NELSA MARIA PINHO GUEDES

COMPORTAMENTO EM POPULAÇÕES DE *SITOPHILUS ZEAMAIS* RESISTENTES A INSETICIDAS

Tese apresentada à Universidade Federal de Viçosa como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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Aprovada: 29 de fevereiro de 2008.

Prof. Eliseu José Guedes Pereira

Dr. João Alfredo Marinho Ferreira

Dra. Madelaine Venzon

Prof. Simon Luke Elliot

Prof. Marcelo Coutinho Picanço
(Presidente da banca)

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RESUMO

GUEDES, Nelsa Maria Pinho, D.Sc., Universidade Federal de Viçosa, fevereiro de 2008. Comportamento em populações de *Sitophilus zeamais* resistentes a inseticidas. Orientador: Raul Narciso Carvalho Guedes. Co-Orientadores: Terezinha Maria C. Della Lucia e José Lino Neto.

O principal método de controle contra o caruncho-do-milho em clima tropical é o uso de inseticidas, particularmente piretróides, devido à falta de métodos alternativos de controle. Custos adaptativos associados à resistência a inseticidas foram observados em linhagens de *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Linhagens de *Sitophilus zeamais* susceptíveis e resistentes a piretróides foram usadas para avaliar o processo comportamental e o resultado da competição larval dentro do grão e, também, a resistência e a plasticidade comportamental de adultos do caruncho-do-milho expostos a doses crescentes de deltametrina. O número de ovos depositados no grão de milho e o número de larvas eclodidas determinam o comportamento larval dentro da semente que pode ser do tipo acomodação ou ataque. O baixo número de adultos emergentes por semente em relação ao número de ovos depositados resultou de um processo de competição larval do tipo ataque, para ambas as linhagens - resistente e susceptível, ocorrendo uma interferência direta das larvas. Não houve redução na massa corpórea dos insetos com o aumento da competição dentro do grão e o agrupamento ótimo de ovos/grão foi igual a dois em ambas as linhagens. A plasticidade alimentar é um componente da plasticidade fenotípica que geralmente não é considerada em linhagens de insetos adaptados a ambiente modificados por inseticidas, contudo esta plasticidade pode tanto acentuar quanto diminuir a resistência a inseticidas. Uma linhagem susceptível e duas linhagens resistentes a piretróides do caruncho-do-milho foram sujeitas a testes de livre escolha e sem chance de escolha em milho tratado com doses crescentes de deltametrina. As linhagens resistentes exibiram uma discriminação à deltametrina maior do que a linhagem susceptível no teste de livre escolha e, também, tiveram um desempenho melhor no teste sem chance de escolha onde exibiram baixos índices de deterrência alimentar (≤ 12%) do que a linhagem susceptível (50%) com o aumento de doses de deltametrina. Houve diferenças na mobilidade das linhagens quando expostas a superfícies tratadas com deltametrina. No experimento de decolagem as respostas foram dependentes da dose em duas linhagens de insetos, exceto para uma linhagem resistente a deltametrina, enquanto que as respostas de caminhamento foram dose-independente para todas as linhagens. A resistência comportamental foi independente da resistência fisiológica com as linhagens resistentes exibindo extremos superiores e inferiores da taxa de decolagem. A mobilidade das fêmeas foi similar para todas as linhagens mas diferente para os machos. O padrão de mobilidade dos machos de cada linhagem teve tendência semelhante aos resultados à de decolagem.

ABSTRACT

GUEDES, Nelsa Maria Pinho, D.Sc., Universidade Federal de Viçosa, February, 2008. Behavior of insecticide-resistant strains of *Sitophilus zeamais*. Advisor: Raul Narciso C. Guedes. Co-advisors: Terezinha Maria de C. Della Lucia and José Lino Neto.

The main control method used against maize weevil infestations in tropic regions is the use of insecticides, particularly pyrethroids, due to the lack of suitable control alternatives. Adaptative costs associated with insecticide resistance were reported in *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Pyrethroid-susceptible and resistant strains of maize weevil were used to assess the behavioral process and the ecological outcome of larval competition inside the grain and also the behavioral resistance and plasticity of adults of maize weevil exposed to increasing doses of deltamethrin. The number of eggs laid in a seed and the consequent number of hatched larvae guide the larval behavior within the seed determining its outcome – scramble or contest. The low adult emergence in the maize weevil resulted from a contest process of competition with direct interference among the larvae for both strains studied. There was no reduction in body mass with increased competition in either strain, and they exhibited similar optimal clutch size (two eggs/seed). Feeding plasticity is a component of phenotypic plasticity not usually considered in insect strains adapted to insecticide-altered environments, but which may either accentuate or mitigate insecticide resistance. One susceptible and two pyrethroid-resistant strains of maize weevil were subjected to free-choice and no-choice tests with maize grains sprayed with increasing doses of deltamethrin. The resistant strains exhibited higher feeding avoidance with increased deltamethrin doses than insects from the susceptible strain when subjected to free-choice tests. In addition, the resistant strains performed better under sprayed grains and exhibited low levels of feeding deterrence (≤ 12%) than the susceptible strain (50%). Locomotion plays a major role determining insecticide exposure. The behavioral responses to deltamethrin-sprayed surfaces differ among the maize weevil strains. Such responses were dose-dependent for flight take-off (reducing with insecticide concentration) in two strains of maize weevil, but not for an insecticide-resistant strain, while walking response was dose-independent for all of the strains. Behavioral resistance was independent from physiological resistance with one resistant strain exhibiting higher rates of flight take-off, and the other resistant strain exhibiting lower flight take-off. Female mobility was similar for all strains, unlike male mobility. Males of each strain exhibited a pattern of mobility following the same trend of flight take-off.

INTRODUÇÃO GERAL

O caruncho-do-milho, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), está entre as pragas mais destrutivas e mundialmente bem distribuídas dentre as de grãos armazenados (Danho & Haubruge, 2003; Danho *et al*., 2002; Rees, 1996). No Brasil, ela é considerada a principal praga do milho armazenado, além de ser considerada importante em cereais armazenados de modo geral (Adda *et al*., 2002). Infestações pelo caruncho-do-milho frequentemente se iniciam a campo, antes do armazenamento (Adda *et al*., 2002; Brown & Lee 2002), o que aliada a sua boa capacidade de vôo (Hagstrum *et al*., 1996) e poder destrutivo, propicia elevada perda na fase pós-colheita de grãos (Santos *et al*., 1986).

Grandes diferenças entre populações tropicais e subtropicais do caruncho-do-milho são resultantes da natureza descontínua do armazenamento de grãos e também do manejo destes insetos-praga (e.g., resistência a inseticidas) dentro de unidades armazenadoras de grãos. Essas unidades provavelmente acentuam os ciclos sazonais dessas populações o que possibilita o estabelecimento rápido de novas populações a partir de um número reduzido de indivíduos (Tanaka, 1990; Tran & Credland, 1995).

Apesar de distinguir entre grãos infestados e não infestados, as fêmeas de *S. zeamais* utilizam uma estratégia reprodutiva que pode vir a prejudicar o desenvolvimento da sua progênie, pois preferem depositar seus ovos na superfície de grãos infestados com ovos de coespecíficos do que em grãos não-infestados (Danho & Haubruge, 2002). Vários ovos depositados em um único grão podem levar as larvas a adotar estratégias distintas de competição larval, seja acomodação ou ataque. As larvas recém-emergidas são incapazes de migrar entre os grãos e uma vez ocorrendo a eclosão dos ovos, elas perfuram o grão, alimentam-se e desenvolvem-se no seu interior até transformarem-se em pupa e eventualmente adultos, que posteriormente deixam o grão (Danho *et al*., 2002; Danho & Haubruge, 2003).

O principal método de controle do caruncho-do-milho é através do uso de inseticidas, já que este geralmente é o método de controle mais simples, rápido e econômico para conter infestações de pragas de produtos armazenados (Guedes 1990 e 1991; White & Leesch, 1996). Contudo o uso frequente de inseticidas na proteção de grãos armazenados resultou no desenvolvimento da resistência de insetos a estes compostos em vários países (Champ & Dyte, 1996; Badmin, 1990; Subramanyam & Hagstrum, 1996), inclusive no Brasil (Guedes *et al.*, 1995; Ribeiro *et al.* 2003).

A seleção intensa e a evolução rápida da resistência a inseticidas em populações naturais tem sido os principais obstáculos a serem superados no manejo satisfatório de insetos-praga (Kence & Jdeidi, 1997), principalmente se forem considerados os ambientes onde o controle químico é o método mais eficiente, como no caso de pragas de grãos armazenados (Guedes *et al.*, 1994, 1995; Ribeiro *et al.*, 2003). Estudos feitos sobre resistência a inseticidas geralmente focalizam o efeito fisiológico do inseticida em detrimento do efeito causado por este composto no comportamento do inseto (Kongmee *et al.*, 2004). A capacidade dos insetos em perceber os inseticidas através de processos sensoriais pode levar a evolução de resistência comportamental a inseticidas (Gould, 1984 e 1991; Haynes, 1988; Hoy *et al.*, 1998). Tanto a resistência fisiológica quanto a comportamental pode comprometer o controle de insetos. Estudos que correlacionam a resistência fisiológica e a comportamental raramente são realizados (Suiter & Gould, 1984), talvez porque o padrão comportamental considerado e a sua dependência da dose possam dificultar estabelecer tal correlação.

Neste contexto é importante observar outro fenômeno pouco estudado que é a plasticidade fenotípica de uma espécie frente a modificação do ambiente por inseticidas. Plasticidade fenotípica é reconhecida quando a expressão de uma determinada característica do organismo varia com o ambiente em que ele vive e com o genótipo através da interação genótipo e ambiente (Sibly *et al.*, 1997; Futuyma, 1998; Stearns & Hoekstra, 2000). Como o comportamento de um indivíduo geralmente varia com o ambiente, o que se espera é que a plasticidade fenotípica em populações de *S. zeamais*, quando tratada com inseticida, seja modificada.

Informações sobre padrões comportamentais de populações resistentes e susceptíveis frente à exposição a inseticidas são essenciais ao desenvolvimento de programas de manejo de resistência a inseticidas objetivando a contenção das perdas decorrentes do fenômeno. Populações brasileiras do caruncho-do-milho apresentam resistência fisiológica a piretróides (Guedes *et al.*, 1995; Ribeiro *et al.*, 2003; Fragoso et al., 2003), contudo ainda não existem estudos comportamentais que correlacionem este fenômeno com a resistência fisiológica a inseticidas.

Para verificar diferenças comportamentais de populações susceptíveis e resistentes a deltametrina, dois tipos de ensaios foram realizados. O primeiro, sem o uso do inseticida, foi a observação do comportamento larval dessas populações dentro do grão, onde suspeita-se que a população resistente (sem custo adaptativo na ausência do inseticida) possa apresentar uma estratégia de competição distinta da susceptível. O segundo conjunto de ensaios seria para observar o comportamento do adulto verificando se existe plasticidade nos padrões comportamentais das diferentes populações frente à exposição a doses crescentes de deltametrina e, também, verificar se existe a possibilidade de ocorrência de resistência comportamental à deltametrina em linhagens do caruncho-do-milho.

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Format: *Animal Behaviour*

Contest-like behavioural process of larval competition within seeds in strains of the maize weevil (*Sitophilus zeamais*)

Running headline: *Behavioural process of larval competition in the maize weevil*

ABSTRACT

Larval competition is a limitation for obligate internally feeding insect larvae unable to leave the seed selected by their mother. The number of eggs laid in a seed and the consequent number of hatched larvae guide the larval behavior within the seed determining its outcome – scramble or contest. Although the scramble outcome of competition prevails in insects, few individuals emerge from seeds infested by the maize weevil. The low adult emergence in the maize weevil may result either from a scramble process with a low optimum egg density, or from a contest competition process with direct interference among the larvae. Both processes may generate a scramble outcome with an optimum egg density, but with a decrease in the body mass of the emerging insects in the first case, which would not take place with a contest process of competition. Therefore, the behavioral process and the ecological outcome of competition were assessed in two strains of the maize weevil. There was no reduction in body mass with increased competition in either strain, and they exhibited similar optimal egg density (two eggs/seed). Despite this scramble outcome, the small egg density suggests that body mass is unlikely to be reduced in the emerging adults compromising the conclusion of a contest-type of behavioral process based on this trait. However, x-ray images of the larvae within the seeds throughout their development indicate direct interference among them and provide evidence of the contest behavioral process of competition taking place within the seeds and generating a scramble ecological outcome with low egg density.

Keywords: competition strategy; Curculionidae; interaction; intraspecific competition; insecticide resistance; life history trait; resource monopolization; scramble competition; *Zea mays*

Local resource competition may lead to the selection of specific adaptative behavior to overcome harmful interference among individuals (Sanz & Gurrea 2000; Alves-Costa & Knogge 2005). The competition among individuals for limited resources is a determinant of both life history and behavior (Smith & Lessells 1985; Smith 1990, 1991). Two distinct strategies of intraspecific competition – scramble and contest – were originally defined by Nicholson (1954) and frequently recognized in terms of resource use (e.g., Varley et al. 1973; Lomnicki 1988). All members of a population have equal access to the limited resource in scramble competition, while only the successful competitor secures as much of the governing resource as needed for its survival and reproduction in contest competition.

The resource-limitation concept of competition stated above is population-based. However, Nicholson (1954) also recognized the process by which competition takes place, which is an individual-based phenomenon. The process of scramble competition takes place when there is accommodation of all competitors within a resource patch allowing increased survival, but with reduced individual resource gain and consequently lower gain of body mass with development in contrast with the process of contest competition (Miller 1967; Giga & Smith 1991; Toquenaga 1993; Lale & Vidal 2001; Guedes et al. 2007). The process of contest competition takes place when there is direct interference among competitors (i.e., behavioral interference, such as active aggression), as if competing for a prize with only one potential winner (Smith & Lessells 1985; Mano et al. 2006; Guedes et al. 2007). The proximal mechanisms of the behavioral process of competition are however only poorly known, particularly for seed beetles (Alves-Costa & Knogge 2005; Guedes et al. 2003, 2007), and the competition strategies are usually recognized by their outcome based on the relationship between the number of (or fitness) surviving individuals versus the initial numbers (Varley et al. 1973; Bellows 1982; Guedes et al. 2003).

The distinction between the behavioral process of competition and the ecological outcome is important, although rarely considered (Smith & Lessells 1985, Smith 1990, Guedes et al. 2007). The behavioral process of competition is a consequence of intrinsically disruptive selection on larval competition strategies, while the ecological outcome of competition also depends on the amount of resources required for development counter-imposed to the resource availability within a patch (Smith & Lessells 1985; Guedes et al. 2007). Therefore, a contest outcome may result even from a scramble process (i.e., without behavioral interference) depending on the resource availability and proximal mechanism involved with consequences on life history evolution and its practical implications (Smith 1990; Colegrave 1994, 1997; Nylin 2001; Guedes et al. 2003, 2007).

A highly selective scenario is predictable in discrete resource units like seeds parasitized by beetle larvae. This is particularly so for seed beetles whose larval stages are spent within a single seed selected by their mother, which results in a closed system where the young are unable to avoid competition with conspecifics if multiple eggs are laid on the same seed (Smith 1990, 1991; Colegrave 1994). Some species of seed beetles, such as the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), have a reported clustered egg-laying behavioral pattern leading to seed superparasitism and further increasing competition within the seed (Smith & Lessells 1985; Danho et al. 2002; Danho & Haubruge 2003).

The maize weevil is an insect-pest of cereals throughout agricultural regions of the world with broad strain differences likely resulting from their management as pests (e.g., insecticide resistance) and the discontinuous nature of the stored seed environment probably accentuating seasonal population cycles and leading to bottlenecks, where only small numbers of individuals establish new populations, which quickly develop from the colonizers (Tanaka 1990; Tran & Credland 1995, Guedes et al. 1997, 2006; Fragoso et al. 2003, 2005; Araújo et al. 2008). Few individuals emerge from seeds infested by larvae of this species (Throne 1994; Danho et al. 2002; Danho & Haubruge 2003), although the scramble outcome of competition prevails in insects (Price 1975; Mano et al. 2007). The low adult emergence in the maize weevil may result either from a scramble process with a low optimum egg density, or from a contest process of competition with direct interference among the larvae. Both processes may generate a scramble outcome with an optimum egg density, but with a decrease in the body mass of the emerging insects in scramble competition but not in contest competition. Therefore, the behavioral process and the ecological outcome of competition were assessed in two strains of the maize weevil. One of the strains is resistant to insecticides, but without fitness disadvantage associated with this trait probably due to its higher intermediate metabolism (Guedes et al. 2006; Araújo et al. 2008), which may allow them to employ a distinct competition strategy.

METHODS

Insect Populations

Two strains of *S. zeamais* were used in the present investigation, one susceptible and one resistant to insecticides (Guedes et al., 1994; Ribeiro et al. 2003), which were termed here as ‘susceptible’ and ‘resistant’. The susceptible strain was collected in the mid-1980’s in Sete Lagoas County (state of Minas Gerais, Brazil). The resistant strain was collected in Jacarezinho County (state of Paraná, Brazil) in the late 1980’s (Guedes et al. 1994, 1995). It is resistant to pyrethroid insecticides, but does not exhibit fitness cost associated with insecticide resistance (Fragoso et al. 2005; Oliveira et al. 2007). Both strains were derived from stock cultures maintained at the Maize and Sorghum Research Center of the Brazilian Agricultural Research Corporation (EMBRAPA Milho & Sorghum, Sete Lagoas, MG, Brazil). The insect cultures were maintained in whole maize grains (13% m.c.) free of insecticides using the same procedures and densities in environmentally controlled conditions of temperature (28 ± 2^{o}C), relative humidity (70 ± 5%) and photoperiod (LD 12:12 h).

Competition Experiments

Maize seeds with different infestation densities were obtained by allowing 25 pairs of 5-day-old adults from each strain to mate for 24 hours, removing the males, and then leaving the females for 24 hours in a new batch of 20 seeds for egg-laying. The seeds then were examined under a dissecting microscope for the presence of eggs, and preliminary egg density was determined. Egg densities ranging from 1 to 7 eggs/seed were obtained and confirmed by digital x-ray imaging, as described later. The location and interaction among larvae within the seeds were digitally recorded throughout their development. The number of replicates (i.e., seeds) per egg density and strain was inversely proportional to the egg density ranging from 3 to 24 to make the within-treatment variance homogeneous among larval densities, as suggested by Giga & Smith (1991). The seeds were maintained separately under controlled conditions until the start of adult emergence, when observations were made daily and the emergence, sex, body mass, and developmental time were recorded for each individual on the day of emergence (Giga & Smith 1991; Guedes et al. 2007). The experiment was established following the completely random design. The emerged adults were sexed using rostrum patterns of shape and texture (Reddy 1951; Tolpo & Morrison 1965) and individually weighed on a UMT2 microbalance (Mettler Toledo

Inc., Columbus, OH, USA). The maize seeds containing eggs were periodically observed using a MX-20 Specimen Radiography System equipped with a 14-bit digital camera (Faxitron X-Ray Corp., Wheeling, IL, USA). The locations and interactions among larvae within the seeds were digitally recorded throughout their development.

Respirometry

Carbon dioxide production was measured in a Micro-Oxymax 10-channel computerized respirometer with dual-range O_2 and CO_2 detectors (Micro-Oxymax, Columbus Instruments, Columbus, OH, USA). A series of 50-ml flasks was used, each flask containing a single maize seed with a single hatched larva (15-days old) in its interior, as verified by x-ray. The respiration rate (µl CO_2 produced/h/larva) was determined for 24 hours in 20 insects of each strain in addition to a control treatment where the respiration rate of only the maize seed without any eggs was determined to correct the values obtained for each strain against seed respiration. After the measurement, the seeds were removed from the flasks and retained in individual wells of 24-well insect tissue culture plates with lid until adult emergence, when the body mass of the newly-emerged adults was determined. The experiment was established following the completely random design. The respiration rate was assessed because it may favor a contest-type of outcome when in higher levels in one of the strains, as observed with the Yemen strain of the cowpea weevil *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) (Daniel & Smith 1994; Guedes et al. 2003). In the present case, the insecticide-resistant strain of maize weevil shows higher intermediate metabolism and higher respiration rate, which potentially mitigates the fitness costs usually associated with insecticide resistance (Guedes et al. 2006; Araújo et al. 2008), and may be related to the behavioral process of larval competition within the seed making such determination necessary.

Data Analyses

Adult emergence data were subjected to regression analysis using TableCurve 2D (SPSS 2000) by fitting adult emergence (y) against egg density (x) using a model derived from Horn (1997), following Guedes et al. (2007): $y = \alpha x^{-\beta}$, where α is a measure of the quality of the host for the seed beetle (i.e., the adult emergence from a seed with a single egg); and β is the competition coefficient (the higher is β, the higher is the competition). Adult body mass, developmental time, and proportion of females (no. females/total no. insects) were subjected to analysis of covariance. Insect strain was the independent variable and the density of eggs was the covariate for the analysis of covariance of proportion of females emerged (PROC GLM; SAS Institute 2002). In contrast, sex and insect strain were the independent variables and the density of eggs was the covariate for the analysis of covariance of adult body mass and developmental time (PROC GLM; SAS Institute 2002). Complementary regression analyses were carried out for each strain when necessary (PROC REG; SAS Institute 2002). The larvae sharing a seed were regarded as pseudo-replicates of the effect of density since their measures are confounded by a common seed environment. Therefore, only mean results from different seeds were considered as replicates.

Fecundity and longevity are both related to body mass and larval survival, and adult body mass at emergence is affected by competition (Anderson 1978; Smith & Lessells 1985). Therefore, these parameters should all be included in measures of fitness, which was achieved in the present work by estimating the insect biomass (mg) produced per seed used as a measure of total larval fitness to generate larval competition curves (Smith & Lessells 1985). The models were tested from the simplest (linear and quadratic) to the alternative models of increasing complexity (non-linear peak models). TableCurve 2D was used to fit these curves (SPSS 2000).The model selection was carried out based on simplicity, high F-values (and mean squares) and steep increase of R^2 with model complexity. Residue distribution was also checked for each analysis on TableCurve 2D to ascertain of parametric assumptions.

RESULTS

Adult Emergence

The adult emergence curves for both insect strains were indistinguishable ($\alpha = 64.48 \pm 4.99$ and 71.79 ± 3.85 and $\beta = 0.97 \pm 0.11$ and 0.92 ± 0.08 for the susceptible and resistant strains respectively in the model $y = \alpha x^{-\beta}$) and therefore their data were pooled together for a single estimated curve of adult emergence (Fig. 1). The higher the density of eggs laid per seed, the lower was the adult emergence with high host quality ($\alpha = 68.20 \pm 3.31\%$) and even higher intensity of competition ($\beta = 0.95 \pm 0.07$).

Body Mass, Developmental Time, and Proportion of Females

Analysis of covariance for adult body mass, developmental time, and proportion of females indicate significant differences for only body mass ($F_{21,101} = 7.19$, $p < 0.0001$). There was no significant effect ($p > 0.05$) of strain and larval density on proportion of females (0.61 ± 0.08), nor for strain, sex, and larval density on developmental time (39.56 ± 0.56 days). Sex and egg density did not affect adult body mass at emergence ($p > 0.05$), but there was significant effect of strain on adult body mass at emergence ($F_{1,101} = 29.27$, $p < 0.0001$) with insects from the resistant strain exhibiting significantly higher body mass (3.61 ± 0.06 mg) than those from the susceptible strain (2.80 ± 0.05 mg).

Larval Competition Curves

The competition curves relate total larval fitness (as adult biomass produced per seed) with density of eggs laid (Fig. 2). Competition curves reaching a plateau at higher densities indicate a contest outcome, while a peak indicates a scramble outcome (Smith & Lessells 1985). Both types of outcome can take place with the scramble process of competition, unlike the contest process of competition which only generates the contest outcome (Smith & Lessells 1985; Smith 1990, 1991). The best model obtained for both strains indicates a peak for both strains at the same egg density – two eggs per seed (Fig. 2), despite the resistant strain exhibiting significantly higher larval fitness than the susceptible strain.

Larval Respiration Rates

The higher body mass and larval fitness of the resistant strain are suggestive of a higher respiration rate. The two-way analysis of variance (strain x sex) for respiration rate (CO_2 production per insect per hour) indicate significant differences for strain only ($F_{1,65} = 3.67$, $p = 0.016$), with the larvae of the resistant strain exhibiting significantly higher respiration rate (21.41 ± 1.57 µl CO_2/insect/h) than the larvae of the susceptible strain (16.13 ± 1.99 µl CO_2/insect/h). However, since the same model described the humped-shaped curve of total larval fitness of both strains, the differences in respiration rate between the strains does not seem to be playing any role in the outcome of competition among maize weevil larvae.

X-Ray Imaging

Adult body mass at emergence is commonly used to preliminarily recognize the process of competition within seeds (Smith & Lessells 1985, Giga & Smith 1991, Guedes et al. 2007). Here however, in addition to such determination, digital x-ray image recording was also carried out throughout the larval development within the seed to recognize the distribution and interaction of the larvae within the seed and therefore directly identify the behavioral process of competition taking place among them. The representative (temporal) sequences of images shown in Figs. 3 and 4 illustrate the main results observed. The image sequence of the larva hatched from a single egg laid in the seed in Fig. 3a illustrates its behavior. The eggs are usually laid near the seed germ and the hatched larva burrows into the seed away from the germ and towards the endosperm. When larger numbers of eggs are laid in a single seed, as in Fig. 3b, the larvae hatched from these eggs located near the germ interfere with one another in the nearby region during early burrowing and while moving away from the germ. Only two larvae usually survive the initial interference from conspecifics and place themselves at the center of the seed and opposite ends of the endosperm away from the germ, where they pupate and eventually emerge as adults.

The interference behavior among larvae within a seed is more clearly shown in the sequences of images in Fig. 4. The 1st sequence of x-ray images shows the interaction between three hatched larvae (Fig. 4a). The larger of three larvae (L_1) approaches one of the others (L_2), which was burrowing in the other half of the seed, but upon coalescence of its burrow with the larva from the same half, the potential interference with the opposite larva is interrupted and the large larva eliminates (probably by direct attack) its within-burrow competitor before moving to the edge of the endosperm. At this site, the larger larva pupates, while the larva from the opposite half of the seed deteriorates probably due to the minor interference (or brief attack) by the larger larva before facing its within-burrow competitor. An even more clear interference and attack is illustrated in the image sequence of Fig. 4b, where from the three early competing larvae, one is soon eliminated (L_3) and one of the remaining two larvae burrows toward the other. They meet 24 days after oviposition and the pursuing larva (L_1) kills and consumes its competitor, eventually emerging alone from the seed. The last sequence of images (Fig. 4c) shows a coalescence of multiple larval burrows around the seed germ region in their initial development with only one larva surviving and moving to the center of the seed. A smaller larva (L_3) hatched from an egg laid in the opposite side of the seed, which shows delayed development, moves towards the extreme edge of the endosperm avoiding interference by the large larva (L_1), but actually pursuing and successfully attacking and killing it upon its pupation. The images obtained provide evidence of active interference by means of aggression among conspecific larvae and cannibalism among them.

DISCUSSION

Larval competition in granivorous species has important effects on the evolution of behavior and the behavior has consequences for the rate of population growth and stability (Smith & Lessells 1985). Adults of internally feeding seed beetles lay eggs on or inside a seed selected by the mother, and the offspring are unable to leave this resource patch leading to larval competition within the seed if multiple eggs are laid per seed. Some species of seed beetles minimize larval competition by laying few (or just one) eggs per seed in a uniform or random distribution, as observed in *Callosobruchus* species (e.g., Smith & Lessells 1985, Smith 1991). In contrast, egg distribution in weevils of the genus *Sitophilus* is generally regarded as clumped, particularly in the case of the maize weevil (Dobie 1974; Legg et al. 1987; Nardon et al. 1988; Fava & Burlando 1995; Stejskal & Kučerová 1996; Danho & Haubruge 2003). Despite this seed superparasitism by the maize weevil, few individuals of this species emerge from seeds multiply infested by their larvae (Throne 1994; Danho et al. 2002; Danho & Haubruge 2003). This is a likely result of intense competition and may take place with or without aggression among individuals within the seed.

Low adult emergence in internally feeding seed beetles can result from either scramble or contest competition depending on resource availability and the behavioral process taking place. Resource availability decreases with egg density, and the clumped egg laying behavior of maize weevil is suggestive of intense competition among larvae. Indeed, the intensity of competition determined in our study by the adult emergence curves as a function of egg density was equally intense for both strains regardless of the higher respiration rate observed in one of them, unlike observed in the cowpea weevil *Callosobruchus maculatus* (Guedes et al. 2003, 2007). Under limited resource availability, scramble outcome of competition may result either from a scramble process of larval competition with a low optimum egg density or from a contest process of competition with direct interference among the larvae (Smith & Lessells 1985; Smith 1991). In contrast, contest outcome of competition is a result of a contest or attack behavior with active interference among the larvae.

The larval fitness curves or competition curves indicate the competition outcome of the strains of maize weevil. If a plateau is reached in the larval fitness curve at higher densities, contest competition takes place, which although less frequent among insect species was reported in a few species such as some strains of *Callosobruchus analis* and *C. maculatus* (Bruchidae), and *Ravena rubiginosa* (Curculionidae) (Toquenaga & Fuji 1990; Alves-Costa & Knogge 2005; Guedes et al. 2007). In contrast, larval fitness curves with a peak of optimum egg density indicate scramble competition (Smith & Lessells 1985). Both strains of maize weevil showed humped larval fitness curves with optimal egg density at two eggs per seed indicating scramble outcome of competition with a low optimal egg density. Such optimal egg density for the maize weevil came as a surprise based on the clumped egg distribution reported for this species (Dobie 1974; Legg et al. 1987; Nardon et al. 1988; Fava & Burlando 1995; Stejskal & Kučerová 1996; Danho & Haubruge 2003). However, these studies were based on laboratory experiments, and the high egg densities observed, together with their pattern of distribution, may be the result of confining the females with limited numbers of kernels. In fact, egg aggregation decreases with egg density and increases with female density (Legg et al. 1987; Danho et al. 2002), and field studies and laboratory investigations circumventing such conditions indicate prevalence of around two eggs per seed in *Sitophilus* spp. (Pedersen 1979; Stubbs & Abood 1983; Throne 1994; Campbell 2002; Niewiada et al. 2005), in accordance with the optimal egg density reported here for the *S. zeamais*. Sex ratio of the maize weevil is not usually affected by environmental conditions (Throne 1994), which was also observed in the present study, we also did not detect significant variation in developmental time of this species.

The scramble outcome of competition with low optimum egg density (two eggs/seed) observed for both strains of maize weevil may result from either a scramble behavioral process of larval competition with a low optimum egg density or a contest behavioral process of competition with direct interference among the larvae. The scramble behavioral process of larval competition is usually inferred from a decrease in body mass of the adult insects emerging from seeds containing increased egg densities, in contrast with the contest behavioral process of competition where no variation in body mass with egg density is expected (Smith & Lessells 1985; Giga & Smith 1991; Guedes et al. 2007). There was no reduction in body mass with increased competition in either strain of maize weevil. Despite this result suggesting a contest-like process of competition, the small egg density observed indicates that body mass is unlikely to be reduced in the emerging adults compromising the conclusion of a contest-type of behavioral process based on this trait. However, x-ray images of the larvae within the seeds throughout their development indicate direct interference among them and provide evidence of the contest behavioral process of competition taking place within the seeds and generating a scramble ecological outcome with low egg density.

The contest behavioral process of larval competition in the maize weevil is likely adaptive even under seed superparasitism. At low egg densities, no interference behavior is likely to take place between the larvae, which will have enough resources for their development and reproduction. In contrast, at high egg densities (i.e., seed superparasitism), interference behavior will take place among the larvae with the survival of only one or two individuals per seed. Such competition between larvae may incur indirect and direct benefits to the maize weevil. The potential indirect benefits of the seed superparasitism are the increase of the genetic diversity among competing individuals and of the number of individual larvae competing within the seed, potentially increasing the offspring fitness. In addition to these indirect benefits, the cannibalism among larvae also evidenced in the present study may enhance the survival of the cannibalistic larva compensating for reproductive costs and ultimately increasing fitness (Danho et al. 2002). This hypothesis is supported by the fact that the quality of survivors does not seem compromised by competition (there was no variation in body mass, developmental time, or sex ratio). Danho & Haubruge (2003) went so far as to suggest, based on preliminary evidence, that females emerging from seeds superparasitized by the maize weevil were more fecund than females emerging from seeds carrying a single egg, as a result of larva cannibalism, supported by evidence from the red flour beetle *Tribolium castaneum* (Tenebrionidae) (Ho & Dawson 1966; Stevens 1989). Such potential benefits of seed superparasitism associated with the contest process of larval competition in seed beetles deserve future attention to understand the simultaneous evolution of both traits with their applied implications for managing such pest species.

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Figure Captions

Figure 1. Adult emergence (%) of two strains of *Sitophilus zeamais*¸ one susceptible and one resistant to insecticides, subjected to increasing density of eggs on maize seeds (and therefore increasing levels of larval competition). Each symbol represents the observed results for each strain [Susceptible (○), Resistant (▽)], which were pooled together to fit the exponential model $y = \alpha\, x^{-\beta}$.

Figure 2. Larval fitness curve [total body mass of adult insects emerged per seed] of two strains of *Sitophilus zeamais*¸one susceptible and one resistant to insecticides, subjected to increasing density of eggs on maize seeds (and therefore increasing levels of larval competition). Each symbol represents the mean of observed results for each strain [Susceptible (○), Resistant (▽)].

Figure 3. Temporal sequence of X-ray pictures showing the movement and interaction of larvae of *Sitophilus zeamais* (insecticide-susceptible strain) within maize seeds with one egg (a) and multiple eggs (b) laid per seed. The larvae interfere with one another particularly during their early development in the seed with multiple eggs (b) and only two larvae were able to pupate and emerge as adults. The developmental stage (either larva or pupa) is indicated (L or P respectively), as well as the individual larva (1, 2, 3).

Figure 4. Temporal sequence of X-ray pictures showing the movement and interaction of larvae of *Sitophilus zeamais* within maize seeds with multiple eggs laid per seed. The sequence (a) shows the dominance of a larger larva directly interfering with two others, leading to their deterioration and its eventual pupation and emergence from the seed. The sequence (b) shows even more clearly the interference among larvae with the prevailing one apparently killing and consuming its competitor before eventually pupating and emerging from the seed as adult. The sequence (c) shows the early coalescence of larval burrows within the seed with the sole surviving larva moving to the center of the seed, where it pupates and is then attacked by the smaller larva occupying the edge of the endosperm, which escaped the early interference with the conspecifics. The developmental stage (either larva, pupa or adult) is indicated (L, P and A respectively), as well as the individual larva (1, 2, 3).

Fig. 1.

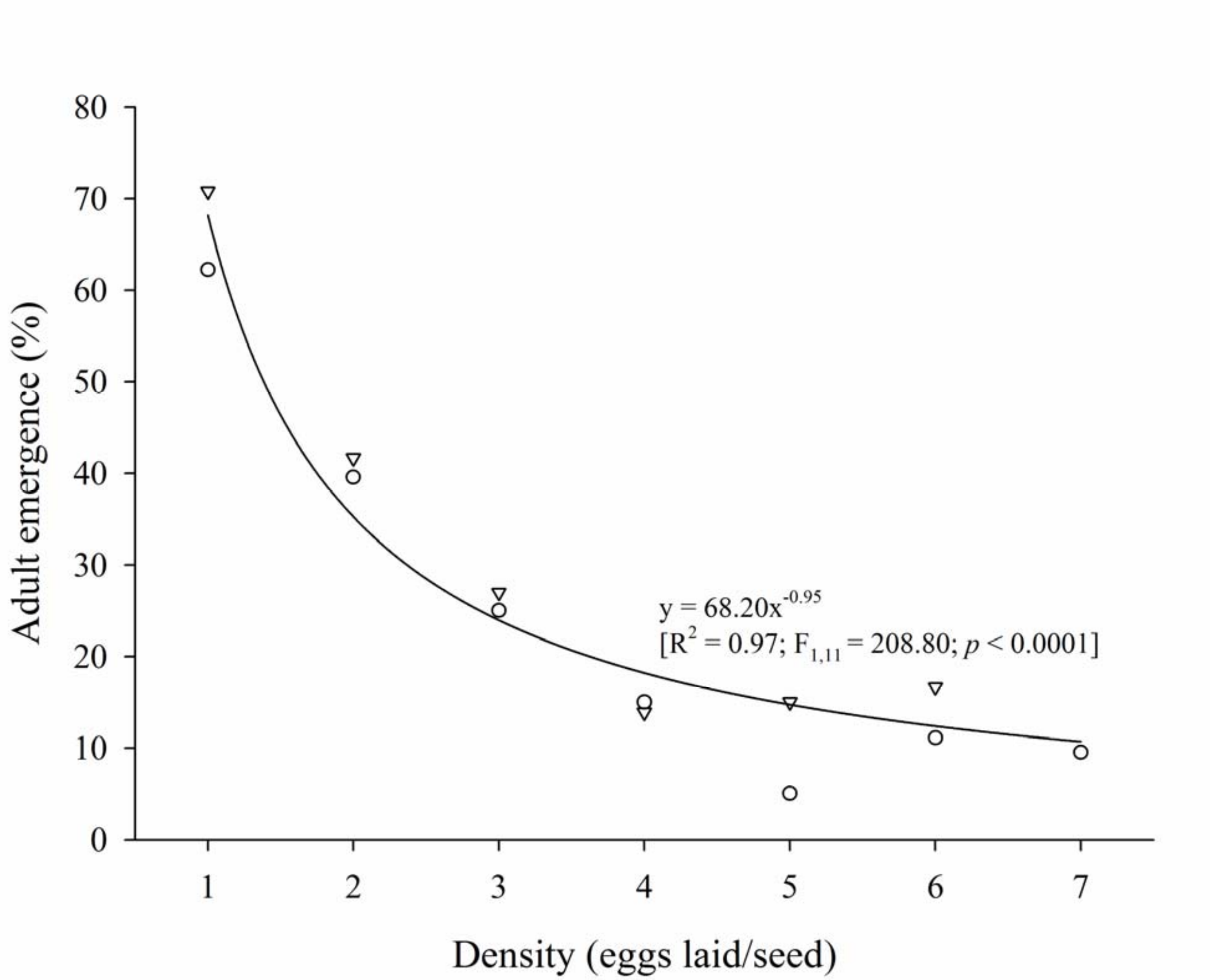

80
70
60
50
40
30
20
10
0
Adult emergence (%)
1
2
3
4
5
6
7
Density (eggs laid/seed)
y = 68.20x^-0.95
[R^2 = 0.97; F_1,11 = 208.80; p < 0.0001]

Fig. 2.

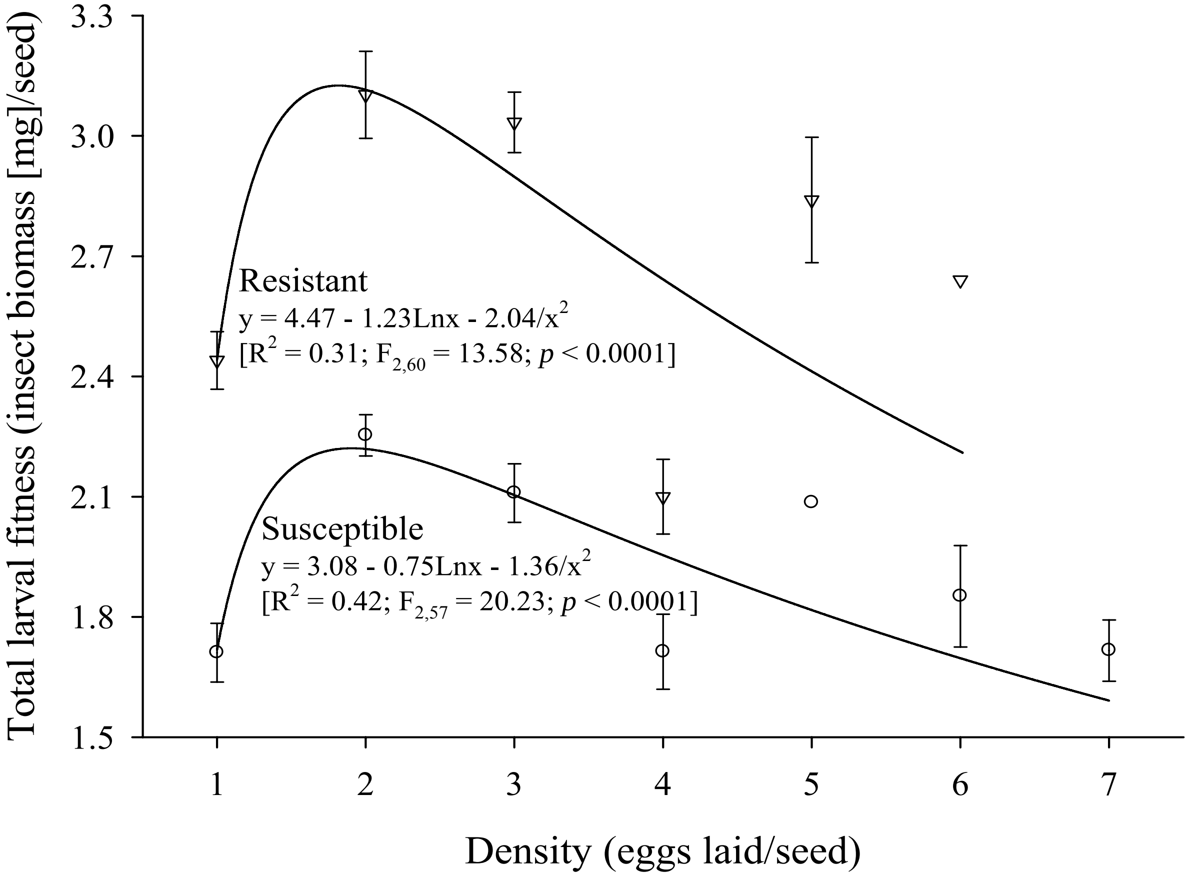

Total larval fitness (insect biomass [mg]/seed)
3.3
3.0
2.7
2.4
2.1
1.8
1.5
1
2
3
4
5
6
7
Density (eggs laid/seed)
Resistant
$y = 4.47 - 1.23\ln x - 2.04/x^2$
$[R^2 = 0.31; F_{2,60} = 13.58; p < 0.0001]$
Susceptible
$y = 3.08 - 0.75\ln x - 1.36/x^2$
$[R^2 = 0.42; F_{2,57} = 20.23; p < 0.0001]$

Fig.3

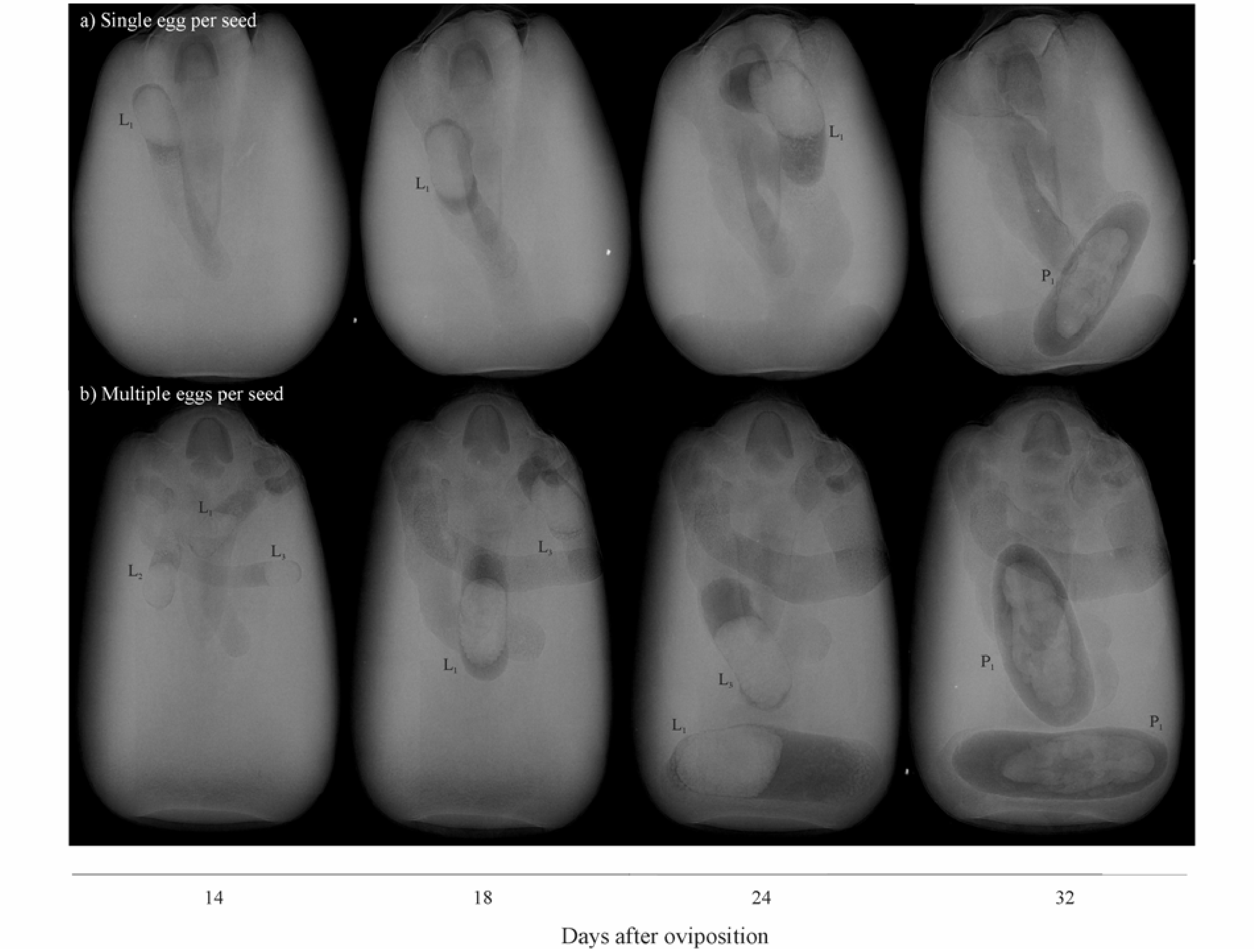
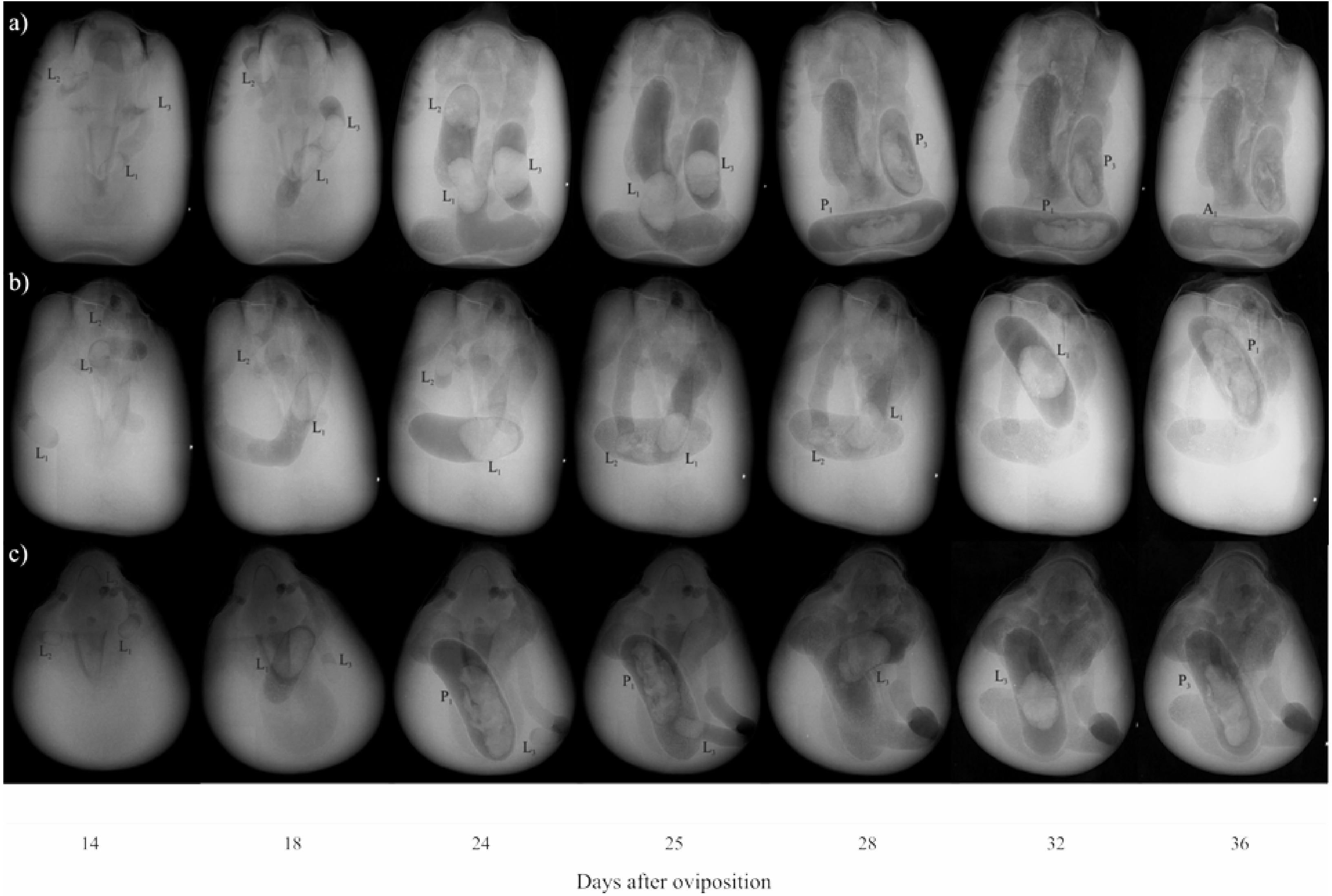

a) Single egg per seed
L_1
L_1
L_1
P_1
b) Multiple eggs per seed
L_1
L_2
L_3
L_3
L_1
L_3
L_1
P_1
P_1
14
18
24
32
Days after oviposition

Fig. 4


a)
L2
L3
L1
L2
L3
L1
L2
L3
L1
L3
L1
P3
P1
P3
P1
A1
b)
L2
L3
L1
L2
L1
L2
L1
L2
L1
L1
L2
L1
P1
c)
L2
L1
L1
L3
P1
L3
P1
L3
L3
L3
P3
14
18
24
25
28
32
36
Days after oviposition

Format: *Entomologia Experimentalis et Applicata*

Deltamethrin-induced feeding plasticity in pyrethroid-susceptible and -resistant strains of the maize weevil, *Sitophilus zeamais*

Running title: Feeding plasticity in pyrethroid-resistant strains of the maize weevil

Key words: behavioral resistance, stored grains, insecticide resistance, feeding behavior, insecticide avoidance, food conversion

Abstract

Phenotypic plasticity contributes to the adaptative evolution of populations exposed to new or altered environments. Feeding plasticity is a component of phenotypic plasticity not usually considered in insect strains adapted to insecticide-altered environments, but which may either accentuate or mitigate insecticide resistance. This is a concern in pyrethroid-resistant strains of the maize weevil *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), and the reason for this study. A pyrethroid-susceptible and two pyrethroid-resistant strains of maize weevil were subjected to free-choice and no-choice tests with maize grains sprayed with increasing doses of the pyrethroid deltamethrin. The insects from the pyrethroid-resistant strains exhibited higher feeding avoidance with increased deltamethrin doses than insects from the susceptible strain when subjected to free-choice tests. In addition, the pyrethroid-resistant strains performed better under sprayed grains and exhibited low levels of feeding deterrence (≤ 12%) than the susceptible strain (50%). The strains of maize weevil physiologically resistant to pyrethroids were also behaviorally resistant to the pyrethroid deltamethrin – an additional management concern. The resistant strains better discriminate deltamethrin-sprayed grains and are less nutritionally-affected by this compound with increasingly divergent responses from the susceptible strain with increased doses of deltamethrin. Furthermore, the higher relative growth rate and consequently higher efficiency of food conversion observed in the insecticide-resistant strains were significant even without insecticide exposure indicating that these traits are stimulus-independent and may persist even without further insecticide selection, potentially limiting the tactics available for their management.

Introduction

Insecticide resistance is a phenomenon of economical and environmental concern and it is also important as a model system for the study of the evolution of newly-adapted phenotypes (Lockwood et al., 1984; Haynes, 1988; Mallet, 1989; Hoy et al., 1998). Insects may withstand insecticide applications either through the evolution of physiological mechanisms allowing them to cope with high insecticide levels on or within the body, or through behavioral mechanisms minimizing their exposure to insecticides (Gould, 1984; Hoy et al., 1998; Jallow & Hoy, 2005). Interest in the latter was stimulated by the use of pyrethroid insecticides exhibiting irritant and repellent effect in insects (Lockwood et al., 1984; Mallet, 1989). However, consistent studies correlating physiological and behavioral resistance to insecticides are rare (Suiter & Gould, 1994; Renou et al., 1997; Kongmee et al., 2004; Jallow & Hoy, 2005).

Insecticide resistance is recognized as a pleiotropic trait whose expression may vary with the individual genotype and its environment (Chevillon et al., 1997; Boivin et al., 2001; Oliveira et al., 2005; Ribeiro et al., 2007). Such plasticity of expression of insecticide resistance may contribute to the adaptative evolution of populations exposed to new or altered environments (Pagliucci & Muren, 2003; Price et al., 2003; Ghalambor et al., 2007). Feeding plasticity is a component of phenotypic plasticity not usually considered in insect strains adapted to insecticide-altered environments, but which may either accentuate or mitigate insecticide resistance. This is a concern in pyrethroid-resistant strains of the maize weevil *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), a major insect-pest of stored grains (USDA, 1980; Rees, 1996; Araújo et al., 2008; Guedes & Pereira, 2008), and the reason for the present study.

The overreliance on insecticide use against stored grain insect-pests led to widespread evolution of insecticide resistance in these pest species (Champ & Dyte, 1976; Badmin, 1990; Subramanyam & Hagstrum, 1996; Pimentel et al., 2007). The maize weevil is no exception and high levels of insecticide resistance, particularly pyrethroid resistance, have been reported (Guedes et al., 1994, 1995; Perez-Mendoza, 1999; Ribeiro et al., 2003; Fragoso et al., 2003). The neurotoxic activity of pyrethroids and their reported association with avoidance behavior in insects are indications of the potential importance of the behavioral response elicited by this group of insecticides in pyrethroid-resistant populations of insect-pests (Dethier et al., 1960; Gammon, 1978; Lockwood et al., 1984; Pekár & Haddad, 2005). Insecticide-induced behavioral responses have also been reported in stored-product insects (Watson & Barson, 1986; Cox et al., 1997), but not their plasticity and association with insecticide resistance. Here we explored the feeding plasticity of pyrethroid-suceptible and –resistant strains of the maize weevil when exposed to deltamethrin-sprayed maize grains at increasing rates in free-choice and no-choice tests. We expected to learn if there is association between physiological and behavioral resistance to pyrethroids in strains of maize weevil and we were expecting increased divergence of response (i.e., feeding plasticity) between the susceptible and resistant strains with exposure to increased doses of deltamethrin.

Material and methods

Insects and insecticides

Three strains of *S. zeamais* were used in the present investigation, which were termed here as 'susceptible', 'resistant cost', and 'resistant no-cost'. The susceptible strain was collected by mid-1980 in Sete Lagoas County (state of Minas Gerais, Brazil) (Guedes et al., 1994, 1995). The resistant cost strain was collected in Juiz de Fora County (state of Minas Gerais, Brazil) in 1999. It is resistant to pyrethroids, but has reduced fitness in the absence of pyrethroid exposure (Fragoso et al., 2003; Guedes et al., 2006; Oliveira et al., 2007). The resistant no-cost strain was collected in Jacarezinho County (state of Paraná, Brazil) in the late 1980's (Guedes et al., 1994, 1995). It is also resistant to pyrethroids, but does not exhibit fitness cost associated with insecticide resistance (Fragoso et al., 2005; Guedes et al., 2006; Oliveira et al., 2007). Both insecticide-resistant strains (i.e., resistant cost and resistant no-cost) exhibit high levels of pyrethroid resistance (> 100x), which are periodically checked (Guedes et al., 1994, 1995; Ribeiro et al., 2003; Araújo et al., 2008). The two resistant strains share the same major insecticide resistance mechanism – altered target-site sensitivity with secondary involvement of enhanced activity of glutathione *S*-transferases (Guedes et al., 1995; Ribeiro et al., 2003; Fragoso et al., 2003, 2007). The three strains were replicated and maintained in large numbers (to minimize effects of genetic drift) in whole maize grains free of insecticides under controlled temperature (25 ± 2°C), relative humidity (70 ± 5%) and photoperiod (LD 12:12 h).

The commercial formulation of the pyrethroid insecticide deltamethrin ([cyano-(3-phenoxyphenyl)-methyl] 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate); K-Obiol® 25 CE; emulsifiable concentrate at 25 g a.i./L; Bayer CropScience Brasil, São Paulo, Brazil) was purchased at the local market. Deltamethrin was sprayed in batches of 1 kg of maize using manual sprayers with their nozzles directed down towards the grains at 20 cm high. A volume of 1 ml was used to spray each grain batch at 3 bar pressure. The manual sprayers were changed for each insecticide application to prevent residue contamination. The sprayed grains were let drying overnight before use.

Free-choice feeding test of preference

Pair-wise free choice tests were carried out using white plastic trays (25 x 15 x 6 cm) with one half containing water-sprayed grains (50 g) and the other half containing deltamethrin-sprayed grains (50 g) at increasing doses. A control with water-sprayed grains on both sides was also used to normalize the results. The trays were coated with Teflon® PTFE (DuPont, Wilmington, DE, USA) to prevent insect escape and covered with a fine fabric (organza) to prevent insect flight. Fifty non-sexed adult insects (one to two weeks old) were released in the center of the tray and insect preference was assessed after one hour by determining the proportion of insects present on the deltamethrin-sprayed grains. The results obtained with one hour exposure were similar to those obtained with extended exposure (up to 24 h) based on preliminary tests. Five replicates were used for each combination of deltamethrin dose and maize weevil strain.

No-choice feeding test

Batches of 10 g of sprayed maize grains were placed in glass jars (0.5 L) with the upper portion of their inner walls coated with Teflon® PTFE. The grains were previously weighted and their moisture content determined. Thirty non-sexed adult insects (one to two weeks old) were released within each jar. After 10 days, the insects were removed, assessed (if alive or not) and weighted. The grain batches were also weighted and their moisture content determined to estimate the loss of grain mass due to water loss within the period. Five replicates were used for each combination of strain and deltamthrin dose. The following nutritional indexes were calculated for each combination of deltamethrin-sprayed dose and maize weevil strain, as described elsewhere (Isman et al., 1990; Huang et al., 1997): relative growth rate (daily weight gain per insect weight), relative consumption rate (daily consumption rate per insect weight), conversion efficiency of ingested food ((relative growth rate/relative consumption rate) x 100), and feeding deterrence index (proportion of grain mass not consumed by the insects).

Statistical analysis

The experiments were established following the completely random design. The overall nutritional results of both strains without insecticide exposure were subjected to multivariate analysis of variance (PROC GLM with MANOVA statement; SAS Institute, 2002), and subsequent individual analysis of variance for each nutritional parameter were carried out, if appropriate (PROC GLM; SAS Institute, 2002). The results of both experiments were subjected to regression analysis with the doses of deltamethrin as independent variable. The models were tested from the simplest (linear and quadratic) to the alternative models of increasing complexity (non-linear peak models). TableCurve 2D was used to fit these curves (SPSS 2000).The model selection was carried out based on simplicity, high F-values (and mean squares) and steep increase of R^2 with model complexity. Residue distribution was also checked for each analysis on TableCurve 2D to ascertain of parametric assumptions.

Results

Free-choice feeding preference

The proportion of insects detecting deltamethrin-sprayed grains varied with the insecticide dose and the insect strain (Figure 1). The curves of discrimination for all three insect strains followed similar trend with the proportion of insects discriminating deltamethrin-sprayed grains increasing with the increase in insecticide dose until reaching a plateau. Therefore, deltamethrin avoidance was stimulus-dependent and differed among maize weevil strains. The insects from the both insecticide-resistant strains showed higher deltamethrin sensitivity than the insecticide-susceptible insects, which is evident for doses lower than the recommended label rate (< 0.5 ppm), where the resistant strains exhibited an approximated 5-fold higher deltamethrin sensitivity than the susceptible strain (Figure 1). The resistant no-cost strain exhibited the highest deltamethrin avoidance with over 60% of the insects discriminating grains with higher residue levels (≥ 2 ppm), while the resistant cost strain exhibited about 50% of discriminating insects at the same residue levels and the susceptible strain exhibited little over 30% of discriminating insects.

No-choice feeding response

Mortality rates and nutritional indexes were calculated for the maize weevil strains exposed for 10 days to maize grains at increasing doses of deltamethrin to verify if the insects from different strains differ in their food conversion and if they employ any strategy to either minimize or compensate insecticide ingestion. The multivariate analysis of variance for the nutritional parameters indicated significant differences among strains without insecticide exposure (Wilks' lambda = 0.1616; $F_{6/20}$ = 4.96; p = 0.002), a likely a result of their different genetic make-up. The univariate analysis of variance for the individual nutritional parameters indicated significant strain differences for relative growth rate ($F_{2,12}$ = 20.44; $p < 0.0001$) and efficiency of conversion of ingested food ($F_{2,12}$ = 16.92; $p = 0.0003$), but there was no significant difference for relative consumption rate among strains (0.72 ± 0.01 mg/mg.day; $F_{2,12}$ = 1.53; $p = 0.26$); as feeding deterrence was standardized based on the control, the strain differences without insecticide exposure were not considered. The relative insect growth rate was higher for the insecticide-resistant strains (0.016 ± 0.0004 and 0.013 ± 0.001 mg/mg.day for the resistant cost and resistant no-cost strains respectively) than for the insecticide-susceptible strain (0.0089 ± 0.0009 mg/mg.day). Similar trend was observed for the efficiency of food conversion (2.17 ± 0.05, 1.89 ± 0.09 and 1.30 ± 0.15 % for the resistant cost, resistant no-cost and susceptible strains, respectively), which was expected since there was no significant strain variation in relative consumption rate and therefore the variation in food conversion was basically determined by the strain differences in relative growth rate.

The mortality curves obtained for each strain reflect their differential susceptibility to the insecticide (Figure 2). There was no significant insect mortality at low deltamethrin doses (≤ 20%), but a sharp increase in mortality took place for the susceptible strain at 0.05 ppm deltamethrin, a dose 10x lower than the recommended label rate (0.5 ppm). At the label rate the mortality level reached near 100%. In contrast, the low mortality of the resistant strains showed significant increase just at the recommended label rate not reaching the 40% mortality level at the highest dose of deltamethrin sprayed (i.e., 5.0 ppm).

Regarding the sub-lethal feeding effects of deltamethrin-sprayed grains, they were significantly plastic among strains for insect growth and consumption rates, food conversion and feeding deterrence. Deltamethrin did not affect the relative growth rate of the resistant no-cost strain, but doses higher than 0.05 ppm led to steady decline trend of relative growth rate for the resistant cost strain and a drastic decline for the susceptible strain (Figure 3). The relative consumption rate of the susceptible strain also declined with increase in deltamethrin dose particularly above 0.05 ppm, in contrast with the increased relative consumption observed with increased deltamethrin dose for both resistant strains (Figure 4). This trend was similar for both resistant strains, but the resistant no-cost strain exhibited consistently higher consumption.

The efficiency of conversion of ingested food, which is obtained from the ratio of relative growth rate and relative consumption rate, showed an overall trend analogous to those of the relative growth rate with the deltamethrin dose not affecting the resistant no-cost strain, but decreasing the efficiency of food conversion of the susceptible and resistant cost strains (Figure 5). The resistant no-cost strain exhibited lower efficiency of food conversion at low doses of deltamethrin, but the trend inverted with increased doses due to the decline in efficiency exhibited by the resistant cost strain above 0.05 ppm and even more so for the susceptible strain. The resistant cost strain exhibited the highest efficiency of food conversion for deltamethrin doses of up to 0.5 ppm, when its decline in efficiency dropped to levels lower than those exhibited by the resistant no-cost strain at 5.0 ppm deltamethrin. Feeding deterrence increased with deltamethrin dose, particularly after 0.005 ppm (Figure 6). Such increase was drastic for the susceptible strain, going from nearly 0% to 60%, but mild for both resistant strains (≤ 10%). The resistant no-cost strain exhibited the lowest levels of feeding deterrence among the three maize weevil strains.

Discussion

Insecticide resistance is a pleitropic trait potentially amenable to phenotypic plasticity (Chevillon et al., 1997; Boivin et al., 2001; Guedes et al., 2006; Ribeiro et al., 2007; Araújo et al., 2008), which is a concern because such plasticity may contribute to the adaptation of insecticide-resistant strains to new or altered environments (Pagliucci & Muren, 2003; Price, 2003; Ghalambor et al., 2007). Nonetheless, the subject has not been object of attention. Here we reported increased divergence of feeding response between insecticide-susceptible and –resistant strains of the maize weevil exposed to increased residues of deltamethrin in sprayed maize grains. This finding characterizes increased feeding plasticity with increased insecticide doses sprayed on the food source, which may further compromise the management of the resistant insects and provide them with further possibilities of adapting to new environments.

An insect's chance of survival to an insecticide may be greatly increased if its behavior is modified to avoid insecticide-sprayed surfaces (Watson & Barson, 1986; Cox et al., 1997). The perception of insecticide presence in the environment may be achieved through the insect learning ability or through genetic modifications in its peripheral receptors or central processing systems leading to the evolution of behavioral resistance to insecticides in some species (Geroghiou, 1972; Haynes, 1988; Mallet, 1989; Gould, 1991; Hoy et al., 1998). Our results with strains of maize weevil in free-choice tests of feeding preference (or avoidance) with insecticide-sprayed maize grains indicate that the physiologically-resistant strains are better able to detect the deltamethrin sprayed grains than the susceptible strain and even more so with higher insecticide residues on the grain. Therefore, the physiologically-resistant strains are also behaviorally-resistant to deltamethrin further compromising the efficacy of this compound, which may also extend to other pyrethroids. Furthermore, the plasticity of response observed among strains suggests that high-dose management tactics, based on use of high doses of insecticides to increase mortality and turn the resistance functionally recessive (Tabashnik & Croft, 1982; Mallet, 1989), is not promising in such case. This is so because the use of higher doses of insecticide will only increase the avoidance behavior exhibited by the already physiologically-resistant insects minimizing the intended mortality. The use of insecticide rotation or mixtures seems more promising management tactics in this case.

The forced exposure of insects from three strains of maize weevil in no-choice feeding tests with maize grains indicated a higher relative growth rate and consequently higher efficiency of food conversion in the insecticide-resistant strains were significant even without insecticide exposure indicating that these traits are stimulus-independent and may persist even without further insecticide selection, potentially limiting the tactics available for their management. In addition, such forced exposure of the insects to deltamethrin-sprayed grains also indicated higher phenotypic plasticity with increased divergence of feeding response between insecticide-susceptible and –resistant strains subjected to grains with increased insecticide residues.

An insecticide may interfere with insect food consumption if it is able to affect one of the following behavioral components: feeding likelihood, feeding frequency, ingestion efficiency and feeding duration (Simpson, 1995). Insecticide-resistant strains of maize weevil are less likely to feed on insecticide-sprayed grains than the insecticide-susceptible strain, if given a choice, as previously discussed. Deltamethrin deterred feeding only slightly for the resistant strains at the highest doses used (≤ 0.5 ppm) on no-choice tests, but drastically deterred feeding by the susceptible strain at high deltamethrin doses. The consumption of insecticide-sprayed grains by the resistant strains increased at the higher doses of deltamethrin, in contrast with a decrease in consumption by the susceptible strains. In addition, the conversion efficiency of insecticide-sprayed grains is more seriously compromised in the susceptible strain than in the resistant cost strain, particularly at higher doses of deltamethrin. Furthermore, the food conversion efficiency of the resistant no-cost strain is not affected by the deltamethrin doses in the range here considered, which reached 10x the recommended label rate.

In summary, we reported here that pyrethroid-resistant strains of maize weevil better discriminate deltamethrin-sprayed grains and are less nutritionally-affected by this compound with increasingly divergent responses from the susceptible strain with increased doses of deltamethrin. The resistant population without fitness costs associated with insecticide resistance exhibited even higher behavioral resistance and was also even less nutritionally-affected by deltamethrin than the resistant strain with fitness disadvantage. Such responses observed against deltamethrin may also extend to other pyrethroids as well, which deserves attention. The potential pyrethoid-induced plasticity of behavioral responses should also be considered in insecticide resistance assessments since it does have important pest management implications. The management of these resistant strains is further compromised by their deltamethrin-induced feeding plasticity and management tactics based on the high-dose approach are unlikely to succeed. The same can be said regarding the temporary suppression of deltamethrin use aimed at the reestablishment of susceptibility due to fitness disadvantage, because it will not occur for strains not exhibiting fitness disadvantage associated with insecticide resistance, as one of the strains used in the present study. Tactics involving insecticide rotation or mixtures seem more promising.

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Figure Captions

Figure 1. Proportion of adults of one pyrethroid-susceptible (○) and two pyrethroid-resistant strains (Resistant cost (□) and Resistant no-cost (▽)) of maize weevil (*Sitophilus zeamais*) discriminating against maize grains sprayed with increasing doses of the pyrethroid insecticide deltamethrin. Each symbol represents the mean of five replicates.

Figure 2. Mortality of adults (%) of one pyrethroid-susceptible (○) and two pyrethroid-resistant strains (Resistant cost (□) and Resistant no-cost (▽)) of maize weevil (*Sitophilus zeamais*) exposed to maize grains sprayed with increasing doses of the pyrethroid insecticide deltamethrin. Each symbol represents the mean of five replicates.

Figure 3. Relative growth rate (mg/mg.day) of one pyrethroid-susceptible (○) and two pyrethroid-resistant strains (Resistant cost (□) and Resistant no-cost (▽)) of maize weevil (*Sitophilus zeamais*) exposed to maize grains sprayed with increasing doses of the pyrethroid insecticide deltamethrin. Each symbol represents the mean of five replicates.

Figure 4. Relative consumption rate (mg/mg.day) of one pyrethroid-susceptible (○) and two pyrethroid-resistant strains (Resistant cost (□) and Resistant no-cost (▽)) of maize weevil (*Sitophilus zeamais*) exposed to maize grains sprayed with increasing doses of the pyrethroid insecticide deltamethrin. Each symbol represents the mean of five replicates.

Figure 5. Efficiency of conversion of ingested food (%) of one pyrethroid-susceptible (○) and two pyrethroid-resistant strains (Resistant cost (□) and Resistant no-cost (▽)) of maize weevil (*Sitophilus zeamais*) exposed to maize grains sprayed with increasing doses of the pyrethroid insecticide deltamethrin. Each symbol represents the mean of five replicates.

Figure 6. Feeding deterrence of one pyrethroid-susceptible (○) and two pyrethroid-resistant strains (Resistant cost (□) and Resistant no-cost (▽)) of maize weevil (*Sitophilus zeamais*) exposed to maize grains sprayed with increasing doses of the pyrethroid insecticide deltamethrin. Each symbol represents the mean of five replicates.

Fig. 1.

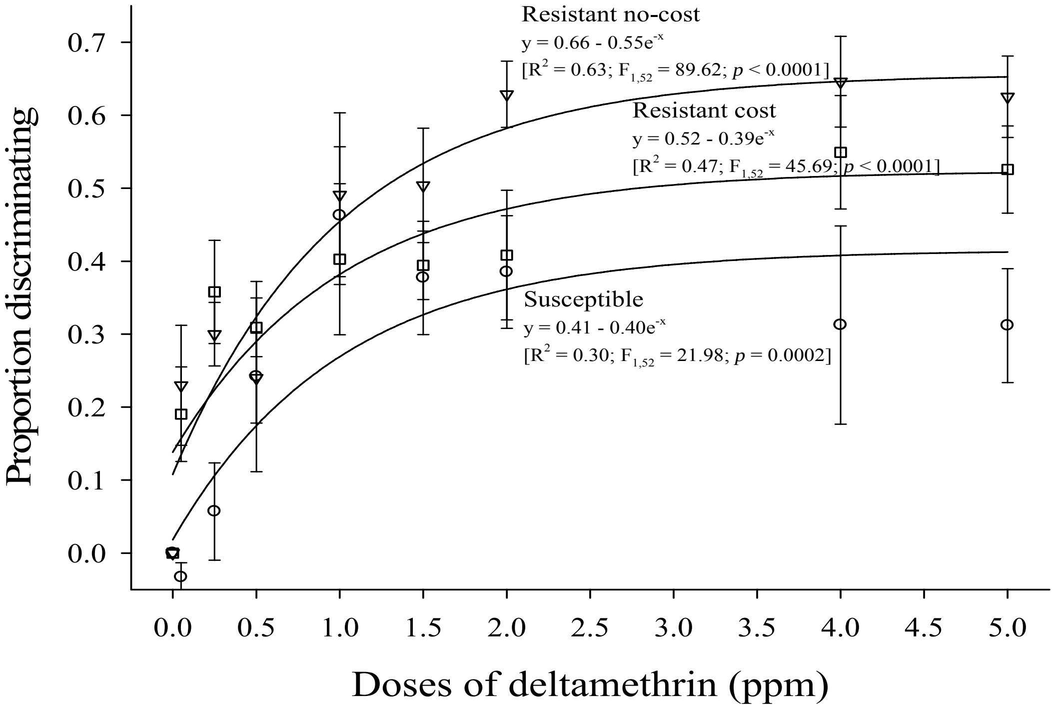

Resistant no-cost
$y = 0.66 - 0.55e^{-x}$
[$R^2 = 0.63$; $F_{1,52} = 89.62$; $p < 0.0001$]
Resistant cost
$y = 0.52 - 0.39e^{-x}$
[$R^2 = 0.47$; $F_{1,52} = 45.69$; $p < 0.0001$]
Susceptible
$y = 0.41 - 0.40e^{-x}$
[$R^2 = 0.30$; $F_{1,52} = 21.98$; $p = 0.0002$]
Proportion discriminating
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Doses of deltamethrin (ppm)

Fig. 2.

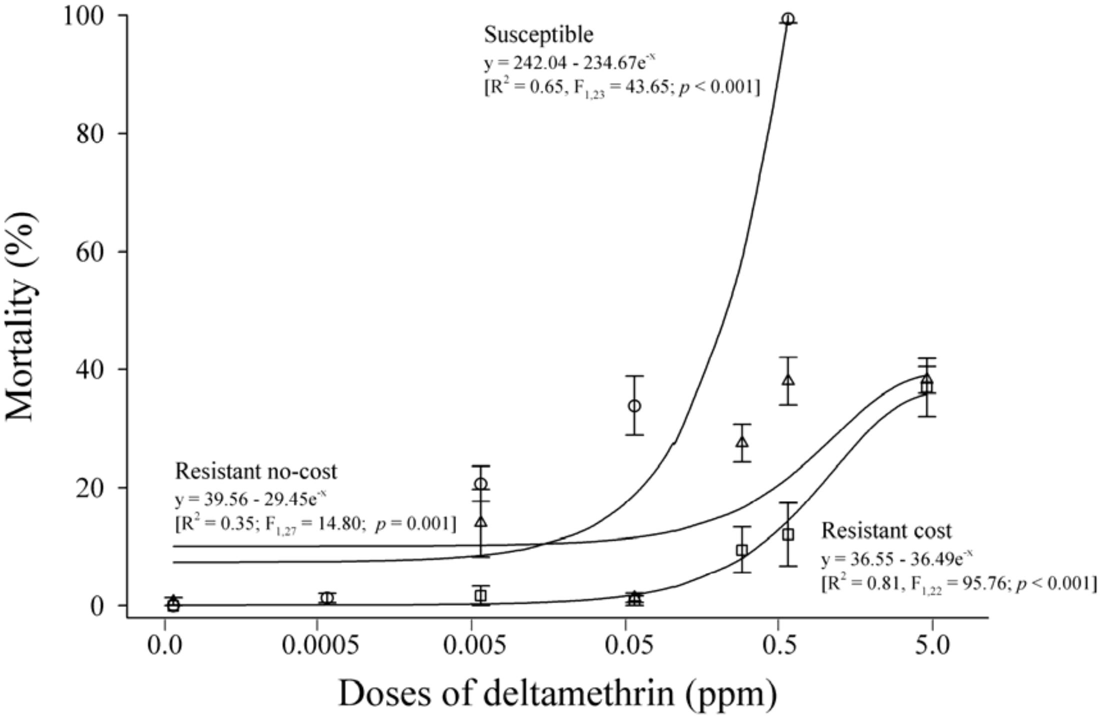

100
80
60
40
20
0
Mortality (%)
Susceptible
$y = 242.04 - 234.67e^{-x}$
[$R^2 = 0.65$, $F_{1,23} = 43.65$; $p < 0.001$]
Resistant no-cost
$y = 39.56 - 29.45e^{-x}$
[$R^2 = 0.35$; $F_{1,27} = 14.80$; $p = 0.001$]
Resistant cost
$y = 36.55 - 36.49e^{-x}$
[$R^2 = 0.81$, $F_{1,22} = 95.76$; $p < 0.001$]
0.0
0.0005
0.005
0.05
0.5
5.0
Doses of deltamethrin (ppm)

Fig. 3.

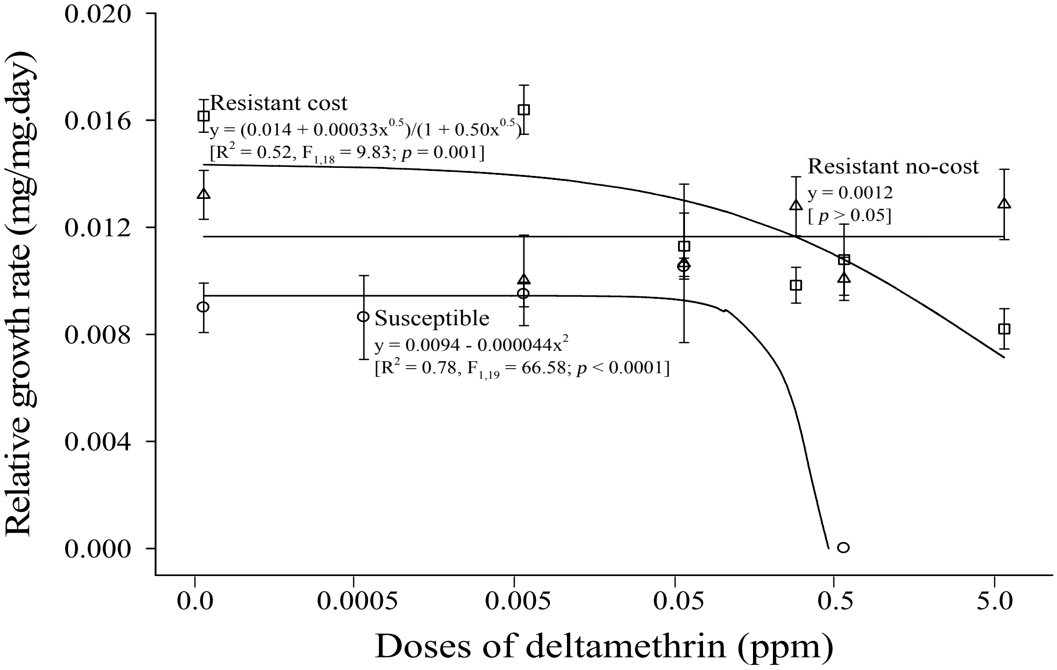
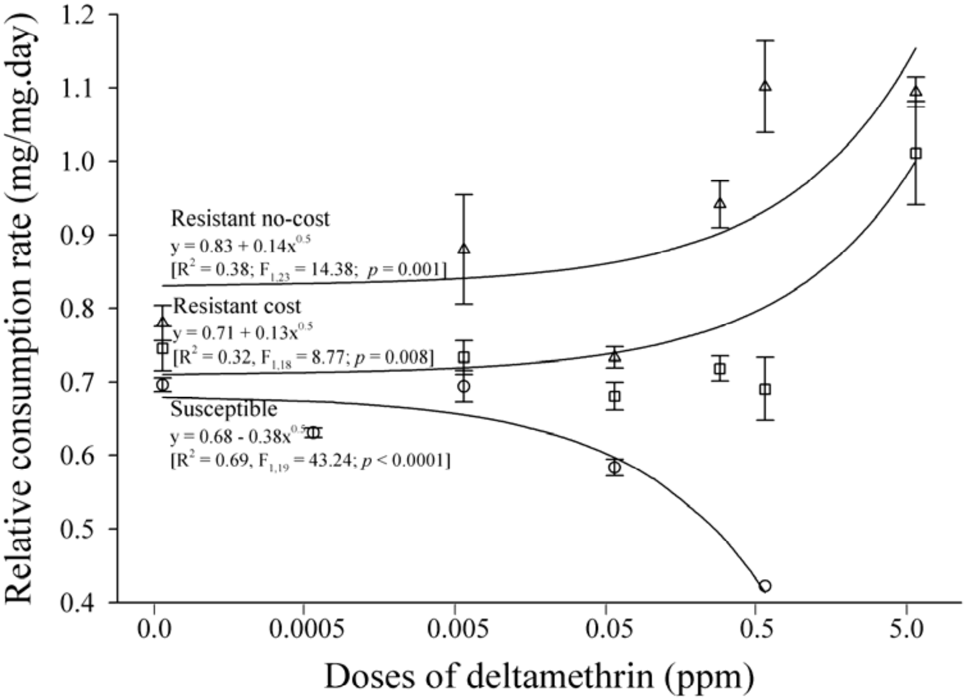

0.020
0.016
0.012
0.008
0.004
0.000
Relative growth rate (mg/mg.day)
Resistant cost
$y = (0.014 + 0.00033x^{0.5})/(1 + 0.50x^{0.5})$
$[R^2 = 0.52, F_{1,18} = 9.83; p = 0.001]$
Resistant no-cost
$y = 0.0012$
$[p > 0.05]$
Susceptible
$y = 0.0094 - 0.000044x^2$
$[R^2 = 0.78, F_{1,19} = 66.58; p < 0.0001]$
0.0
0.0005
0.005
0.05
0.5
5.0
Doses of deltamethrin (ppm)

Fig. 4.



Relative consumption rate (mg/mg.day)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.0
0.0005
0.005
0.05
0.5
5.0
Doses of deltamethrin (ppm)
Resistant no-cost
$y = 0.83 + 0.14x^{0.5}$
$[R^2 = 0.38; F_{1,23} = 14.38; p = 0.001]$
Resistant cost
$y = 0.71 + 0.13x^{0.5}$
$[R^2 = 0.32, F_{1,18} = 8.77; p = 0.008]$
Susceptible
$y = 0.68 - 0.38x^{0.5}$
$[R^2 = 0.69, F_{1,19} = 43.24; p < 0.0001]$

Fig. 5.

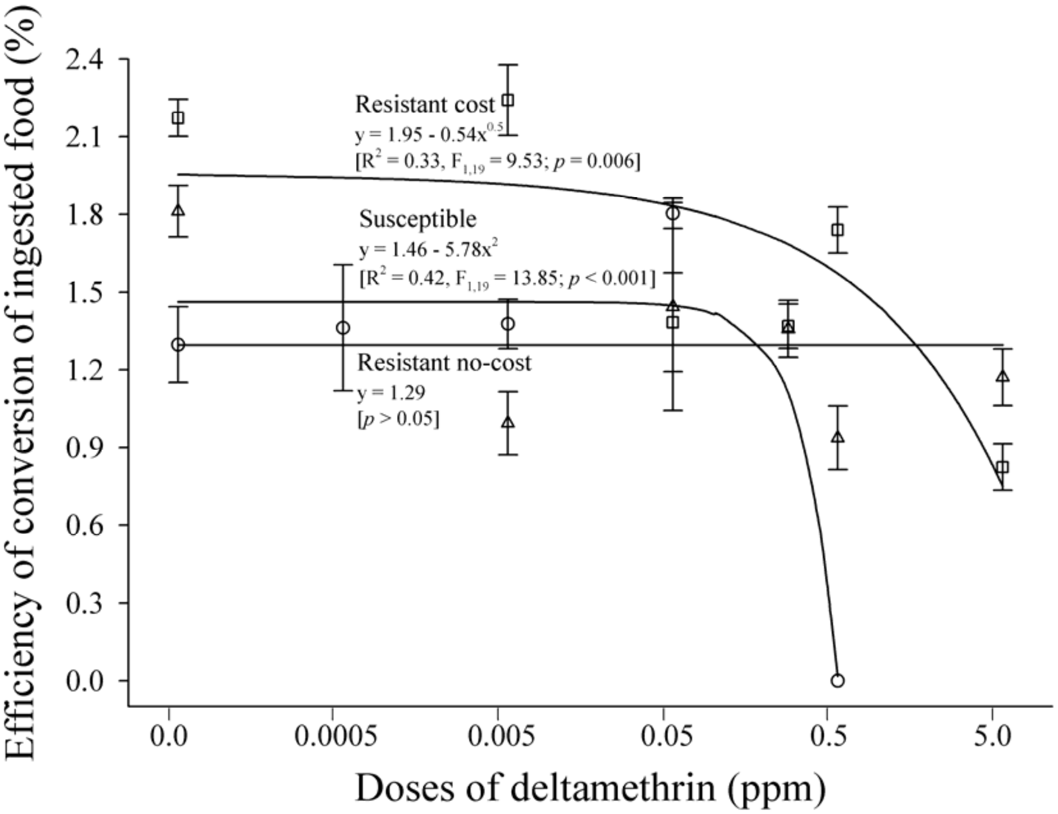
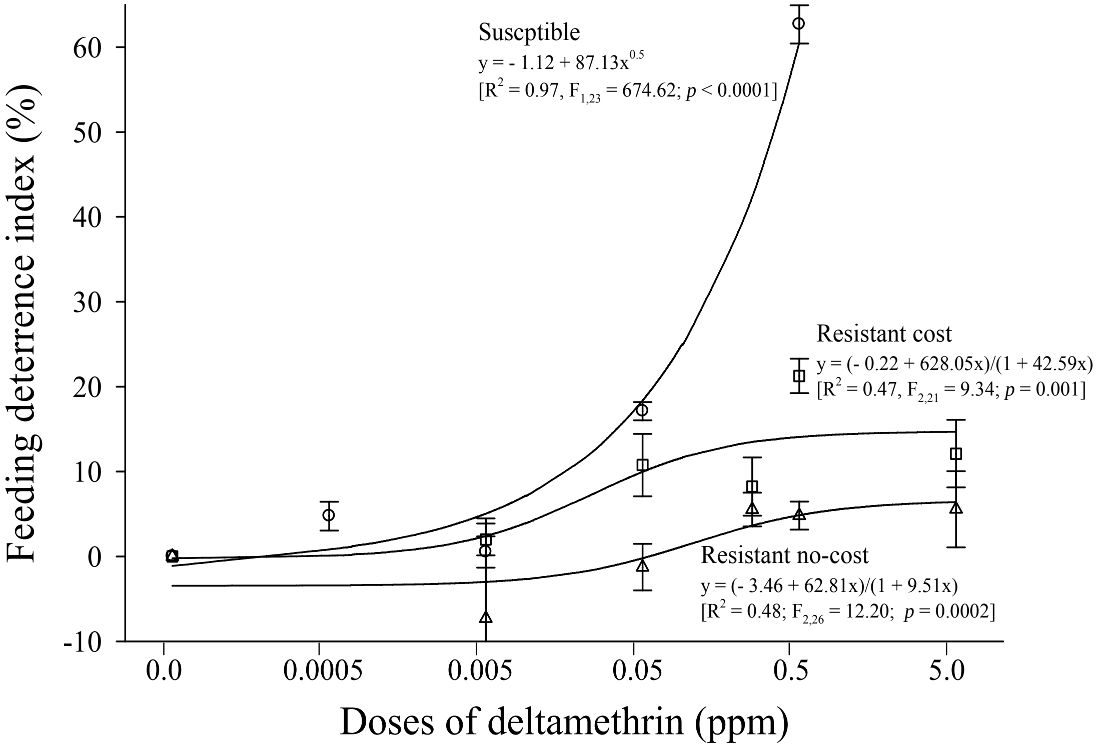

Efficiency of conversion of ingested food (%)
2.4
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0.0
Resistant cost
$y = 1.95 - 0.54x^{0.5}$
[$R^2 = 0.33$, $F_{1,19} = 9.53$; $p = 0.006$]
Susceptible
$y = 1.46 - 5.78x^2$
[$R^2 = 0.42$, $F_{1,19} = 13.85$; $p < 0.001$]
Resistant no-cost
$y = 1.29$
[$p > 0.05$]
0.0
0.0005
0.005
0.05
0.5
5.0
Doses of deltamethrin (ppm)

Fig. 6.



Suscptible
y = - 1.12 + 87.13x^0.5
[R^2 = 0.97, F_1,23 = 674.62; p < 0.0001]
Resistant cost
y = (- 0.22 + 628.05x)/(1 + 42.59x)
[R^2 = 0.47, F_2,21 = 9.34; p = 0.001]
Resistant no-cost
y = (- 3.46 + 62.81x)/(1 + 9.51x)
[R^2 = 0.48; F_2,26 = 12.20; p = 0.0002]
Feeding deterrence index (%)
60
50
40
30
20
10
0
-10
0.0
0.0005
0.005
0.05
0.5
5.0
Doses of deltamethrin (ppm)

Format: *Agricultural and Forest Entomology*

Flight take-off and walking behavior of insecticide-susceptible and –resistant strains of *Sitophilus zeamais* exposed to deltamethrin

Running title: *Locomotory behavior of* Sitophilus zeamais

Keywords: behavioral resistance, insecticide avoidance, insecticide resistance, maize weevil, repellence, mobility, pyrethroid, stored grains.

Abstract

1. Insects have evolved a variety of physiological and behavioral responses to various toxins in natural and managed ecosystems. However, insect behavior is seldom considered in insecticide studies although insects are capable of changing their behavior in response to their sensory perception of insecticides, which may compromise insecticide efficacy. Such perspective is particularly serious for insecticide-resistant pest insects.
2. Locomotion plays a major role determining insecticide exposure and was therefore considered in investigating the behavioral responses of an insecticide-susceptible and two insecticide-resistant strains of the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), a major pest of stored cereals. Different dose-dependent behavioral responses were expected among strains with behavioral resistance less likely to occur in physiologically resistant insects since they are able to withstand higher doses of insecticide.
3. The behavioral responses to deltamethrin-sprayed surfaces differed among the maize weevil strains. Such responses were dose-dependent for flight take-off (reducing with insecticide concentration) in two strains of maize weevil, but not for an insecticide-resistant strain, while walking response was dose-independent for all of the strains.
4. Behavioral resistance was independent from physiological resistance with one resistant strain exhibiting higher rates of flight take-off, and the other resistant strain exhibiting lower flight take-off. Female mobility was similar for all strains, unlike male mobility. Males of each strain exhibited a pattern of mobility following the same trend of flight take-off. Behavioral patterns of response to insecticide are therefore variable among strains, particularly among insecticide-resistant strains, and worth considering in resistance surveys and management programs.

Introduction

Insects have evolved a variety of physiological and behavioral responses to various toxins in natural and managed ecosystems (Jallow & Hoy, 2005). These varied responses can reflect the toxin mode of action and the extent to which they influence the behavior (Hoy et al., 1998). Some insects are capable of changing their behavior is response to their sensory perception of insecticides (Gould, 1984; Lockwood et al., 1984; Haynes, 1988). These behavioral changes may sometimes compromise insecticide efficacy, and consequently standard assays relaying in lethality can overestimate the insecticide impact in a population. Such limitation is particularly serious for insecticide-resistant pest insects.

Insecticide studies usually focus on the insecticide lethality or other direct effects of insecticides, whereas relatively little attention is placed on the behavioral response to insecticidal exposure (Kongmee et al., 2004). One behavioral response of insects to insecticides is the stimulus-dependent avoidance, which refers to the enhanced ability to detect a toxic substance and assumes an irritant or repellent property of the toxicant that elicits avoidance response after detection with or without contact with the insecticide (irritability and repellency respectively) (Georghiou, 1972; Lockwood et al, 1984). Another behavioral response of insects to insecticides is the stimulus-independent avoidance where the insects avoid exposure to a toxicant due to an independent and innate behavioral trait (Lockwood et al., 1984). They are both recognized as behavioral resistant to toxic compounds if such traits are inheritable. If the toxic compound involved is an insecticide and the behavioral trait is enhanced as stimulus-dependent in a population, it is refereed as stimulus-dependent behavioral resistance to the insecticide (Georghiou, 1972; Gould, 1984; Lockwood et al., 1984; Wang et al., 2004). In contrast, if the behavioral trait is not enhanced with increased stimuli in a population, the behavioral response is referred to as stimulus-independent behavioral resistance (Georghiou, 1972; Lockwood et al., 1984).

Behavioral resistance leads to reduced exposure to insecticides and may minimize selection for physiological insecticide resistance (i.e., insecticide resistance in its strict sense) (Gould, 1984; Suiter & Gould, 1994; Jallow & Hoy, 2005). Behavioral resistance to insecticides has been studied in few arthropod species and correlational studies between physiological and behavioral resistance were seldom carried out (Moore, 1977; Lockwood et al., 1984; Moore et al., 1989; Ross, 1993;

Suiter & Gould, 1994). The behavioral pattern considered and its potential dose-dependence relationship makes such correlations difficult to establish and the importance of the behavioral resistance remains largely unrecognized.

Locomotion, either by flight or walk, plays a unique role in determining insecticide exposure. Animals use their freedom of movement to better adapt to their living conditions (Martin & Bateson, 1993). The locomotory activity is linked to and express a synthesis of the animal's physiological processes and its anatomical condition, whilst being central to many aspects of the more complex inter- and intraspecific interaction between animals (Bayley, 2002). Most animals express reactive locomotory responses to both abiotic and biotic environmental stimuli, which are certainly important in assessing hazards posed by environmental conditions, such insecticide-sprayed surfaces.

Animals may alter their physiology and behavior under stressful conditions to maximize their chance of survival (Wingfield, 2003). Stressful conditions resulting from intense and/or prolonged exposure to a given stimuli may lead to a locomotory response impacting the animal fitness (Baatrup & Bayley, 1993). Insecticides may stimulate or depress the general locomotory behavior of arthropods (Haynes, 1988). Such behavioral responses may vary in physiologically resistant populations of insect pests compromising their control, and should therefore be a concern for their management.

Insect pests of stored products frequently show insecticide resistance (e.g., Champ & Dyte, 1976; Badmin, 1990; Subramanyam & Hagstrum, 1996). Behavioral avoidance to insecticides has also been detected in these pest species and their behavioral resistance to insecticides is potentially important, but frequent neglected (Watson & Barson, 1996; Cox et al., 1997; Watson et al., 1997). Brazilian strains of the maize weevil *Sitophilus zemais* Motschulsky (Coleoptera: Curculionidae) show problems of pyrethroid resistance (Guedes et al., 1995; Ribeiro et al., 2003; Fragoso et al., 2003), but the behavioral correlates associated with such strains and their behavioral responses induced by insecticides have yet to be investigated. These were the objectives of the present investigation, which tested the prevalence of a potential negative association between physiological and behavioral resistance to pyrethroids. Different dose-dependent locomotory responses were expected among strains with behavioral resistance less likely to occur in physiologically resistant insects since they are able to withstand higher doses of insecticide. Such expectation is based on the expected relaxation in selection for physiological resistance with the reduced exposure determined by the behavioral resistance, as earlier suggested by Georghiou (1972).

Material and methods

Insects and chemicals

Three strains of *S. zeamais* were used in the present investigation, which were termed here as 'susceptible', 'resistant cost', and 'resistant no-cost'. The susceptible strain was collected in Sete Lagoas county and provided by the National Center of Maize and Sorghum of the Brazilian Agricultural Research Corporation (EMBRAPA Milho e Sorgo). This strain has been maintained for nearly 20 years without insecticide exposure and its susceptibility to pyrethroids and organophosphates is well known and periodically checked (Guedes et al., 1994, 1995; Fragoso et al., 2003; Ribeiro et al., 2003; Araújo et al., 2008).

Both insecticide-resistant strains are pyrethroid-resistant (> 100-fold resistant and subjected to periodical checks before and during the present study) (Guedes et al., 1994, 1995; Ribeiro et al., 2003; Oliveira et al., 2007; Araújo et al., 2008). The resistant no-cost strain was collected in Jacarezinho county (state of Paraná, Brazil) by the late 1980's and the resistant cost strain was collected in Juiz de Fora county (state of Minas Gerais, Brazil) by 1999 (Guedes et al., 1995; Fragoso et al., 2003). The resistant cost strain shows fitness disadvantage in the absence of pyrethroids, unlike the resistant no-cost strain from Jacarezinho (Fragoso et al., 2005; Oliveira et al., 2007). In addition, the insects from the resistant no-cost strain are heavier and show higher energy reserves than the susceptible and the resistant cost strains (Guedes et al., 2006; Oliveira et al., 2007). Both resistant populations share the same major pyrethroid resistance mechanism, altered target-site sensitivity, with secondary involvement of enhanced detoxification by glutathione-*S*-transferases (Guedes et al., 1995; Ribeiro et al., 2003; Fragoso et al., 2003, 2007).

All three insect strains 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). All reagents were purchased from Sigma-Aldrich Química Brasil (São Paulo), except technical grade deltamethrin (99% pure), which was purchased from ChemService (West Chester, PA, USA).

Flight take-off bioassays

Transparent plastic jars (15 cm diameter x 12 cm high) with glue-coated walls were used as experimental units for the flight take-off bioassays, adapting methods from Perez-Mendoza et al. (1999). A filter paper (Whatman no. 1) with dried deltamethrin residue (applied as a 3 ml solution using acetone as solvent and let dry for 30 min) was placed at the bottom of the jar. The bottom of the jar walls received a 2 cm high layer of Teflon® PTFE (DuPont, Paulínia, SP, Brazil) to prevent the insects from climbing them, the remaining of which was coated with sticky glue (Bio Controle, São Paulo, Brazil). Two hundred non-sexed adult insects (one to two weeks old) were released in the center of each jar, which were maintained at 28 ± 2°C for one hour for subsequent recording of the number of insects glued in the walls while taking-off for flight. Five replicates were used for each combination of strain and insecticide concentration (from 0.0, 0.042, 0.169, 0.42, 0.84 to 1.7 mg a.i./cm^2).

Walking behavior bioassays

Two behavioral bioassays were carried out in arenas either fully-treated or half-treated with deltamethrin dissolved in acetone (control treatments were treated with acetone only). Filter papers (Whatman no. 1) with dried deltamethrin residue (applied as 1 ml solution and let dry for 30 min and concentrations of 0, 0.16, 0.41, 0.81, 1.63 mg a.i./cm^2) were placed on Petri dishes (8.5 cm diameter). The inner walls of each Petri dish were coated with Teflon to prevent insects from escaping. Twenty arenas with individual insects were used for each combination of sex, strain and insecticide concentration in each behavioral bioassay (fully- and half-treated arenas), and no mortality was observed within the exposure time used for the behavioral bioassays.

The movement of each insect within the arena during the 30 min trial was recorded and digitally transferred to a computer using an automated video tracking system equipped with a CCD camera (Videomex-One, Columbus Instruments, Columbus, OH, USA). The video images of the arenas were maintained either undivided, for the behavioral bioassay with fully-treated arenas, or divided into two symmetrical zones – one untreated and the other treated with deltamethrin, for the behavioral bioassay with half-treated arenas. The parameters calculated for the fully-treated arenas were walking distance (cm), velocity (cm/s), time spent walking (i.e., walking time; s), time spent immobile (i.e., resting time; s), and time spent stationary while participating in non-forward motion (i.e., stationary time; s). For the half-treated arenas, these same parameters were calculated for each arena zone (i.e., untreated and deltamethrin-treated halves of the arena), in addition to the time spent on the deltamethrin-treated zone (i.e., visiting time).

Statistical analyses

All experiments were established following the completely random design. The results of the flight take-off bioassays were subjected to regression analysis with the doses of deltamethrin as independent variable. The models were tested from the simplest (linear and quadratic) to the alternative models of increasing complexity (non-linear peak models). The software TableCurve 2D was used to fit the regression lines (SPSS, 2000).The model selection was carried out based on simplicity, high F-values (and mean squares) and steep increase of R^2 with model complexity. Residue distribution was also checked for each analysis on TableCurve 2D to ascertain of parametric assumptions. The overall results for fully-treated arenas were subjected to a two-way (sex x strain) multivariate analysis of covariance with deltamethrin concentration as the covariate (PROC GLM with MANOVA statement; SAS Institute, 2002). Individual two-way analyses of covariance for each parameter were eventually carried out followed by Fisher's LSD test, if appropriate ($p < 0.05$) (PROC GLM; SAS Institute, 2002). The results for half-treated arenas were subjected to two distinct sets of analyses. First, the results of untreated x deltamethrin-treated zones of each sex and strain were contrasted using multivariate analyses of variance (PROC GLM with MANOVA statement; SAS Institute, 2002). Second, the results of the deltamethrin-treated half of the arenas were subjected to a two-way (sex x strain) multivariate analysis of covariance with deltamethrin concentration as covariate (PROC GLM with MANOVA statement; SAS Institute, 2002). As with the results for fully-treated arenas, individual analyses of covariance for each parameter assessed in the half-treated arenas were also carried out and followed by Fisher's LSD test, if appropriate ($p < 0.05$) (PROC GLM; SAS Institute, 2002).

Results

Flight take-off from deltamethrin-treated surface

The flight take-off from deltamethrin-treated surfaces was significantly different among strains of the maize weevil (Fig. 1). Flight take-off was concentration-independent for the resistant cost strain ($p > 0.05$), but concentration-dependent for both susceptible and resistant no-cost strains with the rate of insects taking-off from deltamethrin-treated surfaces sharply declining from 0 to 0.2 mg a.i./cm2 for the susceptible strain and steadily declining with insecticide concentration for the resistant no-cost strain. The rate of flight take-off was over 3-fold higher for the resistant cost strain compared with the resistant no-cost strain, while rate of flight take-off for the susceptible strain was intermediate between that of both pyrethroid-resistant strains.

Walking behavior in fully-treated arenas

The overall mobility parameters of the maize weevil strains on the surface treated with increasing deltamethrin concentrations differed for strains ($df_{num/den}$ = 10/610, Wilks' lambda = 0.8385, F = 5.61. $p < 0.0001$), sex ($df_{num/den}$ = 5/305, Wilks' lambda = 0.7289, F = 22.69. $p < 0.0001$), and the interaction strain x sex ($df_{num/den}$ = 10/610, Wilks' lambda = 0.7126, F = 11.26. $p < 0.0001$). Deltamethrin concentration and its interactions with strain and sex were not significant ($p > 0.05$) indicating that the walking behavior is characteristic of the sex and strain, but independent from the insecticide stimuli. Univariate analyses of covariance were therefore carried out for each parameter assessed to determine the main parameters affecting the overall mobility of the three strains. All of the parameters were significant, but there was no significant effect of deltamethrin concentration and its interactions in any of them. The interaction between sex and strain was significant for all parameters ($F_{2,309} > 9.94$, $p < 0.0002$) except resting time ($F_{2,309} = 1.57$, $p = 0.21$), where the effects of sex and strain alone were significant, regardless of each other ($F_{1,309} = 69.93$, $p < 0.0001$ and $F_{2,309} = 12.05$, $p < 0.0001$ respectively). Resting time was significantly higher for the resistant no-cost strain and similarly lower for the susceptible and resistant cost strains (Fig. 2a). Resting time was significantly higher for females rather than males regardless of the strain (Fig. 2b). The remaining mobility parameters showed little variation among females from different strains, which were significantly lower only for velocity and stationary time of the resistant cost strain. In contrast, the overall male mobility parameters were higher than the female parameters with the males from the resistant cost strain exhibiting higher mobility than the resistant no-cost males (Table 1). The susceptible males showed intermediate mobility between both resistant strains (Table 1) following a pattern similar to that observed for flight take-off.

Walking behavior in half-treated arenas

Untreated x deltamethrin-treated halves

The results obtained on both untreated and deltamethrin-treated halves of each arena were constrasted for each strain and sex using deltamethrin concentration as covariate. There was no significant overall difference between untreated and deltamethrin-treated halves of the arena, except for the males of the susceptible strain ($df_{num/den}$ = 5/68, Wilks' lambda = 0.8395, F = 2.60, p = 0.03). The univariate analysis of covariance carried out for each mobility parameter of males of the susceptible strain indicated significant differences only in resting time ($F_{1,72}$ = 9.85, p = 0.002) and visiting time ($F_{1,72}$ = 9.21, p = 0.003) between untreated and deltamethrin-treated halves of the arenas. Susceptible males exhibited higher resting and visiting time on the deltamethrin-treated surfaces (Fig. 3), spending therefore more time on the deltamethrin-treated surface and moving less on such surface than in its untreated portion.

Walking behavior on the deltamethrin-treated half of the arenas

The overall mobility parameters of the maize weevil strains on the deltamethrin-treated zone of half-treated arenas were significantly different for the sex-strain interaction. However, subsequent univariate analyses of covariance for each mobility parameter indicated significant differences only for velocity, where the interaction sex-strain was significant ($F_{2,249}$ = 3.64, p = 0.03). As in the determination for fully-treated arenas, female velocity was indistinguishable among strains and usually smaller than male velocity (except for the susceptible strain) (Table 2). Males from the resistant cost strain exhibited significantly higher velocity than males of the other two strains, which were undistinguishable (Table 2).

Discussion

An insect's chance of survival may be greatly increased if its behavior is modified to avoid insecticide-treated surfaces (Watson & Barson, 1996; Cox et al., 1997). Earlier studies on behavioral resistance to insecticides led to Georghiou's theoretical hypothesis of negative correlation between behavioral and physiological resistance to insecticides (Georghiou, 1972). Such divergent evolution would take place because the most insecticide-susceptible individuals within a population are more likely to show higher avoidance to the insecticide. Insecticide application for successive generations would therefore favor divergent selection with the more susceptible individuals being selected for higher avoidance.

The negative correlation predicted between behavioral and physiological resistance to insecticides was observed in several insect species, but the number of exceptions to this expectation is far from negligible (e.g., Lockwood et al., 1984; Suiter & Gould, 1993; Renou et al., 1997; Kongmee et al., 2004; Wang et al., 2004). The present results with maize weevil strains also challenge this hypothesis. The results of flight take-off and walking behavior on deltamethrin-treated surface do not seem related to physiological resistance to insecticides since the susceptible strain showed intermediate rates of flight take-off and walking behavior between the physiologically resistant strains, in contrast with the initial expectation of an inverse relationship between behavioral and physiological resistance to insecticides.

Behavioral resistance to the pyrethroid deltamethrin does exist in strains of the maize weevil since these strains differ in their exposure to this insecticide, trait which is apparently inheritable and maintained within each strain generation after generation. However, such behavioral resistance is not negatively correlated with physiological resistance to insecticides as earlier suggested by Georghiou (1972), nor is it positively correlated with physiological resistance to insecticides as alternatively suggested by Lockwood et al. (1984) in their proposed coevolution paradigm. Rather, the origin of the (locomotory) behavioral resistance to deltamethrin in strains of maize weevil is independent from physiological resistance to this compound, possibility earlier recognized by Chareonviriyaphap et al. (1997) while studying pesticide avoidance in mosquitoes. The strain and sex differences in locomotion observed in the maize weevil may result from differences in body mass among the strains and sex reported elsewhere, although strain differences in intermediate metabolism are also likely to be important (Guedes et al., 2006; Oliveira et al., 2007; Araújo et al., 2008). As previously reported for the walking activity of insecticide-resistant and susceptible strains of a related species, the granary weevil *Sitophilus granarius* (L.) (Surtees, 1966), heavier maize weevils showed lower rate of flight take-off (represented by the resistant no-cost insects), and lower mobility (represented by females and males particularly from the resistant no-cost strain), what may be important for the spread of insecticide resistance among populations of maize weevil.

Behavioral resistance to insecticides may be either stimulus-dependent or stimulus-independent (Georghiou, 1972; Gould, 1984; Lockwood et al., 1984). Irritability and repellence are regarded as two types of stimulus-dependent behavioral resistance to insecticides (i.e., require sensory stimulation), which has been relatively frequently reported in mosquitoes and other arthropods (e.g., Lockwood et al., 1985; Chareanviriyaphap et al., 1997; Renou et al., 1997; Kongmee et al., 2004; Wang et al., 2004; Jallow & Hoy, 2005; Muenworn et al., 2006; Pothikasikorn et al., 2007). In contrast, stimulus-independent behavioral resistance to insecticides has been object of little attention and only detected in malaria mosquitoes, but with important consequences for their management (Anonymous, 1958; Akiyana, 1973; Ribeiro & Janz, 1990).

The behavioral resistance expressed in the locomotory activity in strains of maize weevil was stimulus-independent because no sensory stimulation was required for its expression (as observed in the untreated surfaces), which was characteristic for each strain (and sex within the strain). In addition, the locomotion-based behavioral resistance in maize weevil strains was independent of the insecticide concentration except for the rate of flight take-off, which reduced with increased deltamethrin concentrations in two strains of maize weevil – a likely consequence of the harmful effect of this insecticide at these concentrations for these two strains. Therefore, the behavioral resistance to deltamethrin expressed in locomotory activity of maize weevil strains was stimulus-independent and largely concentration-independent as a result of the different genetic make-up of the strains allowing some of them to minimize insecticide exposure by their high rate of flight take-off and reduced mobility. These varied behavioral patterns of locomotion leading to behavioral resistance to insecticides are also important for the spread of physiological resistance to insecticides, which may co-exist with behavioral resistance in some strains. Furthermore, these behavioral patterns potentially lead to reduced insecticide exposure compromising the efficacy of such compounds in at least some insect populations and should therefore be considered in insecticide resistance surveys and management programs.

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Table 1 Walking distance, velocity, walking time and stationary time (i.e., time spent on non-forward motion) (± standard error) of both sexes from three strains of the maize weevil *Sitophilus zemais*, an insecticide-susceptible (Susceptible) and two insecticide-resistant strains (Resistant cost and Resistance no-cost), exposed to fully-treated arenas (data from acetone-treated and deltamethrin-treated arenas were pooled together since they did not differ by Fisher's F test from the respective analyses of variance at $p < 0.05$).

Mobility parameter	Sex	Strain		
		Susceptible	Resistant no-cost	Resistant cost
Walking distance (cm)	Female	421.32 ± 55.58 Ba*	411.65 ± 29.98 Aa	332.49 ± 27.32 Ba
	Male	657.28 ± 44.29 Ab	439.41 ± 30.87 Ac	861.78 ± 37.84 Aa
Walking time (s)	Female	585.98 ± 49.44 Ba	599.22 ± 29.98 Aa	577.54 ± 35.97 Ba
	Male	884.78 ± 38.83 Ab	685.70 ± 38.70 Ac	981.98 ± 30.35 Aa
Velocity (cm/s)	Female	0.621 ± 0.027 Ba	0.625 ± 0.016 Aa	0.550 ± 0.013 Bb
	Male	0.712 ± 0.023 Ab	0.637 ± 0.014 Ac	0.866 ± 0.019 Aa
Stationary time (s)	Female	572.46 ± 17.97 Aa	551.53 ± 11.50 Bab	513.40 ± 16.09 Bb
	Male	581.04 ± 22.92 Ab	626.47 ± 21.95 Ab	704.64 ± 17.65 Aa

*Means followed by the same low case letter in a row or the same capital letter in a column, for each mobility parameter, are not significantly different by Fisher's LSD test ($p < 0.05$).

Table 2 Velocity (± standard error) of insects from both sexes from three strains of the maize weevil *Sitophilus zeamais*, an insecticide-susceptible (Susceptible) and two insecticide-resistant strains (Resistant cost and Resistance no-cost), on the treated half of deltamethrin half-treated arenas (data from different insecticide concentrations were pooled together since they did not differ by Fisher's F test from the analysis of variance at $p < 0.05$).

Mobility parameter	Sex	Strain		
		Susceptible	Resistant no-cost	Resistant cost
Velocity (cm/s)	Female	0.676 ± 0.023 Aa*	0.662 ± 0.012 Ba	0.626 ± 0.018 Ba
	Male	0.743 ± 0.019 Ab	0.736 ± 0.014 Ab	0.818 ± 0.024 Aa

*Means followed by the same low case letter in a row, or the same capital letter in a column, are not significantly different by Fisher's LSD test ($p < 0.05$).

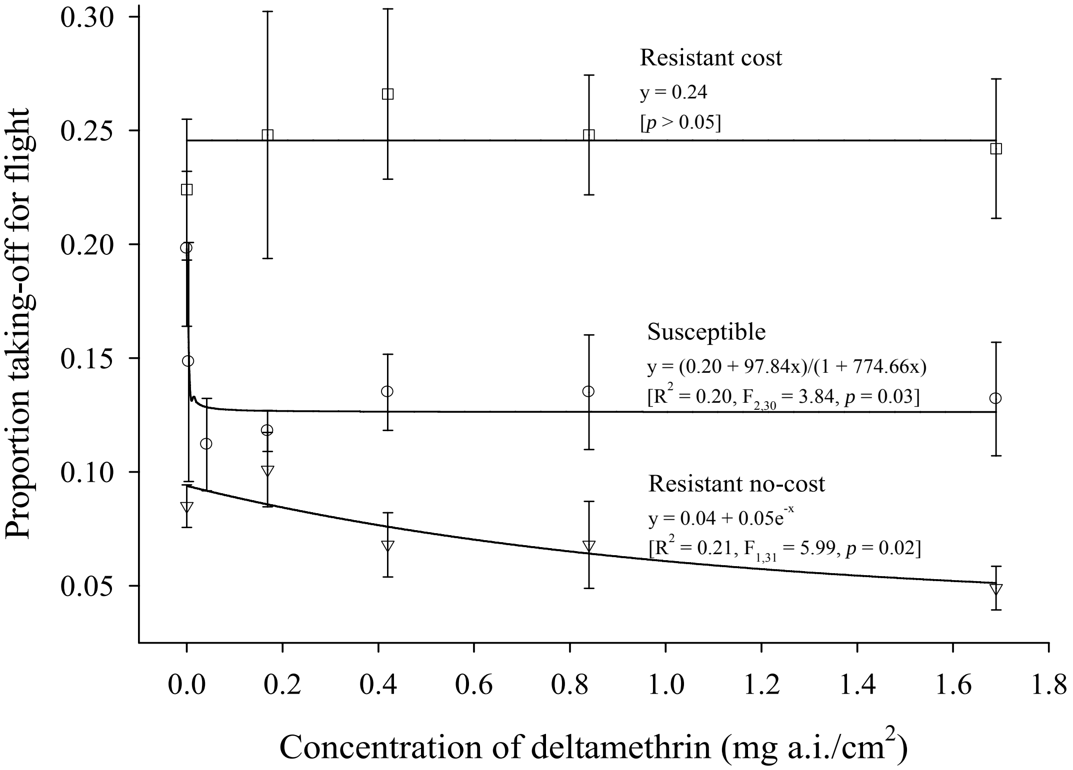
Figure captions

Figure 1 Proportion of adult insects from three strains of the maize weevil *Sitophilus zeamais*, an insecticide-susceptible (Susceptible (○)) and two insecticide-resistant strains (Resistant cost (□) and Resistance no-cost (▽)), taking-off for flight from deltamethrin-treated surfaces. Each symbol represents the mean of five replicates.

Figure 2 Resting time (i.e., time spent immobile) (± standard error) of three strains (a) and both sexes (b) of the maize weevil *Sitophilus zeamais* exposed to arenas fully treated with either solvent (acetone) or deltamethrin. Data from acetone-treated and deltamethrin-treated arenas were pooled together since they did not differ by Fisher's F test from the respective analyses of variance ($p < 0.05$). Histogram bars with the same letter do not significantly different by either Fisher`s LSD test ($p < 0.05$) (a) or Fisher's F test ($p < 0.05$) (b).

Figure 3 Resting time (a) and visiting time (b) (± standard error) of males from an insecticide-susceptible strain of the maize weevil *Sitophilus zeamais* on each half of arenas half-treated with deltamethrin. Histogram bars with the same letter do not significantly different by Fisher`s F test ($p < 0.05$).

Fig. 1



Resistant cost
y = 0.24
[p > 0.05]
Susceptible
y = (0.20 + 97.84x)/(1 + 774.66x)
[R² = 0.20, F2,30 = 3.84, p = 0.03]
Resistant no-cost
y = 0.04 + 0.05e⁻ˣ
[R² = 0.21, F1,31 = 5.99, p = 0.02]
Proportion taking-off for flight
0.30
0.25
0.20
0.15
0.10
0.05
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Concentration of deltamethrin (mg a.i./cm²)

Fig. 2

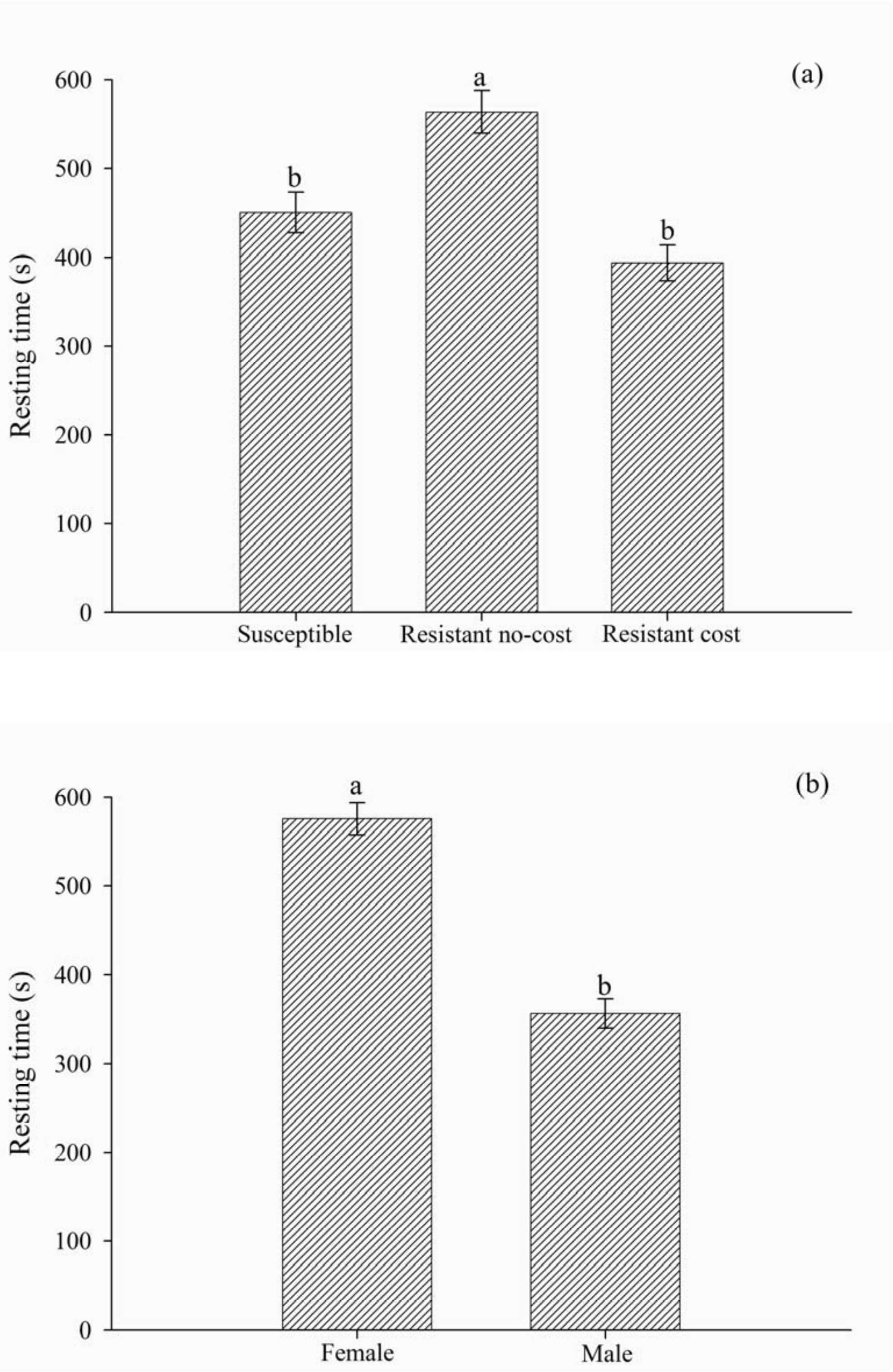
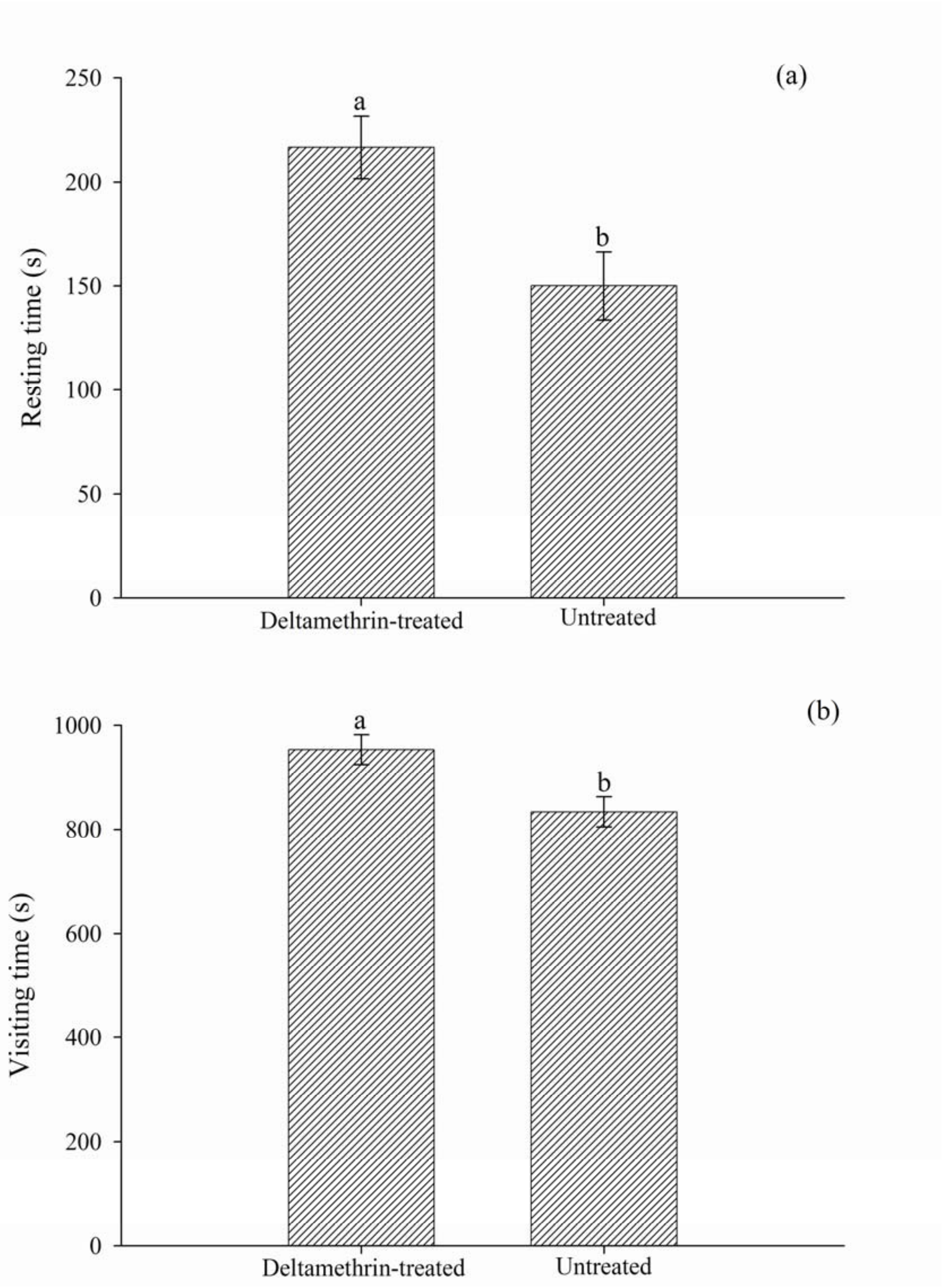

(a)
Resting time (s)
0
100
200
300
400
500
600
b
a
b
Susceptible
Resistant no-cost
Resistant cost
(b)
Resting time (s)
0
100
200
300
400
500
600
a
b
Female
Male

Fig. 3


(a)
Resting time (s)
250
200
150
100
50
0
a
b
Deltamethrin-treated
Untreated
(b)
Visiting time (s)
1000
800
600
400
200
0
a
b
Deltamethrin-treated
Untreated

CONSIDERAÇÕES FINAIS

A competição é particularmente um problema para os insetos cujo estágio larval ocorre dentro de um grão. Este é um exemplo de um sistema fechado em que insetos imaturos são incapazes de evitar competição com co-específicos se múltiplos ovos são colocados em uma mesma semente. A competição larval apresenta duas estratégias distintas de competição – acomodação ou ataque, o que depende a linhagem de inseto e do tipo da semente. Vários trabalhos foram feitos mostrando a competição larval dentro do grão em *Callosobruchus* spp., mas nenhum com *Sitophilus zeamais*. Aqui nós apresentamos resultados que abrangem desde o processo comportamental ao resultado da competição em duas linhagens do caruncho-do-milho (susceptível e resistente a deltametrina) e, também, mostramos uma descrição detalhada, com o uso de raio-x digital, do comportamento larval de *S. zeamais* dentro do grão de milho sendo que o processo de competição adotado pelas duas linhagens é do tipo ataque.

A alimentação de um inseto é considerada como o resultado de uma seqüência de eventos comportamentais. Doses subletais de piretróides afetando o comportamento alimentar de *S. zeamais*, no sentido do inseto evitar ou ingerir menor quantidade de alimento, podem comprometer a sua sobrevivência e produção de progênie já que a alimentação é fundamental para a sua capacidade de locomoção e reprodução. A plasticidade alimentar de um inseto é um componente da plasticidade fenotípica que geralmente não é considerada em estudos sobre linhagens adaptadas ao ambiente modificado por inseticidas, fato este que pode tanto acentuar ou minimizar a resistência a inseticidas. As linhagens fisiologicamente resistentes a deltametrina apresentaram resistência comportamental a este composto já que houve maior repelência por parte delas quando expostas a grãos tratados com este inseticida. Aliado a este resultado, observou-se ainda, baixos níveis de deterrência alimentar nas linhagens resistentes, quando comparadas à susceptível, e uma maior taxa de crescimento relativo e eficiência na conversão alimentar, mesmo na ausência da deltametrina – uma preocupação a mais para o manejo dessas linhagens.

Certos inseticidas estimulam ou diminuem a locomoção do inseto através do caminhamento ou vôo. A locomoção tem uma função determinante com relação a exposição a inseticidas e por esta razão foram feitos experimentos observando a mobilidade das mesmas linhagens resistentes e susceptíveis do caruncho-do-milho, quando expostas a superfície tratada com deltametrina. No experimento de decolagem, houve dependência da dose em duas linhagens de *S. zeamais*, exceto uma linhagem resistente, enquanto as respostas do caminhamento foram independentes da dose em todas as linhagens. A mobilidade das fêmeas foi semelhante em todas as linhagens, diferentemente do que ocorreu com a mobilidade dos machos.

Alguns insetos são capazes de mudar o seu comportamento em resposta a sua percepção sensorial do inseticida o que aumenta a capacidade da população em evitar os efeitos letais do inseticida. Esta mudança de comportamento pode, às vezes, diminuir a eficácia do tratamento com o inseticida e, assim, ensaios padrões para efeitos letais podem superestimar o impacto de um inseticida na população.