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Glyphosate-based herbicides toxicity on life history parameters of zoophytophagous *Podisus nigrispinus* (Heteroptera: Pentatomidae)

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ABSTRACT

The increase of agricultural areas with glyphosate-resistant (GR) crops, and use of this herbicide in Brazil, makes necessary to assess its impacts on non-target organisms. The objective was to evaluate the development, reproduction and life table parameters of *Podisus nigrispinus* (Heteroptera: Pentatomidae) reared on GR-soybean plants treated with glyphosate formulations (Zapp-Qi, Roundup-Transorb-R and Roundup-Original) at the recommended field dose (720 g acid equivalent ha^{-1}). Glyphosate formulations had no affect on nymph and adult weight of this predator. Fourth instar stage was shortest with Zapp Qi. Egg-adult period was similar between treatments (26 days) with a survival over 90%. Zapp-Qi and Roundup-Transorb-R (potassium-salt: K-salt) reduced the egg, posture and nymph number per female, and the longevity and oviposition periods of this predator. *Podisus nigrispinus* net reproductive rate was highest in GR-soybean plants treated with Roundup-Original (isopropylamine-salt: IPA-salt). However, the duration of one generation, intrinsic and finite increase rates, and time to duplicate the population, were similar between treatments. Glyphosate toxicity on *P. nigrispinus* depends of the glyphosate salt type. IPA-salt was least harmless to this predator. Formulations based on K-salt altered its reproductive parameters, however, the development and population dynamic were not affect. Therefore, these glyphosate formulations are compatible with the predator *P. nigrispinus* with GR-soybean crop.

1. Introduction

Pesticides are the main method of controlling insects, diseases and weeds but they may cause environmental impacts, making necessary to search environmentally friendly and cheaper methods for pest control (Silva-Filho et al., 2014). Biological insect control is an important tool of the Integrated Pest Management (IPM) with low environmental impact (Kuar-Gill and Garg, 2014; Alcántara-de la Cruz et al., 2017). Parasitoids, entomopathogens and predators use is becoming a common practice to control agricultural pests (Lenteren et al., 2006; El-Wakeil et al., 2013).

The stink bug predator, *Podisus nigrispinus* Dallas (Heteroptera: Pentatomidae) naturally occurs in soybean crop (*Glicine max* L.) controlling pests (Castro et al., 2013), and it is used for the biological control of agriculture and forest pests (Zanuncio et al., 2016). This insect and other predators feed on plants to supplement their diet obtaining water, what favors their metabolism and predation (Grosman et al., 2005). *Podisus nigrisponus* may change feeding habits in periods of prey scarcity (Holtz et al., 2009).

The expansion of transgenic glyphosate-resistant (GR) crops, such as soybean, has been increasing the use of glyphosate [(N-phosphonomethy) glycine] (Kleter et al., 2011), a systemic non-selective herbicide globally used for over 40 years in weed management (Alcántara-de la Cruz et al., 2016b; Duke, 2017). Glyphosate is not a chemically pure active ingredient, and its formulations may be based on potassium salt (K-salt), ammonium salt (NH_4-salt) or isopopylamine salt (IPA-salt). This herbicide has low adverse effects on wildlife when properly used (Giesy et al., 2000). In Brazil, GR-soybean occupies 20.6 million ha corresponding to 86% of the total area of this crop (Hungria et al., 2014), making the use of glyphosate inevitable, often repeatedly and

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unduly. Under these circumstances, the glyphosate impact on the environment and non-target organisms continues raising concerns (Capinera, 2005).

Biological control can be combined with selective pesticides. The International Organization for the Biological Control (IOBC) has reports about pesticide effects on non-target organisms (Stark et al., 2007), some of them related to herbicide effects on amphibians (Jones et al., 2010), microorganisms (Zaller et al., 2014) or insects (Desneux et al., 2007; Schneider et al., 2009; Evans et al., 2010; Albajes et al., 2011; Szénási et al., 2014). However, conventional toxicological studies may under or overestimate the results, therefore assessment of the pesticide impacts on non-target organisms must integrate ecological parameters (Stark et al., 2007). Life tables indicate population increases, decreases or risks in different scenarios (Zanuncio et al., 2004, 2006).

Podisus nigrispinus exposition to glyphosate treated plants could affect its innate predation. The objective of this work was to evaluate the reproduction and development parameters of *P. nigrispinus* (non-target insect) reared on GR-soybean plants treated with glyphosate formulations through life table parameters.

2. Material and methods

2.1. Biological material

Experiments were conducted in a greenhouse at the Fitotecnia Department of the Univesidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais, Brazil. Five GR-soybean seeds (cultivar CD-219RR) were sown in pots (3 L) with substrate (soil: organic matter) fertilized with ammonium sulphate (equivalent to 50 kg N ha^{-1}). Two plants with 3–4 true leaves were kept after the germination.

Podisus nigrispus individuals were obtained from the Laboratory of Biological Control of Insects (LCBI/BIOAGRO) of UFV. Egg masses of *P. nigrispinus* were maintained in 9 cm Petri dishes in a controlled condition room with 25 ± 2 °C, 75 ± 5% relative humidity, and a photoperiod of 12 h until transferred to the greenhouse.

2.2. Treatments evaluated during the experiment

Three glyphosate formulations were tested: Zapp Qi (62% w/v K-salt; Syngenta Proteção de Cultivos Ltda, Brazil, Roundup Transorb R (58.8% w/v K-salt; Monsanto do Brasil Ltda, Brazil), and Roundup Original (48% w/v IPA-salt; Monsanto do Brasil Ltda, Brazil). Mention of trade names in this publication is solely for the purpose of providing specific information and does not imply their recommendation.

2.3. Nymph development

GR-soybean plants were immersed during five seconds in glyphosate solutions, with 720 g acid equivalent (ae) ha^{-1} in a volume of 200 L ha^{-1}, following guidelines of the International Organization for Biological Control. Control plants were immersed in common water. *Podisus nigrispinus* development reared without plant and without herbicide also was evaluated in laboratory conditions.

Second instar nymphs were transferred to greenhouse. *Podisus nigrispinus* nymphs were placed on a trifoliolated soybean leaf (Zanuncio et al., 2004), in groups of ten individuals (experimental unit) inside of organza bags (20 × 30 cm), two hours after herbicide application. *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae were offered *ad libitum* inside the bags to fed *P. nigrispinus* nymphs. Herbicide can reach the herbivorous insects predated by *P. nigrispinus* in field conditions and, for this reason, 50% of *T. molitor* pupae offered the first time were also immersed in the corresponding glyphosate solution. The organza bags were changed between leaves weekly.

The experiment was arranged in a completely randomized design with eight replications. The survival, weight and duration of each stage of *P. nigrispinus* were daily evaluated. After each molt, nymphs and the newly emerged adults were weighed on an analytical balance (± 0.1 mg). The Kaplan-Meyer model was used to determine the survival curves of *P. nigrispinus* nymphs.

2.4. Life table

Podisus nigrispinus adults with three days old were placed on treated soybean leaves (Zapp Qi, Roundup Transorb R, Roundup Original and water as control), inside organza bags with *T. molitor* pupae supplied *ad libitum*, as described before. Fifty per cent of *T. molitor* pupae offered the first time were immersed in the corresponding glyphosate solution.

Pre-oviposition, oviposition and post-oviposition periods besides the numbers of eggs per female and egg mass, incubation period and female longevity of fifteen *P. nigrispinus* pairs per treatment were daily evaluated, in a completely randomized design. The data were used to construct a *P. nigrispinus* life table. Gross reproductive rate ($G = \Sigma_{x=0}^{y} mx$); net reproductive rate [$Ro = \Sigma_{x=0}^{y} (lx)(mx)$]; the time necessary for the *P. nigrispinus* population to double in size ($DG = [\Sigma_{x=0}^{y} (lx)(mx)]/Ro$); intrinsic increase rate ($rm = \ln(Ro)/DG$); finite increase rate ($\lambda = antilog[(rm)(0.4343)]$); and time required to duplicate the population ($TD = \ln(2)/rm$) were estimated (Krebs, 2009), where: mx = specific fertility (females number produced per surviving female in the age range x), lx = survival rate (survival rate from age zero to the beginning of age x); and x= nymph stage.

2.5. Statistical analysis

Development and reproduction data of *P. nigrispinus* were submitted to ANOVA. The Tukey HSD test at 5% probability was used to separate means. Statistical analysis was performed with Statistix software (version 9.0; Analytical Software, USA). The life table parameters were calculated and analyzed with the SAS statistical program (SAS Institute Inc, USA) according to the Jackknife technique (Maia et al., 2000).

3. Results

3.1. Nymph stage

The glyphosate formulations did not affect *P. nigrispinus* weight of fourth instar and newly emerged adults. Insects reared without plant/herbicide had lowest weight, showing the importance of the plants to this predator (Table 1).

The duration of the fourth instar *P. nigrispinis* nymphs was shorter on plants treated with Zapp Qi and Roundup Transorb R, herbicides based on potassium-salt (K-salt) of glyphosate. However, these variations did not affect the total duration from egg to adult for this predator (Table 2).

The survival rate of *P. nigrispinus* nymphs was 89% in the control (reared on GR-soybean plants immersed in water), 94% in plants treated with Zapp Qi, and those individuals raised on plants treated

Table 1
Weight (mg) of fourth and fifth instar nymph adult males and females *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae, reared on glyphosate-resistant soybean (cultivar CD-219RR) plants immersed in glyphosate solutions. Insects of the control were reared on plants immersed in water and without plant/herbicide.

Treatments	Fourth[ns]	Fifth	Male	Female
Zapp Qi	12.3 ± 0.5	34.7 ± 1.3 a	50.5 ± 1.0 a	72.2 ± 1.6 ab
Roundup Transorb R	12.6 ± 0.5	35.9 ± 1.6 a	48.2 ± 1.0 a	70.2 ± 2.2 ab
Roundup Original	11.1 ± 0.4	36.0 ± 1.5 a	49.5 ± 1.0 a	73.5 ± 1.4 a
Water immersion	11.3 ± 0.5	35.0 ± 1.5 a	48.5 ± 0.9 a	70.3 ± 1.7 ab
Without plant/ herbicide	11.3 ± 0.6	31.0 ± 1.2 c	45.1 ± 1.0 b	68.2 ± 1.2 b

Means with the same letter per column do not differ at 5% probability by Tukey test. [ns] not significant at 5% probability. ± Standard error of the mean (n = 8).