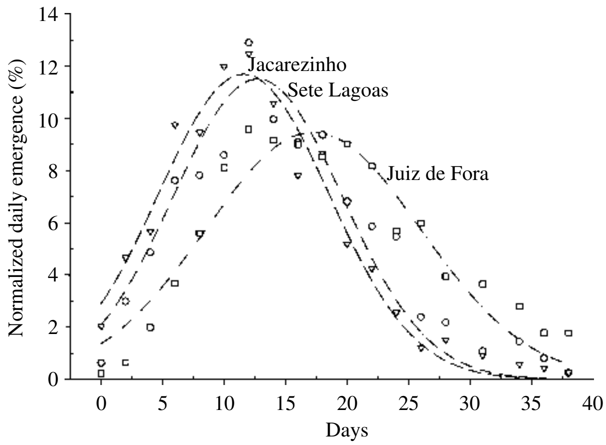

Fig. 1. Normalized daily emergence of three populations of the maize weevil, *Sitophilus zeamais*; Sete Lagoas (○), Jacarezinho (▽) and Juiz de Fora (□). Symbols represent the average of three replicates. ($g.l_{error} = 54$, $P < 0.0001$, $r^2 > 0.80$)

statistical reliability of the analysis (Trematerra *et al.*, 1996). The models proposed by Trematerra *et al.* (1996) for normalized daily emergence were initially used, but the three-parameter Gaussian model [$y = a\ \exp(0.5[(x - b)/c]^2)$] provided a better fit ($P < 0.0001$; $r^2 > 0.80$) and was therefore preferred (Fig. 1). Normalized daily emergence curves for each *S. zeamais* population showed clear differences, with the populations from Sete Lagoas and Jacarezinho closely resembling each other (Fig. 1). Their maximum emergence occurred at approximately the same time (between 12 and 13 days). By contrast, the population from Juiz de Fora showed reduced (approximately 9.0%) and delayed emergence (peak at 17 days after the start of emergence) compared with the other populations.

Respirometry and body mass

Significant differences in respiration rate, measured here as CO_2 production (nmol CO_2 insect^{-1} h^{-1}), were observed among the populations, but there was no significant difference between sexes and the interaction population and sex was also not significant (Table 1). The population from Jacarezinho showed a significantly higher respiration rate (approximately × 2) than the populations from Sete Lagoas and Juiz de Fora, which were similar (Fig. 2).

Analysis of variance for body mass also indicated significant differences among treatments, and the interaction between population and sex was significant at 5% (Table 2). Insects of both sexes from Jacarezinho showed significantly higher body mass than those from the other two populations (Table 2). Females for Sete Lagoas and Juiz de Fora showed similar and smaller body mass than females from Jacarezinho, whereas males from Sete Lagoas were lighter than those from Juiz de Fora, but both were significantly lighter than those from Jacarezinho (Table 2). Males from Jacarezinho and Juiz de Fora were heavier than females from these populations (Table 2). By contrast, males and females from Sete Lagoas showed similar body mass (Table 2).

Cytomorphology of fat body

The area of trophocytes was significantly different for the interaction between populations of *S. zeamais* and sex when subjected to analysis of variance (Table 1). Nonetheless, there were no significant differences between sexes by Tukey's multiple range test ($P = 0.05$), unlike differences among populations (Table 3). The area of trophocytes in insects from Jacarezinho was significantly higher than that in other populations for insects from both sexes (Table 3). Males from Juiz de Fora had trophocytes with an area similar to those of male insects from Jacarezinho, but the females from Juiz de Fora had trophocyte areas resembling the females from Sete Lagoas, whose insects (males and females) always had smaller trophocytes (Table 3).

Histochemical tests in fat body cells showed higher reaction intensity for proteins and carbohydrates in insecticide susceptible females (Sete Lagoas), followed by females from Juiz de Fora and Jacarezinho (Table 4). By contrast, the reaction intensity for proteins and carbohydrates in males was higher in the resistant populations of Jacarezinho and Juiz de Fora, and lower in the susceptible population from Sete Lagoas (Table 4). The reaction intensity for proteins and carbohydrates was higher for females than males from Sete Lagoas, unlike the other two populations (Table 4).

Table 1. Analysis of variance of CO_2 production (nmol CO_2 insect^{-1} h^{-1}), area of trophocytes (μm^2) and body mass of adult insects (mg).

	CO_2 production (nmol CO_2 insect^{-1} h^{-1})			Area of trophocytes (μm^2)			Body mass (mg)		
Sources of variation	d.f	*F*	*P*	d.f	*F*	*P*	d.f	*F*	*P*
Model	5	6.62	0.001*	5	18.99	< 0.001*	5	19.28	< 0.001*
Error	20	–	–	20	–	–	144	–	–
Population (P)	2	15.52	< 0.001*	2	42.84	< 0.001*	2	42.83	< 0.001*
Sex (S)	1	0.24	0.63	1	0.71	0.72	1	0.01	0.96
P × S	2	0.55	0.59	2	4.52	< 0.01*	2	5.37	0.006*

*Significant at $P < 0.05$.

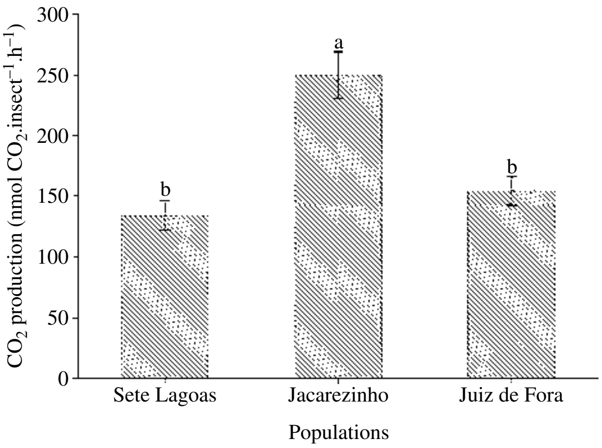

Fig. 2. Production of CO_2 (nmol CO_2 insect^{-1} h^{-1}) (± SE) by adult insects from three populations of the maize weevil, *Sitophilus zeamais*. Means followed by the same letter are not significantly different by Tukey's multiple range test ($P < 0.05$).

Insects from the insecticide-resistant populations, in addition to showing a larger trophocyte area than the susceptible population, also had a higher amount and diversity of vacuoles in these cells (Fig. 3A,B). Curiously, the insects from the resistant populations also showed more protein granules (Fig. 3C,D) and PAS positive (carbohydrate-rich) regions in their cytoplasm (Fig. 3E,F) than the susceptible insects, despite the more intense reaction observed in females from Sete Lagoas compared with females from the insecticide-resistant populations from Jacarezinho and Juiz de Fora, which is likely a consequence of the lower number of vacuoles and smaller trophocytes observed in susceptible insects.

Correlation analysis

Correlation analysis between CO_2 production, body mass and trophocyte area was robust ($r > 0.90$), positive and significant ($P < 0.05$) (Table 5).

Table 2. Body mass (mg) of adult insects (females and males) from three populations of the maize weevil, *Sitophilus zeamais*.

	Body mass ± SE (mg)	
Population/sex	Female	Male
Sete Lagoas	40.36 ± 0.77^{Ab}*	37.85 ± 0.85^{Ac}
Jacarezinho	62.30 ± 0.78^{Ba}	77.50 ± 0.25^{Aa}
Juiz de Fora	39.55 ± 0.59^{Bb}	47.53 ± 0.52^{Ab}

*Means followed by the same capital letter in a row or the same lower case letter in a column are not significantly different by Tukey's multiple range test ($P < 0.05$).

Table 3. Area of trophocytes (μm^2) of adult insects (females and males) from three populations of the maize weevil, *Sitophilus zeamais*.

	Area of trophocytes ± SE (μm^2)	
Population/sex	Female	Male
Sete Lagoas	470.56 ± 23.21^{Ab}*	441.89 ± 58.32^{Ab}
Jacarezinho	844.70 ± 65.93^{Aa}	959.39 ± 91.27^{Aa}
Juiz de Fora	575.29 ± 31.64^{Ab}	701.40 ± 32.22^{Aa}

*Means followed by the same capital letter in a row or the same lower case letter in a column are not significantly different by Tukey's multiple range test ($P < 0.05$).

Discussion

The fat body combines important roles in the insect metabolism resembling those of the liver and adipose tissue of vertebrates. The insect fat body stores carbohydrates, lipids and proteins that are used through energy demand modulated by neurohormones (van der Horst *et al.*, 1997). The existing differences in fat body morphology and insect respiration rate in populations of *S. zeamais* with distinct susceptibility to insecticides suggest a greater mobilization of energy reserves to resist the toxic action of insecticides in the resistant populations of this species. This may be associated with the adaptative costs of insecticide resistance in insecticide-free environments. Insecticide-resistance expression may not be advantageous in the absence of these compounds, where the maintenance of resistance mechanisms may require additional amounts of energy compromising the performance of resistant compared with susceptible individuals (Chevillon *et al.*, 1999; Coustau *et al.*, 2000; Boivin *et al.*, 2001, 2003).

The results of the present study show that the insecticide-susceptible population of *S. zeamais* has smaller fat body cells (i.e. trophocytes) than resistant populations. In addition, the cells from resistant insects have more vacuoles, proteins and carbohydrates than cells from susceptible insects. This suggests that the insecticide-resistant populations have modified the morphology of fat body cells to favour higher stored energy reserves, leading to larger cells. The fat body also acts in the intermediary metabolism in insects storing enzymes involved in the detoxification of toxic molecules, which may also occur in the insecticide resistant-populations from Jacarezinho and Juiz de Fora, as observed in *Spodoptera frugiperda* by Yu *et al.* (2003). The involvement of carbohydrate reserves in the detoxification of insecticide molecules has also been reported by Nath (2000, 2002) and Alaoui *et al.* (1994, 1997). Insecticide target site modifications leading to insecticide resistance may disrupt the pathways within which their targets (or receptors) are involved (Coustau *et al.*, 2000). As a result, a higher metabolic rate may be necessary for resistant individuals to maintain their resistance mechanisms (Hostetler *et al.*, 1994; Chown & Gaston, 1999; Harak *et al.*, 1999). This probably explains the larger trophocytes and higher respiration rate of the resistant weevils from Jacarezinho reported in the present study.

Table 4. Intensity of reaction in histochemical tests carried out in histological sections of trophocytes from populations of the maize weevil, *Sitophilus zeamais*.

	Populations					
	Sete Lagoas		Jacarezinho		Juiz de Fora	
Histochemical tests	Female	Male	Female	Male	Female	Male
Total proteins	++	+/–	+/–	+	+	++
Neutral carbohydrates	++	+/–	+/–	++	+	+

+/–, Weak reaction intensity; +, intermediate reaction intensity; ++, strong reaction intensity.

Lipids, carbohydrates and proteins are the main molecules stored in the fat body that are used as an energy source for insect activities (van der Horst *et al*., 1997). The cytomorphological patterns observed in the trophocytes of the resistant insects suggest a higher availability of neutral carbohydrates and total proteins than in the susceptible population. This higher energy stored in the fat body of resistant insects probably provides a greater mobilization capacity to supply the energy requirements needed to maintain a functional insecticide-resistant mechanism. The results of Park & Keeley (1998) provide support for this hypothesis because the formamidine dimethylchlordimeform leads to a depletion of energy reserves stored in the fat body of the cockroach *Blaberus discoidalis,* increasing their mortality.

The higher intensity of the reaction for total proteins and carbohydrates in the trophocytes in fat bodies of females from the susceptible population suggests that not enough energy is allocated for insecticide resistance mechanisms, although insect development and reproduction is not impaired. It is suspected that the mobilization of energy reserves stored in the fat body of susceptible females does not occur as efficiently as it does in the resistant population, particularly in the population from Jacarezinho. This hypothesis deserves to be tested in future studies.

The respirometry results obtained in the present study show that the Jacarezinho population has a higher respiration rate than the other populations. Studies carried out by Fragoso *et al*. (2003) on these same populations,

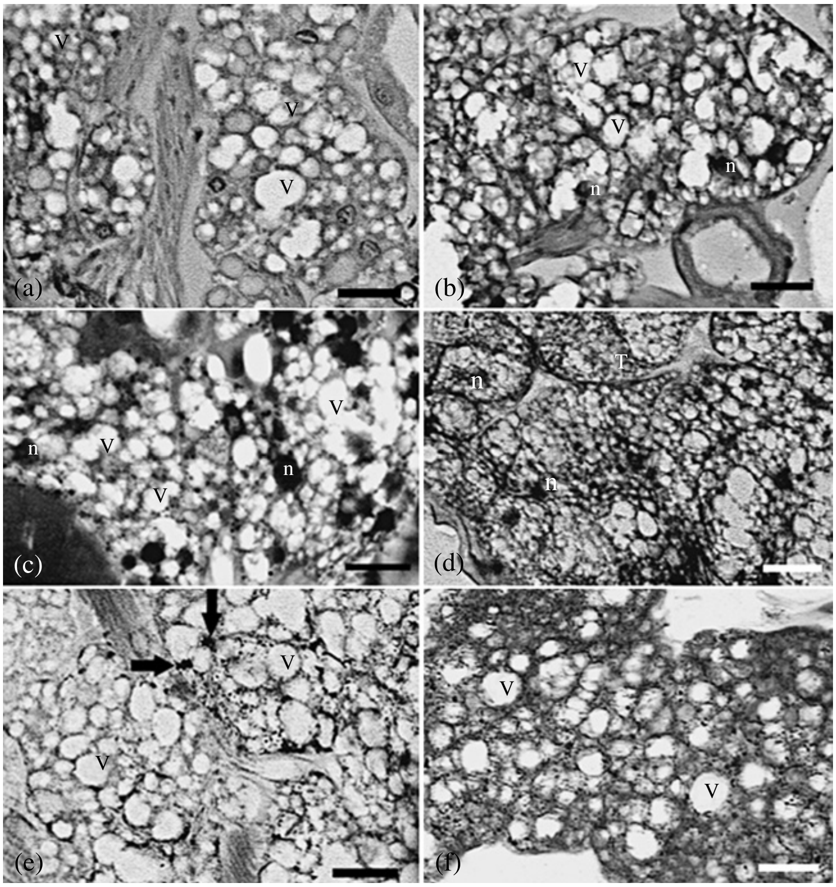

Fig. 3. Fat body from insecticide susceptible and resistant populations of the maize weevil, *Sitophilus zeamais*. (A) Susceptible population with trophocytes presenting small vacuoles (v) in the cytoplasm. (B) Resistant population showing many vacuoles (v) of different sizes. (C, D) Fat body bromophenol blue stained showing trophocytes of the susceptible population (C) with lower amount of positive granules than the resistant population (D). (E, F) Fat body tested by periodic acid–Schiff showing trophocytes from the susceptible population (E) with a lower amount of positive granules (arrows) than the resistant population (F). n, Nucleus. Scale bars = 10 µm.

Table 5. Correlations between CO_2 production (nmol CO_2 insect^{-1} h^{-1}), area of trophocytes (μm^2) and body mass of adult insects (mg) from both sexes and from three different populations of the maize weevil, *Sitophilus zeamais* ($n = 6$).

	CO_2 production (nmol CO_2 insect^{-1} h^{-1})		Area of trophocytes (μm^2)	
Correlations	*r*	*P*	*r*	*P*
Area of trophocytes (μm^2)	0.94	0.005*	–	–
Body mass (mg)	0.98	0.0005*	0.92	0.009*

*Significant at $P < 0.05$.

investigating enzyme activity *in vitro*, indicate a higher activity of glutathione S-transferases and phosphotriesterases in the resistant populations. This higher activity of detoxification enzymes may require a higher respiration rate in the resistant phenotypes, and may have led to an imbalance of gas exchange, resulting in physiological costs due to energy reallocation from other basic physiological processes (Hostetler *et al*., 1994; Chown & Gaston, 1999; Harak *et al*., 1999). In addition, resistance by target site alterations may also incur energy costs (Coustau *et al*., 2000), but this needs more attention.

Despite the initial expectation of an energy imbalance through insecticide resistance leading to physiological costs and a fitness disadvantage to resistant phenotypes in insecticide-free environments, the resistant population from Jacarezinho showed a demographic performance similar to the susceptible population, unlike the resistant population from Juiz de Fora. Fragoso *et al*. (2005) also report a fitness disadvantage in the Juiz de Fora population and a superior performance of the Jacarezinho and susceptible population when assessing their population growth and developmental rate. The better performance of the Jacarezinho population is a likely consequence of its greater body mass, resulting from a more efficient storage of energy reserves. Furthermore, the higher respiration rate observed in the Jacarezinho population suggests that its energy reserves are more efficiently mobilized towards activities of protection against insecticides, development and reproduction. This does not appear to take place as efficiently in the resistant population from Juiz de Fora, which shows a poorer demographic performance. In addition to lower body mass and respiration rate, this indicates that there is a physiological cost associated with insecticide resistance in the Juiz de Fora population.

Fitness differences between the insecticide-resistant populations from Jacarezinho and Juiz de Fora may be due to the genetic origin of their resistance. Routine bioassays for insecticide-resistance diagnosis regularly carried out for these maize weevil populations indicate that the population from Jacarezinho has maintained its resistance for a long time, even in the absence of an insecticide, suggesting that the genes responsible for resistance expression were already modified or replaced by others that are not so costly. This does not appear to have occurred in the Juiz de Fora population (Fragoso *et al*., 2005).

The cost of insecticide resistance in insects is frequently associated with the cost of maintaining the defensive machinery of the organism (Coustau *et al*., 2000). The robust correlations between CO_2 production, area of trophocytes and body mass of the maize weevil, regardless of sex and population, provides evidence of how preferential energy allocation takes place in the insect populations and helps to explain the differences in developmental rates among the populations. In the case of the Jacarezinho population, its greater body mass may lead to more stored energy (larger trophocyte area), resulting in enough energy allocated not only for insect development and reproduction, but also for insecticide resistance. However, in the Juiz de Fora population, even though it has a greater area of trophocytes (but only in males) than the susceptible population from Sete Lagoas, the amount of energy allocated to the insecticide-resistance expression impairs its development and/or reproductive performance.

In summary, the results of the present study in populations of *S. zeamais* demonstrate an association between insecticide resistance and fat body cytomorphology and respiration rate, resulting in higher stored energy that can be readily mobilized for insecticide resistance. Furthermore, the high energy demand for resisting insecticides may carry an additional energy cost that prevents fixation of the resistant phenotype in the absence of an insecticide, unless its capacity of storing energy reserves and mobilizing them is large enough to satisfy potentially conflicting physiological processes (e.g. resistance and development), as appears to be the case for the resistant population from Jacarezinho but not for the resistant population from Juiz de Fora.

Regarding a more applied perspective, it is important to note that some resistant genotypes, such as the population from Jacarezinho, do not demonstrate a fitness cost associated with insecticide resistance. In these cases, resistance-management strategies based on the suppression of insecticide use aiming to re-establish insecticide susceptibility, and the eventual reintroduction of the insecticide, are of little use, unless other fitness costs, not yet detected, are present in these resistant populations (Fragoso *et al*., 2005). By contrast, other resistant genotypes, such as from Juiz de Fora, do show a fitness cost associated with insecticide resistance and the management recommendation described above appears to be sound. The recognition of the prevailing resistant genotypes in a given region should direct the management tactics to be adopted for mitigating insecticide resistance in populations of maize weevil.

Acknowledgements

We would like to thank Dr Jamilton P. Santos (EMBRAPA Maize and Sorghum) for providing the original stocks of the maize weevil populations from Jacarezinho and Sete Lagoas and Mr Anderson K. Santos (Vera Cruz Mills) for field-collecting the population from Juiz de Fora. Financial support provided by the Minas Gerais State Agency for Research Aid (FAPEMIG) and the National Council of Scientific and Technological Development (CNPq) is greatly appreciated. The thoughtful comments and suggestion provided by Professor J. Hardie and two anonymous referees are also greatly appreciated.

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Accepted 17 June 2005