

Effect of Insect Growth Regulator Insecticides Novaluron, Teflubenzuron and Lufenuron on the Morphology and Physiology of *Euschistus heros*

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Abstract

The Neotropical brown stink bug, *Euschistus heros* (F.) (Heteroptera: Pentatomidae), is an important pest that damages soybean, especially in the central-west region of Brazil. The effect of insect growth regulator insecticides on fourth-instar nymphs and adults of the neotropical brown stink bug was evaluated under laboratory and greenhouse conditions, respectively. In the laboratory, the insecticides (doses in g a.i. ha⁻¹) novaluron (20.0 and 40.0), teflubenzuron (26.2 and 52.5), and lufenuron (20.0 and 40.0), in addition to the control (water), were tested on fourth-instar nymphs of *E. heros* and their development were followed to adulthood. Mortality, number of adults with and without deformities, and fecundity were determined. In a greenhouse, the effects of the same insecticides on the adult of the stink bug were evaluated by determining the fecundity and viability of the eggs laid. The fourth-instar nymphs of *E. heros*, when exposed to either dose of the tested insecticides presented mortality and insects with deformations, as well as reduced fecundity of females that reached adulthood. In the trial with adult *E. heros*, all tested insecticides reduced stink bug fecundity. In the same way, the viability of the produced eggs was reduced in all the chemical treatments, except for novaluron at the lowest dose tested, in which egg viability did not differ from the control treatment. Based on the obtained results, one can infer that the growth-regulator insecticides evaluated, although usually more suitable for the control of caterpillars, can interfere negatively in the development and reproduction of the neotropical brown stink bug, thus constituting a complementary alternative for the management of this pest.

Keywords: insect growth regulators, development, fertility, fecundity, Neotropical brown stink bug, soybean

1. Introduction

The crops *Glycine max* (L.) soybean and *Zea mays* L. corn occupy positions of economic prominence in the countries that produce them, and their grains are marketed and consumed worldwide by both humans and animals (CONAB, 2023). Brazil assumed the title of largest producer and exporter of soybean in the world, with production in the 2022/2023 harvest of 153,633.0 million tons (CONAB, 2023). However, numerous phytosanitary problems can negatively affect the productivity of these crops in Brazil, especially diseases and insect pests, which can cause severe damage throughout the crop development cycle (Possebom, Lucini & Panizzi, 2020).

Among the insect pests that attack soybean and corn, the Neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) is considered an important pest, especially in the Brazilian Cerrado region (Bueno, Bortolotto, Pomaris-Fernandes, & França-Neto, 2015); (Ferreira, Peixoto, Oliveira, Quintino, & Bortolani, 2018). At specific population levels, it causes irreversible damage to these crops, reflecting negatively on their productivity (Soares, Cordeiro, Santos, Omoto, & Correa, et al., 2018); (Sosa-Gómez, Corrêa-Ferreira, Kraemer, Pasini, Husch, Vieira, Martinez, & Lopes, 2020). In this way, *E. heros* causes the main damage to the soybean crop, swelling and wrinkling of the grains through the insertion of their stylets, in addition to leaving a hole in the grains that becomes a gateway for pathogens (Schaefer & Panizzi, 2000).

The control of Neotropical stink bugs in soybean crops has mostly been carried out through spraying of chemical insecticides belonging to the groups of neonicotinoids, pyrethroids, carbamates and organophosphates (Castellanos et al., 2019). In corn, the same groups of insecticides are used in spraying, in addition to seed treatment, especially with neonicotinoids (Crosariol Netto et al., 2015). However, continuous exposure of stink bugs to insecticides promotes the development of insect populations resistant to these products (Sosa-Gómez et al., 2001; Sosa-Gómez & Silva, 2010; Guedes, 2017; Tuelher et al., 2018; Somavilla et al., 2019). Thus, alternatives to control phytophagous stink bugs in crops that are compatible with the principles of Integrated Pest Management (IPM) are both economically and environmentally desirable (Liu et al., 2002).

Physiological insecticides, also known as Insect Growth Regulators (IGRs), belong especially to the benzoylurea group and are part of compounds that act on the immature stage of lepidopterans, generally preventing the target species from reaching the adult stage (Sun et al., 2015; Meng et al., 2020). This group of insecticides is normally characterized as selective products for nontarget organisms and have low toxicity for mammals (Khorshidi et al., 2019). Some IGR insecticides can cause negative effects on the reproduction of insects in the orders Coleoptera (Ávila & Nakano 1999; Nakano et al., 2018), Hemiptera (Furiatti et al., 2009; Zantedeschi et al., 2017), Orthoptera (Ghazawy, 2012), and Diptera (Djeghader et al., 2014). Thus, the morphological and physiological effects of IGRs on different groups of pests need to be evaluated to better understand the action of these products on the development of insects.

Therefore, the effects of the growth-regulating insecticides novaluron, teflubenzuron, and lufenuron on fourth-instar nymphs and adults of *E. heros* were evaluated, considering aspects of development and reproduction. A deeper understanding of the effects of IGRs on the development of *E. heros* can be justified by the fact that these insecticides can cause indirect effects on this insect, due to their occurrence in the same soybean structure as the lepidopterans that are direct targets of IGRs.

2. Material and Methods

The experiments were carried out in the Entomology Laboratory and in a greenhouse at Embrapa Agropecuária Oeste, in the 2019/2020 season, Dourados, Mato Grosso do Sul, Brazil (22°16'30" and 54°49'00").

Two experiments were conducted with *E. heros*—one with fourth-instar nymphs under laboratory conditions and the other with adults in a greenhouse. The minimum and maximum dose of the IGRs novaluron, lufenuron, and teflubenzuron recommended for the control of caterpillars in the soybean crop was used as treatments in both trials, in addition to an untreated control (Table 1).

Table 1. Active ingredients, trade name, and doses of growth regulators insecticide used in experiments with *Euschistus heros* nymphs and adults under laboratory and greenhouse conditions, respectively (Dourados, 2019)

Treatments (i.a.)	Commercial name	Dose (g i.a./ha)
Novaluron	Rimon [®] 100 EC	20.0
Novaluron	Rimon [®] 100 EC	40.0
Teflubenzuron	Nomolt [®] 150 SC	26.2
Teflubenzuron	Nomolt [®] 150 SC	52.5
Lufenuron	Match [®] 150 EC	20.0
Lufenuron	Match [®] 150 EC	40.0
Control	-	-

2.1 *Euschistus heros* Rearing

Euschistus heros nymphs and adults used in the tests came from the insect rearing of the Laboratory of Entomology of Embrapa Agropecuária Oeste. The insects were reared under controlled climatic conditions of temperature: 25±1 °C, relative humidity: 70±10%, and photophase: 14 hours.

2.2 IGRs Effect on *Euschistus heros* Nymphs

The experiment was conducted in the laboratory under controlled conditions of temperature: 25±1 °C, relative humidity: 70±10%, and photophase: 14 hours. Fourth-instar nymphs of *E. heros* up to 24 h old, from the laboratory rearing, were used.

The different chemical treatments were sprayed on plastic trays containing filter paper at the bottom, using a CO₂-propelled sprayer equipped with cone-type nozzles, calibrated at a pressure of 45 pounds/pol² to release a

spray volume equivalent to 150 L/ha. In the control treatment, only water was applied. After the sprayed solution dried on the trays, 14 fourth-instar stink bug nymphs were released to walk on the treated surface. As a food source for the nymphs, green beans (*Phaseolus vulgaris* L.), dry soybeans (*Glycine max* L.), peanuts (*Arachis hypogaea* L.), and sunflower seeds (*Helianthus annuus* L.) were placed inside the trays, in addition to a Petri dish containing a cotton soaked in distilled water (Figure 1A). A tulle-like fabric secured with elastic was placed on the upper edge of the trays to keep the insects from escaping (Figure 1B).

After the installation, evaluations were carried out every three days in each tray to record the number of mortality and/or deformed nymphs, as well as the number of insects that reached adulthood in the different treatments. The stink bugs that reached adulthood were sexed by means of the sexual dimorphism existing in the shape of the genitalia of males and females. The males of *E. heros* have a single plate (pygophore), while females have two lateral plates (Corrêa-Ferreira & Panizzi, 1999). Soon after, they formed couples, which were kept in PVC cages with a diameter of 100 mm and provided with the same food source as the nymphs. The PVC cages were closed at the top with a tulle-type fabric and secured at the edges with an elastic band (Figure 1D). As oviposition substrate, raw cotton fabric was glued to the inner walls of the cages (Figure 1C), and the number of eggs laid by insects were recorded. The experimental design used was completely randomized with the seven treatments (insecticide doses + control) in six replications (tray containing 14 fourth-instar nymphs).



Figure 1. (A) Detail of the tray with fourth-instar nymphs of *Euschistus heros* after spraying with IGR insecticides; (B) Trays covered with tulle and fixed with elastic on the edges to containment the nymphs; (C) Interior of the PVC cage with food and oviposition substrate for *E. heros* couples; (D) Detail of the tulle secured with elastic at the top of the cage to containment the *E. heros* adults

2.3 IGRs Effect on *Euschistus heros* Adults

The experiment was done in a greenhouse, under natural environmental conditions. To install the experiment, soybean seeds of the cultivar “Brasmax Potência”, not treated with insecticides, were sown in 5-L plastic pots, containing as a substrate a mixture of soil and organic matter in the proportion of 2:1. After soybean plants emerged, thinning was performed to leave only two plants per pot. The essay was installed when the soybean plants were at the R5.1 stage (beginning of pod filling), which were sprayed with the same treatments previously described for the laboratory test.

After spraying and drying of the spray, the plants were infested with five *E. heros* couples up to 24 hours old, from the breeding at the Entomology Laboratory. All pots containing the treated soybean plants as well as the control treatment were covered with tulle fabric supported by wire cages to contain the insects (Figure 2).

During the essay, the oviposition capacity of the stink bug was evaluated, recording daily the number of eggs deposited on the different parts of the soybean plant. The observed eggs were placed in Petri dishes, containing a portion of cotton soaked in distilled water and maintained in the laboratory under controlled temperature. The eggs were quantified, and their viability was evaluated later in the different treatments of the essay.

The experiment was carried out in a completely randomized design, consisting of seven treatments (insecticide doses + control) and seven replications (pots with two soybean plants).



Figure 2. Detail of pots kept in a greenhouse containing soybean plants sprayed or not with IGRs, in which couples of *Euschistus heros* were released inside

2.4 Statistical Analysis

The data obtained from both the laboratory and greenhouse trails were initially submitted to normality and homoscedasticity tests and later to analysis of variance. When a significant effect of treatment was found by the F test, the means were compared by the Tukey test at 5% probability.

3. Results

Mortality of *E. heros* nymphs was observed in all treatments with different doses of insecticides tested, but only treatments with the highest doses of teflubenzuron and lufenuron showed higher mortality levels than the control treatment (Table 2). Some *E. heros* nymphs, when exposed to the treatments with IGRs, had a darkened body

(Figure 3A), little locomotion, and difficulty returning to their natural position after some disturbance. Similarly, other nymphs had marked deformation of the head and lateral extensions of the pronotum (Figure 3B-3C) or kept their exuvia retained in the posterior region of the body (Figure 3D). In addition, some nymphs presented a liquid blister on their dorsal part, and some adults from the treated nymphs presented deformations or difficulties ridding themselves of their old cuticle (Figures 3E-3F).

The mean number of normal adult *E. heros* from the nymphs exposed to IGRs were lower in all treatments than the control, although without significant difference between the treatments (Table 2). As a result, control of 83.3% to 100% of adult stink bugs were achieved in the treatments with IGRs (Table 2). The *E. heros* females exposed as nymphs to IGRs laid a relatively very low average number of eggs in the two treatments with teflubenzuron compared to the control treatment. Females in the treatments with the other insecticides presented no fecundity (Figure 4).

Table 2. Mortality (+EP) observed (MO), percentage corrected mortality (MC) of nymphs, normal adults, and percentage of control (C) after fourth-instar *Euschistus heros* nymphs were exposed to IGRs in the laboratory. Dourados, 2019

Treatments (g i.a./ha)	Nymphs		Adults	
	MO	MC (%)	Normal	C (%)
Novaluron (20.0)	7.3±1.6 a	28.1	1.7±1.8 b	83.3
Novaluron (40.0)	6.3±2.3 ab	20.7	0.2±0.4 b	98.0
Teflubenzuron (26.2)	6.0±1.5 ab	18.5	0.8±1.6 b	92.2
Teflubenzuron (52.5)	7.7±1.8 a	31.1	0.7±0.8 b	93.1
Lufenuron (20.0)	5.8±1.6 ab	17.0	0.2±0.4 b	98.0
Lufenuron (40.0)	7.8±1.9 a	31.8	0.0±0.0 b	100.0
Control	3.5±2.4 b	-	10.2±1.5 a	-
CV (%)	29.8	-	57.4	-

Note. Means followed by the same letter do not differ statistically by Tukey's test ($p < 0.05$).

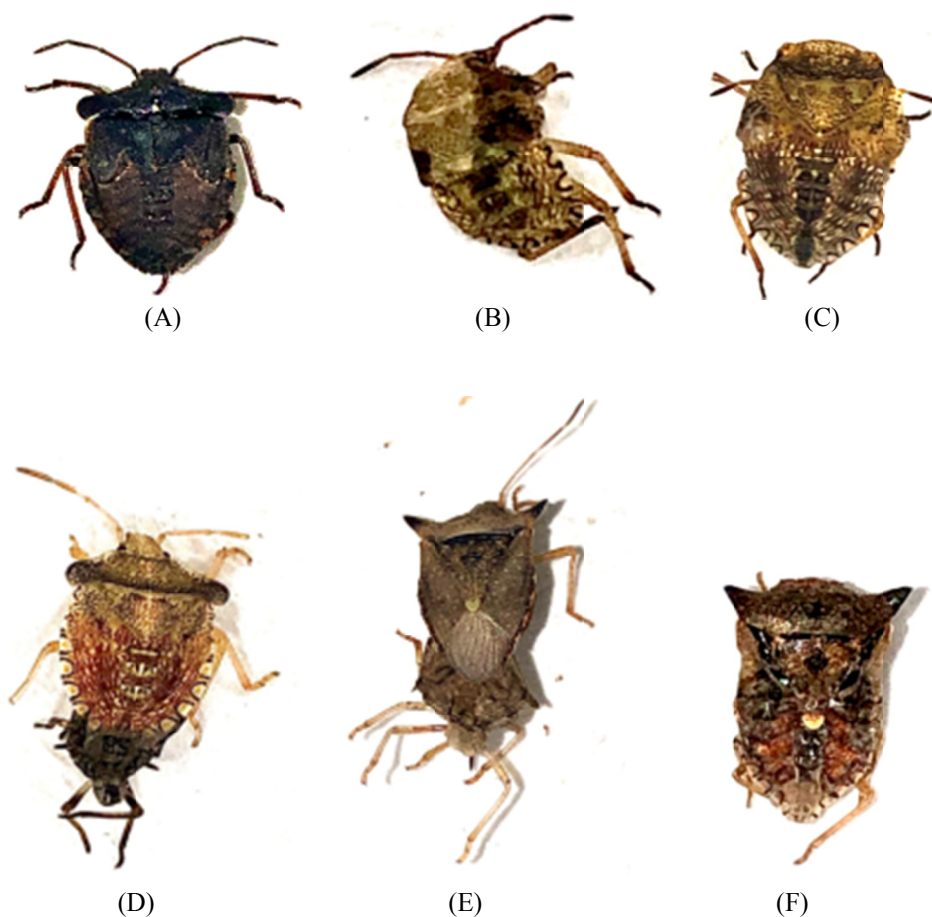


Figure 3. (A) Visual aspect of the darkening of the body of *Euschistus heros* nymphs; (B-C) deformation of the lateral extensions of the pronotum; (D) exuvia retained in the posterior region of the nymph's body; and (E-F) deformed adults from the laboratory test (Dourados, 2019)

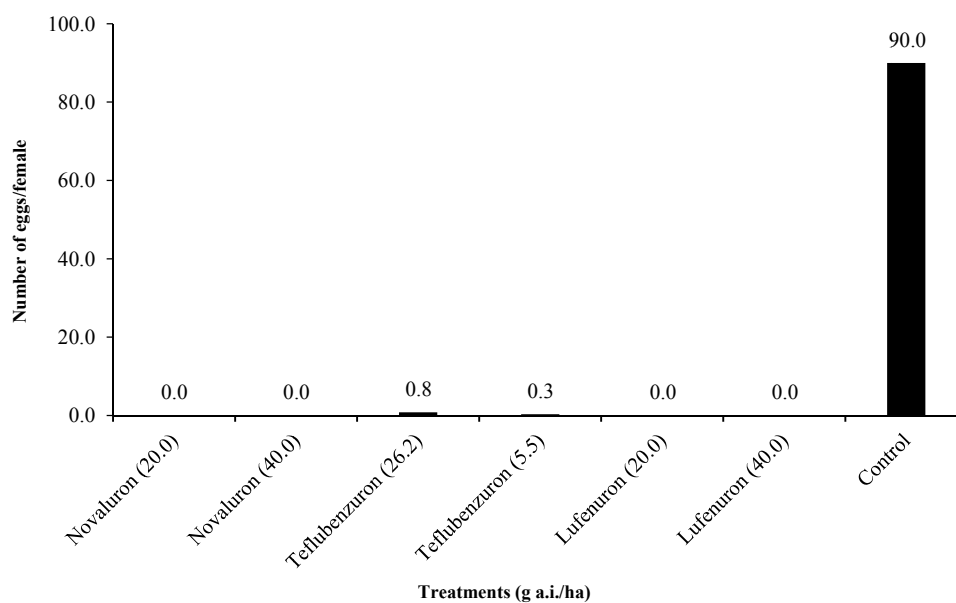


Figure 4. Mean number of eggs from female *Euschistus heros* from fourth-instar nymphs exposed to IGRs in the laboratory (Dourados, 2019)

The *E. heros* couples maintained in contact with soybean plants sprayed with the different IGRs produced a lower number of ovipositions than the control treatment, with the exception of the lufenuron treatment (20.0), in which the oviposition was similar to the unsprayed plants (Table 3). However, the average number of eggs obtained from couples exposed to IGRs was lower in all treatments with insecticides than the control, without however differing between them (Table 3).

Table 3. Average number (+ EP) of egg sacks and eggs obtained after couples of *Euschistus heros* (n = 5) exposed to soybean plants sprayed with different IGRs in a greenhouse (Dourados, MS, 2019)

Treatments (g i. a./ha)	Adults	
	Egg sacks ¹	Eggs ¹
Novaluron (20.0)	5.0±2.0 b	20.9±7.7 b
Novaluron (40.0)	5.7±0.9 b	29.0±6.2 b
Teflubenzuron (26.2)	4.4±1.4 b	16.6±5.5 b
Teflubenzuron (52.5)	8.1±2.7 b	36.4±14.3 b
Lufenuron (20.0)	15.0±1.8 a	46.0±3.2 b
Lufenuron (40.0)	7.9±1.9 b	31.4±6.9 b
Control	20.1±6.1 a	74.6±21.3 a
CV (%)	42.9	42.5

Note. ¹ From 5 couples of *E. heros* confined in soybean plants treated or not in a greenhouse.

Means followed by the same letter do not differ statistically by Tukey's test ($p < 0.05$).

The viability values of eggs obtained from *E. heros* couples that had contact with plants sprayed with IGRs were also lower in most chemical treatments when compared to the control (Figure 5). The exception was the treatment with the lowest dose of novaluron in which the viability of the eggs was similar to the control (Figure 5). Eggs from couples exposed to IGRs generally contained empty interiors or mortatilty embryos, which reflected negatively on their viability.

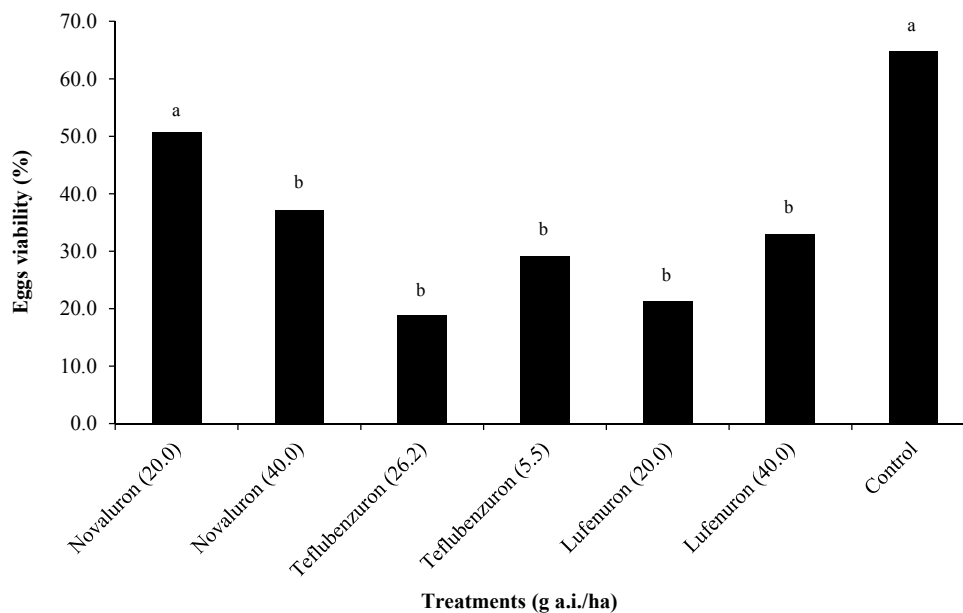


Figure 5. Mean (%) viability of *Euschistus heros* eggs obtained from couples exposed to soybean plants sprayed with different IGRs in a greenhouse. Dourados, MS. 2019. Columns followed by the same letter do not differ statistically by Tukey's test ($p < 0.05$)

4. Discussion

The results obtained with the IGRs tested on *E. heros* generally indicated that they caused deleterious effects on the immature phase of this species, affecting the normal process of ecdysis and causing deformations, and mortality of *E. heros* nymphs.

Studying the effect of different concentrations of lufenuron (250, 300, 350, and 400 mg.ml⁻¹) on *E. heros*, Turchen et al. (2016) observed that this IGR at concentrations of 250 to 400 mg.ml⁻¹ also caused the mortality of the neotropical brown stink bug nymphs as well as reduced the number of nymphs that completed the ecdysis process until adulthood. These authors also verified that lufenuron did not cause adult mortality or interfere with the fecundity and viability of stink bug eggs. These results partially corroborate to those obtained in this work, as the tested doses of lufenuron also caused mortality of nymphs of the stink bug.

Insecticides belonging to the chemical group of benzoylureas are considered inhibitors of chitin synthesis and act on the ecdysis process during the immature phase of lepidopterans, when they prevent the secretion of a new cuticle and exocuticle release in the caterpillars, thus causing their mortality (Aliabadi et al., 2016). Similar results were observed in this work for the nymph ecdysis process, mortality, deformations, changes in the number of ovipositions, egg viability, in *E. heros* nymphs, although these insects are in a different order (Matsumoto et al., 2021; Cremonez et al., 2019). In a study with the insecticides lufenuron (0.187 g a.i. ha⁻¹) and novaluron (0.249 g a.i. ha⁻¹) on the immature phase of *Anticarsia gemmatilis* Hubner 1818 (Lepidoptera: Eribidae), Loeck et al. (2007) found deleterious effects in the prepupal stage and deformations in the wings of adults, as well as a reduction in the females fecundity. Similar results were observed in this work for the fourth-instar nymphs of the Neotropical brown stink bug, when submitted to lufenuron and novaluron, which caused deformation and mortality of *E. heros* nymphs, in addition to the negative effects on the fecundity and viability of eggs produced by this species. For nymphs of *E. heros*, Lufenuron and diflubenzuron caused malformations in the mouthparts, consequently inhibiting feeding and due to this, nutritional deficiency directly affects metamorphosis and also caused mortality, reported by Agüero et al. (2023).

In another study evaluating the effect of the insecticide lufenuron on the green stink bug *Nezara viridula* (Linnaeus 1758) (Hemiptera: Pentatomidae), Furiatti et al. (2009) found that this IGR at a concentration of 0.025% efficiently controlled nymphs, causing more than 80% mortality after 24 hours of contact with this product, result this similar to that found in our study. Study about ingestion of the product novaluron (2.1 mg/l) by *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), Xu et al. (2017) observed deleterious effects of this insecticide on fourth-instar larvae, noting that the inhibition of chitin biosynthesis impaired the pupal stage and consequently the emergence of the adults, which is similar to the results obtained in this work, when fourth-instar nymphs of *E. heros* were exposed to this IGR.

According to Zantedeschi et al. (2017), the insecticide lufenuron at the recommended dose to control caterpillars (100 g a.i. ha⁻¹) had a deleterious effect on the reproduction of *E. heros*, reducing the number of eggs produced per female, similar to what was observed this work for the same species in a greenhouse. Ávila & Nakano (1999) evaluated the effect of the IGR lufenuron on adult *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae) when a 0.033% solution of this product was sprayed on bean plants. Couples of *D. speciosa* fed bean leaves treated with this IGRs had minors fecundity and viability of the eggs produced than untreated couples (control). These authors also observed that the non-viable eggs showed embryonic development, but the larvae did not hatch, results that partially corroborate to those obtained with *E. heros* in this work.

The reproduction of *Schistocerca gregaria* (Foskal, 1775) (Orthoptera: Acrididae) was also affected after application of the insecticide lufenuron (50, 100, 200, 300, and 400 ppm) on fifth-instar nymphs, being observed spermatocyte disorganization as well as the loss of testicular tissue and the *Golgi apparatus* (Ghazawy, 2012). Another study carried out by Mansur et al. (2010) to verify the effects of different doses of lufenuron (7.5 and 15 µg) on female *Rhodnius prolixus* (Stal, 1859) (Hemiptera: Reduviidae), was observed a 30 to 50% reduction in the oviposition rate of this stink bug. The stink bug *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae), when exposed to lufenuron, had a reduction in the viability of eggs, but the fecundity was not affected (Evangelista et al., 2002). The same was observed in the present study, when the stink bug *E. heros* was submitted to a low dose of lufenuron.

The use of insecticides can often cause stressful situations, not only because they are considered toxic in high doses, but in some situations in low doses they can have stimulating or beneficial effects (Guedes et al., 2016); (Cutler, 2013). In the case of males throughout the mating period, they use mechanisms such as: sperm mobility, sperm storage, stimulation of ovulation or oviposition and protection of the eggs, which make it possible to increase the transfer of genetic matter (Avila et al., 2011); (Ge et al., 2010). Males of *E. heros*, when exposed to

under doses, exhibit the behavior mentioned above, and may have the ability to alter or manipulate the secretions of their accessory glands to increase their performance (Yu et al., 2012; Haddi et al., 2016) and they are also encouraged to walk further and find a greater number of females for mating (Gatehouse, 1997). On the other hand, females of *E. heros*, when exposed to sublethal dosages of some insecticides, may have an increase in their reproductive capacity (Harano, 2015; Haddi et al., 2016), because when exposed to stress conditions, they can use this mechanism to increase the perpetuation of the species (Vahed, 2017). The same was observed in our work when the females were exposed to the lowest dose of the insecticide lufenuron, where their oviposition capacity was not altered when exposed to the product.

Studies performed in the laboratory by Tail et al. (2010) with the grasshopper *S. gregaria*, to verify the influence of the ingestion of diflubenzuron (30 mg/ml), the authors found reduced weight of the ovaries after females of this species ingested this IGRs. In a study with adult of *Lygus lineolaris* (Palisot Beauvois, 1818) (Heteroptera: Miridae), which ingested a diet incorporated with novaluron (600 ppm), Catchot et al. (2020) observed a negative effect on oviposition rate, ovarian maturation, and hatching of eggs laid by this species. These results corroborate those obtained in this work when adults of *E. heros* were exposed to soybean leaves treated with novaluron at the highest dose tested, for egg viability, although the target species and exposure mode were different.

Ferreira Agüero et al. (2014) when testing the juvenile physiological insecticide pyriproxyfen (100 g a.i. ha⁻¹) and diflubenzuron (800 g a.i. ha⁻¹) on *N. viridula* nymphs observed that pyriproxyfen reduced fertility and decreased the percentage of hatched nymphs, while diflubenzuron, affected the formation and development of the female and male reproductive system of this species. The same authors also found that the eggs presented low viability or empty embryos due to embryo malformation, similar to the results verified for *E. heros* in the present work.

5. Conclusion

In summary, the results obtained in our work showed that the IGRs tested on *E. heros* nymphs and adults caused deleterious effects in the nymphal ecdysis process, resulting in deformation and mortality of insects. In addition to affecting the reproductive process in the adult phase, producing nonviable eggs (without embryo formation) and reducing the quantity of eggs produced. Thus, we can infer that the insecticides lufenuron, novaluron, and teflubenzuron, although recommended to control caterpillars in soybean, may also be a complementary alternative for the management of *E. heros* in this crop.

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