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# Susceptibility of first instar *Hippodamia convergens* (Coleoptera: Coccinellidae) and *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) to the insecticide sulfoxaflor

Rafael Carlesso Aita<sup>1</sup>, Anh K. Tran<sup>1</sup>, and Robert L. Koch<sup>1,\*</sup>

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## Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), continues to be the most economically important arthropod pest of soybean in the Midwest. Currently, management tactics for *A. glycines* rely on scouting and application of broad-spectrum insecticides. However, broad-spectrum insecticides are toxic to most natural enemies of this aphid. Selective insecticides may provide an alternative strategy for suppressing *A. glycines* populations while conserving populations of its natural enemies. Therefore, the aim of this study was to evaluate the potential lethal and sublethal effects of sulfoxaflor (a relatively new selective insecticide), to 2 of this pest's natural enemies, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) and *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae). Laboratory bioassays were performed on first instars of both predators with residual toxicity evaluated over time until adult emergence. Parameters evaluated were mortality and developmental time for larvae and pupae, and adult body size. Fecundity also was determined for *C. rufilabris*. We found that sulfoxaflor was not toxic to first instar *C. rufilabris*. However, developmental time to adult was significantly delayed after exposure to this insecticide, but fecundity and body size were not negatively affected. For *H. convergens*, sulfoxaflor at 25% of the field rate was toxic to first instars. No significant differences were found with regard to developmental time and body size. It is important to note that sulfoxaflor, though relatively less toxic than some insecticides, is not entirely without consequence if natural enemies are exposed. The present study emphasizes the importance of examining earlier life stages and potential sublethal effects when evaluating the toxicity of insecticides in the presence of natural enemies.

Key Words: integrated pest management; natural enemies; selective insecticides; soybean aphid

## Resumo

O pulgão da soja, *Aphis glycines* Matsumura (Hemiptera: Aphididae), continua a ser o inseto-praga de maior importância econômica da soja no Centro-Oeste americano. Atualmente, as técnicas de manejo de *A. glycines* dependem da amostragem e aplicação de inseticidas de amplo espectro. No entanto, inseticidas de amplo espectro são tóxicos para a maioria dos inimigos naturais deste pulgão. Inseticidas seletivos podem fornecer uma estratégia alternativa para suprimir as populações de *A. glycines*, conservando populações de seus inimigos naturais. Portanto, o objetivo deste estudo foi avaliar os potenciais efeitos letais e subletais do sulfoxaflor (um inseticida seletivo relativamente novo) a 2 dos inimigos naturais desta praga, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) e *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae). Ensaios de laboratório foram realizados nos primeiros instares de ambos os predadores, com toxicidade residual avaliada ao longo do tempo até a emergência dos adultos. Os parâmetros avaliados foram mortalidade e tempo de desenvolvimento de larvas e pupas, e tamanho dos adultos. Fecundidade também foi determinada para *C. rufilabris*. Encontramos que sulfoxaflor não foi tóxico para o primeiro instar de *C. rufilabris*. Entretanto, o tempo de desenvolvimento até a fase adulta foi significativamente maior após a exposição a este inseticida, porém fecundidade e tamanho dos adultos não foram negativamente afetados. Para *H. convergens*, 25% da dose recomendada de sulfoxaflor foi tóxico para os primeiros instares. Não foram encontradas diferenças significativas em relação ao tempo de desenvolvimento e tamanho dos adultos. É importante ressaltar que sulfoxaflor, embora relativamente menos tóxico que alguns inseticidas, ainda apresenta consequências negativas se inimigos naturais forem expostos. O presente estudo enfatiza a importância de se examinar os estágios iniciais de desenvolvimento do inseto e possíveis efeitos subletais ao avaliar-se a toxicidade de inseticidas na presença de inimigos naturais.

Palavras Chaves: manejo integrado de pragas; inimigos naturais; inseticidas seletivos; pulgão da soja

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The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), first detected in North America in 2000, continues to be the most economically important arthropod pest of soybean, *Glycine max* (L.) Merrill (Fabaceae), in the Midwest (Ragsdale et al. 2011; Hurlley & Mitchell 2017). Large infestations of *A. glycines* can significantly reduce photosynthetic rates of infested soybean leaflets by 50% (Macedo et al. 2003), and negatively impact yield components (i.e., seed size, seeds per pod, and pods per plant) (Beckendorf et al. 2008) that cause up to

40% yield loss (Ragsdale et al. 2007). Currently, management tactics for *A. glycines* rely on scouting and application of broad-spectrum insecticides based on an established economic threshold and economic injury level (Ragsdale et al. 2007; Hodgson et al. 2012; Koch et al. 2016). Since the introduction of *A. glycines* in North America, the amount of soybean acreage treated with insecticides in the Midwest increased from < 0.1% in 2000 to > 13% in 2006 (Ragsdale et al. 2011). Non-chemical tactics for managing *A. glycines*, such as host plant resistance (Hill et al.

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2004; Hesler et al. 2013; Hanson & Koch 2018) and biological control (Heimpel et al. 2004), are promising complementary tactics for improving current integrated pest management (IPM) programs for this pest.

Natural enemies have been shown to play a role in suppressing and preventing outbreaks of *A. glycines* (Costamagna et al. 2008; Ragsdale et al. 2011; Koch & Costamagna 2017). In North America, there are over 40 species of predators and parasitoids associated with this pest (Rutledge et al. 2004). Costamagna and Landis (2006) showed that natural enemies significantly reduced population growth and establishment of *A. glycines* in several production systems. Additionally, Fox et al. (2005) found that generalist predators reduced overall survival of this aphid during a 24-h period in 50% of field cage trials performed. Insecticides commonly used for *A. glycines* management (i.e., organophosphates and pyrethroids) (Hodgson et al. 2012) may have lethal and sublethal impacts on beneficial arthropods (Desneux et al. 2007; Seagraves & Lundgren 2012; Guedes et al. 2016). Selective insecticides may provide an alternative for suppressing *A. glycines* populations, while conserving populations of natural enemies (Weinzierl 2009). Integrated pest management programs can be improved with the combination of selective insecticides and biological control agents (Garzón et al. 2015). Previous studies have evaluated the potential role of selective insecticides in *A. glycines* management programs (Ohnesorg et al. 2009; Bahlai et al. 2010; Frewin et al. 2012; Varenhorst & O'Neal 2012; Pezzini & Koch 2015; Tran et al. 2016; Koch et al. 2019). However, it has been shown that some selective insecticides may not be entirely benign to natural enemies (Bahlai et al. 2010; Gentz et al. 2010; Biondi et al. 2012a). Understanding the impacts of insecticides, including sublethal effects, to beneficial arthropods is essential for an integrated pest management program. Sublethal effects are defined as deleterious physiological or behavioral effects on individuals that survive an exposure to a pesticide (Desneux et al. 2007). Previous authors have reported that population dynamics and other reproductive and behavioral traits (e.g., developmental rate, fecundity, fertility, longevity, sex ratio, feeding, and oviposition) of beneficial arthropods may be adversely affected by sublethal concentrations of pesticides (Stark & Banks 2003; Desneux et al. 2007; Biondi et al. 2012b; Cloyd 2012; Moscardini et al. 2013; Guedes et al. 2016).

Sulfoxaflor is in the sulfoximine class of insecticides and is a potential selective chemical tool for management of *A. glycines* (Knodel et al. 2016; Tran et al. 2016). The specific activity of sulfoxaflor on the insect nicotinic acetylcholine receptor (nAChR) is novel and structurally different from neonicotinoids (Babcock et al. 2011; Zhu et al. 2011; Sparks et al. 2013). This factor has resulted in sulfoxamines being classified as Group 4C by the Insecticide Resistance Action Committee (IRAC 2018). Sulfoxaflor is effective against a wide range of sap-feeding insects, such as the rice brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) (Ghosh et al. 2013); plant bugs, *Lygus hesperus* Knight (Joseph & Bolda 2016) and *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) (Siebert et al. 2012); whiteflies, *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (Longhurst et al. 2013); and aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Zhu et al. 2011); as well as *A. glycines* (Hemiptera: Aphididae) (Tran et al. 2016). The high efficacy of this insecticide to control sap-feeding insect pests, reduced toxicity to natural enemies, and the lack of cross-resistance with some insecticides (Babcock et al. 2011; Longhurst et al. 2013; Sparks et al. 2013; Tran et al. 2016; Liao et al. 2019), suggests that sulfoxaflor may provide an effective alternative for integrated pest management and insecticide resistance management programs for pests such as *A. glycines*.

However, the lethal and sublethal impacts of sulfoxaflor on natural enemies are not fully understood. Potential impacts of sulfoxaflor on natural enemies appear to depend on the concentration of the insecti-

cide and the species of natural enemy used in the study. For example, Pan et al. (2017) reported that sulfoxaflor had a negative impact on the growth, feeding, and behavior of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). In addition, Garzón et al. (2015) showed that sulfoxaflor was highly toxic to the late instar larvae of *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae). However, when compared with broad-spectrum insecticides, sulfoxaflor was less impactful to key predators of *A. glycines* (Tran et al. 2016). These studies generally have not examined impacts of sulfoxaflor to first instars of natural enemies, which are often the most susceptible life stage (Kraiss & Cullen 2008; Pezzini & Koch 2015; Prabhaker et al. 2017).

Therefore, to improve the integration of chemical and biological control for *A. glycines*, further understanding is needed of the potential lethal and sublethal effects of sulfoxaflor on natural enemies. The objective of this study was to investigate the potential lethal and sublethal effects of sulfoxaflor after exposure of early instars of 2 representative natural enemies, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) and *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), under laboratory conditions.

## Materials and Methods

### CHRYSOPERLA RUFILABRIS

Laboratory bioassays were performed on first instar *C. rufilabris* and *H. convergens* at the University of Minnesota, St. Paul, Minnesota, USA. *Chrysoperla rufilabris* eggs were purchased from Beneficial Insectary (Redding, California, USA) and shipped overnight. Upon arrival, eggs were removed from the original packaging and transferred into individual 60 × 15-mm plastic Petri dishes. *Chrysoperla rufilabris* eggs were allowed to develop into 2- to 3-d-old larvae in a growth chamber at 25 °C, 75% RH, and a photoperiod of 16:8 h (L:D).

To evaluate insecticide residual toxicity to first instar *C. rufilabris*, a randomized complete block design experiment was used with 3 treatments and 4 replications, with 15 individuals per replication. Treatments consisted of sulfoxaflor (34.8 g a.i. per ha, Transform, Corteva Agriscience, Wilmington, Delaware, USA) (i.e., high end of range of labeled field rates); λ-cyhalothrin (29.1 g a.i. per ha, Warrior II, Syngenta Crop Protection Inc., Basel, Switzerland); and an untreated check. The bioassay methodology was similar to the laboratory bioassay performed by Tran et al. (2016). Treatments were applied to the interior of 60 × 15-mm plastic Petri dishes. After application, dishes were allowed to dry for 1 h and the 2- to 3-d-old first instars of *C. rufilabris* were transferred to treated Petri dishes. Larvae were maintained in the treated dishes for 24 h. After 24 h, *C. rufilabris* larvae were transferred to untreated Petri dishes. Larvae were maintained in a growth chamber under the conditions previously described, and provided with water-moistened florist foam and *Ephesia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs ad libitum as food source until larvae reached the pupal stage. Prior to transferring the larvae into untreated Petri dishes, the exterior surfaces of the dishes were wiped with a cloth sprayed with Static Guard (B&G Foods Inc., Parsippany, New Jersey, USA) to prevent static electricity from interfering with larval transfer and food placement (Amarasekare & Shearer 2013). *Chrysoperla rufilabris* larval mortality was monitored daily and defined as the immobility of the larvae upon stimulation with a fine camel-hair brush. For pupae, mortality was defined as the inability to molt to the next life stage. Developmental time for each life stage was recorded.

Adult *C. rufilabris* that emerged successfully from the pupal stage were transferred to clean Petri dishes, and provisioned with honey and water-moistened floral foam. Honey was used to provision adult

*C. rufilabris*, because artificial diets can affect female fecundity (Hagen 1950; Sundby 1967). Petri dishes were cleaned every other d to prevent mold growth. After 10 d, surviving adults were placed into the freezer at approximately  $-20^{\circ}\text{C}$ . Adult wing lengths were recorded from the base axillary sclerite to the apex of the wing using a dissecting scope and Leica Application Suite software (Version 4.0.0, Leica Microsystems Inc., Buffalo Grove, Illinois, USA). The adults were sexed and dissected in Dulbecco's Phosphate Buffered Saline 1X (DPBS) with calcium and magnesium solution (Mediatech Inc., Manassas, Virginia, USA) to record sex and fecundity (i.e., number of eggs in ovaries) of females.

### HIPPODAMIA CONVERGENS

*Hippodamia convergens* adults were purchased from Arbio Organics (Oro Valley, Arizona, USA) and shipped overnight. Upon arrival, approximately 20 pairs of adults were separated into individual  $60 \times 15\text{-mm}$  Petri dishes for mating, and were maintained in a growth chamber at  $25^{\circ}\text{C}$ , 55% RH, and a photoperiod of 16:8 h (L:D). Live *A. glycines* on soybean leaves were provisioned ad libitum as a food source, and water was provisioned through moistened florist foam. *Aphis glycines* and water were replenished every 48 h or as needed. A filter paper disc also was placed inside each Petri dish to absorb excess humidity, and was replaced if mold was present. After a 7-d mating period, 20 female adult *H. convergens* were separated into individual  $60 \times 15\text{-mm}$  plastic Petri dishes with food and water as described above. Eggs deposited by females were collected by transferring the females to clean Petri dishes and maintaining the eggs in the previous dishes. Eggs remained in the dishes under conditions described above, and were reared to 2- to 3-d-old first instars.

To evaluate insecticide residual toxicity to first instar *H. convergens*, a randomized complete block design experiment was performed with 3 treatments and 3 replications with 10 individuals per replication. Preliminary experiments conducted with a field rate (i.e., low end of range of labeled field rates) and 50% field rate of sulfoxaflor (Transform, 25.8 g a.i. per ha and 12.9 g a.i. per ha, respectively) resulted in high rates of mortality of first instar *H. convergens*. Therefore, sulfoxaflor concentrations were reduced for this experiment. Treatments were 10% field rate of sulfoxaflor (2.6 g a.i. per ha); 25% field rate of sulfoxaflor (6.4 g a.i. per ha); and an untreated check. Methodology for treating dishes and performing the bioassays was the same as described for *C. rufilabris* experiment. However, during the 24-h exposure period to treatments, approximately 0.2 to 0.3 g of frozen *E. kuehniella* eggs were placed inside each treated dish to reduce mortality due to starvation.

*Hippodamia convergens* larvae were maintained in a growth chamber under the conditions described earlier, and provided with water-moistened florist foam and *E. kuehniella* eggs ad libitum as a food source until larvae reached the adult stage. *Hippodamia convergens* larval mortality was monitored daily, and developmental time for each life stage was recorded as described above. Newly emerged adults (1-d-old) were placed in a freezer at a temperature of  $-20^{\circ}\text{C}$  for future measurements. Individuals were sexed based on the shape of the terminal abdominal segment (Heimpel & Lundgren 2000), and body weight was measured using an analytical balance (Sartorius Entris@ 224, Sartorius AG, Göttingen, Germany). Elytral lengths and pronotal widths were measured using a dissecting scope and Leica Application Suite software (Version 4.0.0, Leica Microsystems Inc., Buffalo Grove, Illinois, USA).

### STATISTICAL ANALYSES

Data were analyzed using R version 3.5.2 (R Core Team 2018) and RStudio Desktop version 1.1.463 (RStudio Team 2018). The effect of

treatments on mortality of *H. convergens* and *C. rufilabris* were subjected to a bias-reduced generalized linear model (package: 'brglm2') (Kosmidis 2018) with a binomial response variable (i.e., 1 = alive, 0 = dead). Separate linear mixed-effect models (package: 'lme4') (Bates et al. 2015) were used to test the fixed effect of pesticide treatment on developmental time and fecundity with a random effect for replication. Separate linear mixed-effect models were used to test the fixed effects of pesticide treatment, sex, and their interaction on body weight, pronotal width, elytral length, and wing length, with a random effect for replication. Random effects accounted for location differences in blocking of treatments within growth chambers. Non-significant ( $P > 0.05$ ) interactions were removed from the models. Responses were analyzed on non-transformed scales, except development times for *C. rufilabris* were square-root transformed for analyses. Means were separated using Tukey's honestly significant difference (HSD) test at  $\alpha = 0.05$ .

## Results

### CHRYSOPERLA RUFILABRIS

Proportion mortality of *C. rufilabris* was significantly affected after individuals were treated in the first instar. In particular, treatment with  $\lambda$ -cyhalothrin significantly increased mortality during the first instar compared with the control and sulfoxaflor ( $\chi^2 = 54.33$ ;  $df = 2$ ;  $P < 0.001$ ) (Fig. 1A). In addition, total proportion mortality (i.e., from first instar to adult) was significantly increased by  $\lambda$ -cyhalothrin compared to the control and sulfoxaflor ( $\chi^2 = 33.56$ ;  $df = 2$ ;  $P < 0.001$ ) (Fig. 1A). No significant differences in mortality were found among treatments for the remaining life stages (second instar:  $\chi^2 = 9.11$ ;  $df = 2$ ;  $P = 0.01$ ; third instar:  $\chi^2 = 0.19$ ;  $df = 2$ ;  $P = 0.91$ ; and pupa:  $\chi^2 = 1.52$ ;  $df = 2$ ;  $P = 0.46$ ) (Fig. 1A).

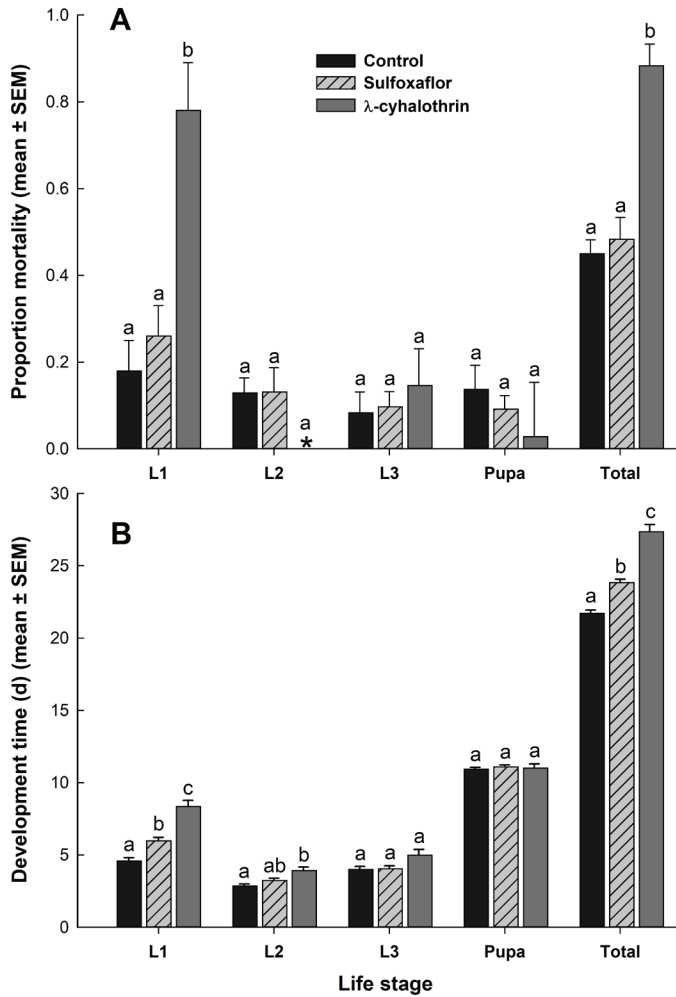
Development time of *C. rufilabris* was significantly affected after individuals were treated in the first instar. In particular,  $\lambda$ -cyhalothrin and sulfoxaflor increased development time of the first instar ( $\chi^2 = 72.51$ ;  $df = 2$ ;  $P < 0.001$ ) and total (i.e., first instar to adult) ( $\chi^2 = 112.92$ ;  $df = 2$ ;  $P < 0.001$ ) (Fig. 1B). In addition,  $\lambda$ -cyhalothrin increased development time of the second instar ( $\chi^2 = 13.54$ ;  $df = 2$ ;  $P = 0.001$ ) (Fig. 1B). No significant differences were found for development times of the third instar ( $\chi^2 = 4.36$ ;  $df = 2$ ;  $P = 0.11$ ) or pupa ( $\chi^2 = 0.78$ ;  $df = 2$ ;  $P = 0.67$ ) (Fig. 1B).

Mean ( $\pm$  SEM) fecundity (i.e., number of eggs in ovaries) of *C. rufilabris* females ranged from  $2.92 \pm 1.26$  to  $4.38 \pm 1.25$  among treatments, but did not differ significantly ( $\chi^2 = 0.9$ ;  $df = 2$ ;  $P = 0.63$ ). Mean wing length of females ( $12.65 \pm 0.16$  mm) was greater than that of males ( $11.72 \pm 0.17$  mm) ( $\chi^2 = 35.49$ ;  $df = 1$ ;  $P < 0.001$ ). However, the effect of treatment on adult wing length was not significant ( $\chi^2 = 4.80$ ;  $df = 2$ ;  $P = 0.9$ ).

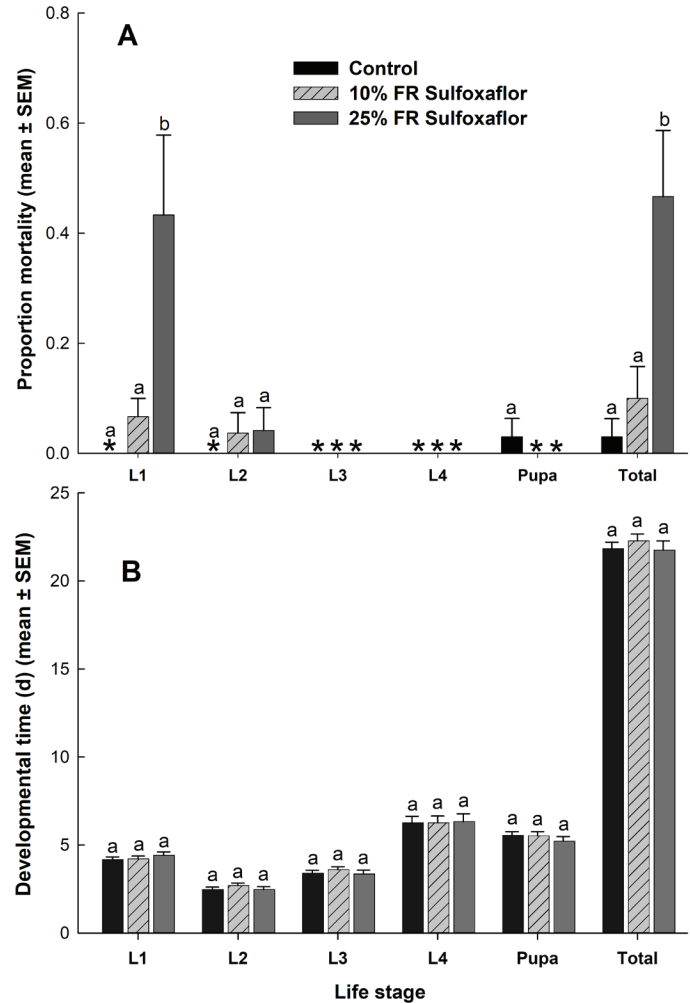
### HIPPODAMIA CONVERGENS

The 25% field rate of sulfoxaflor significantly increased *H. convergens* mortality during the first instar ( $\chi^2 = 24.29$ ;  $df = 2$ ;  $P < 0.001$ ) and total mortality from first instar to adult ( $\chi^2 = 20.34$ ;  $df = 2$ ;  $P < 0.001$ ) compared with the control and 10% sulfoxaflor field rate (Fig. 2A). No significant differences were found for the remaining life stages where mortality occurred (second instar:  $\chi^2 = 0.33$ ;  $df = 2$ ;  $P = 0.84$ ; pupa:  $\chi^2 = 0.10$ ;  $df = 2$ ;  $P = 0.94$ ) (Fig. 2A).

No significant differences were found among treatments for developmental time of *H. convergens* for all life stages (first instar:  $\chi^2 = 1.05$ ;  $df = 2$ ;  $P = 0.58$ ; second instar:  $\chi^2 = 1.45$ ;  $df = 2$ ;  $P = 1.48$ ; third instar:  $\chi^2 = 1.10$ ;  $df = 2$ ;  $P = 0.57$ ; fourth instar:  $\chi^2 = 0.01$ ;  $df = 2$ ;  $P = 0.99$ ; pupa:  $\chi^2 = 0.99$ ;  $df = 2$ ;  $P = 0.6$ ; and total:  $\chi^2 = 0.95$ ;  $df = 2$ ;  $P = 0.62$ ) (Fig.



**Fig. 1.** Proportion of mortality (A) and developmental time (B) of *Chrysoperla rufilabris* life stages after exposure of first instars to dried insecticide residues. After exposure, individuals were reared to adults (L1, L2, and L3 represent first, second, and third instars, respectively, and total represents first instar to adult). Within life stages, treatment means with the same letter are not significantly different (Tukey HSD,  $P > 0.05$ ). Asterisks (\*) indicate zeros.



**Fig. 2.** Proportion of mortality (A) and developmental time (B) of *Hippodamia convergens* life stages after exposure of first instars to dried insecticide residues. After exposure, individuals were reared to adults (L1, L2, L3, and L4 represent first, second, third, and fourth instars, respectively, and total represents first instar to adult). Within life stages, treatment means with the same letter are not significantly different (Tukey HSD,  $P > 0.05$ ). FR = field rate of insecticide. Asterisks (\*) indicate zeros.

2B). Mean body weight of females ( $15.10 \pm 0.36$  mg) was greater than males ( $13.25 \pm 0.37$  mg) ( $\chi^2 = 25.52$ ;  $df = 1$ ;  $P < 0.001$ ). However, the effects of treatment on body weight were not significant ( $\chi^2 = 0.90$ ;  $df = 2$ ;  $P = 0.63$ ). Mean pronotum width and elytra length of females ( $2.36 \pm 0.02$  mm and  $4.49 \pm 0.05$  mm, respectively) were greater than males ( $2.25 \pm 0.02$  mm and  $4.17 \pm 0.05$  mm, respectively) (pronotum width:  $\chi^2 = 26.20$ ;  $df = 1$ ;  $P < 0.001$ ; elytra length:  $\chi^2 = 37.30$ ;  $df = 1$ ;  $P < 0.001$ ). But the effect of treatment on body size was not significant (pronotum width:  $\chi^2 = 1.20$ ;  $df = 2$ ;  $P = 0.54$ ; elytra length:  $\chi^2 = 0.47$ ;  $df = 2$ ;  $P = 0.78$ ).

## Discussion

Our study provides the first examination of the potential lethal and sublethal effects of sulfoxaflor to first instars of *C. rufilabris* and *H. convergens*. Sulfoxaflor had distinct effects to both predators. Although mortality was increased by reduced rates of sulfoxaflor applied to *H. convergens*, a field rate of this insecticide proved to be non-toxic to *C. rufilabris*. Our results for *H. convergens* are in contrast to those of Tran et al. (2016), Colares et al. (2017), and Prabhaker et al. (2017).

However, these authors used later life stages than those used in our study, which may have contributed to the higher rates of mortality reported in the present study. The greater susceptibility of larvae in early instars could be partially explained by their smaller size, presence of a more permeable cuticle, or lower enzymatic detoxifying processes (Stark et al. 2004; Fogel et al. 2013). In addition, under field conditions, the lower mobility of immatures compared to adults, which can fly and potentially avoid insecticidal contact, could further contribute to differences in pesticide susceptibility among life stages (Medina et al. 2004; Garzón et al. 2015). However, when *C. rufilabris* and *H. convergens* were exposed to the insecticide treatments as first instars, lethal and sublethal effects were generally limited to that stage and their total development from first instar to adult.

As stated earlier, exposure of *C. rufilabris* to a field rate of sulfoxaflor in the first instar did not affect mortality but did cause an intermediate increase in developmental time compared with the control and λ-cyhalothrin. In a residual toxicity experiment, Tran et al. (2016) found that sulfoxaflor was harmless to third instars of *C. rufilabris*. Similar results were found by Garzón et al. (2015), where sulfoxaflor was found to be harmless to the third instars of *Chrysoperla carnea*.

(Stephens) (Neuroptera: Chrysopidae). Additionally, sulfoxaflor had no toxicity when first instars of *C. carnea* were exposed via residues on treated leaves (Barbosa et al. 2017). However, larvae of *C. carnea* had slower developmental time compared with the control when ingesting food contaminated with sulfoxaflor (Barbosa et al. 2017). The generally lower susceptibility of *C. rufilabris* to these insecticides compared with *H. convergens* may have been due to generally higher esterase activity in Chrysopidae (Ishaayn & Casida 1981).

Moreover, exposure of *H. convergens* to reduced rates of sulfoxaflor in the first instar affected mortality at the 25% field rate, but not the 10% field rate. In addition, these rates did not affect development time at any life stage or total development time. Lower rates of sulfoxaflor were used in this study because of the high mortality found for first instars at a full field rate. Similarly, a field rate of sulfoxaflor was highly toxic to second instar *H. convergens* exposed to residues on treated leaves (Colares et al. 2017). In addition, sulfoxaflor was highly toxic to fourth instars of *A. bipunctata* (Garzón et al. 2015). The greater insecticide tolerance of *H. convergens* adults compared to larvae is consistent with results for other coccinellids (Galvan et al. 2005; Jalali et al. 2009; Fogel et al. 2013), and may be due to some of the factors described above.

Sulfoxaflor holds promise for improved integration of chemical and biological controls of *A. glycines* and other piercing-sucking pests. Consistent with other studies, some sublethal effects on development time for both predators were found, but none on size or reproductive potential (Garzón et al. 2015; Colares et al. 2017). Therefore, when developing integrated pest management programs it is important to note that the use of sulfoxaflor is not entirely without consequence to natural enemies. The present study emphasizes the importance of examining earlier life stages and potential sublethal effects when evaluating compatibility of insecticides with natural enemies. Additional research should examine the potential consequences of these lethal and sublethal effects on the effectiveness of biological control offered by these predators.

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