PLANT PROTECTION

Age and Time Exposure-Related Toxicity of Fenthion to Male and Female *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae)

EDUARDO HUMERES[1], IVANA B.M. DA CRUZ[2] AND ALICE K. DE OLIVEIRA[3]

[1]Estação Experimental de São Joaquim, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, EPAGRI, Caixa postal 81, 88600-000, São Joaquim, SC.
[2]Faculdade de Biociências/Instituto de Geriatria e Gerontologia, PUCRS, Caixa postal 1429, 90619-000, Porto Alegre, RS.
[3]Departamento de Genética, Instituto de Biociências, UFRGS, Caixa postal 15053, 91501-970, Porto Alegre, RS.

An. Soc. Entomol. Brasil 28(2): 285-292 (1999)

Toxicidade do Fentiom Relacionada à Idade e ao Tempo de Exposição de Machos e Fêmeas de *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae)

RESUMO - O efeito da idade, sexo e tempo de exposição de machos e fêmeas de *Anastrepha fraterculus* (Wied.) à toxicidade do fentiom foi avaliado. A idade das moscas mostrou ser importante para o aparecimento dos primeiros sintomas de intoxicação; machos e fêmeas no pico reprodutivo (30 e 60 dias de idade, respectivamente) são menos susceptíveis ao inseticida que moscas nas demais idades (quatro e 120 dias). Não foram detectadas diferenças de toxicidade relacionadas com o peso corporal dos insetos. O $TL_{50,}$ que variou de 3-7 min, mostrou serem os machos menos sensíveis que as fêmeas em todas as idades testadas. Adultos de ambos os sexos, com 30 dias de idade, submetidos a uma exposição contínua e descontínua ao fentiom, não apresentaram efeito toxicológico cumulativo do inseticida. Análises estatísticas sugerem a possivel ocorrência de um mecanismo geral de desintoxicação (quantitativo e/ou qualitativo) relacionado com o sexo, a idade e o tempo de exposição ao fentiom. Como a espécie apresenta alta mobilidade na natureza sugere-se que estas características biológicas e comportamentais sejam consideradas para a obtenção de resultados mais realistas.

PALAVRAS-CHAVE: Insecta, mosca-das-frutas, inseticida, toxicidade.

ABSTRACT- Effect of age, sex and time exposure of *Anastrepha fraterculus* (Wied.) to toxicity of fenthion was evaluated. The age of the flies was important to the appearance of the first intoxication symptoms; males and females of reproductive ages (30 and 60 days-old, respectively) were less susceptible to insecticide than flies in the remainder ages (four and 120 days-old). The effect of body weight on insect intoxication was not detected. LT_{50} biossay with fenthion (varying from 3-7 minutes) showed a lower susceptibility of males than females at all ages. Adults of both sexes and 30 days-old submitted to continuous and discontinuous exposure to fenthion did not show toxic cumulative effect of the

insecticide. The statistical analysis suggests a possible general detoxification mechanism (quantitative and/or qualitative) to fenthion sex-, age- and time-related. Once the species is highly mobile in nature we suggest that in fruit fly toxicological bioassays, these biological traits need to be observed in order to obtain more realistic data.

KEY WORDS: Insecta, fruit-fly, insecticide, toxicity.

The family Tephritidae contains some of the most important fruit pests in the world, including the broad-range pulp fruit pests such as *Anastrepha fraterculus* (Wied.). The characteristic features of this species are a long adult life, dispersion flights and migrations (Zwölfer, 1983) that usually dictate a quick control response in suppression campaigns, hence the extensive use of aerial applications in the control area (Rossler, 1989).

A recent study in *A. fraterculus* (Da Cruz *et al*. 1997) showed differential fenthion toxicity in three classic bioassays: feeding, topical contact and residual contact. The insects exposed to residual contact were 18 times more susceptible than those exposed by feeding bioassay. One of the suggested explanations for the differences was the effect of the insecticide exposure time. This was continuous in residual contact and discontinuous in the feeding bioassay. In the ecological context, these differences are important since the adult population shows a high mobility and different ages (review in Zwolfer, 1983). Moreover, there are longevity differences between males and females of *A. fraterculus* which makes this species a good model to study age-related modulation of insecticide stress-response.

In order to evaluate the fenthion time exposure effects on sex and age in *A. fraterculus* mortality three bioassays were carried out: 1) the time of exposure necessary to observe the early symptoms of intoxication; 2) exposure time bioassay to kill 50% of population (LT_{50}) and 3) toxicological effect of continuous and discontinuous fenthion exposure in fruit fly mortality.

Material and Methods

The *A . fraterculus* population and biological conditions tested. The *A. fraterculus* population was collected in the town of São Joaquim, SC, Brazil from fruits of guava (*Feijoa sellowiana*). It was reared in conditions described by Da Cruz *et al*. (1997). The experiments were carried out using the first generation of field collected fruit-flies; this procedure avoids loss of genetic variability that may decrease when the population is reared for many generations under laboratory conditions.

Intoxication symptoms and LT_{50} bioassays were measured in independent fruit-fly age-groups (4, 30, 60, 90 and 120 days). For continuous and discontinuous fenthion exposure bioassay 30 day-old flies were used.

Biossay Procedures. The organophosphate "Fenthion" [0,0-dimethyl-0,-(methylmercapte-4-methylphthiophenyl)-thiophosphate 0,0- dimethyl-0-(3-methyl-4-methylthio-phenyl) phosphorothionate] 500 CE was used in a 10 ppm concentration. The insecticide manipulation and glass preparation were made as described by Da Cruz *et al.* (1997) for the residual contact bioassay. Each of the following experiments was replicated five times, including the controls; 10 males and 10 females were used in each replication. Flies of both sexes were grasped by the wings with tweezers and then placed inside the glass containers. After insecticide exposure, fruit flies that did not move any appendage when picked up with tweezers were considered to be dead.

Fenthion intoxication symptoms in *A. fraterculus* are easily characterized. Initially the fly becomes immobilized and this is followed by fast wing beats, circling movements and finally it lies down ventrally. The intoxication effect was estimated using the initial appearance time of the rapid wing beat pattern of behavior. For this analysis, Petri dishes previously treated with fenthion were used as described in residual contact bioassay (Da Cruz *et al*. 1997) . The samples were observed at 5 min intervals until more than 95% showed intoxication effects. When the first intoxication symptoms were seen, the fruit flies were immediately collected and transferred to clean Petri dishes. The mortality was assessed after 24 hours. The weights of all flies was measured before testing to check whether body weight affect fenthion intoxication.

An LT_{50} time-response bioassay was performed using independent sampling of insecticide exposure for a given fixed time (Robertson & Preissler, 1992). A preliminary test was made in order to determine the range of exposure-time. Samples were exposed to at least nine different times of fenthion exposure. Petri dishes treated with alcohol (because alcohol was used as insecticide-diluent) and clean Petri dishes were used as controls. No differences were found between these two control groups. For each test the samples were kept in fenthion-treated Petri dishes during the fixed time intervals and immediately transferred to clean glasses. After 24 hours, mortality was assessed and the LT_{50} evaluated.

The continuous and discontinuous exposure effect of fenthion toxicity was also analyzed. The continuous exposure test was made with insects continuously exposed for one, two and 10 minutes and then transferred to clean glasses. Discontinuous fenthion exposure evaluations were made with insects that were placed for 2 min in glasses treated with the insecticide and then transferred to clean glasses for 10 min. This procedure was repeated until the insects had been exposed to the insecticide for an accumulated 10 min. In order to verify the effects of the manipulation stress caused by glass transference, the same discontinuous experiment was performed using clean glasses. After 24 hours the mortality was evaluated in all samples.

Fenthion intoxication symptoms were analyzed by one way analysis of variance followed by LSD Fisher's test for pairwise comparisons. The differences between sex of each age were also evaluated using the non-parametric Wilcoxon Mann-Whitney test. The effects of sex, age and body weight on fenthion toxicity was determined by multivariate analysis (general linear model, MANOVA). Lethal time bioassay results from all replicates for each sex- and age- samples were pooled and subjected to probit analysis (Finney, 1971). The hypothesis of equality of regression was tested by likelihood ratio test using a computer program [POLO-PC (Le Ora 1987, Berkeley, CA)]. A minimum of 100 insects (20 per treatment) was used in the analysis to ensure reliability in the estimation of LT_{50} values (Robertson *et al*. 1984). The analysis of cumulative effect was made by chi-square goodness fit test using the MINITAB computer package (Ryan *et al*. 1985) following recommendations of Zar (1984).

Results and Discussion

Fenthion intoxication as well as the LT_{50} bioassays results were age- and sex-related. In the first experiment, young and older flies were more responsive when in contact with fenthion than fruit flies in reproductive ages. However, all samples died after the first intoxication effect appear. When we compare the intoxication rate by sex, age and body weight we observed influence of the two first variables in the age groups tested (Table 1, Table 2)

Except for the 60 day-old samples, the intoxication rate in both sex was similar in all ages (Table 1).

Body weight did not affect the toxic response to fenthion. Males and females with significantly different body weight did not show any differences in intoxication rates

Table 1. Mean ± SEM of first fenthion intoxication symptoms and body weight of *A. fraterculus* males and females at different ages (in days).

Age (days)	Sex[1]	Intoxication rate (min) Mean ± SEM	Body weight (mg) Mean ± SEM
4	M	21.20 ± 1.04 a [3]	111.64 ± 1.74 a
	F	22.00 ± 1.06 x	112.60 ± 1.49 x
		ns [2]	ns
30	M	79.00 ± 4.44 b	141.38 ± 2.84 b
	F	76.20 ± 4.13 z	161.62 ± 3.26 z
		ns	P< 0.008
60	M	87.10 ± 4.31 b	131.08 ± 2.54 b
	F	73.00 ± 4.12 z	170.00 ± 4.18 z
		P<0.002	P<0.000
120	M	58.00 ± 5.16 c	134.64 ± 2.36 b
	F	49.30 ± 5.15 w	164.92 ± 3.66 z
		ns	P< 0.000

[1]M= male; F= female

[2]ns = non significant males and females comparison by non parametric Wilcoxon-Man-Whitney test

[3]Means followed with same letter (a, b, c for males and x, y, z for females), are not significantly different by Anova One-Way, Fisher's LSD test;

The number of samples tested in each age group was 100.

(Table 1). Similar results have been described in other organisms. Robertson & Preissler (1992) suggested that the use of body weight variable in toxicity tests is very complicated because generally the data obtained does not present a positive or negative regression. Therefore, we did not consider this biological parameter in the remaining tests.

LT_{50} bioassay presented a dramatic effect in *A. fraterculus* with a range of 3.06 to 7.39 min to kill 50% of the population (Table 3).

Table 2. MANOVA testing of first fenthion intoxication symptoms of *A. fraterculus* to evaluate sex, age and body weight interaction.

Biological parameters	Criterion values and statistic significance				
	Wilk's lambda	Lawley-Hoteling trace	Pillai trace	*F* (df)	*P*
Age	0.725	0.379	0.275	37.36 (4.4)	0.000
Sex	0.988	0.0012	0.012	4.82 (1.4)	0.029
Body weight	0.744	0.344	0.256	0.926 (107.3)	0.676

Reproductive males (30 - 90 day-old) were less responsive than 4 and 120 day-old males as observed in intoxication rate presented in

Table 3. Time-response of *A. fraterculus* (males and females) at different ages exposed to fenthion using residual contact bioassay.

Age (days)	Sex[1]	Slope ± SEM	LT_{50} (95% CL)[3] (min)	LT_{50} sex ratio M/F	χ^2 (df)	P
4	M	2.09±0.28	3.29 (2.39-4.33) a	1.02	8.02 (4)	P<0.05
	F	2.80±0.27	3.23 (3.14-4.14) x		18.17 (10)	P<0.01
		χ^2=0.18[2]				
		ns	ns			
30	M	2.63±0.23	5.37 (4.35-6.41) b	1.34	13.39 (7)	P<0.01
	F	2.89±0.21	4.01 (3.39-4.32) x		7.97 (7)	ns
		χ^2=16.72				
		P<0.000	P<0.005			
60	M	6.52±0.77	7.39 (9.46-7.54) c	1.73	2.10 (6)	ns
	F	3.03±0.27	4.24 (3.37-4.38) x		3.63 (5)	ns
		χ^2=26.54				
		P<0.000	P<0.000			
90	M	3.50±0.35	6.04 (5.13-7.55) c	1.40	11.67 (6)	P<0.05
	F	3.15±0.31	4.32 (3.35-5.27) x		7.73 (5)	ns
		χ^2=0.54				
		ns	ns			
120	M	2.22±0.22	3.15 (2.16-4.21) a	1.03	9.79 (5)	P<0.05
	F	2.19±0.28	3.06 (1.17-4.16) x		3.93 (3)	ns
		χ^2=0.07				
		ns	ns			

[1]M= male; F= female.
[2]Hypothesis test that male and female slopes are the same.
[3]ns = non significant χ^2 goodness-of-fit values of comparison between LT_{50} of males and females at each age.
[4]Data for each sex, followed by the same letter (a, b, c for males and x, y, z for females), do not differ significantly based on the overlap of confidence intervals at LT_{50}.

Table 1, Figure 1. However, females were found not to be time mortality age-related. Gerder comparisons showed that males were more tolerant to fenthion exposure than females at all ages tested. Biologically, these differences observed from Probit regressions may reflect the quality and quantity of enzymes involved in detoxification pathways. Parallel lines may indicate that organisms have qualitatively identical (same detoxification enzymes), but quantitatively different levels of detoxification enzymes (Robertson & Rappaport, 1979). However, a line neither equal nor parallel was seen in 60 days-old males. This may suggest qualitative and quantitative enzyme differences among samples tested.

In the cumulative test we observed significant differences between 10 min continuous and 10 min discontinuous treatments. In 10 min continuous samples the male and female mortality was 63% and 75% respectively, whereas the 10 min discontinuous samples showed similar mortality to those at two min-

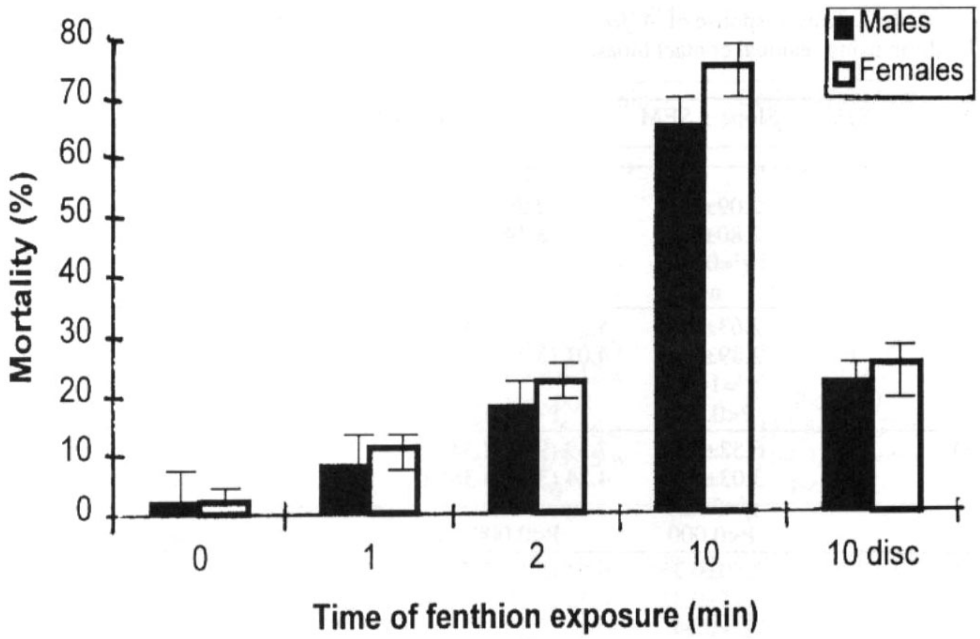

Figure 1. Mortality (in %) of *A. fraterculus* males and females 30 days-old exposured during 1, 2, 10 continuous and 10 discontinuous min to fenthion residual contact bioassay. 0 (control mortality).

utes exposure (Fig. 1).

Although the fenthion LT_{50} was very low in discontinuous test, the differences observed in both fruit fly mortality tests suggest an absence of a cumulative effect on the insects and, at the same time, the presence of general metabolic pathways of detoxification. Since the species is very mobile, the displacement and flying behavior at places with and without insecticide could help metabolic degradation of the insecticide.

In general, there is little information about the nature of male-female mortality differences for most non-human species whether submitted to stress conditions such as insecticide exposure or not. However, several studies suggest that there is a general framework for mortality differences between sexes. The underlying mortality factors are grouped by Carey *et al.* (1995) in three interrelated categories: constitutional endowment, reproductive biology and behavior. This framework can provide insights into how sex differences may contribute to stress or environmental stress response.

Sex differences in traits which have a strong influence on stress response may arise through either natural or sexual selection (Carey & Liedo 1995). Natural selection refers to being able to pass on one's genes to future generations by being able to survive to breeding age and reproduce (Futuyma, 1979; Hoyenga & Hoyenga, 1982; Maynard Smith, 1976). Natural selection pressures on the physiology and behavior of the genders could differ if their reproductive roles are different. Sexual selection refers to being able to pass on one's genes by successfully persuading a member of the opposite sex to engage in reproduction. This can occur through success-

ful competition within one's own gender for mates (Charnov, 1982). There are several pieces of information about sex selection-relation, but differential metabolic responses related to environmental stress are still incipient and need to be understood. Therefore biochemical analysis in male and female groves with different responses observed in the present data need to be made to understand the possible detoxification differences between sex.

The results obtained in the present experiments are important once conventional and alternative insecticides can have reduced effects in nature due to flight behavior. The effect of mobility behavior over insecticide toxicity response was demonstrated in other organisms. Zhai & Robinson (1992) showed that the amount of walking affects the rate of knockdown of German cockroaches placed on a cypermethrin-treated surface. Therefore, we suggest that to obtain more realistic data in fruit fly toxicological bioassays, sex, age and insecticide exposure time must be considered. Furthermore additional biochemical studies of *A. fraterculus* organophosphate detoxification related enzymes such as esterases, as well as further toxicological studies, such as fenthion toxicological activity in *A. fraterculus* preimaginal stages, could help to understand the sex- and age differences observed here. These studies are in course in our laboratory.

Acknowledgements

We thank Jurema C. do Nascimento, Cláudia Bicca and Maristela Taufer for helping in the *A. fraterculus* rearing and experiments. We thank the Conselho Nacional de Desenvolvimento Científico (CNPq) and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) for grants and fellowships.

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Received 15/VII/98. Accepted 12/IV/99.