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Deltamethrin-induced behavioral responses of pyrethroid-resistant and -susceptible populations of the maize weevil (*Sitophilus zeamais*)

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Abstract

Three populations of the maize weevil (*Sitophilus zeamais*), two resistant and another susceptible to pyrethroids, were used to assess behavioral responses induced by deltamethrin exposure. The behavioral responses targeted in the present study were the discrimination of deltamethrin-sprayed maize grains and flight take-off from deltamethrin-treated surfaces. The hypotheses under test with these experiments were – the prevalence of a negative correlation between behavioral and physiological resistance to insecticides or the independence between them. The experiment of discrimination of deltamethrin-sprayed grains indicated that the resistant populations were choosier with a higher proportion of individuals avoiding the sprayed grains than the susceptible population – an evidence that the resistant populations show not only physiological resistance to deltamethrin, but also behavioral resistance to this compound avoiding exposure to it. The response observed was dose-dependent. The results of the flight take-off experiment were different. The susceptible population showed a take-off rate intermediate between both resistant populations. These results indicate independence between behavioral and physiological resistance in contrast with the results from the discrimination of sprayed grains, what deserves further attention.

Key words: Behavioral resistance, insecticide, food discrimination, flight take-off.

Introduction

Insects have evolved a variety of physiological and behavioral responses to various toxins in natural and managed systems (Jallow and 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). Insects may survive insecticide applications by physiological mechanisms, which allow them to cope with high insecticide exposure, or by behavioral mechanisms, which reduce the insecticide exposure (Gould, 1984; Hoy et al., 1998; Jallow and Hoy, 2005).

Insecticide studies usually focus on the direct physiological effects of insecticides, whereas relatively little attention is placed on the behavioral response to exposure (Kongmee et al., 2004). The general behavioral responses of insects to insecticides fall into two broad categories – stimulus-dependent and stimulus-independent avoidance (Georghiou, 1972). The first category, stimulus-dependent avoidance (e.g., irritability, repellency), 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

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detection. The second category, stimulus-independent avoidance (e.g., exophily, zoophily), refers to behavioral patterns that prevent exposure to the toxic substance, in contrast to stimulus-dependent avoidance where contact usually takes place (Georghiou, 1972; Gould, 1984). If the toxic compound involved is an insecticide and the behavioral trait is enhanced by selection, these categories are referred as stimulus-dependent and –independent resistance (to the insecticide), encompassing what is also known as behavioral insecticide resistance (Georghiou, 1972; Gould, 1984; Lockwood et al., 1984; Wang et al., 2004).

Behavioral resistance leads to reduced exposure to insecticides and may minimize selection for physiological resistance (i.e., insecticide resistance in its strict sense) (Gould, 1984; Suiter and Gould, 1994; Jallow and Hoy, 2005). Behavioral resistance has been studied in a few species, including two-spotted spider mites (*Tetranychus urticae*), horn flies (*Haematobia irritans*), diamondback moth (*Plutella xylostella*), German cockroach (*Blatella germanica*) and yellowfever mosquito (*Aedes aegypti*) (Moore, 1977; Lockwood *et al.*, 1984; Moore *et al.*, 1989; Ross, 1993; Suiter and Gould, 1994). Correlational studies between physiological resistance and behavioral resistance were seldom carried out and consistent (e.g., Suiter and Gould, 1984). The behavioral pattern considered and its dose-dependence may make such correlations difficult to establish and the importance of the behavioral resistance remains largely unrecognized.

Both physiological and behavioral resistance may compromise insect control and their relationship should be considered when designing management programs. Insect pests of stored products frequently show insecticide resistance (e.g., Subramanyam and Hagstrum, 1996). Behavioral avoidance to insecticides has also been detected in these pest species and behavioral resistance to insecticides is likely to be important, but frequently neglected (Watson and Barson, 1996; Cox et al., 1997; Watson et al., 1997). Brazilian populations of the maize weevil *Sitophilus zeamais* (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 populations and their behavioral responses induced by insecticides have yet to be investigated. These were the objectives of the present investigation. The hypothesis under test is the prevalence of a negative correlation between physiological resistance to pyrethroids and behavioral resistance, what is expected 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 Brazilian populations of *S. zeamais* were used in this study, one susceptible (Sete Lagoas) and two resistant to pyrethroids (Jacarezinho and Juiz de Fora). The standard susceptible population was obtained from the Embrapa Milho e Sorgo (Sete Lagoas, MG, Brazil), where it has been maintained for over 15 years without insecticide exposure and its susceptibility to pyrethroids and organophosphates is known (Guedes et al., 1994, 1995; Ribeiro et al., 2003). The second population is resistant to DDT and pyrethroids and was collected from infested maize in Jacarezinho County (State of Paraná, Brazil) in the early 1980s; it shows high resistance levels to pyrethroids (Guedes et al., 1994, 1995). The third population was collected in a grain mill in Juiz de Fora County in 1999 and also shows high levels of pyrethroid resistance (Fragoso et al., 2003). Each population was established in laboratory from at least 500 individuals. All populations were reared in whole maize grains free of insecticides and maintained at controlled conditions (28 ± 2 °C and 70 ± 5 % r.h.). Regarding the chemicals used in the present study, acetone (p.a.) was obtained from Cromato

Prod. Quim (Diadema, São Paulo, Brazil), teflon (PTFE 30 fluorocarbon resin) was obtained from DuPont (Wilmington, DE, USA), and technical grade deltamethrin was provided by Bayer CropScience (Paulínia, São Paulo, Brazil).

Free-choice preference test with deltamethrin-sprayed grains

Batchs of whole maize grains (50 g) were sprayed at the rate of 1 ml/kg with increasing concentrations of deltamethrin. 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 and the other half containing deltamethrin-sprayed grains. A control with water-sprayed grains on both sides was also used to normalize the results. Fifty non-sexed adult insects were released in the center of the tray and insect preference was assessed after one hour by counting the proportion of insects remaining on the sprayed grains. The trays were coated with teflon in their edges and covered with organza to prevent insect escape. Six replicates were used for each combination of deltamethrin dose under test and maize weevil population.

Flight take-off from deltamethrin-treated surface

Transparent plastic jars (15 cm diameter x 12 cm high) with glue-coated walls were used as experimental unit for the flight take-off study. 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 on the bottom of the jar. The bottom of the jar walls received a 2 cm layer of teflon to prevent the insects from climbing them, the remaining of which was coated with sticky glue (BioControle, São Paulo, SP, Brazil). Two hundred non-sexed adult insects were released in the center of each jar, which were maintained at 28 ± 2 °C for one hour after what the number of insects glued at the jar walls were counted. Five replicates were used for each combination of deltamethrin concentration and maize weevil population.

Statistical analyses

Data from both experiments were subjected to regression analysis with deltamethrin dose (or concentration) as independent variable and the behavioral response as dependent variable using the curve-fitting procedure of SigmaPlot 2000 (SPSS, 2000).

Results

Free-choice preference test with deltamethrin-sprayed grains

The proportion of insects not discriminating deltamethrin-sprayed grains varied with the deltamethrin dose and the insect population (Figure 1). The proportion of insects not discriminating the treated grain decreased with the grain residue of deltamethrin following a similar trend for all three populations ($y = a + b\ln x$; $R^2 > 0.65$, $p < 0.01$), but with significant differences among them. Insects from the resistant population showed significantly higher deltamethrin-discriminating ability than insects from the susceptible population. The deltamethrin-discriminating ability increased with the deltamethrin residue for all three populations and particularly so for the resistant population from Jacarezinho.

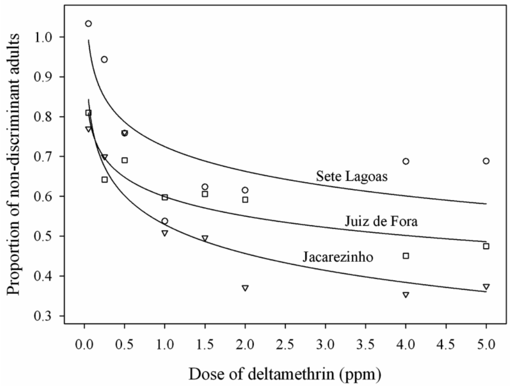

Figure 1. Proportion of adults of one pyrethroid-susceptible (Sete Lagoas) and two pyrethroid-resistant populations (Jacarezinho and Juiz de Fora) of maize weevil (*S. zeamais*) discriminanting against maize grains with increasing doses of deltamethrin ($R^2 > 0.65$, $p < 0.01$).

Flight take-off from deltamethrin-treated surface

The flight take-off from deltamethrin-treated surfaces was also significantly different among the maize weevil populations (Figure 2). Flight take-off was however stimulus-independent for both resistant population (Jacarezinho and Juiz de Fora; $p > 0.05$). The proportion of adults taking-off for flight was 7.42 ± 0.68 % for the resistant population from Jacarezinho and 24.56 ± 1.54 % for the resistant population from Juiz de Fora. In contrast, the susceptible population (Sete Lagoas) showed a take-off rate intermediate between both pyrethroid-resistant populations. In addition, the proportion of adults from the susceptible population taking-off for flight showed a steep decline with increasing deltamethrin residues from 0 to 0.42 mg a.i./cm^2 reaching a plateau at the last concentration onwards ($R^2 = 0.82$, $p = 0.04$).

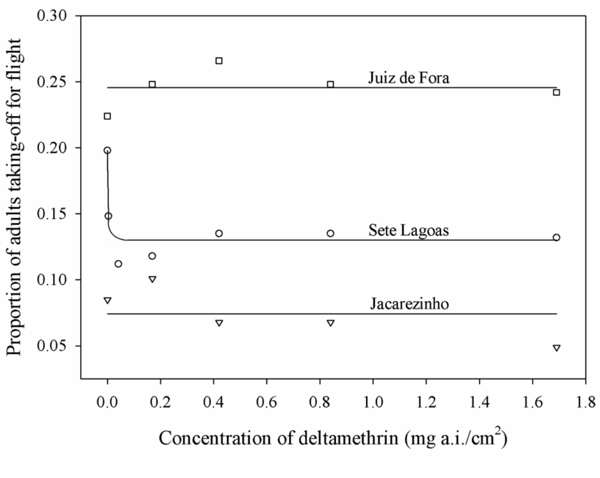

Figure 2. Proportion of adults of one pyrethroid-susceptible (Sete Lagoas) and two pyrethroid-resistant populations (Jacarezinho and Juiz de Fora) of maize weevil (*S. zeamais*) taking-off for flight from surface treated with increasing concentrations of deltamethrin ($R^2 = 0.82$, $p = 0.04$ for the susceptible population; $p > 0.05$ for both resistant populations).

Discussion

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

The negative correlation predicted between behavioral and physiological resistance 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 and Gould, 1993; Renou et al., 1997; Kongmee et al., 2004; Wang et al., 2004). The present results with maize weevil populations also challenge this hypothesis. The free-choice preference test with deltamethrin-sprayed grains indicated that the physiologically resistant populations show higher avoidance to the sprayed grains than the susceptible population – therefore a positive relationship between behavioral and physiological resistance. The results of flight take-off from deltamethrin-treated surface do not seem related to physiological resistance to insecticides since the susceptible population showed intermediate rate of flight take-off, again in contrast with the initial expectation of an inverse relationship between behavior and physiological resistance.

Behavioral resistance to insecticides do exist in populations of the maize weevil, however it is independent or positively correlated with physiological resistance depending on the behavioral trait under investigation. While repellency (or avoidance) to deltamethrin-sprayed grain is a stimulus-dependent response, since the effect increases with the insecticide dose and contact seems to occur, flight take-off from deltamethrin-treated surface is stimulus-independent in the physiologically resistant populations and show concentration-dependence only for the susceptible population under very low residue levels of deltamethrin.

Lockwood et al. (1984) offered an alternative view from Georghiou (1972) regarding the evolution of behavioral and physiological resistance to insecticides. They suggest a coevolution paradigma between behavioral and physiological resistance. The view is rooted in two relationships. First, all behavior has a physiological basis - it is a physical manifestation of physiology, what is beyond dispute. Second, the uniqueness of any resistance mechanism is dependent of its ability to lead to the individual survival (and fitness). Therefore, if a single (physiological) mechanism of insecticide resistance leads to high survival without compromising fitness, the scenario described by Georghiou (1972) of negative correlation between behavioral and physiological resistance is expected. In contrast, the co-occurrence of behavioral and physiological resistance (or multiple physiological mechanisms of resistance for that matter) is expected when a single physiological mechanism does not provide sufficient level of resistance to a given insecticide. In truth, behavior may be the part of the phenotype most likely to become modified in response to changes in the environment and behavioral plasticity is an important avenue of adaptation, particularly considering the spatial and temporal limitation of the selective action of insecticides (Lockwood et al., 1984; Hoy et al., 1998). Therefore, the co-occurrence of behavioral and physiological resistance in populations of maize weevil subjected to intensive selection with insecticides is not a surprise.

There are two possible origins of behavioral resistance as proposed by Chareonviriyaphap et al. (1997) – tandem evolution between behavioral and physiological resistance (but under different genetic control; either positive or negative) and independent evolution. Both situations seem to occur in maize weevil, positive tandem evolution for repellence to sprayed grains and independent evolution for flight take-off. The repellence to sprayed grains is a likely result of insecticide overuse, particularly in Brazilian seed stores until mid 1980’s, what explains the tandem evolution of behavioral and physiological resistance in maize weevil, as suggested by Lockwood et al. (1984). In contrast, flight take-off from treated surfaces probably evolved independently as a result of weight differences on maize weevil populations (Guedes et al., 2006), but this trait may prove important for the spread of insecticide resistance, particularly if flight take-off correlates with flight activity, what deserves future attention. Since behavioral resistance is not commonly assessed and may drastically impair insecticide efficiency, it should be considered in designing management programs for stored product insects.

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