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Article

COMPARATIVE SELECTIVITY OF HERBICIDES USED IN WHEAT CROP ON THE PREDATORS *Chrysoperla externa* AND *Eriopis connexa*

Comparativo da Seletividade de Herbicidas Utilizados na Cultura do Trigo sobre os Predadores ***Chrysoperla externa*** *e* ***Eriopis connexa***

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ABSTRACT **-** The selectivity of eight herbicides registered for use in the wheat crop was assessed on the predators *Chrysoperla externa* and *Eriopis connexa*. Bioassays were conducted in the laboratory by exposing larvae, eggs, and pupae of these predators to dry residues of the herbicides 2,4-D amine, bentazon, glyphosate 1.200, glyphosate 1.440, ammonium glufosinate, iodosulfuron-methyl, metsulfuron-methyl, and pyrimidinedione. The herbicide ammonium glufosinate was classified as moderately harmful (class 3) to the larval stage of both predators. In the egg stage, only metsulfuron-methyl showed a reduction in larval hatching higher than 30% and was classified as slightly harmful (class 2) to eggs of *E. connexa*. The herbicide 2,4-D amine was classified as slightly harmful (class 2) to pupae of *C. externa*, while pyrimidinedione presented the same classification to pupae of *E. connexa*. The herbicides bentazon, glyphosate 1.200, glyphosate 1.440, and iodosulfuron-methyl were considered as innocuous to the stages of larva, egg, and pupa of *C. externa* and *E. connexa* and can be used in the integrated pest management of wheat crop, assisting in predator conservation.

Keywords: pesticides, biological control, chemical control, *Triticum aestivum*.

RESUMO - Avaliou-se a seletividade de oito herbicidas registrados para uso na cultura do trigo sobre os predadores ***Chrysoperla externa*** *e* ***Eriopis connexa****. Os bioensaios foram conduzidos em laboratório, expondo-se larvas, ovos e pupas dos referidos predadores aos resíduos secos dos herbicidas 2,4-D amina, bentazon, glyphosate 1,200, glyphosate 1,440, glufosinato de amônio, iodosulfuron-metil, metsulfuron-metil e pirimidinadiona. O herbicida glufosinato de amônio foi classificado como moderadamente nocivo (classe 3) à fase larval de ambos os predadores. Na fase de ovo, somente metsulfuron-metil apresentou redução na eclosão das larvas superior a 30% e foi classificado como levemente nocivo (classe 2) aos ovos de* ***E. connexa****. O herbicida 2,4-D amina foi classificado como levemente nocivo (classe 2) a pupas de* ***C. externa****, e pirimidinadiona apresentou a mesma classificação a pupas de* ***E. connexa****. Os herbicidas bentazon, glyphosate 1,200, glyphosate 1,440 e iodosulfuron-metil foram considerados inócuos às fases de larva, ovo e pupa de* ***C. externa*** *e* ***E. connexa*** *e podem ser utilizados no Manejo Integrado de Pragas da cultura, auxiliando na conservação dos predadores.*

Palavras-chave: agrotóxicos, controle biológico, controle químico, *Triticum aestivum*.

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INTRODUCTION

Several factors act as limiting to obtain a high productivity in wheat crop, especially weeds, which cause a decrease in yield due to the competition for resources available in the medium (Agostinetto et al., 2008). The main species that compete for resources in this crop are ryegrass (*Lolium multiflorum*), black oat (*Avena strigosa*), wild radish (*Raphanus raphanistrum*), radish (*Raphanus sativus*), black bindweed (*Polygonum convolvulus*), hoary bowlesia (*Bowlesia incana*), hairy fleabane (*Conyza bonariensis*), and horseweed (*Conyza canadensis*) (Mariani and Vargas, 2012; Lamego et al., 2013).

Chemical control with herbicide application is a commonly used method for suppression of these plant species (Lamego et al., 2013). However, herbicide applications are recognized as causes of biological imbalance in agroecosystems (Guedes et al., 2015; Menezes and Soares, 2016). Thus, the use of selective pesticides is of paramount importance to delay or even avoid problems arising from their indiscriminate use. Selectivity is defined as the property that a pesticide presents to control a specific pest (weeds, insects, and diseases) with the least possible impact on its natural enemies in the agroecosystem under the same conditions in which the pest is controlled successfully (Castilhos et al., 2014).

Among the generalist predators of insect pests that are relevant in commercial wheat crops are those of the families Chrysopidae and Coccinellidae (Gassen and Tambasco, 1983). The green lacewing *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) stands out for its wide geographical distribution, occurrence in varied habitats, polyphagia, great search capacity, and high voracity, in addition to a high reproductive potential and tolerance to some phytosanitary products, which make them a high potential for use in biological control programs (Costa et al., 2003). Among the coccinellids, *Eriopis connexa* (German, 1824) (Coleoptera: Coccinellidae) is widely distributed in the neotropical region and it is considered as a control agent of numerous insect pests in several crops. According to Gassen (1988), it is one of the most voracious predators of aphids in wheat crop, being each adult able to consume up to 43 aphids per day. In wheat crop, these two predators are very important because they feed mainly on the aphid complex, which hinders the cultivation of this cereal.

One of the fastest ways to access the adverse effect of an herbicide is through laboratory selectivity tests by using methodologies standardized by the International Organization for Biological and Integrated Control (IOBC). Studies regarding selectivity in wheat crop were performed by Eichler and Reis (1976) by using the predators *Cycloneda sanguinea* (Linnaeus, 1763) (Coleoptera: Coccinellidae) and *E. connexa*. However, that study was conducted in the 1970s with a non-standardized methodology and only with insecticides, without any information on herbicide selectivity. These results are used in the technical recommendations of wheat crop (Cunha et al., 2016). Therefore, obtaining results on the selectivity of herbicides to the predators *C. externa* and *E. connexa* can also be used in technical indications as the basis for the choice of herbicides more adequate for wheat producers.

Thus, this study aimed to determine the selectivity of herbicides used in the wheat crop on larva, egg, and pupa stages of *C. externa* and *E. connexa*, as well as sublethal effects on the reproductive parameters of adults of both predators.

MATERIAL AND METHODS

Rearing and maintenance of *C. externa* and *E. connexa*

The insects used in the bioassays were obtained from a laboratory rearing (temperature of 25 ± 1 °C, relative humidity of 70 ± 10%, and photophase of 14 hours) according a methodology adapted from Carvalho and Souza (2000) and Vogt et al. (2000) for *C. externa* and Silva et al. (2009) for *E. connexa*. Larvae of predators were maintained until pupation in plastic trays (43 x 27 x 13 cm) sprinkled with talc on the sides and capped with a voile fabric. Larvae of both predators were fed ad libitum with eggs of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) reared according to a methodology proposed by Parra (1997). Pupae of *C. externa* were transferred to acrylic cages (15.5 cm in height and 18.5 cm in diameter), closed with a paper towel at both ends, where adults were reared and fed with an artificial diet proposed by Vogt et al. (2000). Eggs were collected from the paper towel on the top surface of the acrylic cage. Pupae of *E. connexa* were packed in pots (9 cm in height and 14 cm in diameter), closed with paper towels at the upper end. Adults from the pupae were fed with flour moth eggs and honey for later assembly of couples. Water was supplied through a cotton wad soaked with distilled water. This cotton wad also served as a substrate for females to lay eggs, which were collected daily.

Herbicides

Eight herbicides registered for wheat (Agrofit, 2015) were assessed on larvae of *C. externa* and *E. connexa*. The following insecticides and active ingredient [commercial product – chemical group (maximum dose of the commercial formulation registered for wheat crop in L ha^{-1} or kg ha^{-1}/ active ingredient concentration in the spray solution, in %)] were used: 2,4-D amine [Aminol 806 – aryloxyalkanoic acid (1.500/)], bentazon [Basagran 600 – benzothiadiazinone (1.600/0.480)], ammonium glufosinate [Finale – substituted homoalanine (2.000/0.200)], iodosulfuron-methyl [Hussar – sulfonylureas (0.100/0.002)], metsulfuron-methyl [Ally – sulfonylureas (0.006/0.001), pyrimidinedione (Heat – saflufenacil (0.070/0.024), glyphosate – isopropylamine salt 1.200 [Stinger – substituted glycine (5.000/1.200), and glyphosate – isopropylamine salt 1.440 [Heat – substituted glycine (6.000/1.440)].

In addition to the tested herbicides, each bioassay was composed of a negative control (absence of herbicide) and a positive control composed of the insecticide Engeo Pleno (lambda-cyhalothrin + thiamethoxam) at a dose of 0.150 L ha^{-1} and concentration of the active ingredient in the spray solution (%) of 0.010 + 0.007 as a standard of recognized toxicity (Zotti et al., 2010). The used doses were the maximum recommended for the wheat crop, adjusted to correspond to a spray solution volume of 200 L ha^{-1}.

Bioassays of residual effect on *C. externa* and *E. connexa* larvae

The insecticides were sprayed on glass plates (50 x 41 cm) by using a CO_2 pressurized sprayer with a nozzle Teejet XR 110015 EVS and working pressure of approximately 50 psi, corresponding to a spray solution deposition of 2 ± 0.2 mg cm^{-2} (Schmuck et al., 2000; Vogt et al., 2000). After drying, the plates were overlaid with another acrylic plate of the same size and with 20 holes of 7.5 cm in diameter. Transparent plastic cups without bottom and with inert talc were coupled to these holes, constituting the exposure arenas.

First instar larvae (1-2 days old) of both predators were conditioned in the arenas, staying in contact with the herbicides until the emergence of adults. Each treatment consisted of two plates with 20 arenas, totalizing 40 insects. Each insect was considered as a replication.

The duration of each stage of development (larval instars, pre-pupa, and pupa) of the predator in the different treatments was assessed daily. Mortality (%) and the number of emerged adults were also determined. In treatments where the accumulated mortality was $\leq$ 50%, an assessment of reproductive performance (fecundity and fertility) of adults was carried out. Adults from the larvae exposed to herbicides were separated into containers with the same dimensions and conditions as those used to rear predators.

One week after the first laying, adults of *C. externa* were sexed and four samples of eggs deposited in a 24 hour interval were collected (Castilhos et al., 2014). The total number of eggs from each collection was measured and divided by the number of females in the cage in order to determine the average fecundity (number of eggs per female and day). After the first laying of *E. connexa*, adults were sexed and 10 collections of the eggs deposited in a 24 hour interval were performed. The total number of eggs from each collection was measured and divided by the number of females in order to determine the average fecundity. A portion of the collected eggs was incubated until larvae hatching of both predators to determine the fertility rate (percentage of hatched larvae). The averages fecundity and fertility obtained from each collection were calculated and compared with those obtained in the control of each bioassay.

Bioassays with eggs of *C. externa* and *E. connexa*

In the bioassay with eggs, four replications with 24 eggs each were used, totaling 96 eggs per treatment. Spraying was carried out through a Potter tower previously calibrated to ensure a deposit of 2 ± 0.2 mg cm^{-2}. After spraying and drying of spray solution, treated eggs were individualized into 96 well cell culture plates and conditioned in a room set to the same conditions in which the insects were reared. After approximately five days, egg viability was assessed and the reduction in larval hatching (RLH) provided by each herbicide was calculated.

Bioassays with pupae of *C. externa* and *E. connexa*

In the bioassay with pupae, four replications with six pupae each were used, totaling 24 pupae per treatment. Spraying was carried out through a Potter tower to obtain a spray solution deposit of 2 ± 0.2 mg cm^{-2}. After spraying and drying of spray solution, treated pupae were individualized into 24 well cell culture plates and conditioned in a room set to the same conditions in which the insects were reared. The viability and reduction in adult emergence (RAE) caused by herbicides were determined after approximately one week. Adults emerged from treated pupae were assessed for possible sublethal effects on fecundity and fertility. In order to assess these reproductive parameters, seven adult couples were grouped in cages (15.5 cm in height x 18.5 cm in diameter) and approximately one week after the first laying, 10 samples of eggs, corresponding to the eggs deposited in a 24 hour period, were collected in 10 consecutive days and incubated to determine the average number of eggs/female/day and the average percentage of larvae hatching.

Selectivity classification

Larval mortality and reduction in larval hatching and adult emergence were corrected as a function of the control by the Schneider-Orelli equation (Püntener, 1981). The total effect of each herbicide for pupae was calculated using the equation proposed by Vogt et al. (1992): $E = 100\% - (100\% - RAE\%) \times R1 \times R2$, where E is the total effect (%), RAE% is the reduction in adult emergence, R1 is the ratio between the daily average of oviposited eggs per treated and untreated female, and R2 is the ratio between the average viability of oviposited eggs per treated and untreated female. Herbicides were classified for eggs according to the reduction in hatching of larvae and larvae and pupae as a function of the total effect, considering the toxicity classes proposed by IOBC: 1) innocuous (<30%), 2) slightly harmful (30-79%), 3) moderately harmful (80-99%), and 4) harmful (>99%).

Statistical analysis

The data regarding the duration of each stage of development of first instar larvae exposed to herbicides, the viability of eggs and pupae, as well as the average fecundity and fertility were analyzed according to their distribution through the Shapiro-Wilk normality test and Bartlett's test for homogeneity variances. When showing a normal distribution, the data were submitted to an analysis of variance (ANOVA). All bioassays used a completely randomized design. The average viability of eggs and pupae from each treatment was compared with the control by the Dunnett test ($p<0.05$), while the Tukey's test ($p<0.05$) was used to compare the average fecundity and fertility. The statistical analyses were performed using the statistical software Assistat version 7.7 (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

Effect on larvae of *C. externa* and *E. connexa*

The herbicide ammonium glufosinate presented a larval mortality of 80% (Table 1) when larvae of *C. externa* were exposed to the residual contact with herbicides, not being possible to safely assess their sublethal effects on the reproductive parameters of adults from larvae exposed to herbicides. In this case, according to IOBC, these parameters should be assessed only when the treatment presents a mortality lower than 50%. The herbicide ammonium glufosinate showed a similar toxicity behavior for larvae of *E. connexa*, causing a larval mortality of 84.62% (Table 2). Larval mortality of *C. externa* and *E. connexa* was small or null in almost all the herbicides tested in the bioassay I (Tables 1 and 2).

Table 1 **-** Cumulative larval mortality (%), fecundity (number of eggs per female/day ± SE), fertility (percentage of hatched larvae ± SE), total effect, and IOBC classification when larvae of *Chrysoperla externa* were exposed to residual contact with herbicides registered for the wheat crop

Treatment	a.i.c. (%)(1)	M (%)	Fecundity(2)	Fertility(2)	E (%)	C
Bioassay I						
Control	–	–	22.82±1.96ab	81.68±1.81a	–	–
2,4-D amine	0.502	0.00	19.33±1.29b	76.13±4.08a	23.40	1
Bentazon	0.480	0.00	27.95±1.95a	85.47±3.38a	0.00	1
Pyrimidinedione	0.024	0.00	23.09±1.72ab	80.91±3.71a	0.00	1
Glyphosate 1.200	1. 200	0.00	27.27±2.30a	78.69±1.32a	0.00	1
Thiamethoxam + lambda-cyhalothrin (standard)	0.010+0.007	100.00	–	–	100.00	4
Bioassay II						
Control	–	–	22.79±2.61a	75.71±4.00a	–	–
Ammonium glufosinate	0.200	80.00	–	–	80.00	3
Iodosulfuron-methyl	0.002	0.00	31.23±2.85a	71.84±3.57a	0.00	1
Metsulfuron-methyl	0.001	0.00	19.58±5.44a	78.49±2.66a	1.03	1
Glyphosate 1.440	1.440	0.00	29.00±1.09a	74.09±2.56a	0.00	1
Thiamethoxam + lambda-cyhalothrin (standard)	0.010+0.007	100.00	–	–	100.00	4

(1) a.i.c. (%) = tested active ingredient concentration (%) in the spray solution; M = larval mortality corrected by Schneider-Orelli; E = total effect; C = IOBC classes, 1 = innocuous (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), 4 = harmful (>99%). (2) Means followed by the same letter in the columns do not differ significantly from each other by the Tukey's test (p>0.05). Bioassay I: Fecundity: F = 4.3305, DF = 4, p = 0.0158; Fertility: F = 1.2903, DF = 4, p = 0.3178. Bioassay II: Fecundity: F = 2.5393, DF = 3, p = 0.1056; Fertility: F = 0.7359, DF = 3, p = 0.5505.

Table 2 **-** Cumulative larval mortality (%), fecundity (number of eggs per female/day ± SE), fertility (percentage of hatched larvae ± SE), total effect, and IOBC classification when larvae of *Eriopis connexa* were exposed to residual contact with herbicides registered for the wheat crop

Treatment	a.i.c. (%)(1)	M (%)	Fecundity(2)	Fertility(2)	E (%)	C
Bioassay I						
Control	–	–	32.73±3.20a	75.57±3.42a	–	–
2,4-D amine	0.502	5.26	26.77±2.98a	78.21±3.12a	19.79	1
Bentazon	0.480	2.63	28.64±3.78a	72.69±4.84a	18.02	1
Pyrimidinedione	0.024	0.00	35.09±3.12a	63.01±10.78a	10.59	1
Glyphosate 1.200	1.200	0.00	28.93±2.04a	72.28±4.25a	15.44	1
Thiamethoxam + lambda-cyhalothrin (standard)	0.010+0.007	100.00	–	–	100.00	4
Bioassay II						
Control	–	–	29.40±2.94a	77.65±3.22a	–	–
Ammonium glufosinate	0.200	84.62	–	–	84.62	3
Iodosulfuron-methyl	0.002	0.00	30.53±3.17a	74.69±4.20a	0.10	1
Metsulfuron-methyl	0.001	0.00	34.21±2.17a	71.35±2.97a	1.16	1
Glyphosate 1.440	1.440	2.56	25.00±3.41a	72.89±1.77a	22.23	1
Thiamethoxam + lambda-cyhalothrin (standard)	0.010+0.007	100.00	–	–	100.00	4

(1) a.i.c. (%) = tested active ingredient concentration (%) in the spray solution; M = larval mortality corrected by Schneider-Orelli; E = total effect; C = IOBC classes, 1 = innocuous (<30%), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), 4 = harmful (>99%). (2) Means followed by the same letter in the columns do not differ significantly from each other by the Tukey's test (p>0.05). Bioassay I: Fecundity: F = 1.2113, DF = 4, p = 0.3266; Fertility: F = 0.9203, DF = 4, p = 0.4651. Bioassay II: Fecundity: F = 1.6426, DF = 4, p = 0.2059; Fertility: F = 0.7326, DF = 4, p = 0.5427.

The results obtained by Ahn et al. (2001) suggest that when first instar larvae of *Chrysopa pallens* (Rambur, 1838) (Neuroptera Chrysopidae) and *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) are exposed to residual contact with the herbicide ammonium glufosinate at concentrations higher than 2.160 ppm, a larval mortality of 40% for *C. pallens* and 100% for *H. axyridis* is observed. These results are in accordance with those of our study, in which *C. externa* and *E. connexa* also showed a high larval mortality when neonate larvae were exposed to residual contact with the herbicide ammonium glufosinate. Castilhos et al. (2013) obtained a larval mortality similar to that observed in our study for glyphosate (1,440 a.i.c %) when testing selectivity of herbicides registered for peach crop on first instar larvae of *C. externa*, being the herbicide classified as innocuous to larvae of the predator, with no differences in fecundity and fertility, which was also observed in our study (Table 1). Schneider et al. (2009) assessed the effect on larvae of *C. externa* fed eggs of *Sitotroga cerealella* Oliver, 1789 (Lepidoptera: Gelechiidae) treated with glyphosate and found short-term effects on predator larvae, with a survival similar to that of the control treatment. However, the development time from larvae to pupae was significantly lower in approximately two days in the treatment with glyphosate and fecundity and fertility of females of *C. externa* were negatively affected.

Regarding fecundity and fertility of adults from larvae exposed to herbicide residues, no significant reduction was observed for females of *C. externa* in bioassays I and II when compared to control treatments (Table 1). In bioassay I, the herbicides bentazon and glyphosate 1.200 presented a significantly higher fecundity when compared to that obtained for 2,4-D amine (Table 1). The herbicides tested on *E. connexa* did not differ statistically from the control regarding the reproductive parameters fecundity and fertility in both bioassays (Table 2).

Michaud and Vargas (2010) tested the effect of the herbicides 2,4-D ester (1.17 L ha^{-1}) and metsulfuron-methyl (70 g ha^{-1}) on wheat crop and applied topically to first instar larvae of the coccinellids *Coleomegilla maculata* (DeGeer, 1775) and *Hippodamia convergens* (Guerin-Meneville, 1842) (Coleoptera: Coccinellidae). According to these authors, 2,4-D ester resulted in mortalities of 25% and 60%, respectively, for both species. According to Sterk et al. (1999), 2,4-D and metsulfuron-methyl are reported as herbicides with little or limited toxicity to natural enemies in residual exposure bioassays.

Regarding the total effect of herbicides, 2,4-D amine, bentazon, glyphosate 1.200, glyphosate 1.440, iodosulfuron-methyl, metsulfuron-methyl, and pyrimidinedione presented a total effect lower than 30%, being classified as innocuous (class 1) to the predators *C. externa* and *E. connexa*. Ammonium glufosinate, however, was classified as moderately harmful (Class 3) according to larval mortality of *C. externa* and *E. connexa* (Tables 1 and 2).

Duration of immature stages of *C. externa* and *E. connexa*

In bioassay I, 2,4-D amine and bentazon differed significantly from the control regarding the duration of pupae stage of *C. externa*. However, when the duration of the larva-adult period was analyzed, it did not differ in relation to the control. In bioassay II, although the herbicide ammonium glufosinate has presented an 80% larval mortality, the remaining larvae were used to calculate the duration of the different immature stages (Table 3). In this case, a significant difference was observed in relation to the control regarding the duration of second instar, pre-pupa and pupa stages, and the larva-adult period. The herbicide metsulfuron-methyl only differed from the control regarding the duration of the pupal period.

The duration of the distinct stages of development when larvae of the predator *E. connexa* were exposed to the herbicides differed significantly from the control treatment (Table 4). In general, the larva-adult period ranged from 13.14 to 14.98 days between treatments of both bioassays. In bioassay I, 2,4-D amine, bentazon, and glyphosate 1.200 presented a duration of the larva-adult development period significantly higher when compared to the control treatment, which was also observed in bioassay II for iodosulfuron-methyl.

According to Castilhos et al. (2013), no differences were observed in the duration of different stages of development of the predator *C. externa* when first instar larvae were submitted to residual contact with herbicide, presenting a similar behavior when compared to that of our study, not differing from the control for the larva-adult period (Table 3). Similar results were

Table 3 **-** Duration of larval instars (number of days ± SE), pre-pupa and pupa stages, and duration of the larva-adult period of *Chrysoperla externa* when the larval stage was exposed to residual contact with herbicides registered for the wheat crop

Treatment	a.i.c. (%)(1)	Duration (days)					
		1st instar	2nd instar	3rd instar	Pre-pupa	Pupa	Larva-adult
Bioassay I							
Control	–	2.15±0.05a	3.00±0.11ab	3.90±0.28ab	3.96±0.17a	7.43±0.08a	19.87±0.17ab
2,4-D amine	0.502	2.17±0.06a	3.20±0.11ab	3.65±0.12ab	3.89±0.07a	6.81±0.10b	19.73±0.16ab
Bentazon	0.480	2.32±0.07a	3.02±0.10ab	3.82±0.18ab	4.31±0.08a	6.71±0.15b	19.94±0.17a
Pyrimidinedione	0.024	2.25±0.06a	3.35±0.09a	3.21±0.14b	3.87±0.14a	7.03±0.09ab	19.18±0.20b
Glyphosate 1.200	1. 200	2.37±0.11a	2.90±0.11b	3.97±0.14a	4.21±0.08a	7.00±0.19ab	19.78±0.22ab
Thiamethoxam+ lambda-cyhalothrin (standard)	0.010+0.007	–	–	–	–	–	–
Bioassay II							
Control	–	2.10±0.04a	2.35±0.07b	3.22±0.06a	3.72±0.07b	7.12±0.09b	18.52±0.18bc
Ammonium glufosinate	0.200	2.10±0.04a	2.70±0.14a	3.54±0.19a	5.73±0.38a	8.33±0.88a	20.50±0.76a
Iodosulfuron-methyl	0.002	2.20±0.07a	2.10±0.05b	3.55±0.12a	3.87±0.11b	6.68±0.14bc	18.40±0.20bc
Metsulfuron-methyl	0.001	2.17±0.06a	2.22±0.06b	3.15±0.12a	3.88±0.13b	6.45±0.15c	17.88±0.23c
Glyphosate 1.440	1. 440	2.20±0.06a	2.17±0.06b	3.55±0.11a	3.82±0.10b	7.10±0.09b	18.85±0.12b
Thiamethoxam+ lambda-cyhalothrin (standard)	0.010+0.007	–	–	–	–	–	–

(1) a.i.c. (%) = tested active ingredient concentration (%) in the spray solution. Means followed by the same letter in the columns do not differ significantly from each other by the Tukey's test (p>0.05). Bioassay I: 1st instar: F = 1.5403, DF = 4, p = 0.5405; 2nd instar: F = 2.4865, DF = 4, p = 0.0373; 3rd instar: F = 2.5040, DF = 4, p = 0.0251; pre-pupa: F = 3.1379, DF = 4, p = 0.0354; pupa: F = 4.27978, DF = 4, p = 0.0001; larva-adult: F = 2.4531, DF = 4, p = 0.0274. Bioassay II: 1st instar: F = 0.6877, DF = 4, p = 0.43023; 2nd instar: F = 7.2738, DF = 4, p = 0.0001; 3rd instar: F = 2.6862, DF = 4, p = 0.0310; pre-pupa: F = 22.8060, DF = 4, p = 0.0001; pupa: F = 8.8920, DF = 4, p = 0.0001; larva-adult: F = 7.7095, DF = 4, p = 0.0001.

obtained by other studies in relation to the duration of the larva-adult development period for 2,4-D amine and metsulfuron-methyl (Table 4). Michaud and Vargas (2010) observed that Ally (metsulfuron-methyl) presented no effect on the development of larvae of *C. maculata* and *H. convergens*, but 2,4-D ester, although not affecting the development period of *H. convergens*, increased significantly by approximately one day the development period in relation to the control for the predator *C. maculata*.

Effect on eggs of *C. externa* and *E. connexa*

The average viability of eggs sprayed with herbicides ranged from 66.66 to 96.87% for *C. externa* and from 48.95 to 78.12% for *E. connexa* (Figure 1). The viability of eggs of *C. externa* was not significantly affected by most of the herbicides, except for ammonium glufosinate, in which the viability was 66.66%. The viability of ladybug eggs was significantly affected by the herbicides metsulfuron-methyl, ammonium glufosinate, and pyrimidinedione, with viability rates of 48.95, 60.41, and 57.29%, respectively (Figure 1).

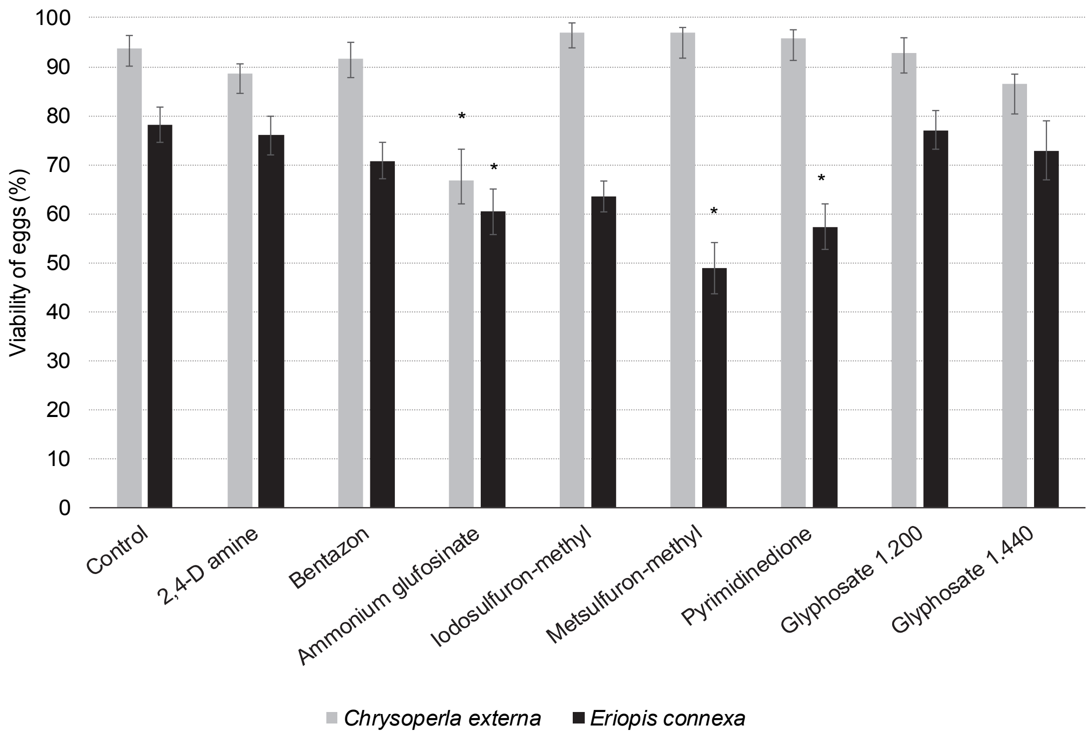
The reduction in larval hatching ranged from 0 to 27.08 for *C. externa* and 1.04 to 37.50 for *E. connexa* (Table 5). For *C. externa*, ammonium glufosinate was the herbicide that presented the highest effect on the reduction in larval hatching, with a value of 27.08%. Therefore, all herbicides were classified as innocuous (class 1) to the predator according to IOBC. Eggs of *E. connexa* were more affected when compared to those of *C. externa*. The herbicides metsulfuron-methyl, ammonium glufosinate, and pyrimidinedione presented more than 20% reduction in the larval hatching of *E. connexa*, while only ammonium glufosinate presented these reduction values for *C. externa* (Table 5). The herbicide metsulfuron-methyl presented the highest reduction in larval hatching (37.50%), being classified as slightly harmful (class 2) to ladybug eggs whereas the other herbicides were considered as innocuous (class 1) to coccinellid eggs.

The results obtained by Ahn et al. (2001) suggest that when eggs of *Orius strigicollis* (Poppius, 1915) (Hemiptera:Anthocoridae) are exposed to residual contact with the herbicide ammonium glufosinate at concentrations of 67.5 to 1,080 ppm a.i., there is a viability of eggs from 57.5 to

Table 4 **-** Duration of larval instars (number of days ± SE), pre-pupa and pupa stages, and duration of the larva-adult period of *Eriopis connexa* when the larval stage was exposed to residual contact with herbicides registered for the wheat crop

Treatment	a.i.c. (%)[(1)]	Duration (days)						
		1st instar	2nd instar	3rd instar	Pre-pupa	Pupa	Larva-adult	1st instar
Bioassay I								
Control	–	2.47±0.09bc	2.05±0.03ab	1.53±0.08a	2.58±0.08c	1.25±0.07a	3.55±0.10ab	13.36±0.09c
2,4-D amine	0.502	2.77±0.06ab	1.62±0.8c	1.82±0.12a	3.56±0.11a	1.10±0.05ab	3.83±0.06a	14.47±0.13a
Bentazon	0.480	2.60±0.9ab	2.02±0.03ab	1.70±0.07a	2.86±0.11bc	1.24±0.07ab	3.59±0.08ab	14.00±0.14ab
Pyrimidinedione	0.024	2.90±0.11a	2.00±0.06b	1.72±0.08a	2.87±0.05bc	1.02±0.03b	3.32±0.11b	13.85±0.17bc
Glyphosate 1.200	1. 200	2.15±0.07c	2.27±0.09a	1.72±0.08a	3.32±0.20ab	1.22±0.06ab	3.40±0.10b	14.05±0.13ab
Thiamethoxam + lambda-cyhalothrin (standard)	0.010+0.007	–	–	–	–	–	–	–
Bioassay II								
Control	–	1.67±0.07c	1.80±0.06b	2.60±0.10a	4.13±0.17ab	1.14±0.08a	3.00±0.22a	14.41±0.33a
Ammonium glufosinate	0.200	2.50±0.09a	1.50±0.09b	2.38±0.18ab	4.03±0.29ab	1.09±0.12a	3.38±0.16a	14.98±0.31a
Iodosulfuron-methyl	0.002	2.10±0.06b	1.77±0.10b	1.85±0.07c	3.58±0.16b	1.11±0.05a	2.71±0.11a	13.14±0.23b
Metsulfuron-methyl	0.001	1.92±0.08bc	1.72±0.07b	2.02±0.04bc	4.35±0.23a	1.17±0.07a	2.70±0.11a	13.90±0.3ab
Glyphosate 1.440	1.440	1.95±0.11bc	2.15±0.09a	1.84±0.10c	4.18±0.11ab	1.11±0.06a	2.73±0.13a	13.87±0.21ab
Thiamethoxam + lambda-cyhalothrin (standard)	0.010+0.007	–	–	–	–	–	–	–

(1) a.i.c. (%) = tested active ingredient concentration (%) in the spray solution. Means followed by the same letter in the columns do not differ significantly from each other by the Tukey's test ($p>0.05$). Bioassay I: 1st instar: F = 11.0829, DF = 4, p = 0.0001; 2nd instar: F = 12.4920, DF = 4, p = 0.0001; 3rd instar: F = 1.2777, DF = 4, p = 0.7090; 4th instar: F = 10.1114, DF = 4, p = 0.0001; pre-pupa: F = 2.8890, DF = 4, p = 0.0392; pupa: F = 4.2640, DF = 4, p = 0.0001; larva-adult: F = 7.9718, DF = 4, p = 0.0001. Bioassay II: 1st instar: F = 12.1937, DF = 4, p = 0.0001; 2nd instar: F = 7.2812, DF = 4, p = 0.0001; 3rd instar: F = 9.0349, DF = 4, p = 0.0001; 4th instar: F = 2.1714, DF = 4, p = 0.9185; pre-pupa: F = 0.1688, DF = 4, p = 0.6128; pupa: F = 2.4737, DF = 4, p = 0.0335; larva-adult: F = 4.4831, DF = 4, p = 0.0001.



*Statistically different when compared to the control by the Dunnett test ($p<0.05$). *Chrysoperla externa*: F = 5.60, DF = 8, p = <0.0002; *Eriopis connexa*: F = 6.05, DF = 8, p = <0.0001.

Figure 1 **-** Viability of eggs of *Chrysoperla externa* and *Eriopis connexa* sprayed with herbicides registered for the wheat crop.

Table 5 **-** Reduction in larval hatching when eggs of *Chrysoperla externa* and *Eriopis connexa* were directly sprayed with herbicides registered for the wheat crop

Treatment	a.i.c. (%)[1]	*Chrysoperla externa*		*Eriopis connexa*	
		RLH[2]	C[3]	RLH[2]	C[3]
Control	–	–	1	–	1
2,4-D amine	0.502	3.12	1	2.08	1
Bentazon	0.480	0.00	1	8.33	1
Ammonium glufosinate	0.200	27.08	1	22.91	1
Iodosulfuron-methyl	0.002	0.00	1	18.75	1
Metsulfuron-methyl	0.001	0.00	1	37.50	2
Pyrimidinedione	0.024	0.00	1	26.04	1
Glyphosate 1.200	1.200	7.29	1	6.25	1
Glyphosate 1.440	1.440	1.04	1	1.04	1

(1) a.i.c. (%) = tested active ingredient concentration (%) in the spray solution; (2) RLH = reduction in larval hatching corrected by the Schneider-Orelli equation (%); (3) C = IOBC classes: 1 = innocuous (<30%), 2 = slightly harmful (30–79%), 3 = moderately harmful (80-99%), and 4 = harmful (>99%).

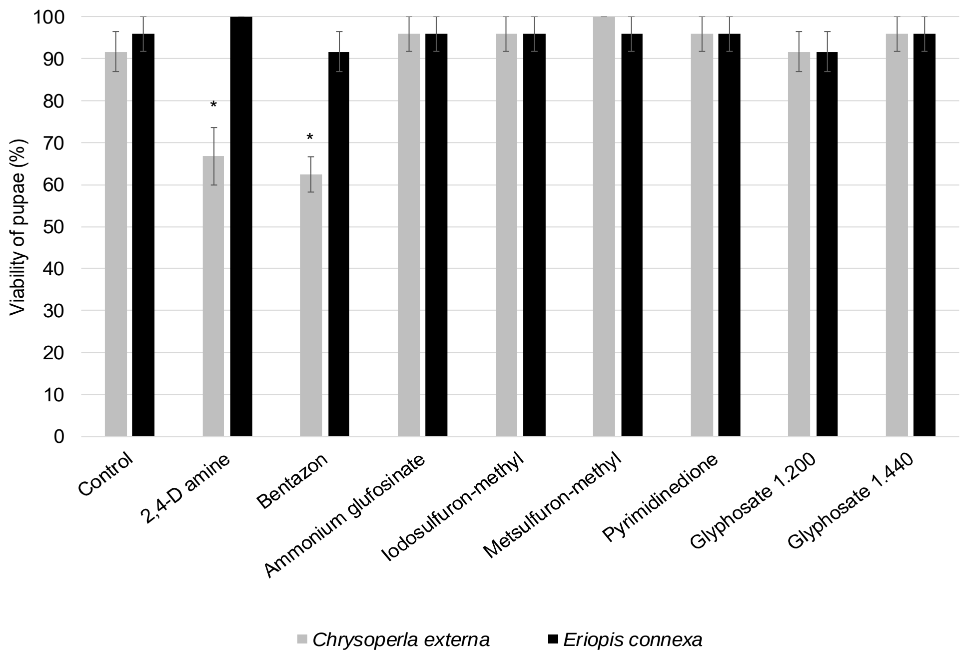
11.5%, respectively, while a total egg mortality occurs at a concentration of 2,160 ppm a.i. However, these authors did not find any effect on eggs of *H. axyridis* when they were exposed to the residual contact with the herbicide ammonium glufosinate at concentrations of 2,160 ppm a.i., which is different from the data obtained in our study in which ammonium glufosinate differed significantly from the control for both *C. externa* and *E. connexa* (Figure 1). Despite the small number of studies that address the effect of herbicides on predator eggs, that of Castilhos et al. (2014) was similar to our study, showing an absence of herbicide effect on egg viability and reduction in larval hatching, also classifying the herbicide as innocuous (class 1) to eggs of this predator (Table 5).

In general, according to Klowden (2007), insect eggs consist of a nucleus, cytoplasm, a layer consisting of waxes, and the corium, which presents an endocorium and exocorium. Corium is a complex structure composed of more than 90% proteins that protect the egg. Therefore, corium constitution, notably the presence of the wax layer, may influence the retention of a certain amount of chemical substances. Thus, although the herbicides metsulfuron-methyl, ammonium glufosinate, and pyrimidinedione have significantly reduced egg viability, they did not cause much toxicity to the larvae of both predators. In addition, in our study, the assessment of herbicide toxicity on eggs was restricted to the verification of reduction in larval hatching. However, sublethal effects such as changes in the duration of the embryonic period and harmful effects in larvae and adults from treated eggs may influence the impact of a certain herbicide on the egg stage of *C. externa* and *E. connexa* in wheat crop, being necessary other studies to determine these possible effects.

Effect on pupae of *C. externa* and *E. connexa*

A significant effect on the viability of pupae of *C. externa* was observed only for the herbicides 2,4-D amine and bentazon, in which 66.66 and 62.5% of the sprayed pupae were viable. For the other herbicides, the viability was similar to that found for the control (Figure 2). The ladybug *E. connexa* did not present a significant difference regarding pupae viability, with all herbicides presenting a similar behavior to that found for control, with values above 90% (Figure 2).

Except for 2,4-D amine and bentazon, the tested herbicides did not cause any reduction in the emergence of adults of *C. externa* (Table 6). For *E. connexa*, bentazon and glyphosate (1,200 and 1,440) caused little reductions in adult emergence (4.16%) (Table 7). Fecundity of adults of *C. externa* emerged from pupae sprayed with herbicides did not differ significantly from those obtained in the control, but fertility was statistically different from the control treatment for the herbicides 2,4-D amine and pyrimidinedione (Table 6). For *E. connexa*, no significant sublethal effect was observed in the reproductive parameters fecundity and fertility (Table 7).



*Statistically different when compared to the control by the Dunnett test (p<0.05). *Chrysoperla externa*: F = 9.5323, DF = 8, p = 0.0001; *Eriopis connexa*: F = 0.3750, DF = 8, p = 0.9246.

Figure 2 **-** Viability of pupae of *Chrysoperla externa* and *Eriopis connexa* sprayed with herbicides registered for the wheat crop.

Table 6 **-** Reduction in adult emergence, fecundity and fertility of emerged adults, total effect, and consequent toxicity classification for pupae of *Chrysoperla externa* sprayed with herbicides registered for the wheat crop

Treatment	a.i.c. (%)(1)	Pupa				
		RAE(2)	Fecundity*	Fertility*	E (%)(3)	C(4)
2,4-D amine	0.502	25.00	22.09±1.70b	76.13±4.08d	51.58	2
Bentazon	0.480	29.16	30.72±1.57a	85.47±3.38bcd	0.00	1
Ammonium glufosinate	0.200	0.00	25.62±1.10ab	96.91±1.04ab	0.00	1
Iodosulfuron-methyl	0.002	0.00	22.62±2.20b	100.00±0.00a	8.72	1
Metsulfuron-methyl	0.001	0.00	25.48±0.79ab	92.70±2.62abc	1.49	1
Pyrimidinedione	0.024	0.00	25.86±1.87ab	80.91±3.71cd	19.59	1
Glyphosate 1.200	1.200	0.00	30.03±2.28ab	89.96±2.29abc	14.68	1
Glyphosate 1.440	1.440	0.00	25.60±1.80ab	93.75±1.20ab	3.15	1
Control	–	0.00	25.59±1.01ab	96.87±1.99ab	–	–

(1) a.i.c. (%) = tested active ingredient concentration (%) in the spray solution; (2) RAE = reduction in adult emergence corrected by the Schneider-Orelli equation (%); (3) E = total effect; (4) C = IOBC classes: 1 = innocuous (<30%), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), and 4 = harmful (>99%). * Means followed by the same letter in the columns do not differ significantly from each other by the Tukey's test (p>0.05). Fecundity: F = 2.9549, DF = 8, p = 0.0164; Fertility: F = 9.4965, DF: 8, p = 0.0001).

Taking into account the total effect calculated for the herbicides, only 2,4-D amine presented harmfulness to pupae of *C. externa* (Table 6) and pyrimidinedione to pupae of *E. connexa* (Table 7), both considered as slightly harmful (class 2) to predators. The other herbicides were considered as innocuous (class 1) to pupae, with a total effect lower than 30%.

The absence of harmful effect to pupae of *C. externa* for most of the tested herbicides are in accordance with other studies, such as that of Castilhos et al. (2014), who studied glyphosate selectivity to pupae of *C. externa* and observed no significant effect on pupal viability and adult emergence of the predator, being classified as innocuous (class 1) to pupae of *C. externa*, as in our study (Table 6),

Table 7 **-** Reduction in adult emergence, fecundity and fertility of emerged adults, total effect, and consequent toxicity classification for pupae of *Eriopis connexa* sprayed with herbicides registered for the wheat crop

Treatment	a.i.c. (%)(1)	Pupa				
		RAE(2)	Fecundity*	Fertility*	E (%)(3)	C(4)
2,4-D amine	0.502	0.00	34.65±4.43a	79.48±6.15a	0.00	1
Bentazon	0.480	4.16	33.51±2.24a	84.63±5.35a	0.00	1
Ammonium glufosinate	0.200	0.00	24.67±3.42a	79.15±2.96a	24.95	1
Iodosulfuron-methyl	0.002	0.00	27.74±2.02a	85.73±3.80a	8.61	1
Metsulfuron-methyl	0.001	0.00	28.11±2.85a	87.01±3.43a	5.99	1
Pyrimidinedione	0.024	0.00	24.10±5.75a	74.63±4.76a	30.88	2
Glyphosate 1.200	1.200	4.16	28.42±2.50a	80.98±1.65a	15.22	1
Glyphosate 1.440	1.440	4.16	27.60±2.36a	82.57±1.06a	16.07	1
Control	–	0.00	30.08±4.43a	86.52±1.94a	–	–

(1) a.i.c. (%) = tested active ingredient concentration (%) in the spray solution; (2) RAE = reduction in adult emergence corrected by the Schneider-Orelli equation (%); (3) E = total effect; (4) C = IOBC classes: 1 = innocuous (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), and 4 = harmful (>99%). *Means followed by the same letter in the columns do not differ significantly from each other by the Tukey's test (p>0.05). Fecundity: F = 0.9969; DF = 8, p = 0.4551; Fertility: F = 1.3291, DF = 8, p = 0.2609.

No difference was found for the adult survival of *E. connexa* from pupae treated with herbicides, suggesting that pupal morphology may have served as a barrier to herbicide penetration, thus protecting insect development (Table 7). According to Croft (1990), among the factors that affect insect susceptibility to chemical substances is integument constitution since it may present, mainly in the pupa stage, a more impermeable cuticular layer, making it difficult product penetration. The results obtained by Ahn et al. (2001) demonstrated a lack of effect for ammonium glufosinate (67.5 ppm a.i.) when tested on pupae of *H. axyridis*, corroborating our results (Table 7). However, when this herbicide was tested at a higher concentration (1,080 ppm a.i.), a larval mortality of the predator of 77.8% was obtained.

Scanning electron micrographs of the silk cocoon surrounding the pupae of *C. externa* were performed by Cosme et al. (2009), revealing that the cocoon presents orifices with 6 ìm in diameter where the insect respiration occurs and possibly herbicides may penetrate and reach the insect inside. However, as observed in our study, only 2,4-D amine and bentazon were able to penetrate through these orifices, significantly reducing the pupal viability of *C. externa* (Figure 2). Most of the herbicides failed to break this pupa barrier.

Although these herbicides target plants, commercial products contain several other chemicals that are not specified on the herbicide label. One of the reasons for this is that the information regarding the surfactant components of the herbicide commercial formulation is often confidential and protected as a manufacturer's right (Chen et al., 2004). These other components present in a herbicide can carry their own toxicity and are reported to cause harmful side effects to egg parasitoids (Stecca et al., 2016), which probably may have caused a toxicity to the predators *C. externa* and *E. connexa*.

Laboratory tests assessing the deleterious effects of herbicides are important to detect harmful effects on natural enemies. The herbicides bentazon, glyphosate 1,200, glyphosate 1,440, and iodosulfuron-methyl, which were considered as innocuous to the larva, egg, and pupa stages of *C. externa* and *E. connexa* in the laboratory, are recommended for use in the integrated pest management of wheat crop. However, those considered as slightly (metsulfuron-methyl to eggs of *E. connexa*, 2,4-D amine to pupae of *C. externa*, and pyrimidinedione to pupae of *E. connexa*) and moderately harmful (ammonium glufosinate to larvae of both predators) should be subsequently tested in the semifield and field stages. Even those herbicides considered as selective may have a side effect on the predation behavior of both predators, affecting the performance in the field. Thus, further studies should be carried out to confirm the true effect of these herbicides on the predators of the wheat crop.

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