

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

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Abstract

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. This is particularly serious for insect pests that are physiologically resistant to insecticides since insecticide avoidance may further compromise their management. Locomotion plays a major role determining insecticide exposure and was, therefore, considered in investigating the behavioral responses of male and female adult insects from 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. The behavioral responses to deltamethrin-sprayed surfaces differed among the maize weevil strains. Such responses were concentration-independent for all of the strains. Stimulus-independent behavioral resistance was unrelated to 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.

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

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Introduction

Insects have evolved a variety of physiological and behavioral responses to various toxins in natural and managed ecosystems (Jallow & Hoy, 2005; Li *et al.*, 2007). These varied responses can reflect the toxin mode of action and the extent to which they influence behavior (Hoy *et al.*, 1998). Some insects are capable of changing their behavior in response to their sensory perception of insecticides (Gould, 1984; Lockwood *et al.*, 1984; Haynes, 1988; Desneux *et al.*, 2007). These behavioral changes may sometimes compromise tests of insecticide efficacy against insect pests; and, consequently, standard assays based on lethality can either overestimate or underestimate the insecticide impact in a population (Moore *et al.*, 1989; Cox *et al.*, 1997; Bayley, 2002). Such limitation is particularly serious for insect pest species with insecticide-resistant populations.

Insecticide studies usually focus on the insecticide lethality or other direct effects of insecticides, whereas relatively little attention is placed on the sublethal behavioral response to insecticidal exposure, particularly in insect pests and in contrast with natural enemies (Kongmee *et al.*, 2004; Desneux *et al.*, 2007). 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 repellence, 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, such as exophily in mosquitos (Anonymous, 1957; Georghiou, 1972; Lockwood *et al.*, 1984). They are both recognized as behavioral resistance to toxic compounds if such traits are inheritable (Georghiou, 1972; Lockwood *et al.*, 1984) and will be used here within the same context, although insecticide resistance may also be more restrictively defined as the result of past insecticide selection. If the toxic compound involved is an insecticide and the behavioral trait is enhanced as stimulus-dependent in a population, it is referred to 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 walking, uniquely determines insecticide exposure by governing the time that animals spend in contact with insecticide. Animals use their

freedom of movement to better adapt to their living conditions (Martin & Bateson, 1993). The locomotory activity is linked to and expresses 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 interactions 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 as insecticide-sprayed surfaces.

Physiological and behavioral responses of animals may change 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; Desneux *et al.*, 2007). Such behavioral responses may vary in physiologically resistant populations of insect pests, further compromising their control, and should, therefore, be a concern for their management (Georghiou, 1972; Gould, 1984; Lockwood *et al.*, 1984).

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 (Watson & Barson, 1996; Cox *et al.*, 1997; Watson *et al.*, 1997). Brazilian strains of the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) show problems of pyrethroid resistance (Guedes *et al.*, 1995; Fragoso *et al.*, 2003; Ribeiro *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 hypothesis 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 & 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 1980s, 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 due to its slower development and reduced progeny production 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; Fragoso *et al.*, 2003, 2007; Ribeiro *et al.*, 2003).

All three insect strains were maintained in whole maize grains free of insecticides under controlled temperature ($25 \pm 2^\circ\text{C}$), relative humidity ($70 \pm 5\%$) and photoperiod (LD 12:12). Acetone was purchased from Cromato Prod. Quím. (Diadema, SP, Brazil) and technical grade deltamethrin (99% pure) was purchased from ChemService (West Chester, PA, USA).

Flight take-off bioassays

Transparent plastic jars (15 cm diameter \times 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 left to 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 $28 \pm 2^\circ\text{C}$ for one hour for subsequent recording of the number of insects glued to the walls while taking-off for flight. Five replicates were used for each combination of strain and insecticide concentration (0.0, 0.004, 0.008, 0.04, 0.17, 0.42 and $0.85 \text{ mg a.i. cm}^{-2}$). These deltamethrin concentrations are in the sublethal range for the strains, and they were selected based on preliminary bioassays and the lowest observed effect concentration (LOEC) established for the susceptible strain ($1.0 \text{ mg a.i. cm}^{-2}$).

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 left to dry for 30 min and concentrations of 0, 0.04, 0.16, 0.39, $0.79 \text{ mg a.i. cm}^{-2}$) were placed on Petri dishes (9.0 cm diameter). The range of deltamethrin concentrations used was established as described above. The inner walls of each Petri dish were coated with Teflon to prevent the 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^{-1}), time spent walking (s), time spent immobile (i.e. resting time (s)), and time spent stationary while participating in non-forward motion (i.e. stationary time (s)). Stationary time was distinguished based on the presence of insect movement, but lack of horizontal body displacement, unlike when either walking (i.e. with horizontal body displacement) or resting (i.e. without any movement). For the half-treated arenas, these same parameters were calculated for each arena zone (i.e. untreated and deltamethrin-treated zones) in addition to the time spent on the deltamethrin-treated zone (i.e. visiting time).

Statistical analyses

The results of the flight take-off bioassays were subjected to analysis of covariance with deltamethrin concentration as covariate (PROC GLM: SAS Institute, 2002). The overall results for fully-treated arenas were subjected to a two-way (sex \times 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 \times deltamethrin-treated zones of each sex and strain were contrasted using multivariate analyses of covariance with deltamethrin concentration as covariate (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 \times strain) multivariate analysis of covariance, again 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 differed among strains of the maize weevil ($F_{2,66} = 51.36$, $P < 0.0001$), but the differences among deltamethrin concentrations and the interaction strain-deltamethrin concentration were not significant ($F_{6,66} = 0.49$, $P = 0.81$ and $F_{8,66} = 0.88$, $P = 0.54$, respectively). The rate of flight take-off was over threefold higher for the resistant cost strain compared

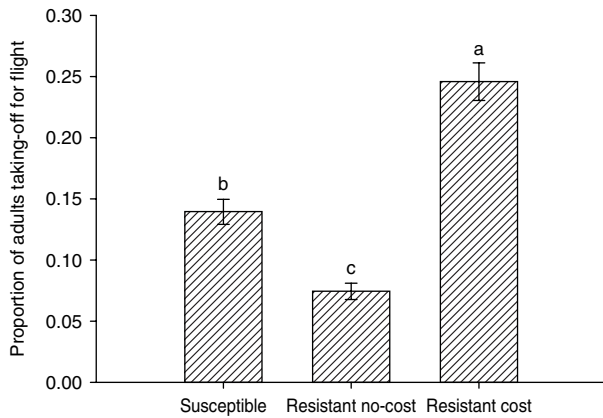


Fig. 1. Proportion of adult insects (\pm SE) from three strains of the maize weevil *Sitophilus zeamais* taking-off for flight from deltamethrin-treated surfaces. Histogram bars with the same letter do not significantly differ by Fisher's LSD test ($P < 0.05$).

with the resistant no-cost strain, while the rate of flight take-off for the susceptible strain was intermediate between that of both pyrethroid-resistant strains, regardless of the deltamethrin concentration to which they were exposed (fig. 1).

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 ($df = 10, 610$, Wilks' lambda = 0.8385, $F = 5.61$, $P < 0.0001$), sex ($df = 5, 305$, Wilks' lambda = 0.7289, $F = 22.69$, $P < 0.0001$) and the interaction strain \times sex ($df = 10, 610$, Wilks' lambda = 0.7126, $F = 11.26$, $P < 0.0001$). Deltamethrin concentration ($df = 20, 1012.5$, Wilks' lambda = 0.9236, $F = 1.23$, $P = 0.22$) and its interactions with strain ($df = 40, 1332.3$, Wilks' lambda = 0.8824, $F = 0.95$, $P = 0.53$), sex ($df = 20, 1012.5$, Wilks' lambda = 0.9003, $F = 1.60$, $P = 0.06$) and both ($df = 40, 1332.3$, Wilks' lambda = 0.8923, $F = 0.88$, $P = 0.68$) were not significant, indicating that the walking behavior is characteristic of the sex and strain but independent from the insecticide stimuli for the range of sublethal insecticide concentrations tested. Univariate analyses of covariance were, therefore, carried out for each parameter measured. There were significant differences for all of the parameters, but there was no significant effect of deltamethrin concentration and its interactions for 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 (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

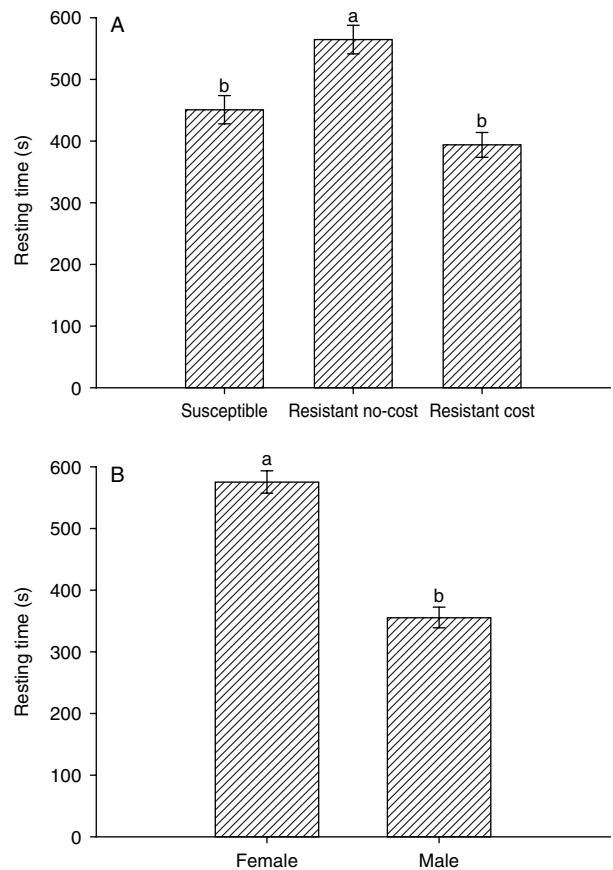


Fig. 2. Resting time (i.e. time spent immobile) (\pm SE) of (A) three strains and (B) both sexes of the maize weevil *Sitophilus zeamais* exposed to arenas fully treated with either solvent (acetone) or deltamethrin. Data from acetone-treated and deltamethrin-treated arenas were pooled together since they did not differ by Fisher's F-test from the respective analyses of covariance ($P < 0.05$). Histogram bars with the same letter do not significantly differ by either (a) Fisher's LSD test ($P < 0.05$) or (b) Fisher's F-test ($P < 0.05$).

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 \times deltamethrin-treated halves

The results obtained on both untreated and deltamethrin-treated zones of each arena were contrasted for each strain and sex using deltamethrin concentration as covariate. There was no significant overall difference between untreated and deltamethrin-treated zones of the arena, except for the males of the susceptible strain ($df = 5, 68$, 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 zones

Table 1. Walking distance, velocity, walking time and stationary time (i.e. time spent on non-forward motion) (\pm SE) of both sexes from three strains of the maize weevil *Sitophilus zeamais*, one 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 \pm 55.58 Ba*	411.65 \pm 29.98 Aa	332.49 \pm 27.32 Ba
	Male	657.28 \pm 44.29 Ab	439.41 \pm 30.87 Ac	861.78 \pm 37.84 Aa
Walking time (s)	Female	585.98 \pm 49.44 Ba	599.22 \pm 29.98 Aa	577.54 \pm 35.97 Ba
	Male	884.78 \pm 38.83 Ab	685.70 \pm 38.70 Ac	981.98 \pm 30.35 Aa
Velocity (cm s ⁻¹)	Female	0.621 \pm 0.027 Ba	0.625 \pm 0.016 Aa	0.550 \pm 0.013 Bb
	Male	0.712 \pm 0.023 Ab	0.637 \pm 0.014 Ac	0.866 \pm 0.019 Aa
Stationary time (s)	Female	572.46 \pm 17.97 Aa	551.53 \pm 11.50 Bab	513.40 \pm 16.09 Bb
	Male	581.04 \pm 22.92 Ab	626.47 \pm 21.95 Ab	704.64 \pm 17.65 Aa

* Means followed by the same lower 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 ($P < 0.05$).

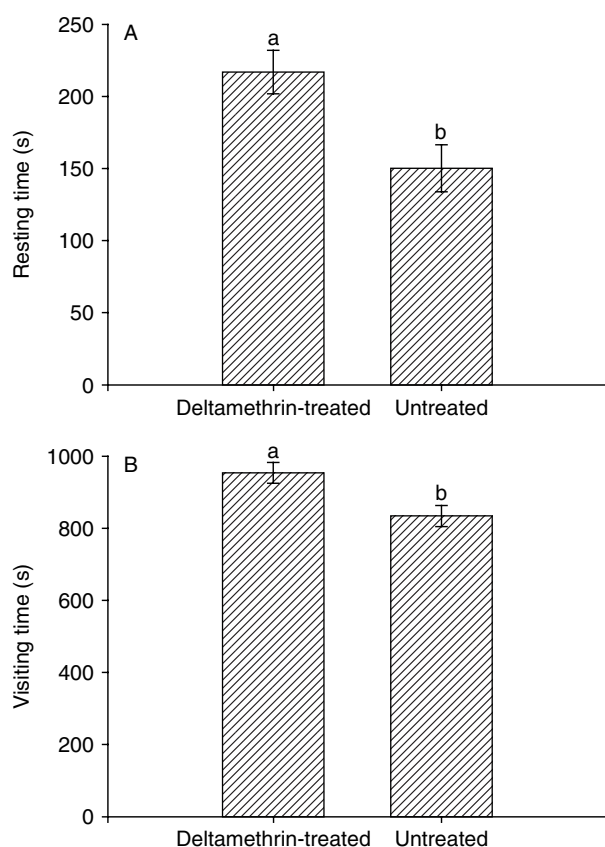


Fig. 3. (A) Resting time and (B) visiting time (\pm SE) of males from an insecticide-susceptible strain of the maize weevil *Sitophilus zeamais* on each half of arenas half-treated with deltamethrin. Histogram bars with the same letter do not significantly differ by Fisher's F-test ($P < 0.05$).

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 a surface than on 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 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, 1994; 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 surfaces 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.

Table 2. Velocity (\pm SE) 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 ⁻¹)	Female	0.676 \pm 0.023 Aa*	0.662 \pm 0.012 Ba	0.626 \pm 0.018 Ba
	Male	0.743 \pm 0.019 Ab	0.736 \pm 0.014 Ab	0.818 \pm 0.024 Aa

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

Stimulus-independent behavioral resistance to the pyrethroid deltamethrin does exist in strains of the maize weevil since these strains differ in their exposure to this insecticide regardless of the presence of insecticide. The trait 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). Rather, the origin of the (locomotory) behavioral resistance in strains of maize weevil is independent from physiological resistance to this compound, a 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 (Guedes *et al.*, 2006; Oliveira *et al.*, 2007), since the heavier strain (i.e. resistant no-cost) and sex (i.e. female) exhibited lower levels of locomotion. However, 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 (females and males particularly from the resistant no-cost strain are heavier). Such lower rate of flight take-off and mobility reduces the gene flow among populations increasing the probability of within-strain mating and may be important for the spread of insecticide resistance among populations of maize weevil (Georghiou, 1972; Gould, 1984).

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; Wang *et al.*, 2004; Muenworn *et al.*, 2006). In contrast, stimulus-independent behavioral resistance to insecticides has been the object of little attention and only detected in malaria mosquitoes, but with important consequences for their management (Anonymous, 1958; Akiyama, 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 low (sublethal) insecticide concentrations. Therefore, the behavioral resistance expressed in locomotory activity of maize weevil strains was stimulus-independent and 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.

The varied behavioral patterns of locomotion leading to stimulus-independent 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.

The stimulus-independent behavioral resistance to deltamethrin was expressed at sublethal insecticide concentrations, which are found in storage facilities after insecticide spraying and the start of the insecticide degradation. Such low residue levels are unlikely to interfere with the regular behavior of the exposed insects, but the diverse (and innate) locomotory behavior among strains will affect insect exposure and should be considered in behaviorally-targeted management strategies (i.e. Push-Pull strategies (Cook *et al.*, 2007)). At higher residue levels of deltamethrin, concentration-dependent behavioral resistance may also exist, which deserves further studies and does also have potential impact on the maize weevil management.

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References

- Akiyama, J.** (1973) Exophily in *Anopheles gambiae* species B in Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 440.
- Anonymous** (1958) Exophily in anophelines and malaria control. *WHO Chronicle* **12**, 81–82.
- Araújo, R.A., Guedes, R.N.C., Oliveira, M.G.A. & Ferreira, G.H.** (2008) Enhanced activity of carbohydrate- and lipid-metabolizing enzymes in insecticide-resistant populations of the maize weevil, *Sitophilus zeamais*. *Bulletin of Entomological Research* **98**, 417–424.
- Baatrup, E. & Bayley, M.** (1993) Quantitative analysis of spider locomotion employing computer-automated video tracking. *Physiology and Behavior* **54**, 83–90.
- Badmin, J.S.** (1990) IRAC survey of resistance of stored grain pests: results and progress. pp. 973–981 in Fleurrat-Lessard, F. & Ducom, P. (Eds) *Proceedings of the 5th International Working Conference on Stored Product Protection*. Bordeaux, France, INRA/SDPV.
- Bayley, M.** (2002) Basic behavior: the use of animal locomotion in behavioural ecotoxicology. pp. 211–230 in Dell’Omo, G. (Ed.) *Behavioural Ecotoxicology*. Chichester, UK, John Wiley & Sons.
- Champ, B.R. & Dyte, C.E.** (1976) *FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests*. 297 pp. Rome, FAO/UN.
- Chareonviriyaphap, T., Roberts, D.R., Andre, R.G., Harlan, H. & Bangs, M.J.** (1997) Pesticide avoidance behavior in *Anopheles albimanus* Wiedmann. *Journal of the American Mosquito Control Association* **13**, 171–183.
- Cook, S.M., Khan, Z.R. & Pickett, J.A.** (2007) The use of Push-Pull strategies in integrated pest management. *Annual Review of Entomology* **52**, 375–400.
- Cox, P.D., Fleming, D.A., Atkinson, J.E., Bannon, K.L. & Whitefield, J.M.** (1997) The effect of behaviour on the survival of *Cryptolestes ferrugineus* in an insecticide-treated laboratory environment. *Journal of Stored Products Research* **33**, 257–269.
- Desneux, N., Decourtye, A. & Delpuech, J.-M.** (2007) The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology* **52**, 81–206.
- Fragoso, D.B., Guedes, R.N.C. & Rezende, S.T.** (2003) Glutathionid S-transferase detoxification as a potential pyrethroid resistance mechanism in the maize weevil, *Sitophilus zeamais*. *Entomologia Experimentalis et Applicata* **109**, 21–29.
- Fragoso, D.B., Guedes, R.N.C. & Peternelli, L.A.** (2005) Developmental rates and population growth of insecticide-resistant and susceptible populations of *Sitophilus zeamais*. *Journal of Stored Products Research* **41**, 271–281.
- Fragoso, D.B., Guedes, R.N.C. & Oliveira, M.G.A.** (2007) Partial characterization of glutathione S-transferases in pyrethroid-resistant and -susceptible populations of the maize weevil, *Sitophilus zeamais*. *Journal of Stored Products Research* **43**, 167–170.
- Georghiou, G.P.** (1972) The evolution of resistance to pesticides. *Annual Review of Ecology and Systematics* **3**, 133–168.
- Gould, F.** (1984) Role of behavior in the evolution of insect adaptation to insecticides and resistant host plants. *Bulletin of the Entomological Society of America* **30**, 34–40.
- Guedes, R.N.C., Lima, J.O.G., Santos, J.P. & Cruz, C.D.** (1994) Inheritance of deltamethrin resistance in a Brazilian strain of maize weevil (*Sitophilus zeamais* Mots.). *International Journal of Pest Management* **40**, 103–106.
- Guedes, R.N.C., Lima, J.O.G., Santos, J.P. & Cruz, C.D.** (1995) Resistance to DDT and pyrethroids in Brazilian populations of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). *Journal of Stored Products Research* **31**, 145–150.
- Guedes, R.N.C., Oliveira, E.E., Guedes, N.M.P., Ribeiro, B. & Serrão, J.E.** (2006) Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. *Physiological Entomology* **31**, 145–150.
- Haynes, K.F.** (1988) Sublethal effects of neurotoxic insecticides on insect behavior. *Annual Review of Entomology* **33**, 149–168.
- Hoy, C.W., Head, G.P. & Hall, F.R.** (1998) Spatial heterogeneity and insect adaptation to toxins. *Annual Review of Entomology* **43**, 571–594.
- Jallow, M.F.A. & Hoy, C.W.** (2005) Phenotypic variation in adult behavioral response and offspring fitness in *Plutella xylostella* (Lepidoptera: Plutellidae) in response to permethrin. *Journal of Economic Entomology* **98**, 2195–2202.
- Kongmee, M., Prabaripai, A., Akratanakul, P., Bangsm, M.J. & Chareonviriyaphap, T.** (2004) Behavioral responses of *Aedes aegypti* (Diptera: Culicidae) exposed to deltamethrin and possible implications for disease control. *Journal of Medical Entomology* **41**, 1055–1063.
- Li, X., Schuler, M.A. & Berenbaum, M.R.** (2007) Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology* **52**, 231–253.
- Lockwood, J.A., Sparks, T.C. & Story, R.N.** (1984) Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior. *Bulletin of Entomological Society of America* **30**, 41–51.
- Lockwood, J.A., Byford, R.L., Story, R.N., Sparks, T.C. & Quisenberry, S.S.** (1985) Behavioral resistance to the pyrethroids in the horn fly, *Hematiobia irritans* (Diptera: Muscidae). *Environmental Entomology* **14**, 873–880.
- Martin, P. & Bateson, P.** (1993) *Measuring Behaviour*. 2nd edn. 222 pp. Cambridge, UK, Cambridge University Press.
- Moore, A., Tabashnik, B.E. & Stark, J.D.** (1989) Leg autotomy: a novel mechanism of protection against insecticide poisoning in the diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* **82**, 1295–1298.
- Moore, C.G.** (1977) Insecticide avoidance by ovipositing *Aedes aegypti*. *Mosquito News* **37**, 291–293.
- Muenworn, V., Akaratanakul, P., Bangs, M.J., Parbaripai, A. & Chareonviriyaphap, T.** (2006) Insecticide-induced behavioral responses in two populations of *Anopheles maculates* and *Anopheles sawadwongporni*, malaria vectors in Thailand. *Journal of the American Mosquito Control Association* **22**, 689–698.
- Oliveira, E.E., Guedes, R.N.C., Tótola, M.R. & De Marco, P. Jr.** (2007) Competition between insecticide-susceptible and -resistant populations of the maize weevil, *Sitophilus zeamais*. *Chemosphere* **67**, 17–24.
- Perez-Mendoza, J., Dover, B.A., Hagstrum, D.W. & Hopkins, T.L.** (1999) Effect of crowding, food deprivation, and diet on flight initiation and lipid reserves of the lesser grain borer, *Rhyzopertha dominica*. *Entomologia Experimentalis et Applicata* **91**, 317–326.
- Renou, M., Henninot-Rodes, E., Delorme, R., Augé, D. & Touton, P.** (1997) Oviposition of resistance and susceptible strains of *Drosophila melanogaster* in the presence of deltamethrin. *Entomologia Experimentalis et Applicata* **84**, 173–181.
- Ribeiro, H. & Janz, J.G.** (1990) Exophagy and exophily in malaria vectors. *Bulletin of the Society of Vector Ecologists* **15**, 185–188.

- Ribeiro, B.M., Guedes, R.N.C., Oliveira, E.E. & Santos, J.P.** (2003) Insecticide resistance and synergism in Brazilian populations of *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Stored Products Research* **39**, 21–31.
- Ross, M.H.** (1993) Laboratory studies on the response of German cockroaches (Dictyoptera: Blattellidae) to an abamectin gel bait. *Journal of Economic Entomology* **86**, 767–771.
- SAS Institute** (2002) SAS/STAT User's Guide. Cary, USA, SAS Institute.
- Subramanyam, Bh. & Hagstrum, D.W.** (1996) Resistance measurement and management. pp. 331–397 in Subramanyam, Bh. & Hagstrum, D.W. (Eds) *Integrated Management of Insects in Stored Products*. New York, Marcel Dekker.
- Suiter, K.A. & Gould, F.** (1994) Physiological and behavioral avoidance responses to residues of four pesticides by six spider mite populations. *Entomologia Experimentalis et Applicata* **71**, 1–14.
- Surtees, G.** (1966) Locomotory behavior of pyrethrum-resistant and susceptible strains of grain weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Animal Behaviour* **14**, 201–203.
- Wang, C., Scharf, M.E. & Bennett, G.W.** (2004) Behavioral and physiological resistance of the German cockroach to gel baits (Blattodea: Blattellidae). *Journal of Economic Entomology* **97**, 2067–2072.
- Watson, E. & Barson, G.** (1996) A laboratory assessment of the behavioural responses of three strains of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) to three insecticides and the insect repellent N,N-diethyl-m-toluamide. *Journal of Stored Products Research* **32**, 59–67.
- Watson, E., Barson, G., Pinniger, D.B., Roberts, G. & Ludlow, A.R.** (1997) Evaluation of the behavioural responses of *Anthrenus verbasci* adults and larvae to permethrin (ec) using a computerized tracking system. *Journal of Stored Products Research* **33**, 335–346.
- Wingfield, J.C.** (2003) Control of behavioural strategies for capricious environments. *Animal Behaviour* **66**, 807–816.