CROP PROTECTION

Developmental Stress by Diflubenzuron in *Haematobia irritans* (L.) (Diptera: Muscidae)

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Estresse por Diflubenzurom no Desenvolvimento de *Haematobia irritans* (L.) (Diptera: Muscidae)

RESUMO - Este trabalho teve como objetivo verificar o efeito de concentrações sub-letais do regulador de desenvolvimento de insetos (IGR), diflubenzurom, no tamanho e na estabilidade do desenvolvimento da mosca-dos-chifres, *Haematobia irritans* (L.), por meio da análise de assimetria flutuante (AF). Adultos criados em substratos com diferentes concentrações sub-letais de diflubenzurom e em meios-controle foram submetidos a medidas morfométricas. Utilizou-se a análise de componentes principais para se obter um índice multivariado de tamanho, a partir de uma matriz de correlação dos caracteres originais. Os níveis de AF e o índice multivariado de tamanho foram comparados entre os tratamentos. Contrariamente ao esperado, a exposição ao diflubenzurom não resultou em aumento significativo e proporcional às concentrações utilizadas nos níveis de AF entre os tratamentos. Entretanto, a redução no tamanho devido ao efeito deste IGR pode estar refletindo a capacidade de *H. irritans* de realizar ajustes fisiológicos e morfológicos, de modo que os fenótipos compensem, pelo menos até certo ponto, as condições ambientais de estresse. Há ainda a possibilidade de o IGR estar selecionando indivíduos mais simétricos e resistentes. Do ponto de vista do controle de pragas, a redução no tamanho de indivíduos submetidos a concentrações sub-letais deste IGR pode ser vantajosa, uma vez que tamanho está usualmente diretamente relacionado à aptidão do indivíduo.

PALAVRAS-CHAVE: Insecta, controle de pragas, mosca-dos-chifres, regulador de crescimento, assimetria flutuante

ABSTRACT - This study aimed to assess the effect of sub-lethal concentrations of the insect growth regulator (IGR), diflubenzuron, on the body size of the horn fly *Haematobia irritans* (L.) and on its developmental stability, by fluctuating asymmetry (FA) analysis. Breeding media with different sub-lethal diflubenzuron concentrations and a control medium, were prepared to obtain morphometric measures on adult individuals. Principal component analysis was used to generate an index of general body size, using the correlation matrix of the original characters. Levels of FA and the multivariate index of size were compared among treatments. Contrary to the expectations, the exposure to diflubenzuron did not result in a significant and concomitant increase in the level of FA across treatments or in the number of individuals showing developmental instability. Nevertheless, a significant reduction of size due to the diflubenzuron may reflect the ability of *H. irritans* to perform physiological and morphological adjustments allowing phenotype compensation, at least to some extent, for stressful environmental conditions. On the other hand, the IGR may be selecting more symmetrical and resistant individuals. From the pest control point of view, the reduction of size may be a positive effect of the IGR since size is usually directly related to fitness.

KEY WORDS: Insecta, pest control, horn fly, insect growth regulator, fluctuating asymmetry

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Fluctuating asymmetry (FA) is defined as random differences between the scores for morphological bilateral traits on the two sides of individuals in a population (Van Valen 1962). The degree of FA provides a measure of developmental instability, which can be influenced by genetic or environmental stress as well as by the ability of the genome to maintain the organized development under conditions of stress (Parsons 1990, Palmer & Strobeck 1996, Møller & Swaddle 1997). Much empirical and theoretical evidences suggest that other kinds of asymmetry, including antisymmetry and directional symmetry may be important markers of developmental instability (Møller & Thornhill 1997). Antisymmetry occurs when a significant difference exists between sides and the larger side is randomly distributed within a sample, and directional asymmetry occurs when there is a consistent bias in a character toward greater development on one side (Palmer & Strobeck 1986).

FA has been attracting the attention of biologists because of reports that it may be negatively correlated with fitness components such as fecundity, growth rate or adult longevity (Mitton & Grant 1984, Leary & Allendorf 1989, Ueno 1994). Fly populations exposed to conventional insecticides may experiment an increase in their FA index (Mckenzie & Clarke 1988, Clarke & Ridsdill-Smith 1990). Nevertheless, because of the capacity of species to show plasticity, an environmental stress may not be sufficiently severe to significantly increase FA levels of the target population (Parson 1992), but may cause reduction in its growth rate which may be also related to decrease in insect fitness (David *et al.* 1994, Santos 2001).

Diflubenzuron is an insect growth regulator (IGR), which causes physiological and morphological changes during insect development, due to its interference with cuticle deposition (Chamberlain 1975, Grosscurt 1978, Graf 1993). Some studies have demonstrated the susceptibility of the horn fly, *Haematobia irritans* (L.) to diflubenzuron (Hopkins & Chamberlain 1976, Kunz & Bay 1977, Silva & Mendes 2002). If this IGR affects horn fly survivorship, concomitant changes in horn fly FA levels and/or on body size may occur. This hypothesis was tested on the present investigation where the effect of sub lethal concentrations of diflubenzuron on the body size of horn fly and on its developmental stability, inferred by FA analysis, were assessed.

Materials and Methods

Collecting Horn Flies and Preparing the Breeding Media. Horn flies were collected using an entomological net, from February 2000 to January 2001 at the farm Douradinho near Uberlândia city, MG, Brazil (18º55’23”S and 48º17’19”W). The flies were transferred to entomological cages (30 x 40 x 33 cm) and taken to the laboratory where a moistened filter paper was placed inside the cage as an egg-laying substratum. Eggs were removed from the filter paper and transferred to flasks containing breeding media. To prepare the medium, diflubenzuron, (95.34%) (Champion Farmoquímico Ltda., Brazil) was diluted in acetone (Merk) and mixed with previously homogenized fresh cattle manure in order to obtain the sub-lethal concentrations of 30 ppb; 25 ppb and 20 ppb (i.e. giving concentrations which are not lethal for at least 30 of the exposed individuals). Equivalent quantities of acetone used to prepare the tested media were added to 1000 g of cattle manure to be used as control. Thereafter, 100 g of treated and control media were transferred separately to the flasks (15 cm x 12 cm) containing a layer of approximately 2 cm of previously sterilized soil. Four replicates of each concentration and respective controls were used. Subsequently, 50 eggs were placed on the surface of the breeding media in each of the containers, which were then maintained in a growth chamber at 25.0 ±± 0.5ºC and 12:12 h of photoperiod. Flasks were monitored daily checking the number of emerged adults up to 20 days, by which time all adults had emerged. Emerged flies were preserved in ethanol 70% until be examined. Details of methods and aspects of horn fly susceptibility to this IGR are given in Silva & Mendes (2002).

Obtaining Morphometric Measures. The wings and legs from around 28 flies (14 females and 14 males) from each of these media concentrations were dry mounted on microscope slides with a cover slip applied. Slides were scanned and images were amplified x 3.0. The measurements were obtained using the computer program CorelDraw, version 5.0 (CorelDraw 1994). The following characters were measured: distance between distal extremity of the vein R$_{2+3}$ and the interception point of dm-cu-M$_1$ (wing measure A); distance between the interception points of dm-cu-M$_1$ and R$_1$-C (wing measure B) (Fig. 1); hind tibia and hind femur length. These characters were chosen because they are essentially plane structures. Each one of these body parts was measured three times. The wing veins provide many well-defined morphological landmarks and are commonly used in morphological analysis (Klingenberg & McIntyre 1998). Measurements of bilateral traits were obtained for both sides in order to evaluate FA for each treatment and control. FA was calculated as the mean difference between the right and left side, i.e. $[(\sum|\text{R-L}|)/n]$, the most common index of FA, according to Palmer & Strobeck (1986).

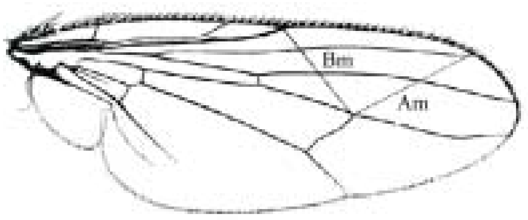

Figure 1. Schematic representation of a *H. irritans* wing, showing the morphological characters measured. Am = wing measure A; Bm = wing measure B.

Statistical Analysis. A two factor analysis of variance (ANOVA) was used to determine whether the between sides variation was significantly larger than the measurement error (Woods *et al.* 1998, Perfectti & Camacho 1999). According to Palmer & Strobeck (1986, 1992) it is necessary to distinguish AF from other kinds of asymmetry. A t-test was performed to verify whether the means of the signed right minus left distribution were not significantly different from zero in order to discard the occurrence of directional asymmetry. Antisymmetry was tested by departures of the right-left frequency distribution from normality using Kolmogorov-Smirnov test. Size dependence of FA was tested for each sample by regressing the unsigned absolute difference of the right minus left measurements on trait size. The controls were pooled in only one group since they did not differ statistically in relation to the amount of FA among the tested characters (Zar 1984). Also, no significant differences between male and female levels of FA were detected. Consequently, a single ANOVA was performed to test for differences in the levels of FA among treatments and control (Palmer & Strobeck, 1986, Preziosi *et al*. 1999). The power of this analysis was also obtained accordingly to Zar (1984).

A PCA multivariate analysis was used to verify the nature and magnitude of variation in morphological characters. An index of general body size (in a multivariate sense) was then estimated using a correlation matrix of the original characters (Manly 1994). The characters used to obtain the scores of the first component were those significantly correlated (wing measure A, femur and tibia, all obtained from the left side). Only 10 males and 10 females were considered in this analysis since some individuals, from which it was not possible to obtain all the measured characters, were discarded. The normality and homoscedasticity of the index of size distribution were verified using respectively the Kolmogorov-Smirnov test and procedures suggested by Zar (1984). Once again, the controls were pooled to form a single group since no differences in size were detected among them (H = 2.86, P = 0.413). Differences between treatments and control were checked using Kruskal-Wallis test and a Tukey-type test for multiple comparisons with equal sample sizes (Zar 1984). All statistical procedures were performed using computer software package Systat for Windows, version 9.0 (Systat 2000).

Results

All distributions were normal (i.e. no evidence of antisymmetry was found) and no correlation between size and FA were observed (Table 1). The asymmetry in the femur as well as in the tibia fluctuated around a mean zero, whereas the FA distributions for the wing measurements were both found to be skewed (Table 1). Since the wing traits do not fulfill the criteria for FA, they were omitted from further parametric analysis. For other variables there was a significant level of FA relative to measurement error, indicated by the highly significant sides x individuals effect (F = 10.462, P < 0.001 for tibia and F = 12.582, P < 0.001 for femur), which means that the measurement error was negligible. The ANOVA indicated no differences in femur and tibia FA levels among treatments (Table 2). The power of the ANOVA for both characters was estimated to be 0.87, indicating a 13% chance of having committed a Type II error in this analysis.

In the PCA, the first component, which is related to the axes that exhibit greatest variance among individuals, explained 57,7% of the total variance (Table 3). In morphological analysis this first component is interpreted as a size representation (Manly 1994). The scores of the size were normally distributed but the treatments showed unequal variances. The nonparametric Kruskal-Wallis analysis indicated differences of size among treatments (H = 13.26, P = 0.004). Individuals emerged from the 25 ppb and 30 ppb treatment were significant smaller than those emerged from 20 ppb treatment as well as from the control group (Fig. 2).

Discussion

Although some studies have reported FA as an indicator of developmental stress in response to insecticide exposure (McKenzie & Clarke 1988, Clarke & Redsdill-Smith 1990), the exposure to diflubenzuron, independent of its concentration, did not result in a significant and concomitant increase in the level of FA. These results are at odds with the prediction that asymmetrical individuals should be more frequent after exposure to diflubenzuron.

Several factors may be influencing the observed results. On the one hand, Parsons (1992) pointed out that in some cases a relatively severe environmental stress was necessary to induce significant FA alterations. In our experiment, the low levels of FA in horn flies after exposure to Diflubenzuron might be due to the peculiar mode of action of this substance, which is very different to that of conventional insecticides (Grosscurt 1978, Graf 1993). On the other hand, the sub-lethal concentrations may be selecting less asymmetric individuals that are also more resistant to diflubenzuron. In these case, severe effects of the IGR were buffered by the individuals ability to cope with it, resulting in a decrease of developmental instability. Other studies show that the absence of correlation between fitness and levels of FA may be the result of

Table 1. Testing asymmetry, antisymmetry and fluctuating asymmetry correlation with size in four characters in *H. irritans.*

Character	Mean ± SE (N) (right minus left value)	Asymmetry		Antisymmetry		Size correlation	
		t	P	D_{max}	P	r	P
Femur	0.002 ± 0.005 (109)	-0.235	0.815	0.078	0.096	-0.158	0.101
Tibia	-0.001 ± 0.004 (109)	-0.181	0.857	0.082	0.067	0.049	0.610
Wing measure A	-0.014 ± 0.002 (116)	-4.871	<0.001	0.062	0.314	-0.072	0.615
Wing measure B	0.005 ± 0.002 (116)	2.811	0.006	0.062	0.311	-0.063	0.500

Table 2. Mean asymmetry values ± standard errors for femur and tibia estimated in treatments and control groups of *H. irrritans* submitted to different concentrations of diflubenzuron.

	FA (mean ± SE)				F	P
	Control	20ppb	25ppb	30ppb		
Femur	0.062 ± 0.010	0.028 ± 0.026	0.039 ± 0.005	0.029 ± 0.005	0.270	0.842
Tibia	0.071 ± 0.011	0.038 ± 0.006	0.032 ± 0.005	0.027 ± 0.004	1.120	0.345

Table 3. Principal components extracted from phenotypic correlation matrix of three morphological characters measured in *H. irritans*, using PCA analysis.

Characters	First component
Wing measure A	0.344
Tibia	0.892
Femur	0.904
Total variance explained (%)	57.708

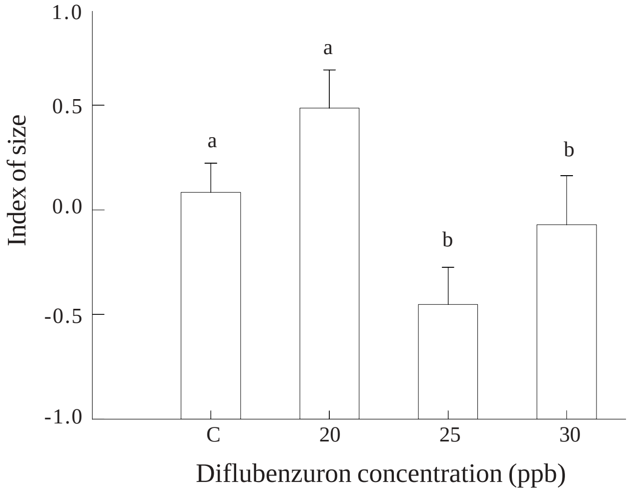

Figure 2. Multivariate index of size (± standard error) of *H. irritans* adults from control (C) and different medium concentration of diflubenzuron. Significant pairwise differences between concentrations obtained using a Tukey-type test ($P < 0.05$) are indicated by different letters.

choosing characters not affected by developmental instability. In fact, there is no concordance of FA for multiple characters among individuals within populations, suggesting a lack of general buffering capacity at the individual level during developmental processes (Eggert & Sakaluk 1994, Evans & Marshall 1996).

Bjorksten *et al*. (2000) verified through an extensive literature review that FA is not only related to a particular trait but it is also stress-specific. Environmental causes of stress, other than toxins, include temperature, parasites and food availability. In addition, genetic causes of developmental instability may be due to mutation or disruption of co-adapted gene complexes produced by inbreeding or hybridization (Scheiner *et al*. 1991, Graham 1992, Møller 1995).

The significant reduction of size due to the effect of diflubenzuron in our populations may reflect the ability of *H. irritans* to perform physiological and morphological adjustments, so that phenotypes compensate, at least to some extent, for stressful environment conditions. According to Cullum *et al*. (2001), biomass or fitness reduction is, in part, explained by the increased metabolic expenditure required to sustain life. Lomônaco & Germanos (2001) have already reported that the levels of FA of the wing of *Musca domestica* L. (Diptera Muscidae) were minimized by the considerable plastic potential for body size reduction and this same pattern of variation was reported by Bubli *et al.* (1998) for *Drosophila* m*elanogaste*r Meigen (Diptera: Drosophilidae), both studies dealing with sources of stress other than insecticides.

Developmental instability due to exposure to insecticides may result in genetic selection for resistance, which may be morphologically expressed by an increase in FA levels (Mckenzie & Clarke 1988), or by a reduction in body size (Bjorksten *et al*. 2000). Given that the exposure of an insect population to sub-lethal concentrations results in development of individuals with smaller size, and since size is usually directly related to fitness (David *et al*. 1994), one can predict that the surviving population will present low fitness. If it is not a starting-point in the selection for a resistant population, this result might be positive from the pest control point of view.

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