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Short and long-term effects of three neurotoxic insecticides on biological and behavioural attributes of the orb-web spider *Alpaida veniliae* (Araneae, Araneidae): implications for IPM programs

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Abstract Soybean pest control in Argentina is done just by chemical control using broad-spectrum pesticides. *Alpaida veniliae* (Araneae, Araneidae) is one of the most abundant spider species of the orb web weaver guild in soybean, and it is considered a very important polyphagous predator, attacking different insects' families. The objective of this study was to determine if neurotoxic insecticides commonly used in soybean crops and a new active ingredient registered in Argentina (spinosad) adversely affected survival, prey consumption, mating behaviour, web building and reproductive capacity of *A. veniliae* females, under standard laboratory conditions. Spinosad was the most harmful insecticide due to high acute toxicity, even at lower concentrations than those registered for its field use and for its sublethal effects also. Cypermethrin caused several sublethal effects although its acute toxicity on spider was lower than other insecticides. It reduced prey consumption, affected web building, caused abnormalities in eggs sacs and decreased drastically the fecundity and fertility at sublethal concentrations. Endosulfan did not reduce prey consumption but it affected web building, caused abnormalities in eggs sacs and egg masses, and decreased the fecundity and fertility. Spinosad was also the compound with the most drastic effect on web building, it did not reduce prey consumption and fecundity, but fertility was reduced and abnormalities in egg sacs and egg masses were observed. The use of these insecticides in IPM programs according to their potential toxicity on spider communities is discussed.

Keywords Spiders · Neurotoxic insecticides · Cypermethrin · Endosulfan · Spinosad

Introduction

In Latin America soybean production has increased significantly over the past 10 years, and is likely to continue increasing in the future (Bindraban et al. 2009). In Argentina, the area sown with this crop is about 19 million ha (61 % of the total cultivated area) with an annual production of 49 million tons (MAGyP 2012). The fast implementation of new technology associated with the introduction of transgenic seeds resistant to glyphosate and no-till agriculture turned Argentina into the third world producer, after the United States and Brazil (MAGyP 2012). As a result, large amounts of pesticides are routinely applied, among which the broad spectrum insecticides are the most commonly used to reduce pest populations. The pyrethroid cypermethrin represents more than half of the total insecticide utilization followed by the organophosphate chlorpyrifos and the organochlorinated endosulfan (Jergentz et al. 2005; CASAFE 2011). Endosulfan and cypermethrin have more than 74 and 119 commercial trademarks, respectively (CASAFE 2011).

Endosulfan is an organochlorinated neurotoxic insecticide which blocks chloride channels in postsynaptic cells, causing excitability, tremors, convulsions, and ultimately the death of the organism (Stenersen 2004). Today it has been banned or severely restricted in more than 30

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countries, but it continues to be widely used in some developing countries (EJF 2002; Wang et al. 2007). In Argentina, through the resolution 511/2001 of SENASA (National Direction of Agrochemicals, Veterinary Products and Food), its importation has been banned since July 2012 and its elaboration and use will banned from the same month of 2013 (SENASA 2013).

Cypermethrin is an a-cyano pyrethroid whose primary target site in the vertebrate nervous system is the sodium channel of the nerve membrane (Stenersen 2004).

Spinosad belongs to biorational insecticides and it is derived from fermentation by the soil-dwelling actinomycete *Saccharopolyspora spinosa* Mertz and Yao. This insecticide is a mixture of the two most active spinosyns (A, 85 % and D, 15 %), and when ingested or topically applied to an insect acts on the nicotinic acetylcholine and GABA receptors (Watson 2001). This product is considered to be safer to non-target organisms than conventional ones (Williams et al. 2003). In Argentina, it has been registered since 2000 decade although its use is incipient yet. Taking into account its selectivity, spinosad could be an option for replacing broad spectrum insecticides in soybean crops, allowing the promotion and integration of other alternatives such as biological control by natural enemies, in IPM programs (Kogan and Jepson 2007).

The soybean crop is inhabited by several herbivore arthropods some of which are pests, and also by natural enemies of herbivores, providing a biological control service of pest populations. Among these organisms, the predator *Alpaida veniliae* Keyserling 1895 (Araneae, Araneidae) is one of the most abundant species of the spider orb web weaving guild (Minervino 1996; Benamú 2010). Previous studies indicated that in addition to its high abundance, its biological and ecological attributes point out the importance of conservation this predator as a natural enemy of many soybean crop pests (Benamú et al. 2011).

The negative impact of pesticides on natural enemies of pests has been well documented (Stark and Banks 2003; Schneider et al. 2003, 2004, 2008, 2009; Desneux et al. 2007; Stark et al. 2007). In particular, detrimental effects of pesticides on spider populations have been registered by several authors (Pekár 1998; Bel'skaya and Esyunin 2003; Frampton and Van den Brink 2007; Benamú et al. 2010).

The objective of this study was to determine if neurotoxic insecticides commonly used in soybean crops and a new active ingredient registered in Argentina (spinosad) adversely affected survival, prey consumption, mating behaviour, web building and reproductive capacity of females of *A. veniliae*, under standard laboratory conditions. The purpose was to assess the implications of applying these insecticides in IPM programs that include the use of natural enemies.

Materials and methods

Spiders

Adults of *A. veniliae* were collected from transgenic soybean crops located at Chivilcoy (35º10'S, 60º60'W) (Buenos Aires, Argentina), from January to March 2006. In the laboratory, mating pairs were held in glass jars (500 ml) to obtain egg sacs, and once juveniles emerged, they were transferred to separate glass vials to rear a laboratory colony for conducting the experiments. Juveniles and adults were fed "ad libitum" with adults of *Drosophila melanogaster* Meigen (Diptera, Drosophilidae) and *Musca domestica* L. (Diptera, Muscidae). Laboratory conditions were 25 ± 2 °C temperature, 75 ± 5 % RH, and a photoperiod of 16:8 (L:D) h.

Insecticides

Commercial products were chosen for the toxicity experiments as follow: Glextrin25® (cypermethrin 25 %, Gleba S.A., Argentina), Endosulfan 35Glex® (endosulfan 35 %, Gleba S.A., Argentina), and Tracer® (spinosad 48 %, Dow Agrosciences, Argentina).

Between four and five concentrations from 1 to 150 mg of active ingredient (a.i.)/L were prepared for each insecticide to evaluate the short-term effects of the insecticides on survival of *A. veniliae*. The full field recommended concentration for each insecticide according to CASAFE (2011) was included in the evaluation (based on the full field recommended rate and a water amount of 1000 L/ha), corresponding 25 mg a.i./L (10 cm^3/hL of commercial product) for cypermethrin, 105 mg a.i./L for endosulfan (30 cm^3/hL of commercial product) and 120 mg a.i./L (25 cm^3/hL of commercial product) for spinosad. Further, sublethal concentrations were chosen to evaluate long-term effects according to the following criteria: (1) mortality was similar to control, and (2) there were no tremors, paralysis or incoordination of movements in survivors (checked under binocular stereoscope during more than 10 min). Accordingly, the concentrations selected were: cypermethrin at 18.75 and 6.25 mg a.i./L (75 and 25 % of full field recommended concentration, respectively); endosulfan at 25 mg a.i./L (23.8 % of the full field recommended concentration,) and spinosad at 3 mg a.i./L (2.5 % of the full field recommended concentration,).

The insecticide solutions were prepared using Analytical Grade fresh acetone as solvent. Controls were treated with acetone alone after recording no mortality in the organisms due to solvent action (the solvent was innocuous).

Short-term effects

Acute mortality: concentration–response assay

Females of *A. veniliae* were treated with fresh insecticide solutions at concentrations ranging from 1 to 150 mg a.i./L, by ingestion of treated prey. The prey (*M. domestica* adults) was treated by dipping in the different insecticides and concentrations for 20 s according to Schneider et al. (2009) and dried under fume cupboard hood for 30 min. Each treatment (insecticide and concentration) had 7–10 females fed during 24 h with treated or untreated prey in the case of control. Treatments were replicated three times. The spiders that not preyed (0 % of consumption) were discarded from the assay. Remains of prey of each treatment were removed after the exposure time and new untreated preys were provided to the females. Cumulative mortality was recorded at 48 h after treatment.

Long-term effects

The exposure way was by ingestion of treated prey following the methodology explained above for short-term effects. After starvation of 1 week, 15 days-old females of *A. veniliae* were placed individually in 6 cm diameter Petri® dishes and fed daily, during 4 days (chronic toxicity), with preys treated with sublethal concentrations of each insecticide corresponding to 25 mg a.i./L for endosulfan, 18.75 and 6.25 mg a.i./L for cypermethrin, and 3 mg a.i./L for spinosad. Controls were fed prey treated with acetone alone (analytical grade). The end-points evaluated in the survivors (≈96 %) were: prey consumption, web building, mating behaviour, reproductive capacity of females (fecundity and fertility) and development time of progeny.

Prey consumption

To determine percentage of prey consumption, residues of prey were daily removed from the Petri dishes and new treated ones were offered. Residues were observed under binocular microscope to verify percentage of prey consumed. For doing so, the prey was arbitrarily divided in ten parts of equal length using an arbitrary scale of 0–95 %, since the remaining 5 % corresponds to parts of the exoskeleton and wings that never are consumed (Benamú et al. 2010). Each experimental unit consisted of one female, and each treatment was replicated 70–75 times.

Web building

After the fourth day, surviving females were randomly selected from each treatment, placed individually in a wooden frame (15 × 10 × 5 cm) surrounded by glass walls (experimental unit) for viewing the process of web building, and fed untreated prey. To register the process of web building, during 20 consecutive days, digital photos were taken. Webs of control females were considered normal and the number of radiuses and spires compared with those of different treatments. Each treatment was replicated 60 times.

Mating behaviour

Once the females described above, started to weave the web, an untreated and virgin male was placed on the bottom of each experimental unit, so that they can detect the female web and perform the courtship and mating. Mating behaviour (response of female to male courtship and mating) was filmed and recorded as qualitative endpoint. Each treatment was replicated 60 times.

Fecundity, fertility and developmental time of progeny (F_2)

After 15 days of mating, 10 females from each treatment were randomly selected and dissected for measuring the diameter of the oocytes from the final portion of the ovaries, under a stereoscopic microscope with a micrometric eyepiece. The remaining females of each treatment were tracked until the third oviposition to calculate fecundity and fertility. Accumulated fecundity and accumulated fertility were calculated as the mean of the total number of eggs per female and the mean of the total number of offspring per female for the first three ovipositions, respectively. In spite that all females were mated not all of them were able to laid eggs, for this reason the number of replicates per treatment varied from 19 to 30 females. Silk egg sacs and egg masses from control females were considered normal for comparison with those of the insecticide treatments. After dispersion of instar IV (spiderlings) from the egg sacs, these were opened for counting the number of eggs per egg sac, the number of hatched eggs, and the number of abnormal (malformed, dried or decomposed) eggs.

The developmental time of progeny was estimated as other long-term endpoint and calculated as the number of days from oviposition to progeny dispersion (instar IV) per each treatment.

Statistical analysis

The mortality was analysed as a binary response variable. For each pesticide, the probit of mortality was regressed against the base 10 logarithm of concentration (mg a.i/L). When probit regression model did not fit to the data, a logit regression model was used. Resulting equations of the lines were used to predict the lethal concentration LC_{50} (which corresponds to 50 % of the population killed). These models were fitted using the method of maximum likelihood (Statistica 7.0 StatSoft 2004, Chi 1997).

Differences in the percentage of prey consumed among treatments, previous arcsine transformations, were compared by one-way repeated measures analysis of variance (ANOVA). Previously, Mauchley's Sphericity test was used to test the assumption of circularity. When this assumption could not be met the adjustment of the F-statistic degrees of freedom was performed by the Green Greenhouse-Geisser method (Scheiner and Gurevitch 2001). To assess the effects of treatments on the web number of radiuses and spires, oocite diameter, developmental time of progeny, and accumulated fecundity and fertility, a one-way ANOVA was used. When necessary, data were transformed to meet requirements of the analyses on normality and homoscedasticity of variances. Means were separated using Fisher least significant difference test. When ANOVA assumptions were violated a Kruskal–Wallis test was used, and medians were separated using the Box and Wisker plot method. Normal and abnormal webs, egg sacs and egg masses were analyzed by two-way contingency tables using Chi square analysis (Zar 1996). A 0.05 significance level was chosen for all statistical analysis.

Results

Short-term effects

Acute mortality: concentration–response assay

Based on the concentration-mortality relationship for *A. veniliae* exposed by ingestion to each insecticide the LC_{50} was estimated, resulting in: 51.33, 34.60 and 30.29 mg a.i./L for endosulfan, spinosad and cypermethrin, respectively. Accordingly, spinosad was the most toxic insecticide for this spider, causing 10–93.3 % mortality from 10 to 120 mg a.i./L and its LC_{50} corresponded to 29 % of the full field recommended concentration (Fig. 1a).

Endosulfan caused 23.3–87 % mortality of spiders from 25 to 150 mg a.i./L and its LC_{50} corresponded to 49 % of the full field recommended concentration (Fig. 1b).

However, cypermethrin showed a moderate toxicity to *A. veniliae* compared to spinosad and endosulfan, exhibiting mortalities from 10 to 63 % from 1 to 50 mg a.i./L

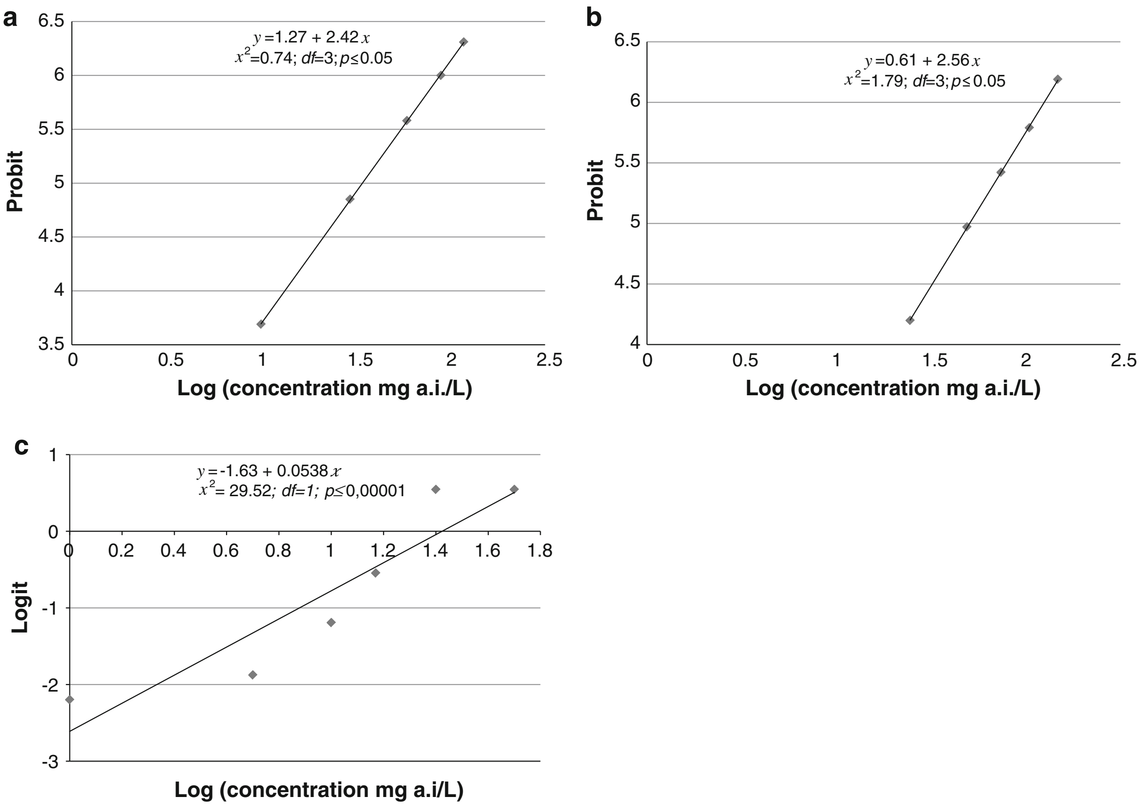

Fig. 1 Short-term effects of three neurotoxic insecticides on survival of *Alpaida veniliae* females at 48 h post-treatment: **a** spinosad, **b** endosulfan, **c** cypermethrin. Linear equations, and Chi square Tests are shown ($p \leq 0.05$)

Table 1 Results of repeated-measures ANOVA for the comparison of prey consumption by *Alpaida veniliae* among treatments

Source of variation	df effect	df error	*F*	*p* value	df G–G effect[a]	df G–G error[a]	Adj. *p* value
Treatment	4	295	327	0.0001			
Time	3	885	1.50	0.22	2.85	840.36	0.22
Interaction	12	885	3.20	0.0001	11.40	840.36	0.0002

[a] df adjusted by Greenhouse–Geisser test; ε = 0.95

(Fig. 1c), where the maximum mortality observed corresponded to the full field recommended concentration and it remained constant at twice of it. Moreover, its LC_{50} correspond to 122 % of the full field recommended concentration.

In relation to the celerity action of the three insecticides, it was observed that endosulfan was faster (individuals died a few hours after the treatment) than spinosad (individuals began to die after 24 h post-treatment) and cypermethrin (individual began to die after 38 h post-treatment) at their full field concentrations. Moreover, females exposed to endosulfan and spinosad showed tremors, paralysis and uncoordinated movements even at 10 mg a.i./L (9.52 and 8.33 % of full field recommended concentration, respectively).

Long-term effects

Prey consumption

Prey consumption differed between treatments but not over time, and there was a significant interaction among factors (Table 1). The mean percentages of prey consumption at 24, 48, 72 and 96 h in the different treatments are shown in Fig. 2. Rates of prey consumption in endosulfan and spinosad treatments did not differ from control and reached values higher than 90 %.

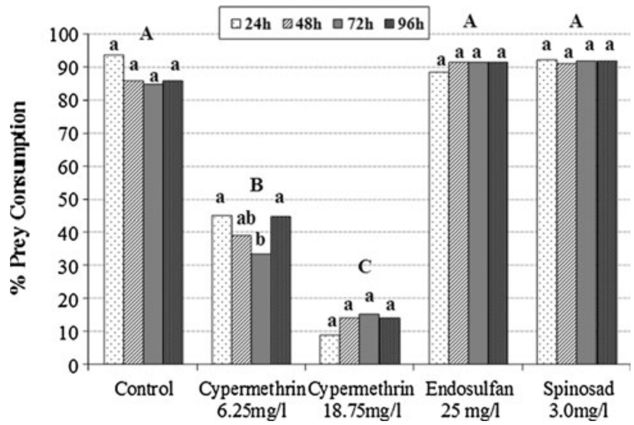

Fig. 2 Percentages of prey consumption of *Alpaida veniliae* over time in each treatment. Capital letters indicate differences between treatments and lowercase within each treatment

On the contrary, in both cypermethrin treatments, prey consumption was much lower than in any other treatments (partial consumption). The lowest consumption was at 18.75 mg a.i./L, where females consumed less than 20 % of the treated prey. In the cypermethrin 6.25 mg a.i./L (25 % of field concentration) treatment there was an interaction between the percentage of prey consumed and time, exhibiting a pattern to decrease from the first to the third day and then a new increase at the fourth day.

Web building

Insecticides affected the number of females that were able to build the web ($X^2 = 97.27$, $p < 0.001$). All females from control treatment built a normal web at the following day of being introduced in the wooden frame. Cypermethrin was the insecticide that had the highest negative impact on web building. At 6.25 and 18.75 mg a.i./L, 44 and 7 % of the treated females, respectively, were able to build a web. Moreover, females began to weave the web on day 20 post-treatment, while in the other treatments they started on day 9. The effects of spinosad and endosulfan were similar, 80 and 74 % of females built the web, respectively. In general, webs made by treated females were defective and had significantly lower number of radiuses and spires than control, lacking the capture spires in some cases (Fig. 3). Webs from females treated with spinosad had the lowest number of radiuses and spires (Table 2).

Mating behaviour

No sublethal effects during mating were observed. Courtship was always successful and even when webs were imperfect, not having the typical orbicular shape, males courted females and they responded to the courtship. Mating was carried out in the "mating thread" placed by the male close to the female web, and all the females were mated.

Fecundity, fertility and developmental time of progeny (F_2)

All dissected insecticide treated females exhibited fatty granules surrounding the oocytes but they were absent in

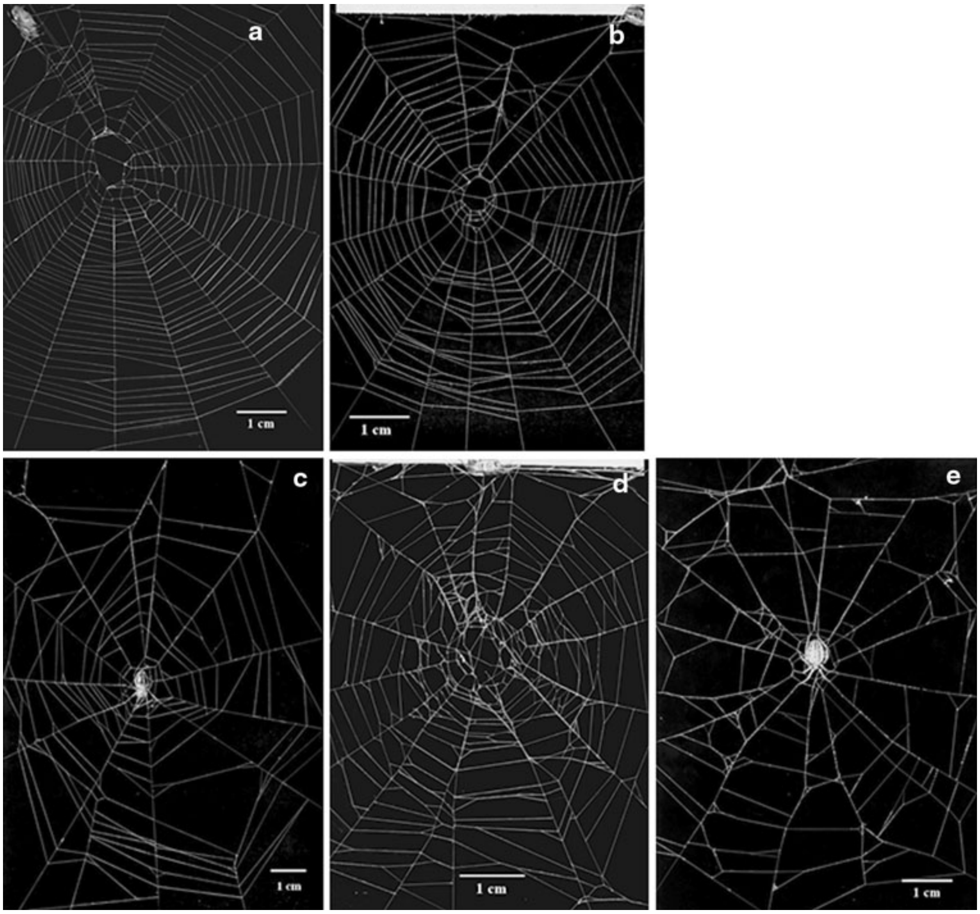

Fig. 3 Orbicular webs of *Alpaida veniliae* exposed to three neurotoxic insecticides. **a** Normal web (control). **b** cypermethrin 8.25 mg a.i./L (25 % of full field recommended concentration). **c** cypermetrhin18.75 mg a.i./L (75 % of full field recommended concentration). **d** endosulfan 25 mg a.i./L (23.8 % of full field recommended concentration). **e** spinosad (2.5 % of full field recommended concentration)

Table 2 Number of radius (mean ± SE) and spires of the *Alpaida ve*niliae webs, built by females treated with different insecticides

Orb web	Treatments					Statistical test
	Control	Cypermethrin 6.25 mg/L	Cypermethrin1 8.75 mg/L	Endosulfan 25 mg/L	Spinosad 3 mg/L	
Radius	19.14 ± 0.41a	18.1 ± 0.50a	13.02 ± 0.32b	15.81 ± 0.39c	12.45 ± 0.41b	H = 105.86, $p < 0.001$
Spires	29.72 ± 0.76a	25.35 ± 0.92b	20.46 ± 0.59c	22.91 ± 0.73b	17.59 ± 0.76d	H = 781.88, $p < 0.001$

Means within each line followed by a different letter were significantly different ($p < 0.001$; Kruskall–Wallis test)

the control. The mean oocyte diameter from the final part of ovaries differed significantly between treatments ($H = 81.26$, $p < 0.001$). The oocyte diameter (mean ± SE) was 0.23 ± 0.02, 0.20 ± 0.02, 0.18 ± 0.03, 0.16 ± 0.02 for the control, cypermethrin (18.75 mg a.i./L), endosulfan and spinosad, respectively.

Abnormalities in egg sacs and egg masses were observed in all treatments, except in control. There was a significant number of abnormal egg sacs and egg masses in insecticide treatments (egg sac: $X^2 = 87.41$, $p < 0.001$; egg mass: $X^2 = 31$, $p < 0.001$). The silk threads of control egg sacs were more dense and tight than those of the different insecticide treatments. The egg masses were affected adversely by the insecticides, but to a lesser extent than the egg sacs, and even within each treatment, except spinosad, the number of normal egg masses was higher than abnormal ones (Fig. 4).

A significant toxic insecticide effect was observed in accumulated fecundity for the first three ovipositions ($F = 5.93$; $df = 4$, 132; $p = 0.0002$) and fertility ($F = 10.52$; $df = 4$, 132; $p \leq 0.001$) (Fig. 5). It was reduced drastically by cypermethrin at 18.75 mg a.i./L (75 % of the full field recommended concentration) and endosulfan, causing a reduction of 45 and 29 % of reproductive capacity, respectively. Spinosad and cypermethrin at 6.25 seemed to be less toxic and did not cause any

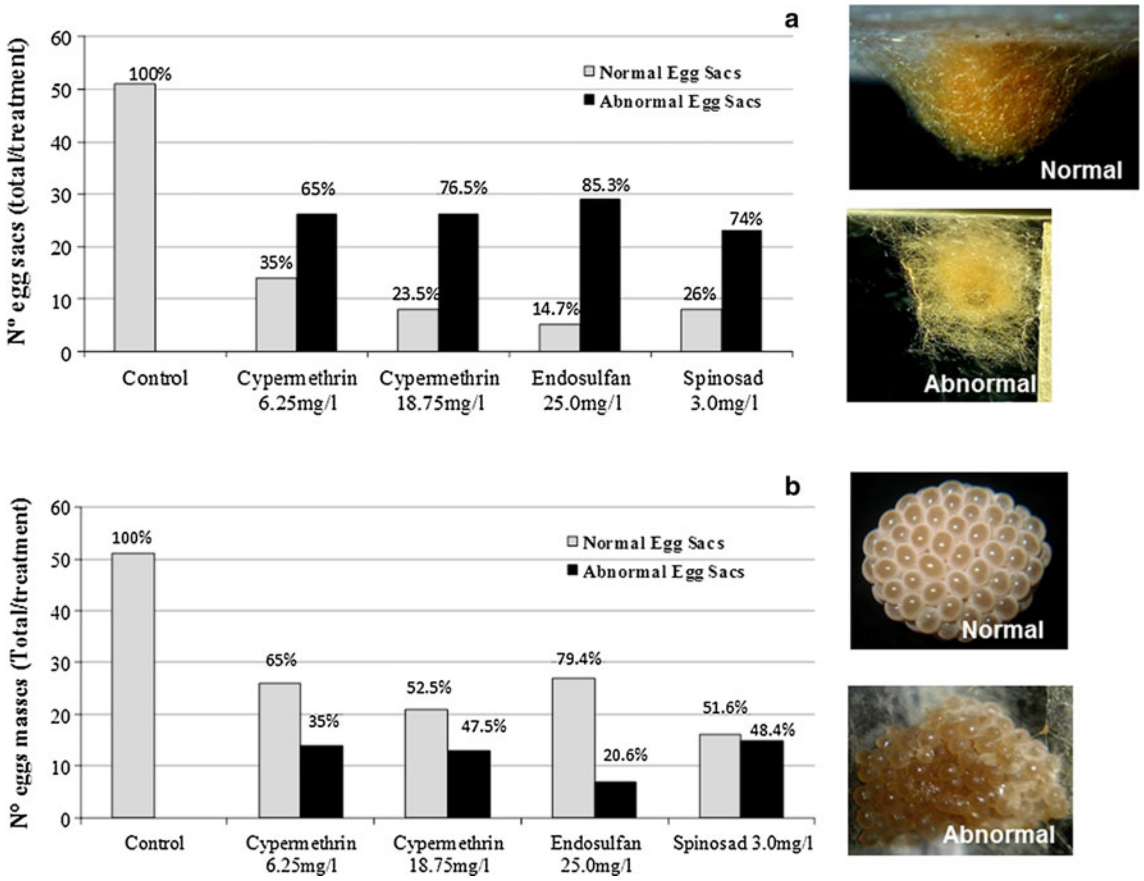

Fig. 4 Abnormalities in silk egg sacs and egg masses of *Alpaida veniliae* females treated by ingestion with three neurotoxic insecticides. **a** Effects on silk eggs sacs. **b** Effects on egg masses. The percentage on each bar denotes the proportion of abnormalities versus normalities in the egg sacs and egg masses respectively. The pictures show the abnormalities in the egg sacs and egg masses, respectively

detrimental effects on fecundity, recording values similar to control. In contrast, fertility was reduced to a greater extent by all insecticides (Fig. 5). Cypermethrin at 18.75 mg a.i./L produced the most toxic effect decreasing the fertility 65 % in relation to control, followed by endosulfan (47 %), spinosad (39 %) and cypermethrin at 6.25 mg a.i./L (24 %).

Developmental time of progeny, from oviposition to the third molt, differed between treatments ($H = 42.21$, $p < 0.001$). The longest developmental time (mean ± SE) was exhibited by the progeny of females treated with endosulfan (11.97 ± 0.25 days), while those treated with spinosad the shortest (8.64 ± 0.39 days). Cypermethrin (10.53 ± 0.37 days) was similar to control (10.45 ± 0.27 days).

Discussion

This study demonstrated deleterious short and long term effects on the spider *A. veniliae* of conventional neurotoxic insecticides and of a new active ingredient used in soybean crops in Argentina. The high direct or acute toxicity of neurotoxic insecticides to arthropods has been deeply documented (Stenersen 2004), and sublethal effects on insect behaviour have also been reviewed by Haynes (1988). Lethal and sublethal concentrations of the insecticides used in this study had differential effects on *A. veniliae*, according to the insecticide used.

Concerning to lethal effects, spinosad and endosulfan caused high mortality in *A. veniliae.* The moderate acute toxicity of cypermethrin at maximum field recommended concentration in relation to the other two insecticides probably was due to the lower prey consumption observed in this treatment.

Short-term effects of endosulfan, cypermethrin and spinosad are consistent with those reported by several authors in predators and parasitoids of agroecosystems (Filgus et al. 1999; Haseeb et al. 2004; Galvan et al. 2005; Ahmed and Maqsood 2006; Desneux et al. 2004, 2006; Agrawal and Brar 2006; Shaw et al. 2006; Mahdian et al. 2007; Ventura Garcia et al. 2009; Dağh and Bahşi 2009; Jalali et al. 2009; Rimoldi et al. 2008, 2012).

Acute toxicity of spinosad was high even at lower concentrations than that recommended for field application.

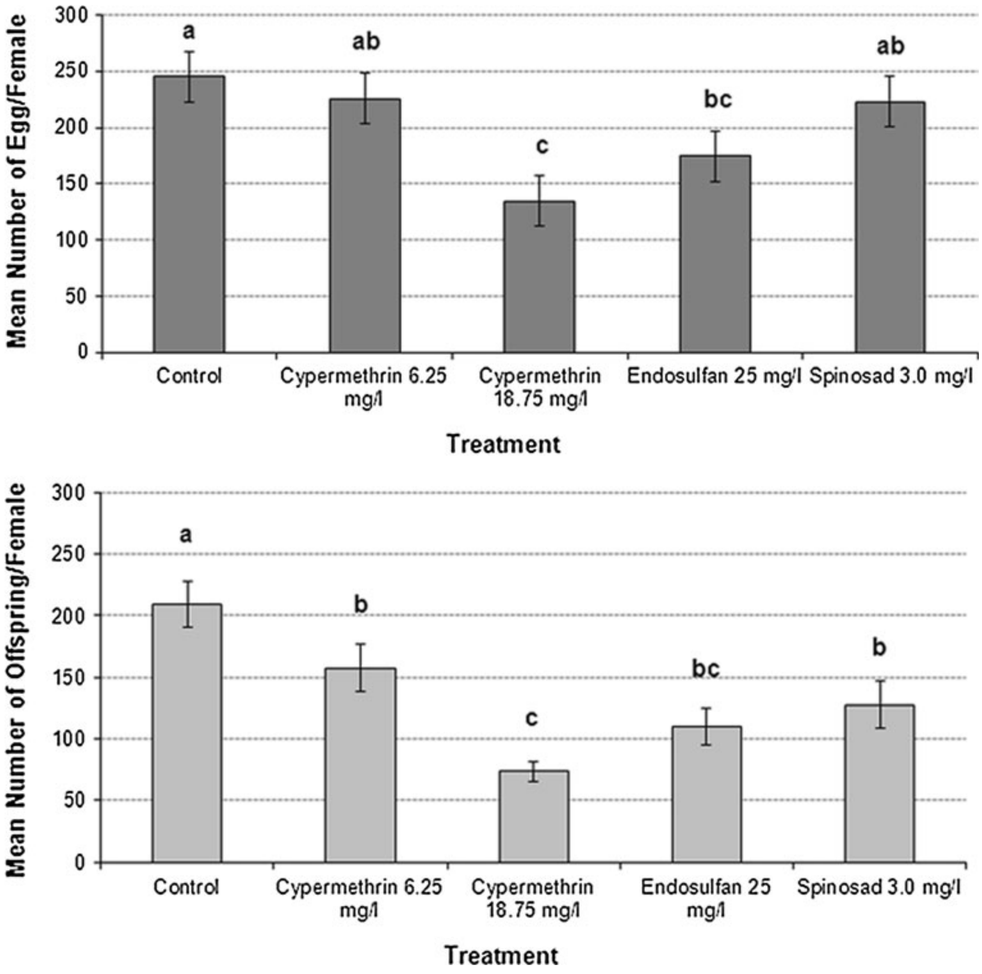

Fig. 5 Long-term effect of insecticides on fecundity and fertility of *Alpaida veniliae* females exposed by ingestion through chronic toxicity experiments. Data are mean ± SE. Bars with the same letter do not differ significantly ($p \leq 0.05$)

The short term effects of this insecticide registered through survival rates differ widely in the literature, having been classified as harmless or harmful, according to the organism considered. In general, low mortality rates were observed in chrysopid, pentatomid, mirid, anthocorid and coccinellid predators, whereas moderate and high toxicity was cited for hymenopteran parasitoids (Williams et al. 2003; Schneider et al. 2003, 2004; Haseeb et al. 2004; Galvan et al. 2005; Kim et al. 2006; Ahmed and Maqsood 2006; Mahdian et al. 2007; Jalali et al. 2009; Dağh and Bahşi 2009; Rimoldi et al., 2008, 2012). Hence, the susceptibility of *A. veniliae* to this insecticide could be comparable to that of hymenopteran parasitoids.

In relation to chronic toxicity at sublethal concentrations of each insecticide, all of them caused detrimental effects on adult females of *A. veniliae*, although in different ways and extent. Our results agree with those of Haynes (1988) who reviewed the sublethal effects of neurotoxic insecticides on insect behaviour.

Cypermethrin was the only insecticide that significantly decreased consumption rate, and the effect was more conspicuous at higher concentration, where only 10 % of the prey was consumed. The mechanisms responsible for reducing consumption or rejection of contaminated prey on spiders have been little studied. Wiles and Jepson (1994) showed that larvae and adults of *Coccinella septempunctata* L. ate less dimethoate treated aphids, suggesting a repellent effect. Likewise, repellent effects of pyrethroids such as deltamethrin on the foraging behaviour of the parasitoid *Aphidius rhopalosiphi* (DeStefani-Perez) have been reported (Longley and Jepson 1996).

All insecticides adversely affected web building, not only the number of radiuses and spires, but also increasing the time a female spent for building it. As in prey consumption, cypermethrin had the highest effect. Under field conditions, this effect would decrease prey capture, reducing their consumption rate. Samu et al. (1992) and Samu and Vollrath (1992) recorded a similar effect of cypermethrin in *Araneus diadematus* (Clerck), in the field. Dinter and Phoehling (1995) also reported a negative impact of pyrethroids in other web building spider.

The intake of neurotoxic products influences the instinctive web-building and predatory behaviour of spiders, because they damage the central nervous system (Haynes 1988; Foelix 1996; Hesselberg and Vollrath

2004). Similar biological process in arthropods involving silk glands have been reported by Schneider et al. (2004). These authors found that some neurotoxic insecticides, such as spinosad, interfere in the cocoon building by the last larval instar of the parasitoid *Hyposoter didymator* (Thunberg), mainly due to the damage of the nervous system.

The insecticides negatively affected both egg sacs and egg masses, although it seems than the last ones were affected in a lesser degree. The presence of abnormal oocytes and fatty granules around them in treated females could suggest anomalous hormonal process (Nation 2002a) induced by insecticides.

Cypermethrin at the highest concentration, followed by endosulfan, had the greatest effect in reducing fecundity of *A. veniliae*. However, fertility was reduced by all insecticides to the same extent. Our findings are consistent with those observed in other animals, including beneficial arthropods, snails and amphibians. Tabanor and Hyslop (2005) observed changes in the reproductive capacity of the snails *Melanoides tuberculata* (Muller) and *Thiara granifera* (Lamarck) by exposure at sublethal concentrations of endosulfan. Likewise, reduction of reproductive capacity was found in the parasitoids *Eretmocerus mundus* (Mercet), *E. tejanus* (Rose and Zolnerowich) *H. didymator*, and *Trichogramma cordubensis* (Berliner), when treated with endosulfan, spinosad and pyrethroids through different exposure ways (Jones et al. 1998; Schneider et al. 2004; Ventura Garcia et al. 2009).

There is not enough information on the effects of insecticides on post-embryonic development of spiders, so it is difficult to attribute the possible reasons for the speed of progeny development produced by endosulfan and spinosad. However, it could be related to hormonal and metabolic processes affected by neurotoxic insecticides, taking into account the role of ecdysone and juvenile hormones in the metamorphosis of arthropods (Nation 2002b).

Our laboratory experiments indicated that the neurotoxic insecticides evaluated in this study negatively affected attributes of *A. veniliae* directly related to its fitness and predatory capacity. As a result, its potential as a natural control factor will be limited, and will impact negatively in the implementation of IPM programs aiming to include the predation of this spider as a natural mortality factor of soybean pests. Further, although spinosad belongs to the biorational insecticides and it is considered to be safer to non-target organisms than conventional ones (Williams et al. 2003), our laboratory study indicated lethal and sublethal effects of this insecticide on *A. veniliae,* even at lower concentrations. Therefore, the use of spinosad into soybean IPM programs should be considered with caution and more bioassays should be conducted to complete its toxicological profile under field conditions and through other exposure ways.

This research provides new insights into the effects of neurotoxic insecticides on survival, consumption, web building, mating behaviour and reproduction of the spider *A. veniliae* and highlights the importance of long term effect assessments in ecotoxicological studies. Moreover, it is the first report about side effects of spinosad on spiders.

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Conflicts of interest The authors declare that they have no conflict of interest.

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