Physiological and behavioral resistance to esfenvalerate + fenitrothion in populations of the maize weevil, *Sitophilus zeamais*

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DOI: 10.5073/jka.2010.425.185

Abstract

The maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) is considered the main pest of stored maize in Brazil and its control is achieved mainly by insecticides. The massive and intensive use of these compounds may lead to selection of resistant populations and consequently compromise the control efficacy of this insect pest in Brazilian storage facilities. Therefore, we surveyed physiological and behavioral resistance to the insecticide mixture esfenvalerate + fenitrothion in 27 populations of *S. zeamais* collected in several Brazilian counties and Paraguay, and also investigated possible costs associated with this phenomenon. The insects were subjected to concentration-mortality bioassays to determine the lethal concentrations LC_{50} and LC_{95}. The populations were also subjected to two walking trials on surfaces fully-treated and partially-treated with dried insecticide residues for detection of behavioral resistance. We also determined the instantaneous rate of population increase (r_i), and body mass of individuals of each population. The concentration-mortality bioassays indicated resistance ratios (at LC_{50}) ranging from 1.00 to 5.02x for the insecticide mixture esfenvalerate + fenitrothion compared with the susceptible standard population (Sete Lagoas). Although the resistance ratios were modest at LC_{50}, they reached up to 232x at LC_{95} (Votuporanga county, São Paulo, Brazil). The behavioral trait of walking in treated arena varied among populations and sex, but there was no significant avoidance to the insecticide mixture. There was no correlation between physiological and behavioral resistance, indicating that physiological resistance is independent of behavioral resistance in the populations tested. There was no significant difference in the instantaneous rate of increase (r_i), and body mass among the insects. Therefore, we conclude that there was no fitness cost associated with the levels of resistance observed in the populations studied.

Keywords: Insecticide resistance, Locomotion, Behavioral avoidance, Fitness cost, Rate of population growth

1. Introduction

A major cause of losses in stored maize throughout the warm regions around the world is the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), which frequently requires control measures to reduce its infestation (Rees, 1996; White and Leesch, 1996). Pyrethroid and organophosphate insecticides are heavily used for maize weevil control (Guedes et al., 1995; Fragoso et al., 2003), due to the lack of other suitable control alternatives in warm climates (White and Leesch, 1996). The over-reliance on insecticides for controlling the maize weevil and other stored-grain pests in tropical areas turned insecticide resistance into a frequent problem (Champ and Dyte, 1976; Subramanyam and Hagstrum, 1996). The earlier use of individual organophosphate and pyrethroid insecticides was replaced by the use of tank mixtures of organophosphate + pyrethroid by the 1990's and more recently the commercial fenitrothion + esfenvalerate mixture became available for stored product use in Brazil (Braga et al., 1991; Ministério da Agricultura, Pecuária e Abastecimento, 2009). However, studies of resistance to such an insecticide mixture have yet to be carried out.

Insecticide resistance results from the enhancement of biochemical-physiological barriers to intoxication and decrease in target-site sensitivity (physiological mechanisms of resistance), or behavioral modifications leading to reduced insecticide exposure (behavioral mechanism of resistance) (Georghiou, 1972). However, most studies usually focus on biochemical-physiological mechanisms (here referred as physiological resistance), whereas relatively little attention is placed on behavioral responses leading to reduced insecticidal exposure. Behavioral resistance to insecticides is frequently neglected in studies of storedproduct insect-pests, although potentially important for their control and design of suitable management programs (Georghiou, 1972; Lockwood et al., 1984; Guedes et al., 2009).

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 a fitness cost, since 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; Guedes et al., 2006). However, there are cases without apparent disadvantages, or rather fitness advantages, observed in insecticide resistant individuals of some insect species, including *S. zeamais* (Guedes et al., 2006).

In this study, we carried out a survey of physiological and behavioral resistance to the insecticide mixture of an organophosphate (fenitrothion) with a pyrethroid (esfenvalerate) insecticide in representative populations of *S. zeamais*, which has not been a focus of attention so far. In addition, we determined behavioral responses of the maize weevil populations, their demographic performance, and correlated these responses with the observed levels of physiological resistance.

2. Materials and methods

2.1. Insects

Twenty seven populations of *S. zeamais* collected in eight Brazilian states and Paraguay were used in this study. These populations were randomly obtained from stored maize grains in representative stored product units between March and September of 2007. The standard susceptible population used was obtained from the EMBRAPA National Research Center of Maize and Sorghum (EMBRAPA Milho and Sorgo, Sete Lagoas, MG, Brazil), where it has been maintained for over 20 yrs in the absence of insecticides. They were maintained in glass containers (1.5 L) within growth chambers (25 ± 2°C, 70 ± 10% r.h., 12 h:12 h photoperiod), and reared on insecticide-free whole maize grains. The bioassays were carried out under these same environmental conditions, except for the behavioral trials, which were carried out at room temperature.

2.2. Concentration-mortality bioassays

Bioassays using technical grade fenitrothion (96.8% pure, Iharabrás/Sumitomo Corporation, São Paulo, SP, Brazil) and esfenvarelate (96.9% pure, Iharabrás/Sumitomo Corporation, São Paulo, SP, Brazil) were carried out using a completely randomized experimental design with five replicates. The insecticide mixture was used at the Brazilian registered and recommended proportion of 20 parts fenitrothion to 1 part esfenvalerate. Each replicate was constituted of a 20 mL glass vial treated with its inner walls covered with insecticide residue following Fragoso et al. (2003). Control vials were treated with the solvent only (acetone). Mortality was recorded after 48-h exposure considering dead those insects unable to walk when prodded with a fine hair brush.

2.3. Behavioral bioassays

The methods used were adapted from those of Guedes et al. (2009) and Pereira et al. (2009). Two behavioral bioassays were carried out – one with the whole filter paper disc treated with insecticide (and an untreated control where only acetone was used), and one with only half of the of filter paper disc treated with the insecticide mixture (the other half was treated with acetone only). These arenas were placed under a video tracking system (Viewpoint LifeSciences, Montreal, Canada). One adult of *S. zeamais* was released on the center of the arena and its movement behavior was tracked for 30 min, after an initial 1 min of waiting period. Measurements taken by the tracking system included the distance walked, ambulatory time (i.e. time walking), resting time, stationary time (time moving without walking), and the total time spent in each half of the arena. The walking speed was calculated by dividing the distance walked by the time spent walking. The experimental design was completely randomized with 16 replicates for each sex and insect population.

2.4. Instantaneous rate of population increase (r_i), and body mass

The experiment was carried out in glass jars (1.5 L) containing 250 g of whole maize with 14.4% m.c. (free of insecticide residues). Fifty unsexed adults of *S. zeamais* (2 wk old) were released in each jar and removed after 100 d. Three replicates were used for each population. The number of live insects, as well as grain weight and its moisture content (13.3%), were recorded after the storage period (100 d). The instantaneous rate of population increase (r_i) was calculated using the formula $r_i = [\ln(Nf/Ni]/\Delta T$, where Nf and Ni are respectively the final and initial number of live insects, and ΔT is the duration of the experiment in days (Walthall and Stark, 1997). Sixty insects of each sex were randomly removed each population and individually weighed on an analytical balance for determining their (wet) body mass (Sartorius BP 210D, Göttingen, Germany).

2.5. Statistical analyses

Concentration-mortality data were subjected to probit analysis using the procedure PROBIT of SAS (SAS Institute, 2002). The resistance ratios with their 95% confidence intervals were calculated based on LC_{50} values for the insecticides (Robertson and Preisler, 1992). The resistance ratio is considered significant ($p < 0.05$) when its confidence limits do not include the value one (Robertson and Preisler, 1992).

Measurements on the locomotory behavior of different populations of *S. zeamais* on treated surfaces were subjected to multivariate analysis of variance (PROC GLM, MANOVA; SAS Institute, 2002), followed by univariate analysis of variance and Tukey's HSD test, when appropriate (PROC GLM; SAS Institute, 2002). Data on population growth and insect body mass were subjected to univariate analysis of variance, and the means were again compared using Tukey's HSD test (PROC GLM; SAS Institute, 2002). Pearson's correlation analyses between physiological and behavioral resistance to the insecticide mixture were also carried out to recognize any significant association between them (PROC CORR; SAS Institute, 2002). The assumptions of normality and homogeneity of variances were checked (PROC UNIVARIATE; PROC GPLOT; SAS Institute, 2002), and no data transformation was necessary.

3. Results

3.1. Physiological resistance

The probit model was suitable for the analyses and estimation of the intended toxicological parameters based on the low χ^2-values (< 11.00) and high p-values (> 0.05) obtained (Table 1). The insect populations exhibited low levels of resistance to the insecticide mixture, which ranged from 1.0 to 5.0-fold at LC_{50}. However, over a third of the tested population were significantly resistant to the mixture, and due to the low slope on the probit curves estimated for some populations, the resistance ratios at LC_{95} observed for some of them were fairly high (up to 232-fold, as observed for the Votuporanga population).

Population	No. individuals tested	Slope ± SEM	LC_{50} (FI 95%) μg/cm^2	RR_{50} (IC 95%)	LC_{95} (FI 95%) μg/cm^2	RR_{95} (IC 95%)	χ^2	P
Sete Lagoas	600	2.21 ± 0.39	0.0052 (0.0032 - 0.0066)	1.00 (0.75 - 1.34)	0.029 (0.018 - 0.105)	1.00 (0.51 - 1.98)	7.85	0.10
Piracicaba	780	3.61 ± 0.30	0.0052 (0.0047 - 0.0055)	1.00 (0.80 - 1.24)	0.014 (0.012 - 0.017)	0.51 (0.31 - 0.85)	9.03	0.17
Amambay	780	1.93 ± 0.32	0.0053 (0.0037 - 0.0067)	1.03 (0.77 - 1.36)	0.038 (0.022 - 0.136)	1.32 (0.63 - 2.79)	10.42	0.06
Rio Verde	700	2.17 ± 0.19	0.0057 (0.0049 - 0.0064)	1.09 (0.86 - 1.39)	0.032 (0.025 - 0.045)	2.20 (0.64 - 1.96)	3.20	0.67
Sacramento	700	2.16 ± 0.22	0.0057 (0.0050 - 0.0063)	1.10 (0.87 - 1.39)	0.033 (0.025 - 0.049)	1.15 (0.64 - 2.05)	8.96	0.11
Dourados - Bororó	540	1.40 ± 0.36	0.0060 (0.0019 - 0.0086)	1.15 (0.79 - 1.68)	0.089 (0.032 - 40.46)	3.08 (0.83 - 11.59)	8.49	0.08
Canarana	680	1.93 ± 0.29	0.0062 (0.0043 - 0.0079)	1.20 (0.89 - 1.61)	0.044 (0.026 - 0.131)	1.53 (0.75 - 3.11)	10.92	0.05
Maracaju	740	2.20 ± 0.19	0.0063 (0.0055 - 0.0070)	1.21 (0.95 - 1.53)	0.035 (0.027 - 0.048)	1.21 (0.70 - 2.11)	8.66	0.19
Guaxupé	600	2.02 ± 0.23	0.0064 (0.0057 - 0.0073)	1.24 (0.98 - 1.58)	0.042 (0.029 - 0.072)	1.45 (0.76 - 2.77)	5.15	0.27
Viçosa	640	1.81 ± 0.21	0.0069 (0.0049 - 0.0091)	1.33 (0.98 - 1.81)	0.056 (0.035 - 0.123)	1.93 (1.01 - 3.71)	9.82	0.08
São José do Rio Pardo	600	1.63 ± 0.26	0.0071 (0.0045 - 0.0100)	1.38 (1.00 - 1.90)	0.073 (0.036 - 0.390)	2.53 (1.10 - 5.84)	9.09	0.06
São João	760	2.55 ± 0.21	0.0075 (0.0068 - 0.0082)	1.44 (1.15 - 1.81)	0.033 (0.026 - 0.044)	1.15 (0.67 - 1.97)	4.50	0.61
Nova Era	600	1.62 ± 0.25	0.0076 (0.0047 - 0.0105)	1.46 (1.05 - 2.03)	0.078 (0.040 - 0.395)	2.71 (1.19 - 6.17)	8.91	0.06
Espírito Santo do Pinhal	580	1.15 ± 0.16	0.0076 (0.0057 - 0.0095)	1.46 (1.07 - 2.01)	0.204 (0.108 - 0.590)	7.03 (2.80 - 17.90)	1.22	0.88
Jacuí	600	0.75 ± 0.15	0.0076 (0.0045 - 0.0105)	1.47 (0.96 - 2.24)	1.165 (0.309 - 25.45)	40.20 (5.97 - 277.15)	6.74	0.15
Vicentina	700	1.75 ± 0.20	0.0078 (0.0068 - 0.0089)	1.50 (1.18 - 1.91)	0.067 (0.044 - 0.131)	2.33 (1.15 - 4.74)	9.19	0.10
Machado	700	1.07 ± 0.14	0.0084 (0.0066 - 0.0103)	1.62 (1.20 - 2.18)	0.291 (0.142 - 0.978)	10.06 (3.65 - 28.13)	9.09	0.11
Abre Campo	700	1.56 ± 0.16	0.0085 (0.0072 - 0.0098)	1.64 (1.27 - 2.11)	0.096 (0.064 - 0.173)	3.32 (1.69 - 6.57)	8.18	0.15
Juiz de Fora	600	2.58 ± 0.20	0.0096 (0.0086 - 0.0106)	1.84 (1.46 - 2.33)	0.041 (0.033 - 0.055)	1.44 (0.84 - 2.48)	5.42	0.25
Tunapolis	660	1.36 ± 0.15	0.0121 (0.0102 - 0.0146)	2.33 (1.78 - 3.05)	0.195 (0.112 - 0.463)	6.75 (2.96 - 15.57)	8.75	0.12
Dourados – Barrerinha	580	1.05 ± 0.16	0.0127 (0.0100 - 0.0161)	2.45 (1.80 - 3.33)	0.468 (0.201 - 2.183)	16.14 (4.93 - 53.64)	5.26	0.26
Guarapuava	700	0.93 ± 0.14	0.0175 (0.0138 - 0.0242)	3.37 (2.40 - 4.73)	1.020 (0.364 - 6.506)	35.20 (8.66 - 145.56)	2.70	0.75
Pedro Juan Caballero	600	1.21 ± 0.16	0.0177 (0.0145 - 0.0226)	3.41 (2.53 - 4.60)	0.407 (0.197 - 1.367)	14.06 (5.06 - 39.50)	5.14	0.27
Votuporanga	600	0.64 ± 0.15	0.0185 (0.0128 - 0.0320)	3.55 (2.27 - 5.56)	6.751 (0.923 -1402.00)	232.82 (12.47 - 4474.61)	5.99	0.20
Dourados – Proterito	780	1.36 ± 0.13	0.0204 (0.0170 - 0.0258)	3.93 (2.94 - 5.26)	0.331 (0.187 - 0.753)	11.41 (5.01 - 26.21)	2.58	0.86
Jacarezinho	560	1.18 ± 0.22	0.0211 (0.0128 - 0.0395)	4.06 (2.73 - 6.04)	0.519 (0.155 - 18.36)	17.91 (4.66 - 69.65)	9.44	0.05
Unaí	760	1.40 ± 0.13	0.0261 (0.0214 - 0.0340)	5.02 (3.70 - 6.83)	0.395 (0.221 - 0.913)	13.63 (5.92 - 31.64)	6.48	0.37

Table 1 Relative toxicity of insecticide mixture fenitrothion + esfenvalerate to 27 *Sitophilus zeamais* populations.

3.2. Behavioral resistance

The overall walking behavior of the insect population on fully-treated arenas was significantly different among populations ($df_{num/den}$ = 130/14781; Wilks' lambda = 0.83, F = 4.2; $p < 0.001$) and even sex ($df_{num/den}$ = 5/2999; Wilks' lambda = 0.99, F = 3.1; $p < 0.001$). The interaction population x sex was also significant ($df_{num/den}$ = 130/14781; Wilks' lambda = 0.95, F = 1.3; $p < 0.03$) based on the multivariate analysis of variance carried out. There was a highly significant correlation between distance walked and the other walking parameters observed (n = 27; r > |0.4|; $p < 0.001$). Therefore, distance walked was used to represent the observed trend (Fig. 1). Distance walked was significantly affected by sex and population ($p < 0.05$), although no significant difference was observed between sex in the arena without insecticide application ($p > 0.5$) (Fig. 1).

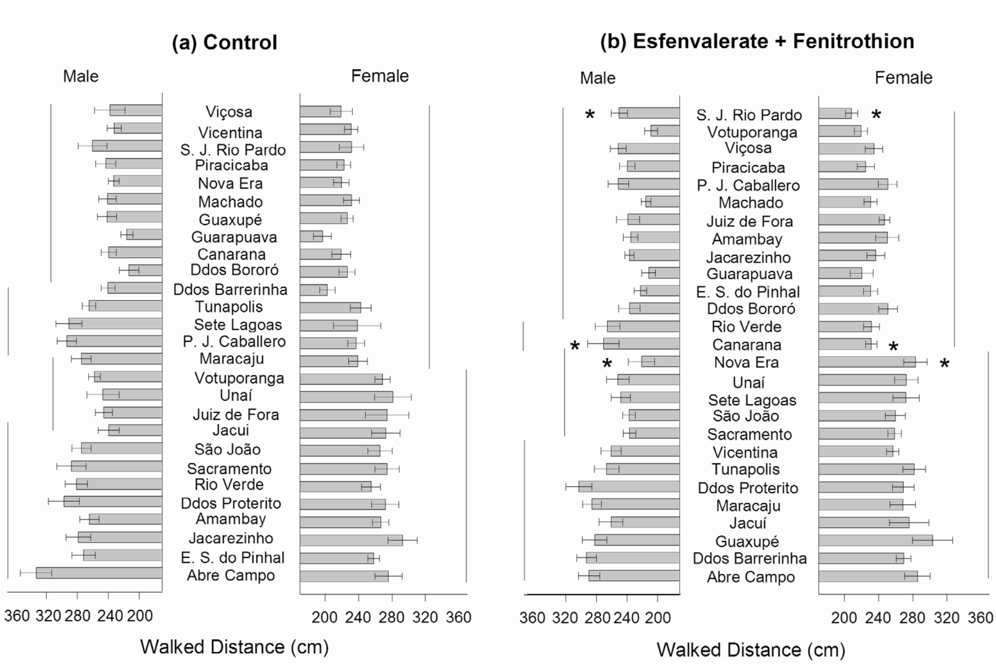

Figure 1 Distance walked (cm) during 10 min (± SEM) by individual insects from 27 populations of *Sitophilus zeamais* in arenas fully-treated with the insecticide mixture fenitrothion + esfenvalerate or not treated (control). Means grouped by the same vertical bar are not significantly different by Scott-Knott groupment analysis test ($p < 0.05$). The asterisk indicates sex differences for the given population also based on Scott-Knott groupment analysis test ($p < 0.05$).

Regarding the avoidance behavior of the maize weevil populations when exposed to partially-treated arenas, there were significant differences among sex and just for three insect populations ($p < 0.05$) (Fig. 2) There was no significant correlation ($p > 0.05$) between physiological and behavioral resistance to the fenitrothion-esfenvalerate mixture (between the resistance ratios and the differences in behavioral walking traits).

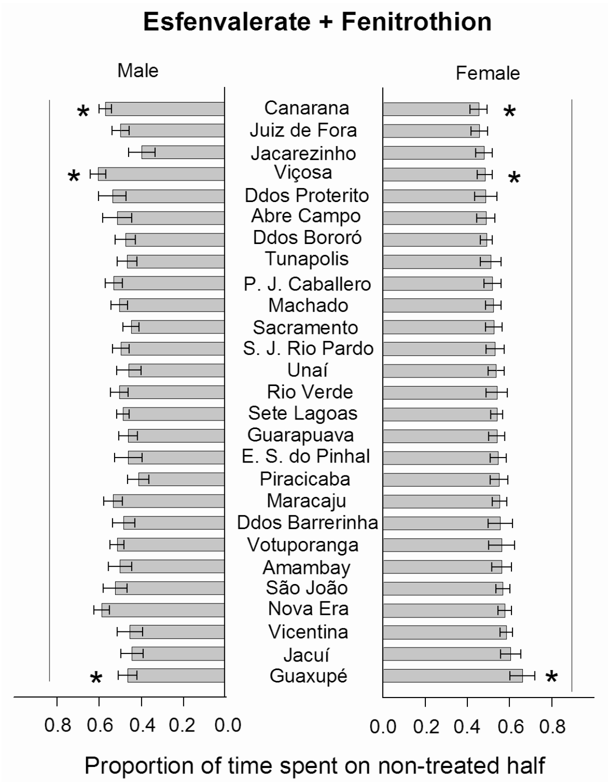

Figure 2 Proportion of time spent on the non-treated half of arenas partially treated with the insecticide mixture fenitrothion + esfenvalerate. Means grouped by the same vertical bar are not significantly different by Scott-Knott groupment analysis test ($p < 0.05$). The asterisk indicates sex differences for the given population also based on Scott-Knott groupment analysis test ($p < 0.05$).

3.3. Instantaneous rate of population increase (r_i), and body mass

The instantaneous rate of population growth (r_i) was not significantly affected by maize weevil strain (0.0213 ± 0.0004 insects/day; $F_{26,54} = 0.73$; $p = 0.80$). In addition, the observed differences in individual insect body mass were not significant either (2.96 ± 0.02 mg; $F_{53,108} = 0.94$; $p = 0.80$).

4. Discussion

Our results of concentration-response bioassays with the insecticide mixture fenitrothion + esfenvalerate indicate emerging problems of resistance to this insecticide mixture. The resistance levels were significant, although low at LC_{50}, among the populations of maize weevil. However, the high variation in response against the mixture in some populations led to high levels of resistance at the LC_{95} level suggesting likely control problems in the field in the near future if insecticide resistance management strategies were not employed. This should not come as a surprise given the history of control failures due to insecticide resistance in maize weevil populations in Brazil – earlier on with DDT and pyrethroids and more recently with organophosphates (Guedes et al., 1995; Fragoso et al., 2003; Pereira et al., 2009).

Locomotion plays a major role determining insecticide exposure and was therefore considered in investigating the behavioral responses of maize weevil populations to insecticide in fully- and partially-treated arenas. The overall mobility parameters of the maize weevils on the insecticide-treated surfaces varied among the populations, although they were not significantly repelled by the insecticides. These varied responses probably reflect the insecticide mode of action and the extent to which they influence the behavior, which varied with the insect population (Hoy et al., 1998). The inter-population variation observed in our tests might reflect differences in the insects' sensory perception of insecticides and could lead to the development of behavioral resistance if such differences are inheritable. Behavioral avoidance to insecticides has yet to be detected in *S. zeamais* and, although potentially important, it is frequently neglected.

We did not detect significant correlation between the levels of physiological resistance of the populations and their mobility parameters. However, the low levels of physiological resistance in the *S. zeamais* populations probably contributed to their undetected correlation with the mobility parameters.

The body mass of individual insects and demographic performance was similar among the maize weevil populations. As a likely result, significant correlations between these variables and resistance ratios were not observed and so far there is no indication of fitness costs associated with resistance to the fenitrothion-esfenvalerate mixture in maize weevil. Such association may however take place with increased levels of insecticide resistance, as earlier reported for stored grain insects (e.g., Pimentel et al., 2007).

In summary, this research shows that the levels of resistance to the fenitrothion-esfenvalerate mixture in populations of *S. zeamais* are low and not yet associated with fitness costs. Such insecticide mixture remains a viable alternative in managing pyrethroid-resistant populations of *S. zeamais*. However, care must be taken to prevent insecticide over-reliance and consequently overuse against populations of the maize weevil, otherwise the current low frequency and low levels of resistance to this insecticide mixture is likely to increase, compromising its future use. This study is one of the few that investigated, in addition to insecticide lethality, the behavioral responses of different insect populations to insecticidal exposure. The variation in the behavioral responses observed were small, but may also evolve to behavioral resistance and reduce insecticide exposure compromising even further the future efficacy of such compounds. Behavioral responses should therefore be considered in future insecticide resistance surveys and management programs.

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