



Atrazine and nicosulfuron affect the reproductive fitness of the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae)

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ABSTRACT

Herbicides can impact non-target metabolic pathways in natural enemies and lead to the reduction of these populations in the field. Behavioral characteristics, morphology and histology of reproductive structures and reproduction of females of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) were evaluated under the effect of the herbicides atrazine, nicosulfuron and the mixture of both. The number of mature oocytes per ovary was lower in females exposed to the herbicides atrazine, nicosulfuron and the mixture of both. Herbicides did not affect the longevity and mortality of *P. nigrispinus*, therefore, they are selective for this predator. On the other hand, herbicides can cause sublethal effects by affecting the reproduction of predators.

Key words: behavior, histology, morphology, reproduction, herbicides.

INTRODUCTION

Weeds are for many beneficial insects, the first source of resources (Norris and Kogan 2000, Msebah and El-Husseini 2009, Camilo et al. 2016). This situation exposes these insects to herbicides by direct contact or ingestion of prey and sap of contaminated plants (Marshall et al. 2003, Mahdian et al. 2007, Cloyd and Bethke 2011, de Castro et al. 2013). Herbicides can cause interference

in metabolic pathways of insects and alter their reproductive capacity and population dynamics (Menezes et al. 2012, Souza et al. 2014).

Hemiptera predators occur naturally in several agroecosystems. *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) is a natural enemy of more than 30 pests (Zanuncio et al. 2003, Freitas et al. 2006, Torres et al. 2006). In addition, it is possible to reduce insecticides with releases of this natural enemy (Lacerda et al. 2004, Lundgren 2011, de Jesus et al. 2014).

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Herbicides limit the permanence of the natural enemy in the environment (Menezes et al. 2012) caused by changes in behavior and interference in female's fitness (Torres et al. 2006, Desneux et al. 2007, Msebah and El-Husseini 2009, Martinou et al. 2014). However, the effects of these agrochemicals on insects tend to reduce the ability of these agents to control pests in the field (Carmo et al. 2010, Menezes et al. 2012).

The objective of this work was to evaluate the ecotoxicological effects of herbicides used in corn crop, by studying the behavioral characteristics, morphology and histology of the reproductive structures and reproduction of females of *P. nigrispinus* predators.

MATERIALS AND METHODS

OBTAINING FEMALES OF *Podisus nigrispinus*

The *P. nigrispinus* eggs, coming from the rearing of the Laboratory of Biological Control of Insects (LCBI) of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), where this predator was maintained at $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity and photoperiod of 12 hours.

Two hundred eggs with about 24 hours were collected and placed in 500 mL plastic pots with moistened cotton. In the third stage, the nymphs of *P. nigrispinus* were separated into groups of five.

Throughout the experiment, nymphs and adults of this predator were fed with pupae of *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) *ad libitum* from LCBI of UFVJM. Adults of *P. nigrispinus* were sexed by the external appearance of the genitalia on the day of the emergence.

COMPOSITION OF TREATMENTS

The treatments were arranged in a completely randomized design with fifteen replicates for the herbicide atrazine (Primoleo[®] 400g ia / L), fifteen for nicosulfuron (Sanson[®] 40g ia / L), fifteen for the mixture (atrazine (Primoleo[®] 400g ia / L)

+ nicosulfuron (Sanson[®] 40g / L)) and a control sample (distilled water).

APPLICATION OF TREATMENTS

Fifteen females of *P. nigrispinus*, four days old, were individualized in 500 mL plastic pots and lined with corn leaves, to avoid contact with excess product in the treatments. A 1 mL syringe with a needle adapted for spraying was used in each pot, in order to spray directly in the female. According to each treatment, the syringe was filled with 0.26 mL of solution with atrazine (12 gL^{-1}); nicosulfuron (0.3 gL^{-1}); the mixture (atrazine 12 gL^{-1} + nicosulfuron 0.3 gL^{-1}); or 0.26 mL of distilled water, as a control (herbicide free) (Menezes et al. 2012). The concentrations were calculated basing on pot area (0.016 m^2) and commercial recommendation, being 6 Lha^{-1} of Primoleo[®] (400 gL^{-1} of atrazine) and 1.5 Lha^{-1} of Sanson[®] (40 gL^{-1} of nicosulfuron). After six hours, the time required for herbicide to penetrate corn leaves, females of *P. nigrispinus* were individualized in Petri dishes (10 cm) with a male of this predator.

COPULA BEHAVIOR

After application of treatments, couples of *P. nigrispinus* were maintained individually in Petri dishes, in a completely randomized design with fifteen replicates. The predisposition of females to copulation and the copulation duration of this predator were observed for 24 hours. After this period, the longevity and fertility of the females was evaluated. The data were submitted to analysis of variance and compared by the Tukey Test at 5% probability with software R.

MORPHOLOGICAL ANALYSIS

Seven days after its emergence, three females of *P. nigrispinus* per treatment were dissected in saline solution at the Laboratory of Histology of the UFVJM. The development and maturation of oocytes by ovaries was observed, as well as the

numbers of mature and immature oocytes by ovaries of this predator were counted (Soares et al. 2011). The ovaries of *P. nigrispinus* were photographed with a 16.1 megapixel digital camera coupled to a stereoscopic microscope. After extraction, the ovaries of this predator were transferred immediately to Bouwin fixative solution for 24 hours in environment temperature.

HISTOLOGICAL ANALYSIS

After fixation, the ovaries of *P. nigrispinus* were dehydrated in increasing series of ethanol and included in HistoResin (Leica). Subsequently, the samples were sectioned at 5µm and stained in aqueous hematoxylin and eosin solution. Sections were analyzed and photographed under an optical microscope. Histological studies were performed with three individuals per treatment.

REPRODUCTION OF *Podisus nigrispinus*

Twelve couples by treatment were chosen randomly of the test of copula behavior and were maintained to evaluate the longevity of females, periods of pre-oviposition, oviposition and post-oviposition, number of eggs per treatment, and fecundity (total viable eggs). The data were submitted to analysis of variance and compared by the Tukey Test at 5% with software R.

RESULTS

COPULA BEHAVIOR

The predisposition of females to copulation was similar in treatments with the isolated herbicides. Of the females exposed to the mixture (atrazine and nicosulfuron), only 13% presented predisposition to copula after 11 hours.

The longevity (15.0 to 22.08 days) and the copulation duration (10 to 15 hours) were not altered by the effect of the isolated herbicides or the mixture (Table I).

MORPHOLOGY OF THE OVARIES OF *Podisus nigrispinus*

The morphology of the reproductive system of *P. nigrispinus* females did not differ between treatments. Regardless of the treatment, each ovary of *P. nigrispinus* had seven ovarioles, joining through the terminal filaments and forming a compact bundle-shaped structure (Fig. 1).

Herbicides reduced the number of mature oocytes of *P. nigrispinus* females. The increase in the maturation period of the oocytes interfered with the number of eggs per female, but not with the viability (Table II). A compact mass of fat body was observed in the reproductive tract of *P. nigrispinus* females exposed to the herbicides, which was not observed in the control sample (Fig. 1).

HISTOLOGY OF THE OVARIES OF *Podisus nigrispinus*

The herbicides atrazine, nicosulfuron and the mixture of both, did not alter the cells of the tissues of the ovaries of *P. nigrispinus*. The ovaries presented the intrinsic characteristics of a telotrophic meroistic ovary type, with each ovary was presented morphologically divided into terminal filament, trophic (nourishing chamber), vitellary and pedicel (Fig. 2a).

Oocytes undergo morph-functional changes in the vitellary, resulting in complete development or maturation of the eggs. The oocytes follow through

TABLE I
Number of females (No.), longevity (Long. (days)), duration of copulation (Dur. (hours) (day ± standard deviation) and number of females mated in 24 hours (Mated (%)) of *Podisus nigrispinus* (Hemiptera: Pentatomidae) exposed to atrazine and nicosulfuron.

Treatment	Long. (days)	Dur. (hours)	Mated (%)
Control	22.08 ± 11.41a	13.8 ± 4.15a	83% a
Atrazine	15.00 ± 8.34a	15.14 ± 2.26a	58% a
Nicosulfuron	22.08 ± 8.81a	11.1 ± 4.14a	83% a
Mixture	18.75 ± 10.21a	10.0 ± 9.89a	13% b

Means followed by the same letter in the columns do not differ according to the Tukey Test at 5% probability.

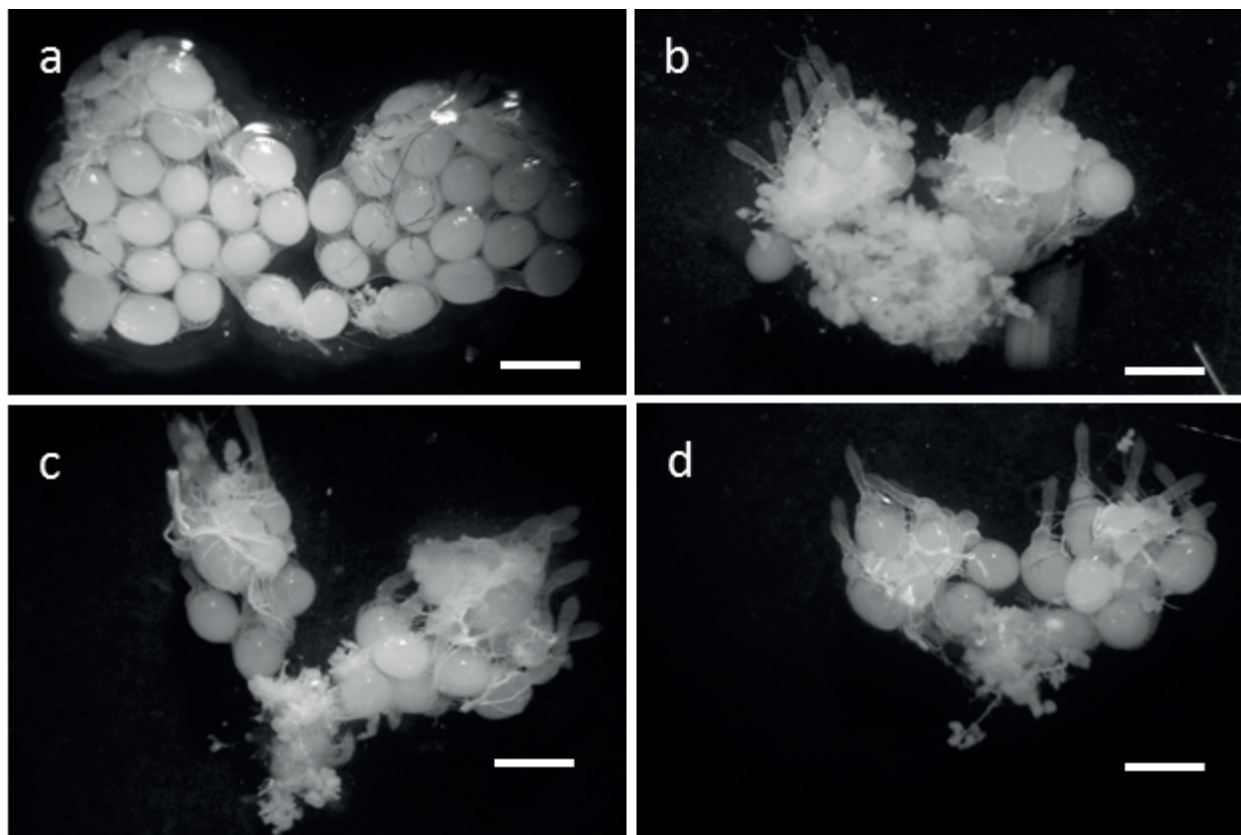


Figure 1 - Ovary of *Podisus nigrispinus* (Hemiptera: Pentatomidae) in treatments: control sample (a), atrazine (b), mixture (atrazine + nicosulfuron) (c), nicosulfuron (d). Bars = 2 mm.

the processes of vitellogenesis and chorionogenesis, in a linear arrangement, with the more developed ones presenting an increase in volume and a greater amount of vitellogenic reserves (Fig. 2b).

The germ cell (Fig. 2c) is the site of division and differentiation where the formation of cysts occurs (a group of differentiated germ cells), from which the trophocytes and oocytes originate with the somatic cells, forming the ovarian follicles.

Follicular cells surround the oocytes in a single layer (Fig. 2d). The cells of the surrounding layer exhibit an interruption represented by nutrient channels to the oocytes. Oocytes at an advanced stage of development, with more compact follicular cells and greater thickness due to their growth were observed in all treatments (Fig. 2d). The metabolism of follicular cells intensified during the vitellogenesis process evidenced by the follicle

cells at different stages of development (Fig. 2e). The follicular cell death was not observed in the ovarioles of females exposed to the herbicides.

REPRODUCTIVE CHARACTERISTICS

The pre-oviposition period was higher with nicosulfuron (Table II). The oviposition and post-oviposition periods and egg viability were similar between treatments (Table II). The number of eggs per female was higher in the control sample than in the treatments with the herbicides (Table II).

DISCUSSION

The knowledge on oogenesis in natural enemies is important for the applied biological control (Andrade et al. 2012). There are few studies on the effects of agrochemicals on the behavior and reproductive structures of *Podisus* spp. females

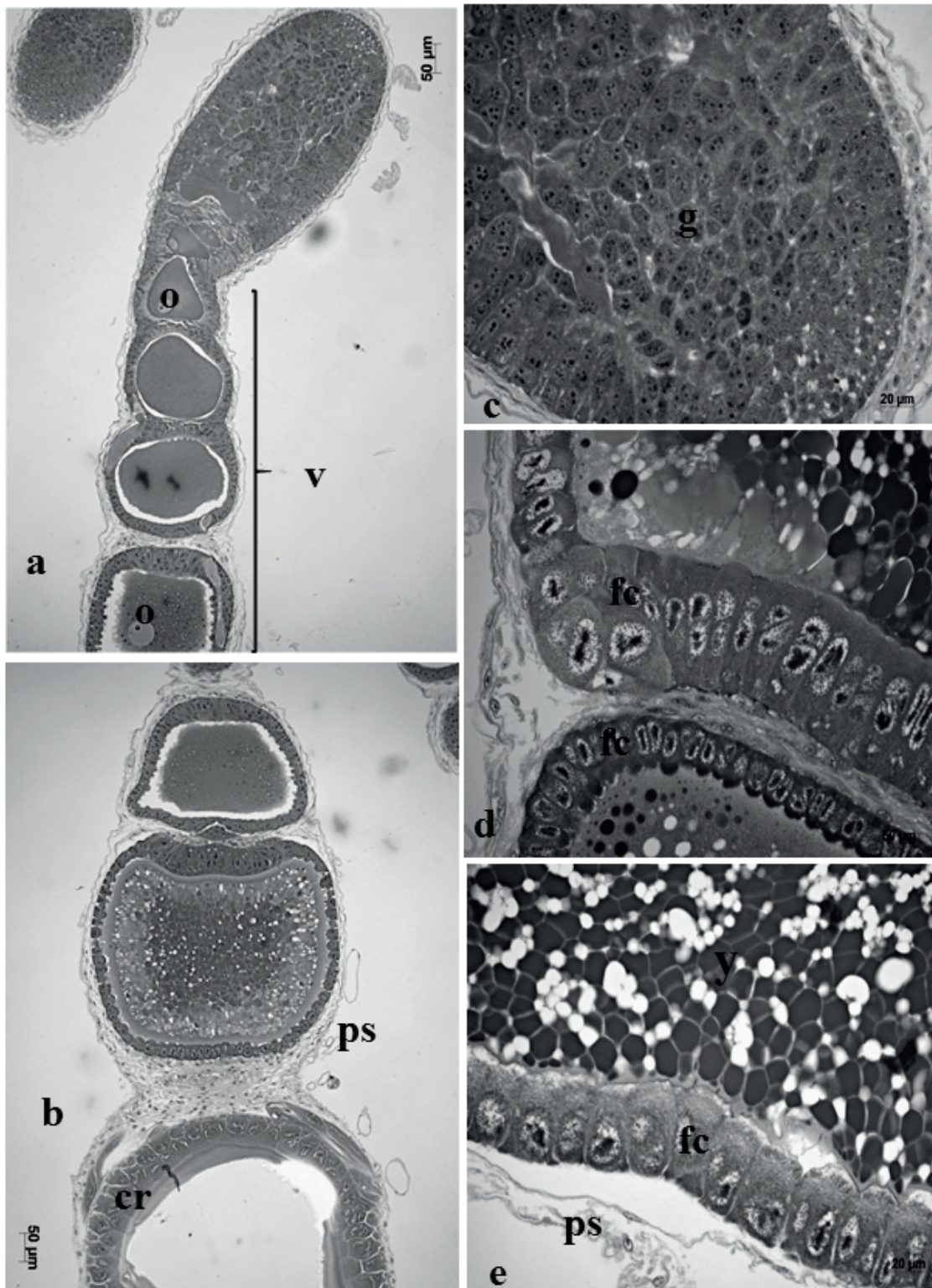


Figure 2 - Longitudinal cut of the ovariole of the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae) with oocytes at different stages of development. **a, b**) oocytes at different stages of development and linear arrangement. **c**) Germ region. **d, e**) Details of follicle cells at different stages of development. **g**, germ; **o**, oocyte; **v**, vitellary; **fc**, follicle cells; **y**, gem; **ps**, peritoneal layer; **cr**, corion.

TABLE II

Pre-oviposition (Pre), post-oviposition (Post), oviposition (Ovip.) periods in days and the number of eggs per female (No.) and the numbers of mature (Mat.) and immature (Imat.) oocytes and the viability of eggs (Viab.) (%) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) in the control sample (Cont.) and the samples exposed to atrazine (Atraz.), nicosulfuron (Nicos.) or a mixture of these herbicides (Mixt.).

Treat.	Pre.	Post.	Ovip.	No.	Mat.	Imat.	Viab.
Cont.	4.67 ± 2.45a	5.44 ± 2.40a	16.67 ± 9.68a	173.0a	25.0 ± 5.19 a	9.33 ± 3.78 a	76 a
Atraz.	7.22 ± 4.41a	2.33 ± 2.50a	8.22 ± 3.63a	74.5b	3.33 ± 2.88 b	14.0 ± 3.60 a	62 a
Nicos.	8.60 ± 2.67b	4.00 ± 2.40a	13.00 ± 7.85a	92.8b	5.67 ± 5.93 b	11.3 ± 1.53 a	71 a
Mixt.	6.90 ± 2.38a	1.7 ± 2.36a	12.10 ± 9.52a	81.2b	7.33 ± 6.42 b	13.33 ± 2.08 a	72 a

Means per column followed by the same letter do not differ according to the Tukey Test at 5% probability.

Pentatomidae predators with potential for use in integrated pest management - MIP (Desneux et al. 2007, Lundgren 2011).

The effects of the herbicides in this study on the copulation behavior of *P. nigrispinus* females were more evident in the first 24 hours with the herbicide mixture, reducing the copula. The use of agrochemicals can affect the chemical communication between arthropods, reducing their predatory instinct and the ability to locate their partners (He et al. 2012, de Castro et al. 2012). The juvenile hormone affects the overall metabolism in most insects, causing changes in histogenesis, polymorphism types and sexual behavior (Griesinger et al. 2011, He et al. 2012). This effect may indicate adaptive behavior, with females exhibiting less mobility (and copulation behavior) to reduce exposure to toxic wastes (Campos et al. 2011). However, lower mobility may increase the vulnerability to others natural enemies in the field (Kunkel et al. 2001). Imidacloprid benefited the mobility of beneficial arthropods, but with different effects over time (Suchail et al. 2001) according to the dose of this compound (Desneux et al. 2007). This shows that the exposure of organisms to agrochemicals can provoke physiological and biochemical responses, including detoxification or metabolism of the product by enzymes (Oliveira et al. 2012).

The predisposition to copulation of the females exposed to the isolated herbicides suggests

tolerance to the isolated application and indicates interactions among the active principles of the herbicides in the treatment composed by the mixture. These interactions may be toxicokinetic and toxicodynamic (Anderson and Lydy 2002). Moreover, the interaction of herbicide formulation chemicals, such as solvents, surfactants and wetting agents, can modify the effect of the herbicide acid equivalent on organisms and explain this behavior (Malkomes 2000, Santos et al. 2004).

The similar copulation duration in the first 24 hours in the different treatments shows that the herbicides should not have affected the gain in genetic and nutritional material of the females by males. The copula of *P. nigrispinus* has a mean duration of 12 hours, time related to the amount of transferred material (Carvalho et al. 1994, Soares et al. 2011) by the male to ensure that its gametes migrate to the spermatheca (Chapman et al. 2000). Agrochemicals trigger distinct responses in *P. nigrispinus* females, since azadirachtin increased the duration of the copula period and the transfer of materials to females (Oliveira et al. 2012).

The longevity of *P. nigrispinus* females varies with prey and environmental conditions. Nymphs of this predator were more vulnerable to these agrochemicals, with less survival until the fourth stage (Menezes et al. 2012). Females of this predator can present longevity around 34 days in satisfactory conditions (Torres et al. 2006).

The morphological and histological similarity of *P. nigrispinus* ovaries in all treatments showed ovarioles with oocytes in continuous development. Each ovariole of the same ovary presents a similar stage of development (Büning 1994, Lemos et al. 2005). The lower number of mature oocytes in females exposed to herbicides, isolated or in combination, hypothesizes the side effects of these compounds on the juvenile hormone (JH), leading to the accumulation of pre-vitellogenic oocytes (Davey 2000). The absence of the JH can affect the spaces between the follicular cells, not allowing greater deposition of vitellogenin. However, oogenesis is reestablished by normalization of the JH supply (Kotaki 1996, Davey 2000, 2007). The observation of the fat body compact mass tissue in the ovaries of *P. nigrispinus* hypothesizes a greater activity in the production of the fatty tissue to detoxification of the organism. Thus, the *P. nigrispinus* fat body being used for detoxification of herbicides could also reduce the production of vitellogenin in the maturation of the oocytes. The synthesis of vitellogenin is controlled by the JH and has action stimulated by the fat body (Davey 2007).

The highest pre-oviposition period of *P. nigrispinus* with nicosulfuron shows an impact on this predator as observed for *Supputius cincticeps* (Stål) (Hemiptera: Pentatomidae) exposed to sublethal concentrations of permethrin (Lemos et al. 2005). The tolerance of Asopinae to agrochemicals has been attributed to the lower penetration rate of these products in the cuticle of these predators or their rapid metabolism (Yu 1988).

The *P. nigrispinus* females maintained copula behavior, maturation of their oocytes and reproduction with the herbicides atrazine, nicosulfuron and the mixture of both, but with lower values. Physiological changes in the reproductive parameters of *P. nigrispinus*, with less reproduction in the presence of the herbicides, show the impact of the herbicides on the coordination between the

nervous and hormonal systems of insects, with physiological and behavioral events related to oviposition.

Agrochemicals compatible with MIP have been selected with mortality tests. However, the sublethal impact of these compounds on the population of *P. nigrispinus* can be better evaluated, as well as methods that this predator uses to ensure their survival.

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