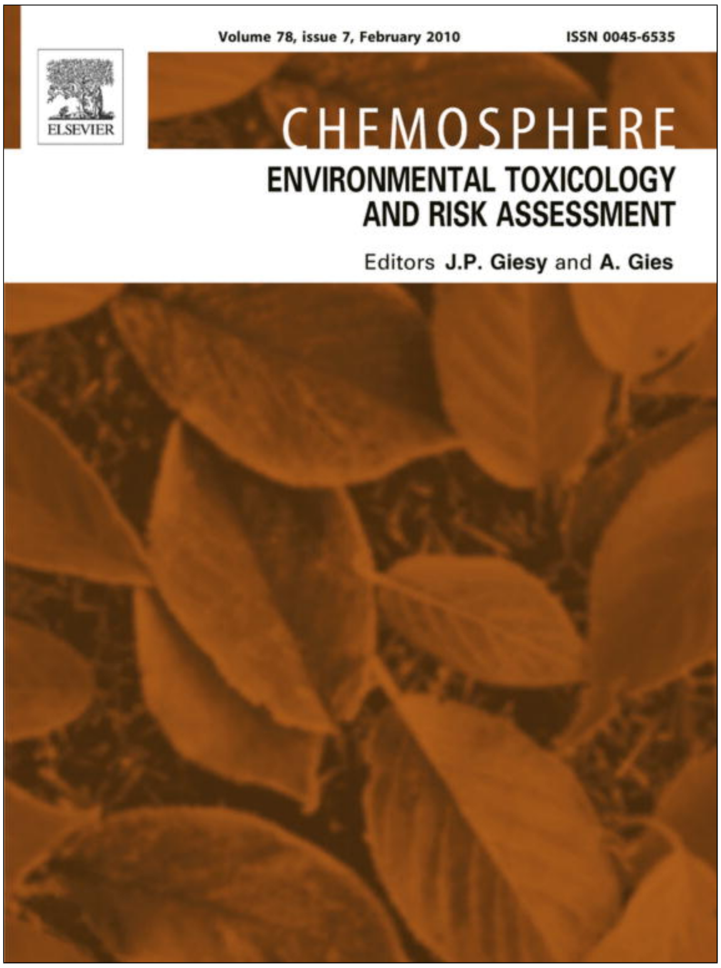
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Effects of the herbicide glyphosate on biological attributes of *Alpaida veniliae* (Araneae, Araneidae), in laboratory

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A B S T R A C T

In the past decades there has been increasing interest in the study of arthropod predators as effective potential natural enemies to be used in the biological control of agricultural pests. In Argentina, transgenic soybean crops (Round-up Ready, RR) are inhabit by many spider species, some of them in high abundance, being indicative of an import potential for pest predation. This crop is associated with the use of glyphosate, a broad-spectrum herbicide, with low environmental impact, even though since the 80's, several negative effects have been deeply documented on mammals, fishes, amphibians, snails, earthworms, insects, etc. Nowadays, the effects on arthropod physiology, behavior and life history traits as end-points in ecotoxicological evaluations are being recognized. In transgenic soybean crops of Buenos Aires province (Argentina), *Alpaida veniliae* (Araneae, Araneidae) is one of the most abundant orb web weaver spiders. The purpose of this study was to address the effects of glyphosate on some biological attributes of *A. veniliae*, in laboratory. Results of this study showed no lethal direct effects of Glifoglex® on this spider, but it is the first report in literature about sublethal effects of this herbicide on a spider's biological attributes. Negative effects on prey consumption, web building, fecundity, fertility and developmental time of progeny were observed. Although sublethal effects have received less attention than direct lethal effects, they are relevant from an ecological point of view, since the reduction of the arthropod performance may create risks to arthropod biodiversity conservation in agroecosystems.

1. Introduction

In the past decades there has been increasing interest in the study of arthropod predators as effective potential natural enemies to be used in the biological control of agricultural pests (Riechert and Lockley, 1984; Riechert and Bishop, 1990; Rypstra and Carter, 1995; Symondson et al., 2002).

In Argentina, transgenic soybean crops (Round-up Ready, RR) are inhabit by many spider species, some of them in high abundance, being indicative of an import potential for pest predation (Minervino, 1996). This crop is associated with the use of glyphosate [(N-phosphonomethyl) glycine], a broad-spectrum herbicide with high water solubility, relatively nonselective, and very effective on deeply rooted perennial species of grasses, sedges, and broadleaf weeds (Blackburn and Boutin, 2003). According to their mode of action, no detrimental effects on other organisms were expected and declared by first manufacturers (www.monsanto.com.ar/h/archivos/1-herbicidas-glifosato.pdf).

However, studies with a glyphosate formulation (Round-up®) on mammals including humans, showed several physiological and biochemical alterations in cells (umbilical, embryonic and placental) (Walsh et al., 2000; Richard et al., 2005; Benachour and Seralini, 2009). In addition, development, morphological, physiological, immunological and biochemical alterations were registered on fishes, amphibians, snails, earthworms, etc. (Tate et al., 2000; Smith, 2001; Lajmanovich et al., 2003; Cauble and Wagner, 2005; Glusczak et al., 2006; Yasmin and D'Souza, 2007; Achiorno et al., 2008).

Another very important but scarce studied topic was reported by Arregui et al. (2004) in relation to the presence of glyphosate residues (aminomethylphosphonic acid) in soybean leaves and grains.

Studies on the direct and indirect effects of glyphosate on arthropods are limited (Tanigoshi and Congdon, 1983; Yokoyama et al., 1984; Paoletti and Pimentel, 2000; Manzoni et al., 2006). Among the few investigations on spiders are those of Haughton et al. (1999, 2001a) reporting neither toxic direct effects of glyphosate spraying in arable field margins nor in the number of wandering spiders, but a reduction in the density of the two most abundant web-spinners, *Gonatium rubens* (Blackwall) and *Lepthyphantes tenuis* (Blackwall) (Araneae, Linyphiidae). As in laboratory the same authors (Haughton et al., 2001b) did not find lethal effects of technical glyphosate (620 g L^{-1} glyphosate–isopropylamine; Monsanto,

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Louvain-La Neuve, Belgium) on the spider *L. tenuis*, they speculated that under field conditions, changes in vegetation structure and microclimate caused by the glyphosate were implicated in the reduction of numbers of spiders in plots receiving the highest rate of herbicide. Likewise, Bell et al. (2002) observed the lack of any effect on species richness, but a significantly reduction in numbers which attributed to the decline in vegetation height and the increase in the percentage of dead vegetation cover, concluding that the spider community is detrimentally affected by applications of this herbicide. Symondson et al. (2002) also reported for changes in spider dispersion, and availability of refuges and places to web building in response to changes of vegetation structure.

Although habitat quality and structure have a profound effect on arthropod populations, this not precludes the existence of a direct and/or indirect effect of applications of glyphosate on the spider populations. Hence, a controversy and debate there still exits about the negative impact of glyphosate on non-target organisms, and more studies are required.

Conservation Biological Control involves adopting practices to manipulate the environment to maintain or enhance the fitness of entomophagous arthropods, in order to increase their effectiveness as natural enemies of pests (Landis et al., 2000). In this sense, it is necessary to protect them from the harmful effects of pesticides.

Conventionally, the impact of pesticides to beneficial arthropods has been measured estimating the acute median lethal dose (LD_{50}). However, this is only a partial appraisal since sublethal effects on arthropod physiology, behavior, and life history traits should also be considered to have a more complete evaluation of their impact (Stark and Banks, 2003; Desneux et al., 2007; Stark et al., 2007). Recent studies have shown a significant detrimental effect of a glyphosate formulation on development, fecundity, fertility and demography of the predator *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) (Schneider et al., 2009).

In transgenic soybean crops of Buenos Aires province (Argentina), *Alpaida veniliae* (Keyserling) (Araneae, Araneidae) is one of the most abundant spider species of the orb web weaver guild. Accordingly, the assessment of side-effects of glyphosate is relevant to conservation of this species in soybean agroecosystems.

The purpose of this study was to determine the side-effects of glyphosate (short-term and long-term effects) on some biological attributes of *A. veniliae*, in laboratory. We examined the effect on mortality, prey consumption, web building, female fecundity and fertility, and developmental time of progeny.

2. Materials and methods

2.1. Spiders

The laboratory colony was started with individuals of *A. veniliae* collected from transgenic soybean crops located at Chivilcoy (35°01′S, 60°06′W) (Buenos Aires, Argentina), from January to March 2006. Adults were reared in 15 × 15 cm height and width glass jars (500 mL), and maintained in the laboratory to obtain egg sacs. Juveniles emerging from the egg sacs were followed to obtain a mass-rearing population. Juveniles and adults were fed *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) and *Musca domestica* L. (Diptera: Muscidae) adults, "*ad libitum*". Laboratory conditions were 25 ± 2 °C temperature, 75 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

2.2. Herbicide solutions and treatments

The commercial Glifoglex 48® (48% glyphosate, Gleba SA, Buenos Aires, Argentina) was used in toxicity bioassays. Fresh solutions with 192 mg L^{-1} a.i. (maximum field registered nominal concentration) (CASAFE, 2007) were prepared using acetone (Analytical Grade) as solvent to assure the evaporation of herbicide solution, considering that spiders avoid feeding on wet preys. The exposure route was by ingestion "through the treated prey" and the chronic toxicity was analyzed. The prey (*M. domestica* adults) was treated by dipping during 20 s according to Schneider et al. (2009), and dried under fume cupboard. The controls were fed prey treated with acetone alone. The mean (±SE) weight of adult preys of both treatments was 11.62 ± 0.05 mg.

2.3. Mortality and prey consumption

Fifteen-d-old adult females from the colony were fed freshly glyphosate treated preys, previous starvation of 1 week, during 4 d. Then, females were fed untreated prey. Control and treated females were kept individually in 6 cm diameter Petri® dishes and each treatment was replicated 60 times. Spiders were monitored every 24 h and up to 96 h to check mortality. Rests of prey were daily removed and new treated preys were offered. Remnants were observed under binocular microscope to determine the percentage of prey consumed. For doing so the prey was arbitrarily divided in ten parts of equal length. The winds and exoskeleton never were consumed.

2.4. Web building

Females from control and glyphosate treatments were individually placed in wooden frames (15 × 10 × 5 cm) surrounded by glass to allow viewing web building, which was daily recorded via digital photos for 20 consecutive days. Orb webs from control females were considered normal and the number of radius and spires compared with those from glyphosate treatment. Once females started to weave the web, a non treated and virgin male was introduced in each experimental unit to observe mating.

2.5. Development of ovaries

A set of 10 presumed mated females from each treatment, were randomly selected. After 15 d, they were dissected and the diameter of the oocites from the final portion of the ovaries was measured using a stereoscopic microscope and a micrometric eyepiece. Measures are given in millimeters.

2.6. Fecundity and fertility

The remaining mated females (five from each treatment) were followed until control females have laid five egg sacs. During this time from glyphosate females laid up to three. In this species, the first three instars develop within the egg sac and the fourth instar, called spiderlings, starts the dispersion. Egg sacs were maintained until the dispersion of spiderlings and then they were dissected to count the number of normal egg masses. Egg sacs and egg masses from control females were considered normal for comparison with those of the glyphosate treatment. Each egg mass was opened to observe malformed, dried or decomposed eggs. The mean number of eggs per egg sac and the mean percentage of egg hatched per egg sac, from the first to the last oviposition, were registered. Mean total fecundity (mean number of eggs) and fertility (mean number of offspring) per female and standard errors were estimated per each treatment.

2.7. Developmental time of progeny

The number of days (mean ± SE) from oviposition to dispersion (fourth instar) of the progeny of each treatment was estimated.

2.8. Statistical analysis

Analysis of variance (ANOVA) was used previous transformations of data to arcsin or ln(x + 1) to meet requirements of the analysis on normality and homocedasticity of variances to test for differences in oocite diameter, number of web radius and spires, and developmental time from oviposition to the third molt between control and glyphosate treatments, Means were separated using Fisher least significant difference (LSD) test or Bonferroni test when the F form analysis was non significant (Scheiner and Gurevitch, 2001). When ANOVA assumptions were violated a Kruskal–Wallis test was used. Normal and abnormal web building, egg sacs and egg masses were analyzed in two-way contingency tables using Fisher's exact test (Zar, 1996). The percentage of prey consumed, the mean number of eggs laid and the mean proportion of egg hatched per egg sac over time were compared by one-way repeated measures analysis of variance (ANOVA). Previously, Mauchley's Sphericity Test was used to tests the assumption of circularity. When this assumption could not be met the adjustment of the F-statistic degrees of freedom was performed by the Greenhouse–Geisser method (Scheiner and Gurevitch, 2001). A 0.05 significance level was chosen for all statistical analysis.

3. Results

3.1. Mortality and prey consumption

Not any mortality was not registered along the 96 h of the experiment in either treatment, and no differential mortality pattern was observed between treatments over time.

Prey consumption did not differ between treatments and over time, but the interaction was significant (Table 1). The mean percentages of prey consumption at 24, 48, 72 and 96 h in both treatments are shown in Fig. 1. Consumption pattern in control exhibited higher values than in glyphosate treatment, experiencing a clear decrease from 24 h post treatment up to 48, 72 and 96 h. Glyphosate treatment showed very little variation over time, being differences between treatments, at 24 h post treatment, at the limit of significance (P = 0.07).

3.2. Web building

Females from glyphosate treatment started web building 17 d later than in control, and only 20% of them wove a normal web (P = 0.001) (Fig. 2A). The number of radius and spires were significantly lower in glyphosate treatment compared to the control

Table 1
Results of repeats-measured ANOVA of percentages of prey consumption, number of eggs laid, and percentages of hatched eggs over time, in control and glyphosate treatments.

	df Effect	df Error	P	df Effect[a]	df Error[a]	F	Adjusted P
Prey consumption							
Treatment	1	118	0.57			0.32	0.57
Time	3	354	0.38	1.86	219.42	1.02	0.36
Interaction	3	354	<0.001	1.86	219.42	10.20	0.0001
Number of eggs laid							
Treatment	1	97	0.003			9.16	0.003
Time	4	388	<0.001	2.36	229.30	240.68	<0.001
Interaction	4	388	0.14	2.36	229.30	1.76	0.17
Percentages of hatched eggs							
Treatment	1	97	0.003			9.16	0.003
Time	4	388	<0.001	2.36	229.30	240.68	<0.001
Interaction	4	388	0.14	2.36	229.30	1.76	0.17

[a] df adjusted by Greenhouse–Geisser test: ε = 0.62 (percentages of prey consumption); ε = 0.59 (number of eggs laid); ε = 0.37 (percentages of hatched eggs).

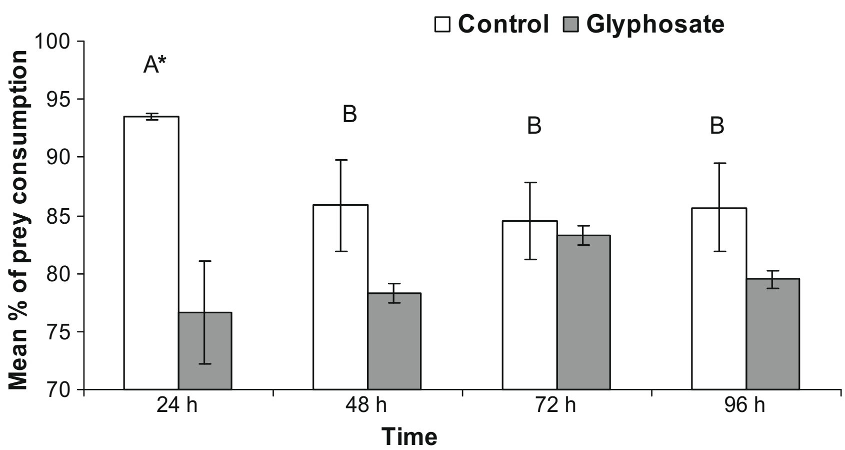

Fig. 1. Effect of glyphosate at 24, 48, 72 and 96 h after treated and control prey consumption. Vertical lines indicate standard errors. Capital letters indicate differences within control treatment. * Indicates significant differences between control and glyphosate treatments at P = 0.007.

(Fig. 2B). Since most of them wove only draglines they were not able to capture preys. However, mating was not affected because in this species the male build a "mating thread". The number of radius (mean ± SE) was 19.14 ± 0.24 and 9.96 ± 0.44 (H = 43.71;

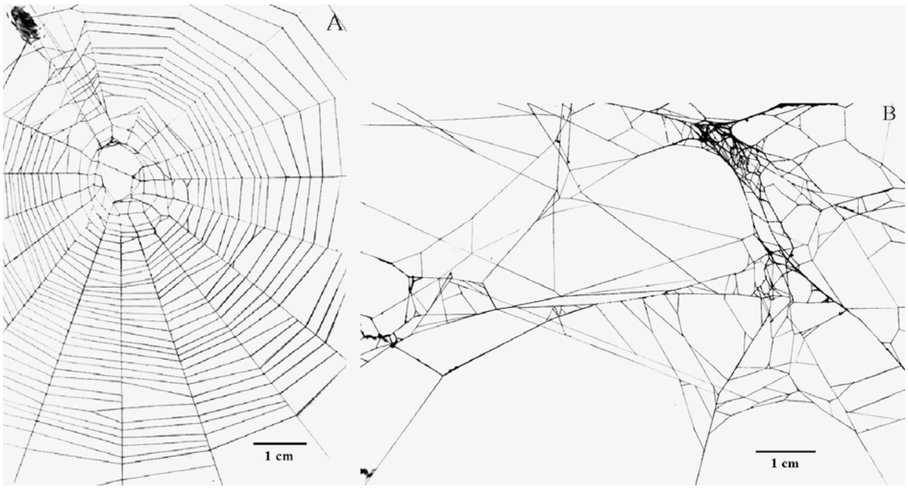

Fig. 2. Photographs of web of A. veniliae from control (A) and glyphosate treatments (B).

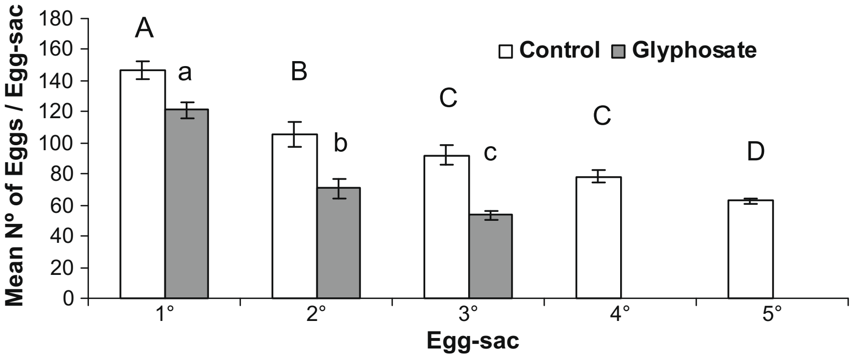

Fig. 3. Effect of glyphosate on the mean number of eggs per egg sac, in control and glyphosate treatments. Vertical lines indicate standard errors. Capital letters indicate differences within control treatment, and lowercase within glyphosate treatment.

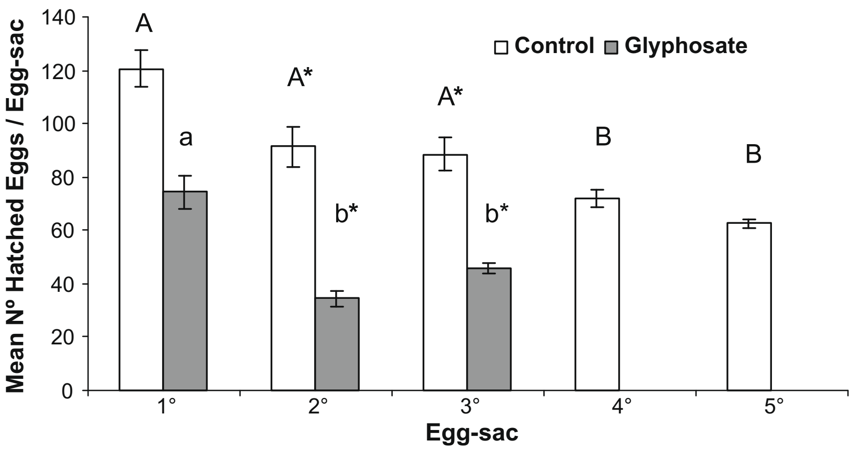

Fig. 4. Effect of glyphosate on mean the number of eggs hatched per egg sac, in control and in glyphosate treatments. Vertical lines indicate standard errors. Capital letters indicate differences within control treatment, and lowercase within glyphosate treatment. * Indicates significant differences between control and glyphosate treatments (P = 0.05).

$P < 0.01$) and the number of espires (mean ± SE) was 29.72 ± 0.40 and 14.34 ± 0.86 ($H = 43.13$; $P < 0.01$), for the control and glyphosate treatments, respectively.

3.3. Development of ovaries and egg sacs

Dissected females from glyphosate treatment showed abnormal ovaries with scarce development of oocites and fatty granules around them. The mean oocite diameter from the final part of ovaries was significantly lower in glyphosate treatment ($F_{1, 68} = 91.93$; $P < 0.001$) 15 d after mating. The diameter (mean ± SE) was 0.18 ± 0.02 and 0.23 ± 0.02 mm in the glyphosate and control treatments, respectively.

The number of abnormal egg sacs and egg masses in each oviposition, as well as in total ovipositions was significantly higher in glyphosate treatment than in control (egg sacs: $P < 0.001$; egg masses: $P < 0.001$).

3.4. Fecundity and fertility

Females from glyphosate and control treatments were able to lay egg up to three and five times, respectively, along the experiment. Glyphosate had a negative effect on fecundity and fertility. Both, treatments and time significantly affected the number of eggs laid and the number of egg hatched (Table 1). There was not significant interactions between treatment and time in fecundity while in fertility it was at the significance limit ($P = 0.06$). There was a tendency to reduce the number of eggs laid (Fig. 3) and in the proportion of egg hatched (Fig. 4) both treatments, from the first to the last oviposition.

Fecundity and fertility were negatively affected by glyphosate. Total fecundity (mean ± SE) was 260.72 ± 14.80 and 168.89 ± 8.90 eggs per female ($F_{1, 97} = 25.36$; $P < 0.001$), and total fertility (mean ± SE) was 224.12 ± 13.00 and 67.10 ± 6.58 hatched eggs per female ($F_{1, 182} = 177.47$; $P < 0.001$) in control and glyphosate treatments, respectively.

3.5. Developmental time of progeny

Developmental time of progeny, from oviposition to the third molt, was significantly longer in glyphosate treatment ($H = 10.34$; $P = 0.001$). The means (±SE) were 10.44 ± 0.09 and 11.83 ± 0.41 d, in control and glyphosate and treatments, respectively.

4. Discussion

The results of this study showed no lethal direct effects of a commercial glyphosate formulation on the spider *A. veniliae*. They are coincident with those of Tanigoshi and Congdon (1983) and Yokoyama et al. (1984) who reported non short-term effect in the mite predator *Euseius hibisci* (Chang) (Acari: Phytoseiidae) and the bug predator *Geocoris pallens* (Stäl) (Hemiptera: Geocoridae) when acute herbicide's toxicity was evaluated. Contrary, high mortalities were recorded in tadpoles and amphibian larvae chronically exposed even to lower concentrations (Lajmanovich et al., 2003; Cauble and Wagner, 2005).

However, in our study we found very clear sublethal effects of glyphosate in the laboratory on prey consumption, web building, fecundity, fertility and developmental time of progeny of *A. veniliae*. These results are coincident with those of Schneider et al. (2009) on the chrysopid *C. externa*, another arthropod predator very common in soybean crops.

Although prey consumption was not affected by treatment over time, the interaction between the treatment and time found could be related to the differences observed in consumption at 24 h post treatment. A possible explanation could be that by treatment, probably because after one week of starvation, control females increased its normal consumption rates when preys were offered. Later on, due to satiation feeling, rates of consumption declined and remained almost constant during 96 h. On the contrary, females from glyphosate treatment had at 24 h, although in the limit of statistical significance, a lower consumption rate than control, probably owing to a rejection of contaminated prey, in spite of the starvation period. Consumption rates in this treatment remained low, with values similar to those of control, until 96 h. Mechanisms responsible of the reduction or avoiding of consumption of toxic prey in the studied arachnid is not yet clear. Smell or taste may act as deterrents, perhaps as signals of unpalatability or toxicity (Toft, 1999). In this sense, Wiles and Jepson (1994) demonstrated that larval and adult stages of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) consumed fewer dimethoate treated aphids, suggesting a repellent effect. The negative effect on web building could affect the efficiency of females to intercept preys under field conditions. Moreover, in foraging behavior, predators and preys exchange information utilizing the same vibratory, visual, and chemical cues that are used in social interactions (Riechert and Luczak, 1982). Predator–prey interactions may be disturbed through a change in foraging behavior of predators as well as in the activity of preys, triggered by glyphosate applications. The interference of pesticides with feeding behavior of exposed insect may include different mechanisms (Desneux et al., 2007). Consumption of toxic prey may consequently be reduced by induced an aversion, probably associated with both prey taste and behavior. However, avoiding toxic prey seems to be limited because aversions are short-lasting and some toxic preys do not induce an aversion (Toft, 1999).

The detrimental effects observed in *A. veniliae* from assays through contaminated preys, could be related to physiological and biochemical alterations produced by the herbicide formulation. This pathway was also hypothesized in other animals and humans (Walsh et al., 2000; Richard et al., 2005; Benachour and Seralini, 2009), with substantial deleterious effects on metabolic and endocrine process.

In this study, fecundity and fertility were the most affected traits. Schneider et al. (2009) also reported substantial reductions in fecundity and fertility, with consequent diminution in the Intrinsic Rate of Increase (r) and Net Reproductive Rate (R_0) of *C. externa* populations after one generation. Similarly, a decrease in fecundity also was reported in the earthworm, *Eisenia fetida* (Savigny) (Haplotaxida: Lumbricidae) (Yasmin and D'Souza, 2007), and in the parasitism by *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) females exposed to glyphosate residues (Manzoni et al., 2006).

It has been suggested that some herbicides may mimic specific hormones or disrupt endocrine systems (Cauble and Wagner, 2005). In this sense, we can hypothesize that the fecundity alterations in arthropods could be related with disruption in hormonal system (ecdysteroid and juvenile hormones) linked with reproductive events, such as ovariole differentiation, oogenesis and vitellogenesis.

Females from glyphosate treatment not only showed abnormal ovaries with scarce development of oocites, but also the mean oocite size was significantly lower. These attributes could be additionally affected by a poor nutrition of females because of the delayed of adult females to start web building and the lower efficiency to capture preys under field conditions. Females poorly fed will be affected in their survival, fecundity and fertility, therefore, natural populations of this spider would be seriously affected in its capacity to grow and persist in natural conditions.

Although sublethal effects have received less attention than direct lethal effects, they are relevant from an ecological point of view, since the reduction of the arthropod performance may create risks to arthropod biodiversity conservation. In the same direction, and in relation to mammalian health effects of pesticides or chemicals, Séralini et al. (2009) appeal to the need to investigate chronic and subchronic effects, which are usually neglected.

Finally, there is a great controversy about the toxicity of surfactants included in the herbicide-formulation. In this sense, several works reported more toxic effects of surfactants than herbicide itself (Mann and Bidwell, 1999; Glusczak et al., 2006). Accordingly, the toxicity of Round-up has been attributed to its surfactant polyethoxylated tallow amine (POEA) (Peixoto, 2005; Glusczak et al., 2006; Mann et al., 2009). Nevertheless, it has being demonstrated through several studies in invertebrates and humans that glyphosate formulation (Round-up) and the active ingredient alone caused similar deleterious effects (Achiorno et al., 2008; Benachour and Seralini, 2009; Gasnier et al., 2009).

Our data provide new insights on the side-effects of glyphosate on biological traits of spiders. In addition, this work highlights once again the relevance of sublethal effect evaluations in ecotoxicological studies to reformulate pests control strategies in agroecosystems. Therefore, in view of the ecotoxicological hazards of glyphosate on non-target organisms, it is imperative to conduct future experiments under more field-related conditions.

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