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Original Article

Phenotypic plasticity in adults of *Anticarsia gemmatalis* exposed to sub-doses of Bt-based bioinsecticide

Plasticidade fenotípica morfométrica em adultos de *Anticarsia gemmatalis* Hübner, 1818 expostos a subdoses de *Bacillus thuringiensis* Berliner, 1911

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Abstract

Anticarsia gemmatalis Hünber, 1818 is one of the main defoliating species in the soybean crop. *Bacillus thuringiensis* Berliner, 1915, is a bacterium used in the biological control of this pest species. Resistant populations and their sublethal effects caused by the use of the bacteria have already been reported; however, there are no studies on phenotypic plasticity in adulthood exposed to Bt-based bioinsecticide sub-doses. This study aimed to evaluate the morphometry of *A. gemmatalis* adults under laboratory conditions submitted to the Bt-based bioinsecticide Dipel® over the three generations. The body segments mensuread were width, length, and area of the anterior and posterior wings, the weight of the integument, chest, abdomen, wings, and the whole adult of males and females. Among the treatments, LC_5 in the first generation and LC_{10} in the second generation were those with lower thresholds in relation to the weight of the chest and abdomen, considering the proportions of the body smaller than the females. The female's weight adulthood was reduced by 10% about males, and, only in the first generation. Males have larger body size and more pronounced phenotypic plasticity than females. Here, we demonstrate the first study assessing the phenotypic plasticity of *A. gemmatalis* adults.

Keywords: allometry measure, morphometry, velvet bean caterpillar, *Bacillus thuringiensis*, phenotype.

Resumo

Anticarsia gemmatalis Hünber, 1818 é uma das principais espécies desfolhadoras da cultura da soja. *Bacillus thuringiensis* Berliner, 1915, é uma bactéria utilizada no controle biológico dessa espécie de praga. Populações resistentes e seus efeitos subletais causados pelo uso da bactéria já foram relatados, no entanto, não há estudos sobre a plasticidade fenotípica na idade adulta exposta a subdoses de bioinseticida à base de Bt. Este trabalho teve como objetivo avaliar a morfometria de adultos de *A. gemmatalis* em condições de laboratório submetidos ao bioinseticida Dipel® ao longo de três gerações. Os segmentos corporais mensuráveis eram largura, comprimento e área das asas anterior e posterior, o peso do tegumento, tórax, abdômen, asas e todo o adulto de machos e fêmeas. Dentre os tratamentos, CL_5 na primeira geração e CL_{10} na segunda geração foram aqueles com limiares mais baixos em relação ao peso do tórax e abdômen, considerando as proporções do corpo menores que as do sexo feminino. O peso da fêmea na idade adulta foi reduzido em 10% em relação aos machos e, apenas na primeira geração. Os machos têm tamanho corporal maior e plasticidade fenotípica mais pronunciada do que as fêmeas. Este estudo demonstra o primeiro estudo avaliando a plasticidade fenotípica de adultos de *A. gemmatalis*.

Palavras-chave: medida de alometria, morfometria, lagarta da soja, *Bacillus thuringiensis*, fenótipo.

1. Introduction

Anticarsia gemmatalis Hünber, 1818 is a polyphagous species, and one of the main defoliating species of the soybean crop on the American continent (Ford et al., 1975; Pashley and Johnson, 1986; Haase et al., 2015; Fernandes et al., 2018). The insect pest's permanence in tropical and subtropical environments is attributed

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to continuous cultivation throughout the year, which favors the formation of green bridges (Oliveira et al., 2014; Fernandes et al., 2020).

Among the existing control methods, *Bacillus thuringiensis* Berliner, 1915, is a bacterium used in the biological control of this pest species. In the form of Bt-based bioinsecticides or biotechnology with the insertion of Cry genes into plants to provide resistance to insects, known as transgenic plants or Bt plants (Konecka et al., 2018; Souza et al., 2021).

Resistant populations and their sub-lethal effects caused by the use of the bacteria have already been reported (Sedaratian et al., 2013; Janmaat et al., 2014; Souza et al., 2019; Rabelo et al., 2020; Fernandes et al., 2021). However, there are no studies on phenotypic plasticity in adulthood, according to the exposure of the underdosage of Bt-based bioinsecticides.

Phenotypic plasticity is the ability of an organism to respond to environmental stresses with changes in form, state, movement or activity (Brisson, 2010; West-Eberhard, 2003). In addition to being considered an important escape tool for survival in unstable environments or disturbed by human action (Gotthard and Nylin, 1995).

Studies demonstrate that these morphological adaptations allow organisms to adapt better to disturbed environments over short time scales, without changes in genotype (West-Eberhard, 2003; Hayes et al., 2019).

However, due to the lack of studies on the phenotypic plasticity of *A. gemmatalis* exposed to underdoses of Bt-based bioinsecticides, this study aimed to evaluate the phenotypic plasticity, based on the morphometry of *A. gemmatalis* adults submitted to the Bt-based bioinsecticides Dipel® in the laboratory over three generations.

2. Material and Methods

2.1. Insect rearing

The population of *A. gemmatalis* used in the bioassays was maintained on an artificial diet (Greene et al., 1976) at the Laboratory of Microbial Control of Arthropod Pests of the State University of São Paulo "Júlio de Mesquita Filho" (UNESP – Jaboticabal). The insects were maintained at 25 ± 1 °C, 70 ± 10% RH, and 12 h photophase.

2.2. Sublethal concentrations

The formulation toxicity was evaluated using the spore-crystal suspensions of the Bt-based bioinsecticide (Dipel®). The suspensions were defined by plating on nutrient agar to determine the CFU, which was evaluated after seven days (Sedaratian et al., 2013). The curve response was estimated using the Six Error Problems analysis (SAS Institute Inc., 2014). 200 µL on the surface of the artificial diet (4.8 cm^3) were previously distributed in polyethylene cups (3.5 cm Ø). A hundred insects were evaluaded to estimate a response curve for each treatment, distributed in 10 repetitions. Deionized water was applied in equal volume as a control. The bioassay evaluations were kept after seven days.

2.3. Assessment of sublethal effects

The surviving caterpillars in each treatment/generation were evaluated daily and were sexed when they reached the pupal stage (Butt and Cantu, 1962). The newly emerged adults were separated into couples, totaling 100 couples, and placed in PVC cages (10 × 20 cm), lined with white A4 sulfite paper (used as an oviposition substrate). At the bottom, a Petri dish with filter paper was used and the top was sealed with *voile* fabric.

The adults were fed with a 10% honey solution moistened with cotton wool placed in a polyethylene petri dish (49 x 12 mm) at the cages (Fernandes et al., 2017). The papers used as a laying substrate were removed, exchanged daily, packed in plastic pots (14.0 cm Ø, 10 cm h), and with the hatching of the larvae, these were used to originate the subsequent generations (Kalvnadi et al., 2018).

2.4. Morphometry

The adults of *A. gemmatalis* exposed to the Bt-based bioinsecticide sub-doses were weighted within 24 hours. The different parts of the individuals were separated with the aid of fine-tipped surgical scissors, and then weighed on an analytical balance (Belmark – 210A). The weighing was performed with the tegument, thorax, abdomen, wings and whole adult.

After mounting on the lamina, coverslip and sealed with a thin layer of colorless nail polish dried for two hours. The measurements of the length, width and area of the anterior and posterior wings were obtained with the aid of a stereoscope microscope with an attached camera (Leica S9 i), according to the technique described by Di Mare and Corseuil (2004).

2.5. Experimental design and data analysis

The mortality data from virulence tests were submitted to Probit regression analysis and sublethal concentration values LC_5, LC_{10}, LC_{15} and LC_{20} (0.20509, 0.38126, 0.57929 and 0.80776 µg Bt.mL diet^{-1}) were obtained using the SAS software (P> 95%) (SAS Institute Inc., 2014). We used a completely randomized design (CRD) with ten repetitions per sex and the treatments arranged in a 3 x 5 x 22 factorial arrangement. There were three generations (F_1, F_2, F_3), five treatments (LC_5, LC_{10}, LC_{15}, LC_{20} and control) and 22 variables being the weight of tegument, thorax, abdomen, wings, whole adult, and width, length and area of the anterior and posterior wings of both sexes.

Statistical analysis was performed on the GENES software (Cruz, 2001). The data were subjected to analysis of variance by F test and the stratified linear correlation was performed per generation and treatment. The Tukey test dismembered the variables that showed an interaction between treatment and the generation at 5% probability.

3. Results

3.1. Males morphometry

In the weight of the tegument of the males, treatments LC_5, LC_{10} and LC_{15} differed in the first generation; the treatments LC_5, LC_{10} and LC_{20} in the second generation and only LC_{15} in the third generation. The chest weight between all treatments obtained significance between the control in the first generation, with no significant differences being observed in the second and third generations. In the abdomen, the weight in the LC_{20} in the first generation reached the highest average, differing significantly among all treatments. In subsequent generations, differences were observed only in the LC_{10} treatment (Table 1).

The weight of the wings showed significance only to the LC_{10} treatment in the first generation. In the total weight of the males, treatments LC_{20} in the first generation and LC_5 in the third generation reached higher averages, not differing significantly from the control. The length of the anterior wings in the LC_5 treatment reached lower averages over the three generations, with significance being observed for LC_{10} in the first generation, LC_{15} in the second generation and LC_{10}, LC_{15} and LC_{20} third generation. The same was not observed in the width of the anterior wings of the males. Therefore, the length of the posterior wings of the males obtained higher averages for the control, differing significantly between all treatments in the first generation and LC_5, LC_{15} and LC_{20} in the second generation. In the last generation, only LC_{10} showed significance among all treatments. The same was not observed in the width of the posterior wings of the males (Table 1).

The area of the anterior wing of the males obtained averages superior to the control in the three generations, differing significantly from the treatments LC_5, LC_{10} and LC_{15} in the first generation and LC_5, LC_{10}, LC_{15} and LC_{20} in the third generation. The same was not observed about the area of the anterior wing of the males (Table 1).

3.2. Females morphometry

In weight of the tegument of the females, the treatment LC_5 in the first generation, LC_{10} in the second generation and LC_{15} in the third generation reached higher averages. The weight of the chest of treatment LC_5 in the first generation, LC_{10}, LC_{15}, LC20 and control in the second generation and LC_5, LC_{15} and LC_{20} in the third generation had the highest averages, not significantly different between both (Table 2).

In the abdomen, LC_{15} and LC_{20} in the first generation, LC_5 and LC_{20} in the second generation and LC_{20} in the third generation reached lower averages among all other treatments. However, weight of the wings, did not show significance between treatments and generations. The total weight of the females revealed that the LC_5 in the first generation differed among all treatments. The same was not observed in the second and third generations (Table 2).

In the length of the anterior wing, LC_{10} and control in the first and second generation reached the highest averages, with significance with the treatments LC_5 and LC_{20}, respectively. In the third generation, there was no significance between treatments. In the width of the anterior wing, it was observed that only in the second generation, the treatments did not differ between both (Table 2).

The length of the posterior wing showed higher averages at LC_{10} and control over the first and second generation, with significance for the LC_5 treatment. In the third generation, there was no significant difference between treatments. Regarding the width of the posterior wing, LC_5 presented lower averages, differing significantly from the control treatment.

The area of the anterior wing in the LC_5 treatment in the first and second generation reached lower averages than the control treatment, differing significantly between both. In the third generation, there was no significant difference between treatments. The same was not observed in the area of the posterior wing of the females (Table 2).

Variations were observed in the wing area of males in all generations, with an increase in the area of the anterior and posterior wings according to the increase in the exposed sub-dose. The same fact was not observed in the LC_{20} treatment in the second and third generations in males (Table 1). In females, the same does not occur, but after the second generation, the area of the anterior wings became more stable, with no significant difference in the third generation. About the posterior wing, the fact occurred only in the second generation in females (Table 2).

3.3. Linear correlation stratifies

In the first generation males, the parameters tegument + abdomen, thorax + healthy adult, and wing + healthy adult achieved moderate positive linear correlations to the LC_5 and control treatments. However, females in the parameters tegument + thorax, tegument + abdomen, intact adult + abdomen, inferior length + superior width, thorax + intact adult and intact adult + integument, reached, predominantly, moderate to strong positive linear correlations to the LC_{15} and LC_{20} treatments, respectively. The same was not observed in the second generation of males, with moderate linear correlations for LC_5 and LC_{10} in the parameters tegument + abdomen, chest + abdomen and healthy adults + abdomen. In females, the treatments that presented strong linear correlations were LC_{10} and LC_{15} to the parameters integument + abdomen, integument + healthy adults and healthy adults + abdomen (Figure 1).

In the third generation, the males in the control treatment showed moderate positive linear correlations to the parameters wing + intact adult, integument + upper wing, wing + upper width and upper width + whole adult. Unlike females who obtained moderate negative linear correlations to the control treatment in the parameters thorax + upper size width, lower size length + thorax, integument + wing, integument + healthy adult, wing + abdomen, healthy adult + abdomen and thorax + abdomen. Therefore, a greater number of moderate positive correlations to treatment LC_5 with the parameters integument + abdomen, integument + intact adult, intact adult + abdomen, thorax + abdomen and integument + thorax, respectively (Figure 1).

Table 1. Body weight (N), wing size and wing area of *Anticarsia gemmatalis* submitted to sublethal doses of the bioinsecticide Dipel® over three generations at 25 ± 1 °C, 70 ± 10% RH and photoperiod L12: D12 h.

Treatments	Male										
	Body Weight (mg)					Wing Sizes (mm)				Wing Area (mm)	
						Anterior		Posterior			
	Tegument	thorax	Abdome	Wing	Full Adult	Length	Width	Length	Width	Anterior	Posterior
1st Generation											
LC_5	69.87 ± 0.05cC[1 2]	40.22 ± 0.03cC	29.65 ± 0.05cB	44.36 ± 0.05aA	114.22 ± 0.07bAB	17.36 ± 0.39cA	9.52 ± 0.29bB	12.12 ± 0.33cC	9.51 ± 0.37bC	67.44 ± 36.47cB	56.14 ± 35.36dB
LC_{10}	112.71 ± 0.02bA	51.05 ± 0.02bA	61.66 ± 0.02bA	11.23 ± 0.02bA	123.94 ± 0.02bA	18.42 ± 0.19bA	9.34 ± 0.14cC	13.46 ± 0.16bB	9.47 ± 0.16bC	71.68 ± 13.14bcC	57.53 ± 10.54cdB
LC_{15}	64.96 ± 0.02cB	20.59 ± 0.02dB	44.37 ± 0.03bcA	41.49 ± 0.02aA	106.45 ± 0.01bA	17.51 ± 0.17cB	10.52 ± 0.17aA	13.51 ± 0.17bA	10.52 ± 0.16aAB	72.52 ± 9.80bcC	64.45 ± 8.97bcB
LC_{20}	126.22 ± 0.04aA	49.16 ± 0.01bA	87.06 ± 0.04aA	40.25 ± 0.04aA	176.47 ± 0.05aA	17.93 ± 0.33bcA	9.83 ± 0.23bB	11.83 ± 0.37cB	9.81 ± 0.30bB	75.98 ± 30.68aB	64.68 ± 30.94aB
Control	129.75 ± 0.04aA	67.34 ± 0.04aA	56.42 ± 0.04bB	39.72 ± 0.04aA	163.47 ± 0.04aA	19.55 ± 0.18aA	14.60 ± 0.14aA	14.54 ± 0.16aA	9.54 ± 0.16bB	75.49 ± 17.28aB	63.97 ± 16.27aB
2nd Generation											
LC_5	91.29 ± 0.02cB	50.68 ± 0.01aB	68.61 ± 0.02aB	14.07 ± 0.03aB	105.36 ± 0.04bB	17.57 ± 0.17bA	9.85 ± 0.22bB	13.58 ± 0.18cB	10.38 ± 0.18abB	84.03 ± 17.05aA	67.67 ± 15.25cA
LC_{10}	93.39 ± 0.01cB	53.32 ± 0.01aA	40.06 ± 0.01bB	13.14 ± 0.01aA	106.53 ± 0.01bA	17.93 ± 0.19bA	10.13 ± 0.21abB	14.40 ± 0.17abA	10.41 ± 0.18abB	85.88 ± 17.05aB	72.01 ± 15.25bA
LC_{15}	113.14 ± 0.02abA	58.77 ± 0.02aA	54.37 ± 0.03abA	7.86 ± 0.02aB	121.00 ± 0.02bA	18.87 ± 0.22aA	10.46 ± 0.15aA	13.65 ± 0.15bcA	9.96 ± 0.25bB	89.78 ± 21.85aA	74.54 ± 17.02abA
LC_{20}	106.02 ± 0.03bcB	52.68 ± 0.01aA	53.34 ± 0.02abB	6.81 ± 0.01aB	112.83 ± 0.03bB	17.85 ± 0.24bA	10.42 ± 0.14aA	13.96 ± 0.26bcA	10.86 ± 0.21aA	88.56 ± 9.10aA	76.88 ± 19.58aA
Control	127.22 ± 0.05aA	58.52 ± 0.00aB	40.70 ± 0.05bAB	21.02 ± 0.04aB	148.24 ± 0.05aAB	19.32 ± 0.14aA	10.53 ± 0.16aB	14.93 ± 0.22aA	10.54 ± 0.20abA	89.87 ± 13.51aA	75.21 ± 17.05aA
3rd Generation											
LC_5	119.68 ± 0.01aA	59.54 ± 0.02aA	60.13 ± 0.02abA	9.74 ± 0.01aB	129.41 ± 0.01abA	16.96 ± 0.29cB	10.05 ± 0.21bA	12.90 ± 0.22cA	10.48 ± 0.17bA	79.01 ± 18.24bA	66.59 ± 20.86cA
LC_{10}	106.20 ± 0.02abAB	50.97 ± 0.02aA	55.22 ± 0.03bAB	9.35 ± 0.02aA	115.55 ± 0.02bA	19.35 ± 0.34aA	10.90 ± 0.27aA	15.01 ± 0.25aA	11.55 ± 0.22aA	85.26 ± 15.98bA	74.13 ± 16.55bA
LC_{15}	102.07 ± 0.02bA	51.29 ± 0.02aA	76.79 ± 0.03aA	6.23 ± 0.02aB	108.31 ± 0.02bA	17.96 ± 0.15bB	10.74 ± 0.15aA	13.08 ± 0.15cA	11.09 ± 0.15abA	80.20 ± 23.50bB	76.71 ± 31.92abA
LC_{20}	107.38 ± 0.02abB	49.08 ± 0.02aA	58.30 ± 0.03abB	6.81 ± 0.02aB	114.19 ± 0.02bB	17.58 ± 0.17bcA	10.47 ± 0.16abB	13.59 ± 0.24bcA	10.54 ± 0.15bA	76.84 ± 25.18bB	69.92 ± 18.87bcB
Control	122.09 ± 0.02aA	46.05 ± 0.01aC	50.04 ± 0.02bA	18.73 ± 0.03aB	140.82 ± 0.02aB	17.40 ± 0.23bcB	9.46 ± 0.20cC	13.93 ± 0.23bB	10.58 ± 0.16bA	95.77 ± 17.47aA	77.58 ± 15.27aA

[1]Mean ± standard error; [2]Means followed by the same lower case letter between treatments and upper case letters between generations do not differ significantly by the Tukey test (P <0.05). N = 10 insects evaluated.

Table 2. Body weight (N), wing size and wing area of *Anticarsia gemmatalis* submitted to sublethal doses of the bioinsecticide Dipel® over three generations at 25 ± 1 °C, 70 ± 10% RH and photoperiod L12: D12 h.

Treatments	Female										
	Body Weight (mg)					Wing Sizes (mm)				Wing Area (mm)	
						Anterior		Posterior			
	Tegument	thorax	Abdome	Wing	Full Adult	Length	Width	Length	Width	Anterior	Posterior
1st Generation											
LC_5	131.81 ± 0.03aA[1 2]	68.98 ± 0.03aA	62.82 ± 0.02aA	18.62 ± 0.03aA	150.42 ± 0.03aA	16.89 ± 0.22bA	9.37 ± 0.29bA	11.97 ± 0.25bcB	9.06 ± 0.28bB	57.63 ± 22.25dC	69.54 ± 23.64aA
LC_{10}	108.05 ± 0.03bA	49.56 ± 0.02bcA	58.49 ± 0.02abA	16.38 ± 0.03aA	124.43 ± 0.03bA	18.37 ± 0.27aA	10.34 ± 0.16aA	14.32 ± 0.27aA	10.22 ± 0.31aA	80.51 ± 18.64aAB	62.52 ± 23.00bA
LC_{15}	78.50 ± 0.4cB	35.87 ± 0.03dB	42.63 ± 0.03cB	5.31 ± 0.01aA	83.81 ± 0.04cC	17.16 ± 0.35bA	9.09 ± 0.32bB	12.70 ± 0.31bA	10.38 ± 0.29aAB	72.77 ± 27.18bB	59.01 ± 29.85bcB
LC_{20}	86.96 ± 0.03cA	42.93 ± 0.02cdA	44.02 ± 0.02cA	5.97 ± 0.01aA	92.93 ± 0.03cA	16.51 ± 0.27bB	9.46 ± 0.30bA	11.57 ± 0.33cB	10.46 ± 0.29aA	66.83 ± 25.26cB	56.30 ± 22.69cB
Control	108.11 ± 0.02bA	56.05 ± 0.04bA	52.06 ± 0.03bcB	14.87 ± 0.02aA	122.98 ± 0.02bA	19.03 ± 0.33aA	10.77 ± 0.38aA	14.22 ± 0.24aA	10.27 ± 0.33aA	81.59 ± 19.25aAB	66.24 ± 21.05bA
2nd Generation											
LC_5	87.79 ± 0.00cA	44.28 ± 0.01bB	43.51 ± 0.03bB	7.81 ± 0.01aA	95.60 ± 0.03bC	17.50 ± 0.16bA	9.83 ± 0.23aA	12.57 ± 0.17cAB	8.95 ± 0.25cB	68.65 ± 24.39bB	58.81 ± 15.72bB
LC_{10}	115.63 ± 0.03abB	54.62 ± 0.01aA	61.01 ± 0.03aA	3.44 ± 0.01aA	119.07 ± 0.03aA	18.39 ± 0.13aA	10.04 ± 0.22aAB	13.47 ± 0.18abB	10.32 ± 0.14abA	84.42 ± 8.08aA	66.46 ± 6.43aA
LC_{15}	101.35 ± 0.03bcA	49.89 ± 0.02abA	51.46 ± 0.02abA	3.06 ± 0.01aA	104.41 ± 0.03abB	17.73 ± 0.23abA	10.46 ± 0.17aA	12.72 ± 0.23bcA	9.77 ± 0.23bcB	84.13 ± 18.53aA	65.20 ± 8.73aA
LC_{20}	86.06 ± 0.02cA	45.49 ± 0.01abA	40.57 ± 0.02bA	7.25 ± 0.01aA	93.32 ± 0.02bA	17.48 ± 0.17bA	9.81 ± 0.23aA	12.91 ± 0.22bcA	10.36 ± 0.15abA	82.11 ± 7.71aA	64.67 ± 9.92aA
Control	117.32 ± 0.00aA	52.90 ± 0.00abAB	64.42 ± 0.02aA	3.58 ± 0.01aA	120.90 ± 0.02aA	18.44 ± 0.16aA	10.54 ± 0.23aA	13.92 ± 0.17aA	10.97 ± 0.25aA	87.42 ± 15.61aA	68.29 ± 22.25aA
3rd Generation											
LC_5	98.18 ± 0.03bA	47.14 ± 0.03abB	51.04 ± 0.02abB	16.20 ± 0.03aA	114.38 ± 0.04abB	17.48 ± 0.28aA	9.54 ± 0.25bA	13.10 ± 0.23aB	9.51 ± 0.20bA	76.75 ± 11.05aA	55.79 ± 10.53cB
LC_{10}	86.14 ± 0.03bB	39.39 ± 0.02bB	46.75 ± 0.02bB	24.84 ± 0.01aA	110.99 ± 0.03abA	17.39 ± 0.22aB	9.86 ± 0.16abB	13.11 ± 0.15aA	11.00 ± 0.17aA	78.92 ± 20.01aB	65.08 ± 16.44abA
LC_{15}	114.24 ± 0.03aA	53.80 ± 0.02aA	60.44 ± 0.02aA	13.91 ± 0.01aA	128.15 ± 0.03aA	16.85 ± 0.16aA	9.49 ± 0.16bA	12.62 ± 0.23aC	10.45 ± 0.15aA	75.24 ± 15.76aB	62.70 ± 21.31bAB
LC_{20}	87.80 ± 0.03bA	45.04 ± 0.02abA	42.76 ± 0.02bA	9.03 ± 0.01aA	96.83 ± 0.03bA	17.51 ± 0.25aAB	10.40 ± 0.24aA	13.01 ± 0.15aA	10.58 ± 0.31aB	79.72 ± 18.88aA	68.62 ± 14.62aA
Control	92.25 ± 0.02bB	46.11 ± 0.01abC	46.14 ± 0.02bB	38.32 ± 0.03aA	130.57 ± 0.03aA	17.19 ± 0.18aB	9.95 ± 0.17abB	12.57 ± 0.23aA	11.07 ± 0.15aB	74.36 ± 16.52aB	60.50 ± 22.97bA

[1]Mean ± standard error; [2]Means followed by the same lower case letter between treatments and upper case letters between generations do not differ significantly by the Tukey test ($P < 0.05$). N = 10 insects evaluated.

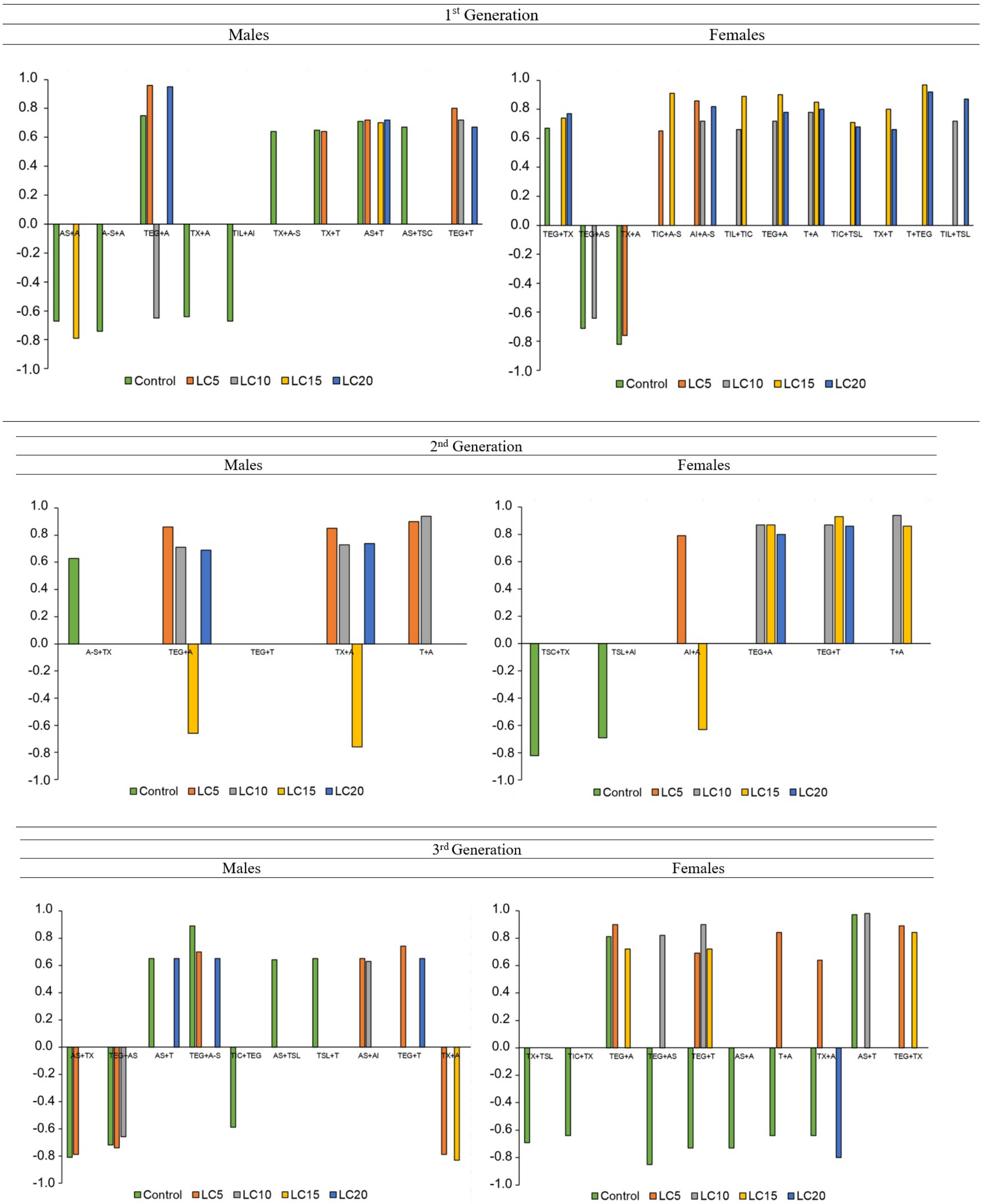

Figure 1. Linear stratified morphometric correlation of *Anticarsia gemmatalis* submitted to sublethal doses of the bioinsecticide Dipel® over three generations at 25 ± 1 °C, 70 ± 10% RH and photoperiod L12: D12 h. Tegument (TEG), thorax (TX), abdomen (A), wing (AS), upper wing (A-S), whole adult (T), upper wing length (TSC), lower wing length (TIC), upper wing width (TSL), bottom wing width (TIL), lower wing area (AI).

4. Discussion

The sublethal effect of Dipel® sub-doses on *A. gemmatalis* morphometry varied according to the concentration of the bioinsecticide. The sublethal effects observed in the bodyweight of adults may be associated with the differential susceptibility between the sexes exposed to sub-doses of the bioinsecticide based on *B. thuringiensis*, in addition to the physiological changes that are reflected in the adult phase (Retnakaran et al., 1983; Alix et al., 2001; Desneux et al., 2007; Sedaratian et al., 2013).

The chest weight was higher than that of females in both generations. In field conditions, one should consider the higher energy expenditure of males to locate and court females and, therefore, the greater need for chest muscles to be developed (Srygley and Chai, 1990). The relative speed of flight in insects is correlated with the chest mass, and the sublethal effects caused by the bioinsecticide can interfere with the formation of muscles essential to flight. This region concentrates phasic muscles, which commonly work to move appendages in the exoskeleton (Howland, 1974). This arrangement of muscles within the insects' rib cage is directly related to weight, because the larger it is inferred that the male will have better physical conditioning (Srygley and Chai, 1990). Individuals who have these morphometric characteristics exhibit, for example, a higher frequency of copulations, better biological and even physical conditioning (Di Mare and Corseuil, 2004).

The abdomen is another fundamental structure for the proper functioning of all insect functions. This structure is responsible for energy reserves and the weight parameter is linked to the amount of this reserve. However, the balance between chest and abdomen must exist for the insect to perform the basic functions for survival (Srygley and Thomas, 2002). The hovering flight that insects present is a major component of the energy cost, requiring a greater energy reserve in the abdomen (Srygley and Chai, 1990). This type of flight has advantages because it allows the insect to escape from predators through high-speed flights (Marden and Chai, 1991).

Among the treatments, LC_5 in the first generation and CL_{10} in the second generation were those with lower thresholds in relation to the weight of the chest and abdomen, considering the proportions of the body smaller than the females. Body size significantly affects most of the physiological characters linked to survival and reproduction, one of the most important quantitative characteristics subject to evolution (Darwin, 1859; Schmidt-Nielsen, 1984; Roff, 1992; Stearns, 1992).

Smaller individuals are potentially less likely to perpetuate their offspring, due to competitive disadvantages compared to other males and the lower acceptability of females (Stearns, 1976). The choice for the female, in this case, can occur, in such a way, that each female has its optimum male size to copulate. This fact, is closely linked to the hypothesis of the physiological capacity of insects to define patterns of allometric measurements (Borgia, 1979).

This optimal size would be the result of a trade-off between the negative influences that the female has with large males on fertility and the advantages of large males for the biological conditioning of the offspring (Clutton-Brock and Parker, 1992; Andersson, 1994). However, even individuals who presented smaller sizes such as LC_5 and LC_{15} in the first generation, LC_5 and LC_{10} in the second generation and LC_{15} and LC_{20} in the third generation may not perpetuate their offspring, considering that the larger body size generally increases the pairing success due to intraspecific competition or female choice (Clutton-Brock and Parker, 1992; Andersson, 1994).

Wing proportions are influenced, according to the size of the rib cage, as individuals with larger wings have more developed muscles (Marden and Chai, 1991). Morphometry studies confirm that the insects' anterior wings have an important allometric measurement in determining size and shape (Di Mare and Corseuil, 2004; Sane, 2003). This fact is called phenotypic plasticity (Gotthard and Nylin, 1995; Loh et al., 2008), and does not justify the great variation only in the wings, but in all the dimensioned segments of this study.

Anterior wings perform the aerodynamic capacity and are closely related to the flight speed. However, the posterior wings function as an airfoil that regulates the direction and maneuverability of the flight (Di Mare and Corseuil, 2004; Dudley, 2000). *A. gemmatalis* lives in open agroecosystems and travels over long distances, thus requiring a relatively larger wing area (Di Mare and Corseuil, 2004). Studies monitoring populations of *A. gemmatalis* have shown that these adults can migrate great distances, even crossing entire states in the USA (Buschman et al., 1977). The species is known to be unable to survive the winter in the continental USA. On many occasions, insect pest populations fly dozens of kilometers in search of favorable conditions for development (Buschman et al., 1977; Sosa-Gómez, 2004).

Studies evaluating the morphometry of adults in Pieridae, Nymphalidae, and Papilionidae families have shown positive correlations between the flight speed and chest weight, but negatively for the abdomen weight that has the function of storing energy and the reproductive organs (Srygley and Chai, 1990). Thus, the influence of weight distribution between the chest and abdomen may interfere with the allometric measurements of *A. gemmatalis* due to exposure to the bioinsecticide sub-dose based on *B. thuringiensis* (Sih, 1987; Srygley and Chai, 1990).

The parameters abdomen + intact adults and abdomen + integument in females had a predominance of positive correlations. Biologically, males aim to develop and fertilize females; in turn, females have the function of producing eggs, storing male sperm until the eggs are ready to be fertilized, generating offspring and perpetuating the species (Milano et al., 2008). The region where the female reproductive system is located is in the abdomen and requires that all basic functions communicate and have a good functioning to generate viable offspring, also, the minimum size is of great relevance for the perpetuation of the species (Milano et al., 2010).

In the integument + healthy adult parameters in both sexes, they reinforce the strong correlation between the balance of the segments, between weight and adequate wing size. The morphology of insect wings has a direct effect on a flight and, therefore, on the ability of flying species to explore their environment efficiently. The need to maneuver, hover, accelerate and fly at a low energy cost should affect the shape of the wing and lead to the diversification of wing morphometry, according to the stress exposed to the host (Meresman et al., 2020).

In insects, the variation in wing morphometry suggests that different selective pressures, such as bioinsecticides, act non-uniformly in different regions of the wings, probably due to differences associated with body size (Bai et al., 2012; Tocco et al., 2019; Le-Roy et al., 2019). Therefore, this can influence the ecology and physiology of the population and even the organization of the community.

Additional effects can also occur in the type of defense used to prevent predators, parasitoids, entomopathogens and in the development and fertility rates of the insect pest (Srygley and Chai, 1990).

Here, we demonstrate the phenotypic plasticity of *A. gemmatalis* adults submitted to sub-doses of the bioinsecticide based on *B. thuringiensis*. Due to the possible difference in susceptibility between the sexes, males have larger body size and more pronounced phenotypic plasticity than females.

The common sense that biopesticides are intrinsically related to their lethal effects (death) restricts, to a few studies, a more holistic and detailed view that would be provided by the assessment of the sub-lethal effects of these products. It is noteworthy that these sub-lethal effects affect the insect population structure target and interfere with their ecological interactions. There is the possibility of being implemented in integrated pest management as one of the methods to assess possible resistant populations under field conditions.

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