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Effect of selective and non-selective insecticides on survival and feeding behavior of the spiders *Hogna cf. bivittata* and *Lycosa poliostoma* (Araneae: Lycosidae)

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Abstract. An important aspect of implementing Integrated Pest Management programs is evaluating the compatibility of insecticides with the biological attributes of the natural enemies used for pest control. In this study, we evaluated the effects of selective and non-selective insecticides on the survival and feeding behavior of *Hogna cf. bivittata* and *Lycosa poliostoma* (C. L. Koch, 1847), present in soybean crops. We used residual exposure to contaminate adult females of both spider species with the broad-spectrum insecticide thiamethoxam+lambda-cyhalothrin and the selective insecticide methoxyfenozide using concentrations of 20%, 10% and 5% of the maximum recommended field concentration. The survival rate after 120 hours post treatment and the prey acceptance rate in survivors were assessed as ecotoxicological parameters. Methoxyfenozide did not show any disruption to survival of either spider at any of the concentrations evaluated. We did not find significant differences related to mortality between the evaluated concentrations of methoxyfenozide and the control groups for both species. Nevertheless, all the concentrations of broad-spectrum insecticides we used caused mortality rates significantly higher when compared to the selective insecticide. The highest percentages with thiamethoxam+lambda-cyhalothrin were recorded for 20% and 10% concentration. Although we observed moderate mortality rates for the lowest concentrations, the exposed spiders showed a lower acceptance rate when compared to all the concentrations of methoxyfenozide and the control group; the two latter treatments were not significantly different. These results suggest that the selective insecticide can be used in different concentrations without affecting the survival or feeding behavior of either spider species.

Keywords: Wolf spider, soybean crops, pesticides, Uruguay

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Spiders are one of the most common groups of natural enemies found in agroecosystems, and yet they can also be beneficial organisms (Riechert & Lockley 1984; Sunderland & Samu 2000). Although spiders are true predators and have a relevant role as natural enemies in several crops (Nyffeler & Sunderland 2003), their role as biological control organisms against local pests has been poorly explored in South America (Benamú & Aguilar 2001; González et al. 2009; Benamú 2010; Avalos et al. 2013). Among cursorial spiders, wolf spiders (Lycosidae) are considered a dominant group in several crops (Armendano & González 2010; Rendon et al. 2015). Besides their abundance, it has been shown that wolf spiders can act as biocontrol agents by reducing population densities of pests and herbivory damages in crops caused by local pests (Nyffeler & Benz 1988; Suenaga & Hamamura 2015); this aspect has been recently explored in wolf spider species from Uruguay (García et al. 2021). In Uruguayan soybean crops, two species are commonly found: *Hogna cf. bivittata* and *Lycosa poliostoma* (C. L. Koch, 1847). *Hogna cf. bivittata* is a small species (mean body length: 10 mm) that is often found on soybean leaves and the lower part of the plant (Lacava 2014). *Lycosa poliostoma* is a nocturnal mid-sized species (mean body length: 21mm). Females and small juveniles live in the soil by digging or under rocks (Capocasale 2001), and males are mainly wandering individuals (Benamú 2010; Armendano & González 2011). *Lycosa poliostoma* has also been reported as a dominant species in some South American crops such as alfalfa and wheat (Armendano & González 2010, 2011).

Soybean production area has dramatically increased in Uruguay and has led to the importation of various products including fertilizers, herbicides and pesticides (Blum et al. 2008a,b). The excessive use of pesticides has caused a series of environmental problems that include the contamination of water bodies and several health issues reported both in Uruguay and in neighboring countries (Ronco et al. 2008; Etchegoyen et al. 2017). However, in Uruguay, ecological and health problems caused by the use of pesticides have been poorly explored (Céspedes-Payret et al. 2009).

One of the ways to assess the negative effects of insecticides is based on the evaluation of how these affect non-target organisms, including pollinators and natural enemies (Wolfenbarger et al. 2008). For example, life history parameters, such as feeding and reproduction, of native parasitoids and predators present in crops are negatively affected by insecticides, reducing its potential for biological control (Koss et al. 2005; Fernandes et al. 2010; Blibech et al. 2015; Tahir et al. 2019). Broad-spectrum insecticides have been shown to similarly affect both target and non-target organisms and, in some cases, beneficial organisms (Cloyd 2012). In contrast,

Table 1.—Active ingredients (mg/ml) present in the broad-spectrum and selective insecticide and their corresponding field concentration. "Percent concentration" = percentage of the maximum recommended field concentration.

	Broad-spectrum insecticide		Selective insecticide
Percent Concentration	Thiamethoxam mg/ml	Lambda-cyhalothrin mg/ml	Methoxyfenozide mg/ml
75%	1.06	0.79	0.18
50%	0.71	0.53	0.12
25%	0.35	0.27	0.06
20%	0.28	0.21	0.05
10%	0.14	0.11	0.02
5%	0.07	0.05	0.01

more specific or selective insecticides are considered to be an alternative and safe way to attack only specific pests and are often used along with natural enemies (Gentz et al. 2010). Nevertheless, in some cases the use of selective insecticides might be controversial, since they could be safe to natural enemies in the short term but have long-term impacts, for example by reducing feeding capability, as has been shown for philodromid spiders (Řezáč et al. 2010).

Side effects of several pesticides on web-building spiders have been documented (Pekár 2002, 2012; Benamú et al. 2007, 2010, 2013); for example, contact application of neonicotinoids affects the mechanical properties, nanostructures, and amino acid compositions of silk production (Benamú et al. 2017). Nevertheless, there is little information available about the side effects of pesticides used in soybean crops, on wandering spiders in South America. The aim of this study was to evaluate the lethal and sublethal effects of the selective insecticide Intrepid® (methoxyfenozide) and the non-selective insecticide Geonex® (thiamethoxam-lambda-cyhalothrin) on the survival and feeding behavior of *Hogna cf. bivittata* and *L. poliostoma*.

METHODS

Spiders.—We selected the species *L. poliostoma* and *Hogna cf. bivittata* as model species, based on their abundance in soybean crops (Lacava, unpublished data). *Lycosa poliostoma* is a mid-sized species that lives in cracks underground, while *Hogna cf. bivittata* is a small-sized species that lives within vegetation. Juveniles of both species were collected in natural grasslands from the department of San José to ensure that the individuals used would be free from any pesticide. The juvenile spiders were individually housed in Petri® dishes (10 × 1.5 cm) and maintained under laboratory conditions (temperature: 25 ± 5 °C, humidity: 75 ± 5% RH, and photoperiod: 12:12 h [L: D]) until they reached adulthood. Spiders were habituated to laboratory conditions, fed once per week with *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) and watered *ad libitum*. Individuals were maintained by this procedure until they reached adulthood. Fifteen days after the last molt, the *Hogna cf. bivittata* and *L. poliostoma* adults were used for bioassays.

Insecticide solutions and application.—As a broad-spectrum insecticide, we used Geonex® (thiamethoxam 141mg/ml + lambda-cyhalothrin 106 mg/ml, Tafirel S.A., Uruguay), which is composed of two complementary active ingredients: neonicotinoid thiamethoxan and the pyrethroid lambda-cyhalothrin. Thiamethoxam is a synthetic organic insecticide that is effective at controlling most sucking and chewing insect pests. It is sprayed in plants via foliar application and ingested by sucking/chewing insects while feeding. Lambda-cyhalothrin acts in a similar way as thiamethoxam, except it targets a wider variety of insects. As a selective insecticide, we used the accelerating compound Intrepid® SC (methoxyfenozide 24 mg/ml, Dow AgroSciences, Argentina). Broad-spectrum insecticide is primarily used for pest control in Uruguayan soybean crops (INIA 2018).

Effect of insecticides on the LC_{50} of spiders.—The median lethal concentration (LC_{50}) was calculated for *L. poliostoma* and *Hogna cf. bivittata* using different pesticide concentrations corresponding to 75%, 50%, 25%, 10% and 5% of the recommended field concentrations for Geonex and Intrepid insecticides (Table 1). For the control, we applied acetone only. For each insecticide concentration and control group, we used 40 spiders (20 males and 20 females) for a total of 240 spiders per species (*Hogna cf. bivittata* female mean mass ± standard error: 0.0970 g ± 0.0014, *Hogna cf. bivittata* male mean mass: 0.0752 g ± 0.0001 SE, *L. poliostoma* female mean mass ± standard error: 0.898 g ± 0.017, *L. poliostoma* male mean mass: 0.756 g ± 0.016).

For contamination, spiders were previously anesthetized by cold, and a droplet of 1.0 µl of the pesticide solution was applied dorsally to each spider's abdomen. For applications, we used acetone (analytical grade) for all products to ensure rapid evaporation and a homogeneous disposition of the pesticide solution on the cuticle of the organism (Benamú et al. 2010). In the control groups, only acetone was applied to spiders.

Mortality for the insecticides and spider species used was recorded 24 hours after application. To evaluate the effect of each concentration and spider species on mortality, we used a binomial generalized linear model (GLM) with a probit as the link function. Quasibinomial family was used when the model presented over/under dispersion.

Sublethal effect of insecticides.—Besides lethal effects, we also evaluated the sublethal effects of insecticides using dilutions of 5, 10 and 20% of the maximum recommended field concentrations for each insecticide (Table 1).

Insecticide exposure.—For evaluating the effect of pesticides, we randomly assigned 20 individuals (10 males, 10 females) to each insecticide, which included the three concentrations for each pesticide used, as well as to the control group. A total of 140 individuals per species was used (*Hogna cf. bivittata* female mean mass ± standard error: 0.0647 g ± 0.0005, *Hogna cf. bivittata* male mean mass: 0.0626 g ± 0.0006 SE, *L. poliostoma* female mean mass ± standard error: 0.913 g ± 0.006, *L. poliostoma* male mean mass: 0.873 g ± 0.005)

Before the spiders were exposed, a piece of filter paper (10 × 10 cm) was dipped for five seconds into a previously prepared solution of each insecticide concentration until it was completely wet. For each treatment, distilled water was used as solvent.

Table 2.—Median lethal concentration (LC_{50}) of broad-spectrum (Geonex: Thiamethoxam+lambda cyhalothrin) and selective (Intrepid: Methoxyfenozide) insecticides against *Lycosa poliostoma* and *Hogna cf. bivittata* based on field concentration used for each insecticide. Reported parameters were estimated using a binomial GLM.

Species	LC_{50} of field concentration (%) for broad-spectrum insecticide (mean ± standard error)	LC_{50} of field concentration (%) for selective insecticide (mean ± standard error)
Lycosa poliostoma	10.04 ± 1.06	66.22 ± 1.13
Hogna cf. bivittata	7.60 ± 1.08	66.72 ± 1.19

Afterwards, the paper was dried at room temperature (25 ± 3 °C) for 30 minutes and placed into a Petri® dish (1.5 cm X 10 cm diameter) with a small hole on the top for ventilation (2 × 2 cm), covered with a fine mesh. The contamination procedure for the control group was the same as that used for the insecticide-exposed spiders, but we used a filter paper wet with distilled water.

Spiders were placed in Petri® dishes (10 × 1.5 cm) with a piece of contaminated filter paper for 30 minutes, following the protocol suggested by Řezáč et al. (2010). After exposure, the spiders were returned to clean Petri® dishes, and we recorded survival and prey consumption afterwards.

Survival analyses.—Immediately after being exposed, spider survival was recorded every 24 hours for five days. The spiders were not fed during this period. For comparing the survival between treatments for each species, we made a survival regression analysis with an exponential distribution, with each insecticide treatment and the death time as explanatory variables, while the survival was used as the response variable. Post-hoc comparisons were made using the Tukey test from the lsmeans package (Lenth 2016).

The overall survival between sexes was also compared. To do this, we recorded the survival for each sex after the fifth day and compared the values using a binomial GLM, with the sex and treatment as explanatory variables and the survival as the response variable. This process was made for each species separately. Quasibinomial family was used when the model presented over/under dispersion (Pekár & Brabec 2016).

Effect of insecticides on prey acceptance.—Spiders that survived the previous treatments were used for this bioassay. During the sixth day after exposure, one *T. molitor* was offered to each spider, and we recorded if it was consumed or not 30 minutes after being supplied. In order to avoid bias because of the prey size, we used *T. molitor* equivalent to the spider prosoma length. For each treatment, the acceptance rate was estimated as the ratio between the accepted prey and the total number of offered prey. We only used individuals from treatments where five or more individuals survived or where the spiders were able to walk

Acceptance was compared between treatments for each species separately by using a binomial GLM with the treatment as the explanatory variable and the acceptance as the response variable. Quasibinomial family was used when the model presented over/under dispersion. All statistical comparisons were made with a significance level of 95%.

Voucher individuals used in experiments are preserved in the Laboratory of Ecotoxicology and Terrestrial Invertebrates from the Centro Universitario de Rivera (CUR)-Universidad de la República, Uruguay.

RESULTS

Determination of LC_{50}.—We found a significant effect of concentration on mortality for both insecticides (X^2_{28} = 69.945, $P<0.01$); however, broad-spectrum insecticide showed a significantly lower LC_{50} (X^2_{29} = 74.58, $P<0.01$) when compared to the selective insecticide (Table 2). The mortality in both species was similar against the evaluated insecticides ($X^2_{30} = 0.41$, $P = 0.673$); therefore, we used the same insecticide concentrations to study sublethal effects.

Survival analyses.—The survival showed a similar trend in both species, since it was significantly different between treatments both in *L. poliostoma* (X^2_5 = 80.81, $P<0.001$) and *Hogna cf. bivittata* ($X^2_5 = 77.02$, $P<0.001$). For *Hogna cf. bivittata*, we found similar survival rates between the control group and all of the concentrations evaluated for the selective insecticide, where a low mortality was recorded (Fig. 1A). In contrast, a significantly lower survival was recorded for the different concentrations of the broad-spectrum insecticide used, compared to the selective insecticide. Similarly, we found significant differences between the highest concentration of broad-spectrum insecticide compared with the other concentrations used for the same insecticide.

A different trend was observed for *L. poliostoma*. This species had a low mortality for all of the treatments of the selective insecticide and the lowest concentration of the broad-spectrum insecticide, no mortality was recorded in the control groups. Nevertheless, mortality increased significantly for the mid and highest concentrations of the broad-spectrum insecticide (Fig. 1B).

When comparing mortality rates, we found a significant interaction between sex and treatment in *H. cf. bivittata* (X^2_5 = 15.22, $P < 0.001$); overall males had a significantly higher mortality ($X^2_1 = 13.07$, $P < 0.001$), and it was especially high in treatments that included the broad-spectrum insecticide (Fig. 2A). For *L. poliostoma*, we did not find a significant interaction between sex and treatment ($X^2_5 = 5.45$, $P = 0.25$); males and females survived in similar proportions ($X^2_{|} = 2.68$, $P = 0.07$) in all treatments (Fig. 2B). Nevertheless, in *L. poliostoma* we found significant differences regarding mortality across treatments; it was higher in broad-spectrum insecticide when compared to the control and selective insecticide ($X^2_5 = 66.42$, $P < 0.001$).

Prey acceptance.—For this experiment, besides discarding treatments with fewer than five surviving individuals, we excluded *L. poliostoma* individuals exposed to the 10% field concentration of the broad-spectrum insecticide, since these were unable to walk. Prey acceptance for the surviving individuals (Table 3) showed a similar trend in both species (Figs. 3A, B). While none of the concentrations used for the selective insecticides affected the acceptance, the prey accep-

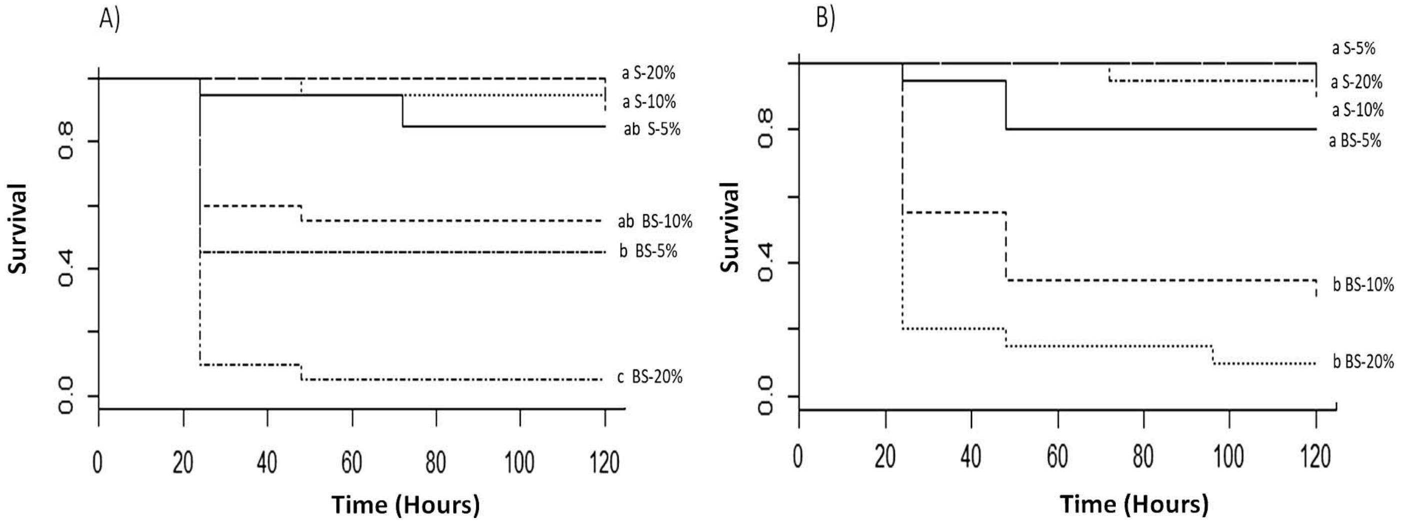

Figure 1.—Survival regression analysis for (A) *Hogna cf. bivittata* and (B) *Lycosa poliostoma* after exposure to insecticides residues (S: selective insecticide, BS: broad-spectrum insecticide). Different letters represent significant differences between treatments ($P<0.05$).

tance for spiders exposed to the broad-spectrum insecticide was significantly lower in *Hogna cf. bivittata* ($X^2_5 = 14.50$, $P < 0.05$) and *L. poliostoma* ($X^2_4 = 20.09$, $P < 0.001$) when compared to the control group.

DISCUSSION

Our results show that the broad-spectrum insecticide affected the survival and feeding aspects in both wolf spider species, while the selective insecticide seemed to be innocuous. In the particular case of the evaluated insecticides, it has been shown that thiamethoxam and the lambda-cyhalothrin have negative effects on natural enemies (Benamú et al. 2017). While thiamethoxam affects the survival and reproduction of a wide variety of predators and parasitoids, including spiders (Prabhaker et al. 2011; Yao et al. 2015; Řezáč et al. 2019), lambda-cyhalothrin has been shown to be slightly harmful for some predators like bugs (Cloyd 2012). Nevertheless, this insecticide reduced the feeding efficiency on acarophagous mites (Cloyd 2012).

In the particular case of spiders, Rodrigues et al. (2013) showed that the usage of lambda-cyhalothrin on rice

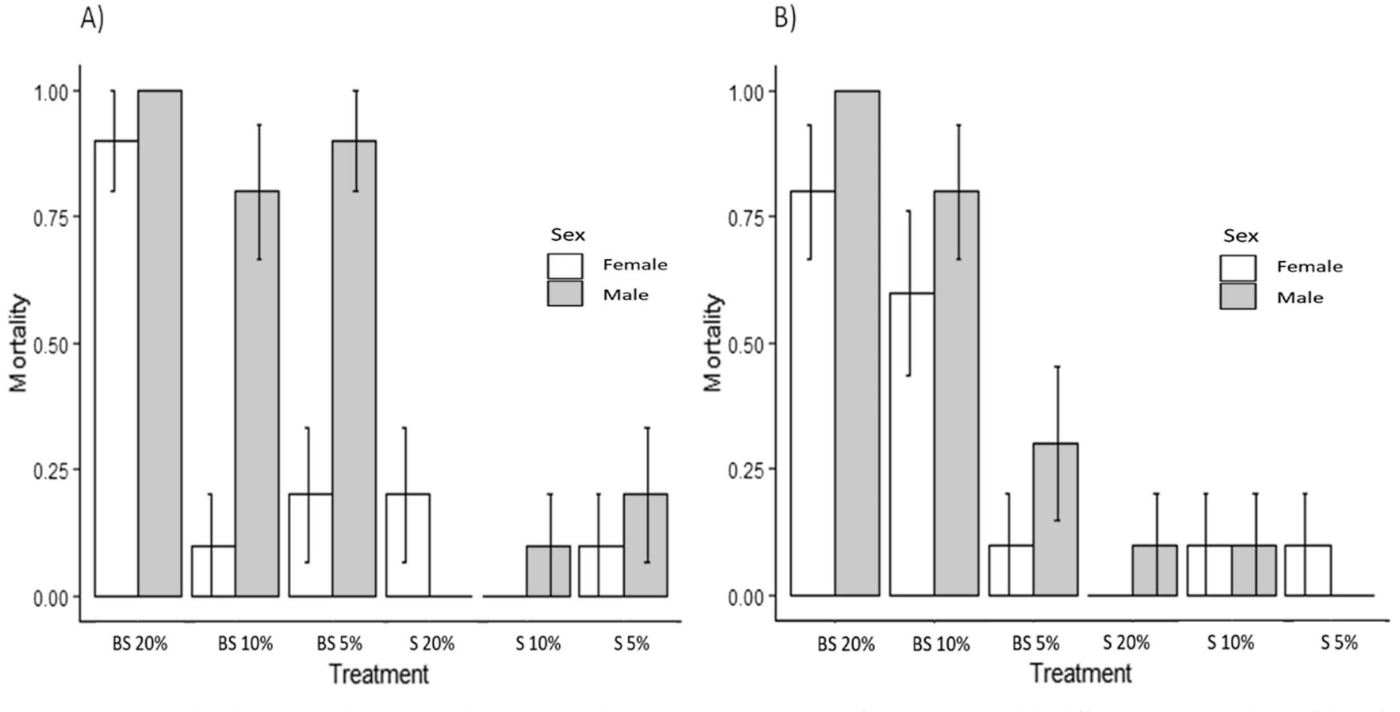

Figure 2.—Survival by sex in (A) *Hogna cf. bivittata* and (B) *Lycosa poliostoma* after treatment with different concentrations of broad-spectrum and selective insecticides (S: selective insecticide, BS: broad-spectrum insecticide). Bars represent means and whiskers the standard error. Some treatments were not plotted because they did not cause any mortality.

Table 3.—Number of surviving males and females of both species for the different experimental groups. An initial number of 10 individuals was used for each treatment and sex.

Species Treatment	Field concentration	*Lycosa poliostoma* Male	*Lycosa poliostoma* Female	*Hogna cf. bivitatta* Male	*Hogna cf. bivitatta* Female
Broad- spectrum insecticide	5%	7	9	1	8
	10%	2	4	2	9
	20%	0	2	0	1
Selective insecticide	5%	10	9	8	9
	10%	9	9	9	10
	20%	9	10	10	8
Control	0	10	10	10	10

decreased the abundance, richness and diversity of several spider families on plots fumigated with this insecticide when compared to insecticide-free plots. Similarly, lambda-cyhalothrin showed negative effects on the spider *Neoscona theisi* (Walckenaer, 1841) by reducing their predatory activity and increasing mortality (Tahir et al. 2019). In spite of this, only few studies have explored the side effects of the combined insecticide thiamethoxam+lambda-cyhalothrin on natural enemies. Asogwa et al. (2011) showed that, when used together, the thiamethoxam+lambda-cyhalothrin induced mortality on predatory mirid bugs, even at low concentrations, which is consistent with our observations regarding a low survival of spiders at 20% and 10% of the field concentrations.

When comparing the mortality for each sex, we found it was higher for males than females in *Hogna cf. bivittata*, especially for broad-spectrum insecticides. These results agree with previous observations that have shown that males from other wolf spider species are more susceptible than females to insecticides (Van Erp et al. 2002). Although we expected that heavier individuals of *L. poliostoma* would be more resistant to broad-spectrum insecticides given their higher mass, this was not the case. Interestingly, females of the smaller *Hogna cf. bivittata* displayed a higher survival rates than females and males of *L. poliostoma,* at a 10% concentration of broad-spectrum insecticides. This suggests that *Hogna cf. bivittata* may have a higher resistance to insecticides and possibly better detoxification capabilities. This could possibly be because some insecticide detoxification genes that have been identified in other wolf spider species, such as *Pardosa pseudoannulata* (Bösenberg & Strand, 1906) (Meng et al. 2015).

Because of the effects of the broad-spectrum insecticide, we were able to use only one concentration of this insecticide on prey-capture experiments. We found it decreased prey acceptance, while most of the concentrations of selective insecticide did not affect feeding behavior. The disruption of the feeding behavior of predators is a common negative trait caused by broad-spectrum insecticides (Benamú et al. 2007, 2013; Hanna & Hanna 2013). In the particular case of neonicotinoids, it has been shown that thiamethoxam+lambda-cyhalothrin can alter the nervous system by disrupting acetylcholine receptors (Martinou & Stavrinides 2015). Consequently, some behavioral responses like prey capture could be altered (Benamú et al. 2013), which might explain the lower prey acceptance rate of spiders contaminated with this insecticide.

The innocuous effects recorded for the selective Intrepid®SC (methoxyfenozide) agree with previous studies that suggested

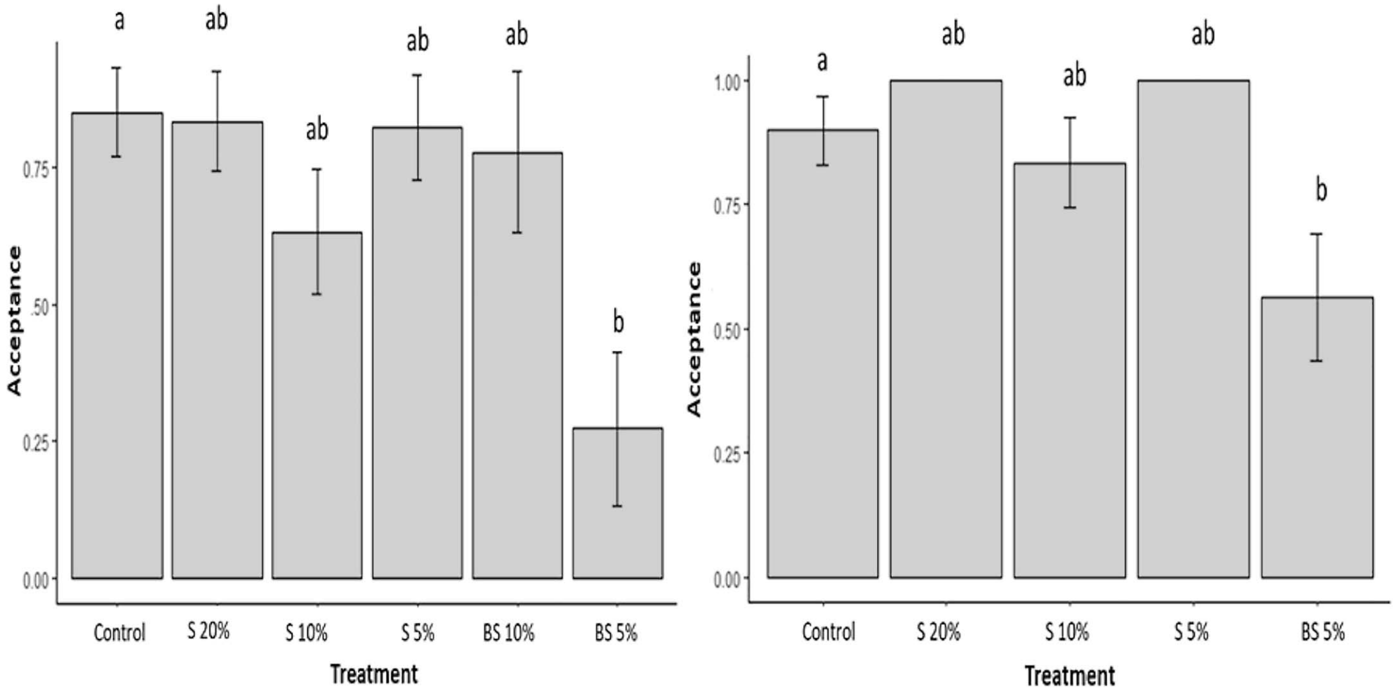

Figure 3.—Proportion of prey captured (prey acceptance) by (A) *Hogna cf. bivittata* and (B) *Lycosa poliostoma* after being treated with different concentrations of broad-spectrum and selective insecticides (S: selective insecticide, BS: broad-spectrum insecticide). Different letters represent significant differences between treatments. Bars represent means and lines the standard error.

its compatibility with some groups of natural enemies. For example, it has been shown that methoxyfenozide does not affect survival or reproduction when evaluated in some parasitoids and predators (Brunner et al. 2001; Rimoldi et al. 2012). In addition, the fact that this insecticide acts mainly by ingestion might have mitigated its effect on the spiders. Our results suggest that the residual effects of methoxyfenozide appear to be harmless to spiders, suggesting a possible compatibility between this insecticide and wolf spiders, as has been shown in other groups of natural enemies (Gentz et al. 2010).

In spite of this, additional aspects should be analyzed. Since methoxyfenozide mimics molting hormones from arthropods (Giuggia et al. 2011), further studies should evaluate whether it affects spider development. Also, other exposure routes (e.g., topical or ingestion) should be explored in the two insecticides used, since these might have stronger sublethal effects on the spider due to its mode of action.

In conclusion, our results suggest that the usage of the broad-spectrum Geonex® (thiamethoxam 14.1% + lambda-cyhalothrin 10.6%, Tafirel S.A., Uruguay) negatively affects two species of native predators and might be potentially noxious to other groups. Therefore, its usage must be limited and applied only when necessary. In contrast, the insecticide Intrepid®SC did not affect survival or foraging behavior in the species studied. We recommend more experiments to fully understand the indirect lethal and sub-lethal impacts of selective insecticide use on other non-target invertebrates (see Lacava et al. 2021). This work highlights once again the relevance of sublethal effect evaluations in ecotoxicological studies to reformulate pest control strategies in agroecosystems. Since these results are potentially applicable to IPM programs in Uruguay, further studies should evaluate the effect of this insecticide over other life history parameters of both species and other groups of natural enemies.

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