PEST MANAGEMENT

Flight Activity of *Sitophilus oryzae* (L) and *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) and its Relationship with Susceptibility to Insecticides

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Atividade de Vôo de *Sitophilus oryzae* (L) e *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) e a sua Relação com a Susceptibilidade a Inseticidas

RESUMO - A mobilidade de insetos pragas pode influenciar sua susceptibilidade aos inseticidas usados no seu controle. O objetivo do presente trabalho foi avaliar a atividade de vôo em populações de *Sitophilus oryzae* (L) e *Sitophilus zeamais* criadas em grãos de milho e trigo, e a sua relação com a susceptibilidade a inseticidas. Insetos adultos não-sexados, com idades entre 10 e 20 dias, foram colocados sobre bandeja plástica. Uma lâmpada de luz incandescente de 40 watts foi posicionada a 50 cm de altura em relação à bandeja. A atividade de vôo foi avaliada durante 30 min. Nos bioensaios para estimar a CL_{50} os insetos foram expostos a resíduos secos do organofosforado fenitrotiom e do piretroide esfenvalerato sobre a superfície interna de tubos de vidro. A avaliação da mortalidade foi feita 24h após a instalação dos experimentos. *Sitophilus zeamais* apresentou maior atividade de vôo quando comparada a *S. oryzae*; outrossim, os insetos criados no milho apresentaram maior atividade de vôo do que aqueles criados no trigo. *Sitophilus oryzae* foi menos susceptível aos inseticidas estudados do que *S. zeamais*. Os adultos procedentes da criação em trigo foram mais susceptíveis aos inseticidas do que aqueles procedentes da criação em milho. O presente trabalho demonstra que a menor atividade de vôo de *S. oryzae* está relacionada a sua maior tolerância a inseticidas.

PALAVRAS-CHAVE: Grão armazenado, controle químico, tolerância, resistência, fluxo gênico

ABSTRACT - Insect-pest mobility can influence insect susceptibility to the insecticides used to control them. The objective of this work was to evaluate the flight activity of *Sitophilus oryzae* (L) and *Sitophilus zeamais* Motsch populations reared on corn and wheat grains, and its relationship with insecticide susceptibility. Unsexed adult insects with ages between 10 and 20 days were placed on a plastic tray. A 40-watt incandescent light bulb was positioned at a 50 cm height relative to the tray. Flight activity was evaluated during 30 min. In the LC_{50} estimation bioassays, the insects were exposed to dry residues of the organophosphorus insecticide fenitrothion and of the pyrethroid insecticide esfenvalerate on the internal surface of glass vials. Mortality was evaluated 24h after installation of the experiments. *Sitophilus zeamais* showed greater flight activity when compared with *S. oryzae*; likewise, insects reared on corn had greater flight activity than those reared on wheat. *Sitophilus oryzae* was less susceptible to the insecticides studied than *S. zeamais*. Adults reared on wheat were more susceptible to the insecticides than those reared on corn. This study demonstrates that the lower flight activity of *S. oryzae* is related to its greater tolerance to insecticides.

KEY WORDS: Stored grain, chemical control, tolerance, resistance, gene flow

Stored cereals are attacked by pests that cause both quantitative (Nyambo 1993) and qualitative losses (Hagstrum *et al* 1999). According to data estimated by FAO and by the Brazilian Ministério da Agricultura, Pecuária e Abastecimento, these losses amount to 10% of the grain yield in Brazil (Beskow & Deckers 2002).

Due to its high biotic potential and to the fact that infestations start in the field and are brought into the store, *Sitophilus oryzae* (L) and *Sitophilus zeamais* Motsch are considered the most destructive pests that attack stored corn and wheat grain in Brazil (Gallo *et al* 2002). Although both species can develop on both types of grain, *S. oryzae* has a marked preference for wheat, while *S. zeamais* strongly prefers corn (Rossetto 1969, Athié & Paula 2002).

Both species can fly, particularly *S. zeamais*, whose infestation in the field during the pre-harvest period constitutes an important event for the infestation that takes place in the stores (Hodges *et al* 1998, Likhayo & Hodges 2000). In the case of *S. oryzae*, field infestations are rarely observed (Champ & Cribb 1965). In the other hand a *S. zeamais* population increased three days after corn sheath opening occurred (Taylor 1971).

Among the factors that affect infestation of corn fields by *S. zeamais*, proximity and intensity of the infestation source (usually represented by infested cereals in the store) and the degree of ear husk cover of the variety grown seem to be the most important (Giles & Ashman 1971). The peak flight activity in *S. zeamais* occurs between 15:00h and 17:00h, and is influenced by environmental conditions, especially temperature (Giles 1969). Because of these behavioral characteristics, cases of resistance to insecticides in *S. oryzae* populations have occurred earlier than those verified for *S. zeamais*, since the great gene flow observed in the latter species would favor a dilution of resistance (Champ & Cribb 1965). The objective of this study was to evaluate the flight activity of Brazilian populations of *S. oryzae* and *S. zeamais* reared on corn and wheat grains, and their relationship with insecticide susceptibility.

Material and Methods

Insects. *Sitophilus oryzae* and *S. zeamais* specimens were obtained from the insect rearing laboratories at the Centro de Energia Nuclear na Agricultura – CENA/USP, in Piracicaba, SP and from the Centro Nacional de Pesquisa de Milho e Sorgo – CNPMS/EMBRAPA, in Sete Lagoas, MG, respectively. The populations of both *S. oryzae* and *S. zeamais* were kept in culture in these laboratories for over 20 years in the absence of selective pressure of insecticides. Therefore, they were considered reference susceptible lines. Both species were reared on insecticide-free corn and wheat grains until installation of the experiments.

Flight activity. Unsexed 10-20-day old adults were placed on plastic trays (40 cm long, 25 cm wide, 7 cm high). A 40-watt incandescent light bulb was positioned at 50 cm above the tray, and flight activity was evaluated for 30 min. Flight was defined as the insect movement in the air, regardless of the distance traveled. Individuals that engaged into flight activity were immediately eliminated and immersed in ethanol. Four replicates each containing 25 insects were installed for each rearing substrate (corn and wheat). The experiments were conducted between 3 p.m. and 5 p.m., the period in which the species under study have their most intense flight activity (Giles 1969, Taylor 1971). Temperature and relative humidity during the tests were 25 ± 1°C and 75%, respectively. Because the variable response of the experiment had a binomial distribution, the data were analyzed by means of logistic regression, using the SAS (1999) logistic procedure.

LC_{50}. Fenitrothion (99.1% a.i.) and esfenvalerate (100% a.i.) analytical standards were used (Iharabras S.A., Sorocaba, SP, Brazil). The bioassays to estimate LC_{50} were adapted from studies on resistance to insecticides of *Rhyzopertha dominica* (F) (Coleoptera: Bostrichidae) (Guedes *et al* 1996). Unsexed 10-20-day old adults were exposed to dry residues of the insecticides on the internal surface of glass vials (2.3 cm diameter × 4 cm height). Five ml of the insecticidal solutions were transferred to the vials and then evaporated by moving air previously dried through a blue silica gel desiccant filter. Afterwards, 25 individuals were placed inside the vials and maintained under controlled conditions (25 ± 1°C temperature and 74% RH). In order to prevent the insects from leaving the treated area, a fine layer of liquid vaseline was spread on the border between the treated and untreated areas of the vial. Mortality was evaluated 24h after installation of the experiments. Still individuals, deemed incapable of moving even when encouraged by an incandescent light source placed a few centimeters from them were considered to be dead. Each bioassay consisted of five or six concentrations, with four replicates per concentration. A control treatment was also included in which the solvent alone (acetone) was applied, with replicates. The concentration-mortality data were submitted to Probit analysis (LeOra Software 1987).

Results and Discussion

Flight activity. The complete model, in which the effects of the main pest species, the grain type and the interaction between them were observed, provided a good fit of data. The residual deviance was 3.80 and the residual X^2 statistic was 4.02 - both non-significant ($P > 0.98$) when compared with the 12 degrees of freedom of the residue. There was a significant effect ($P < 0.01$) for the main pest species and grain type effects, but not for the interaction between these two factors ($P = 0.0731$), indicating that the pest species effect is independent from grain type and vice versa (Table 1). *Sitophilus zeamais* had greater flight activity than *S. oryzae* (Fig 1). Likewise, insects reared on corn had greater flight activity than those reared on wheat. The behavioral differences observed between both species suggest *S. zeamais* has a greater natural dispersal capacity, with a potential to migrate from the storage areas to the field and vice versa (Giles & Ashman 1971, Chesnut 1972). Such migration

Table 1 Deviance analysis for the binomial model with a logistic link function.

Cause of variation	Degrees of freedom	Deviance	$P > \chi^2$
Pest species	1	14.66	0.0001
Grain type	1	11.77	0.0006
Species × grain type	1	3.21	0.0731
Residue	12	3.80	0.9868

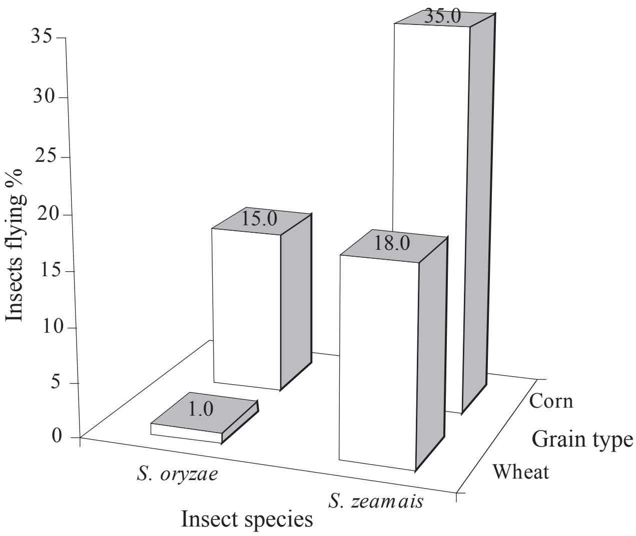

Fig 1 Mean percentage of *Sitophilus oryzae* and *S. zeamais* flying due to grain type and insect species.

encourages the infestation of grains even before they are harvested, and may thus cause significant losses during the storage period. On the other hand, migration also encourages crosses between populations from storage facilities (either family-owned or commercial), where they are exposed to different levels of selection pressure by insecticides and field-inhabiting populations, which do not have contact with these chemical products. Consequently, high gene flow might dilute resistance, facilitating the chemical control of this pest species. The contrary occurs with *S. oryzae* which, because of its reduced flight activity, remains restricted to the storage environment, where a constant selection pressure by insecticides might encourage the development of resistance.

An apparent stimulating effect of the corn kernel was observed on the flight activity of both species, meaning that infestations in the field may occur by migration of populations from stores that contain corn. When *S. oryzae* was reared on corn grains it showed a similar flight activity as *S. zeamais* reared on wheat grains. Under laboratory conditions, both species can be reared on both types of grains, but under storage conditions there is a marked preference of *S. oryzae* for wheat and of *S. zeamais* for corn (Athié & Paula 2002). In this respect, Rossetto (1969) evaluated corn samples collected in the State of São Paulo and found 169 samples infested with *S. zeamais*, 11 samples infested with both *S. oryzae* and *S. zeamais*, and a single sample infested with *S. oryzae*. When both species infest the same mass of grains, competition between them results in the elimination of *S. oryzae* in corn and of *S. zeamais* in wheat (Birch 1954 apud Coombs & Porter 1986). Consequently, the infestation in corn in the field will almost exclusively occur due to *S. zeamais*. Many studies have shown that *S. zeamais* is the only species in this genus that infests corn in the field (Chesnut 1972, Hodges *et al* 1998). Similarly, a greater flight activity of this species has been reported inside stores (Likhayo & Hodges 2000) and under laboratory conditions (Coombs & Porter 1986).

LC_{50}. The mortality in the control treatment was about 5%. Both species were significantly more susceptible to fenitrothion than to esfenvalerate. For fenitrothion, *S. oryzae* was slightly more tolerant than *S. zeamais*; for esfenvalerate, however, the difference in tolerance between the species was much broader (Table 2). One explanation for these results is the great gene flow that occurs in *S. zeamais*, which might dilute resistance, and the low migration capacity of *S. oryzae*, which might favor development of this phenomenon.

Through time, this trait seems to have encouraged greater tolerance to insecticides in *S. oryzae* than in *S. zeamais*. In this respect, greater tolerance of *S. oryzae* to organophosphorus compounds (Samson & Parker 1989) and greater genetic variability of *S. zeamais* (Grenier *et al* 1994) have been reported**.** Although several studies have demonstrated a high effectiveness of organophosphorus compounds to control *Sitophilus* spp. (Collins *et al* 1993, Sgarbiero *et al* 2003), the resistance of Brazilian *S. oryzae* populations to this class

Table 2 Characterization of *Sitophilus oryzae* and *Sitophilus zeamais* susceptibility when reared on corn and wheat grains, to the insecticides fenitrothion and esfenvalerate by means of a dry film bioassay.

Species / grain type	LC_{50} µg A.I./ml	Confidence interval (95%)	Slope coefficient ± standard error	X^2	D.F. P > 0.05	Hetero-geneity
			Fenitrothion			
S. oryzae / corn	0.17	0.15 – 0.19	5.77 ± 0.43	5.93	3	1.98
S. oryzae / wheat	0.09	0.06 – 0.10	3.74 ± 0.39	5.70	3	1.90
S. zeamais / corn	0.08	0.07 – 0.10	4.69 ± 0.59	10.17	3	3.39
S. zeamais / wheat	0.06	0.05 – 0.07	10.07 ± 0.98	10.88	3	3.63
			Esfenvalerate			
S. oryzae / corn	3.54	2.84 – 4.94	2.39 ± 0.24	3.43	3	1.14
S. oryzae / wheat	1.80	1.57 – 2.08	1.86 ± 0.17	0.88	4	0.22
S. zeamais / corn	0.26	0.20 – 0.34	2.17 ± 0.16	8.99	4	2.25
S. zeamais / wheat	0.30	0.27 – 0.34	2.18 ± 0.16	3.95	4	0.99

of insecticides was demonstrated (Pacheco *et al* 1993). This situation is opposed to that observed in *S. zeamais*, where only a slight resistance to chlorpyrifos methyl was found until recently (Ribeiro *et al* 2003). Similar results were obtained by Samson & Parker (1989) and Vásquez-Castro (2006), who recorded better control of *S. zeamais* than of *S. oryzae* when organophosphorus insecticides were used. Although esfenvalerate has never been used against the populations under study, it was less toxic than fenitrothion. This was possibly due to cross resistance with other pyrethroids such as deltamethrin and with DDT, an insecticide much used in the past to control pests in stores, since both DDT and pyrethroids share the same mechanism of action. This phenomenon was verified in *Sitophilus granarius* (L) by Prickett (1980), in *S. oryzae* by Heather (1986) and in *S. zeamais* by Guedes (1993).

The insects reared on wheat were more susceptible to the insecticides than those reared on corn, except for *S. zeamais* exposed to esfenvalerate, in which both populations showed the same degree of susceptibility. Thus, the *S. oryzae* population reared on corn was two-fold more tolerant to fenitrothion and esfenvalerate than the population reared on wheat, while the *S. zeamais* population reared on corn was 1.3-fold more tolerant to fenitrothion than the one reared on wheat. These results demonstrate the influence of food type on the susceptibility of pests to insecticides; therefore, it is important to take this factor into consideration in the establishment of insecticide resistance management programs for stored grain insect pests, since apparently different doses should be used initially to treat corn and wheat.

In conclusion, *S. zeamais* shows greater flight activity than *S. oryzae* and therefore is more susceptible to the insecticides used in stored grain protection. On the other hand, insects reared on corn show greater tolerance to the insecticides than those reared on wheat.

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