Toxicity of indoxacarb and spinosad to the multicolored Asian lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), via three routes of exposure

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Abstract: The use of selective insecticides may improve conservation of natural enemies and therefore contribute to the success of integrated pest management (IPM) programs. In this study, the toxicity of two commonly used selective insecticides, indoxacarb and spinosad, to the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), was evaluated. Third instars and adults of *H. axyridis* were exposed to indoxacarb at 50 and 100% of the field rate (FR), to spinosad at 100% FR and to water (untreated check) under laboratory conditions via three routes of exposure. Treatments were applied directly on insects (i.e., topical application), on Petri dishes (i.e., residues), or on soybean aphids, *Aphis glycines* Matsumara (i.e., treated prey). Mortality of exposed individuals in each life stage was recorded 2 and 7 days after treatment. Logistic regression indicated that indoxacarb at 100% FR, followed by indoxacarb at 50% FR, was more insecticidal than spinosad to third instars. Mortality was higher when *H. axyridis* were exposed to both insecticides via residues followed by treated prey. Indoxacarb at 100 or 50% FR was insecticidal to adults. Adults were tolerant to spinosad via all routes of exposure. The present results suggest that indoxacarb may decrease *H. axyridis* field populations by causing mortality to larvae and adults via all routes of exposure. Implications of the toxicity of indoxacarb to *H. axyridis* within an IPM context and possible reasons for the differences in susceptibility of *H. axyridis* for each route of exposure are discussed.

Keywords: *Harmonia axyridis*; route of exposure; spinosad; indoxacarb; conservation biological control

1 INTRODUCTION

Conservation of natural enemies in integrated pest management (IPM) programs is enhanced through habitat manipulation or the use of selective insecticides that can increase natural enemy populations or attack rates on pests.[1] Insecticide selectivity can occur through ecological or physiological methods.[2] The former is attained by reducing the exposure of natural enemies to insecticide application.[2,3] The latter is attained by using insecticides that are harmful to insect pests but relatively harmless to natural enemies.[3–5] Both ecological and physiological selectivity have the ultimate goal of increasing the effectiveness of biological control. By conserving natural enemies through the use of selective insecticides, the likelihood of pest resurgence may be reduced[6] and the number of insecticide applications may be decreased.[7]

Both laboratory and field studies can be used to evaluate the level of toxicity of selective insecticides to natural enemies.[8,9] However, since field studies can be limited by abiotic and biotic factors, laboratory studies are more common owing to greater experimental control. A shortcoming of laboratory studies is the artificial exposure of natural enemies to insecticides. In the field, insects are normally exposed via at least three routes: topical application, insecticide residues and treated prey.[10] However, most laboratory studies expose natural enemies to insecticides via only one[11,12] or two[13,14] routes of exposure.

Recent insecticide chemistries, such as indoxacarb and spinosad, have proven effective against several pest species[7,15,16] while showing low toxicity to natural enemies.[9,17,18] Hence, these insecticides have been labeled as 'reduced risk' insecticides.[7] For example, in a previous laboratory study it was reported by the present authors that indoxacarb and spinosad were less insecticidal to the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), than conventional insecticides such as bifenthrin and carbaryl.[9] However, in this study, *H. axyridis* larvae and adults were exposed to insecticides via topical application only. Results of studies using only one route of exposure may produce biased estimates of the overall risk of selective insecticides to natural enemies.

In this paper, the toxicities of indoxacarb and spinosad to third-instar and adult *H. axyridis* were evaluated under laboratory conditions using topical application, insecticide residues and treated prey. *Harmonia axyridis*, a dominant predator in many agricultural systems,[19] was used in these studies to continue the authors' research on incorporating this predator into a sweetcorn IPM program.[9,20] Two

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questions were addressed in the study. First, do these two selective insecticides differ in toxicity to *H. axyridis*? Second, does route of exposure alter insecticide toxicity to *H. axyridis*?

2 MATERIALS AND METHODS

2.1 Insects

Harmonia axyridis adults were obtained from a laboratory colony founded from adults collected during October 2004 at the University of Minnesota Outreach, Research and Education (UMORE) Park, Rosemount, MN. Beetles were identified using a diagnostic guide[21] and voucher specimens in the Insect Museum (Department of Entomology, University of Minnesota). Following collection, beetles were held in 1.96 L plastic dishes with ∼200 beetles (males and females combined) per dish, and maintained at 10 ± 1 °C with a 16:8 h light:dark photoperiod. Prior to experimentation, the dishes containing beetles were warmed to 25 ± 1 °C with the same photoperiod (these rearing conditions were used throughout the rest of the studies), and the beetles were allowed to mate for 14 days. The beetles were provided an *ad libitum* supply of live soybean aphids, *Aphis glycines* Matsumara, a diet made from freeze-dried drone honey bee, *Apis mellifera* L., pupae,[22] and water in 0.5 mL plastic microcentrifuge tubes plugged with cotton. After the mating period, adult females were maintained individually in plastic Petri dishes (60×15 mm) lined with 55 mm filter paper disks. The Petri dishes containing females were checked daily for oviposition. If eggs were found, females were removed and transferred to new Petri dishes (60×15 mm) provisioned with food and water. After egg hatch and dispersal of larvae from egg clusters (i.e. ∼1 day after hatching), individuals were placed individually into separate plastic Petri dishes (60×15 mm) and were reared to the desired developmental stages (i.e., third instar or adult) on a diet of freeze-dried *A. mellifera* drone pupae.

2.2 Experimentation

The experiments were conducted as separate randomized complete block designs for third instars (24 ± 1 h after molting) and adults (14 ± 2 days after emergence) of *H. axyridis*. The experiment for each stage consisted of 12 factorial treatments (four levels of the spray factor and three levels of the route of exposure factor), four replications over time and five individuals per replication of each treatment. Levels of the spray factor included: indoxacarb 300 g kg^{-1} WG (Avaunt® WG; EI du Pont de Nemours and Company, Wilmington, DE) at 100% of the field rate (FR) [0.062 kg AI ha^{-1} (0.055 lb AI acre^{-1})]; indoxacarb at 50% FR [0.031 kg AI ha^{-1} (0.0275 lb AI acre^{-1})]; spinosad 240 g L^{-1} SC (SpinTor™ 2SC; Dow AgroSciences LLC, Indianapolis, IN) at 100% FR [0.11 kg AI ha^{-1} (0.094 lb AI acre^{-1})]; and an untreated check (i.e., water). Treatments were applied using a motorized spray chamber (Department of Agronomy, University of Minnesota) with a single XR-Teejet 8002 flat fan nozzle.[9,20] The sprayer was calibrated to deliver the equivalent of 233.9 L ha^{-1} (25 gal acre^{-1}) at 242.3 kPa (35 psi).

Levels of the route of exposure factor included: topical application, insecticide residues and treated prey. For each replication of topical application, five individuals of larval or adult stages were placed into plastic Petri dish bottoms (150×15 mm) covered with a fine metal mesh to retain the insects in the dish, and then placed in the spray chamber. After treatment, the Petri dish bottoms were removed from the spray chamber and allowed to dry for 1 h before the treated individuals were transferred individually to five clean plastic Petri dishes (60×15 mm). For each replication of insecticide residues, insecticides or water were applied inside the tops and bottoms of five plastic Petri dishes (60×15 mm) using the spray chamber. After treatment, the tops and bottoms of Petri dishes were removed from the spray chamber and allowed to dry for 1 h before one third instar or adult was transferred to each treated Petri dish. For each replication of treated prey, late instars and apterous adults of *A. glycines* were killed by placing them at −80 °C for 15 min. The freeze-killed aphids were then transferred into plastic Petri dish bottoms (150×15 mm), and the bottoms were placed into the spray chamber. After treatment, the Petri dish bottoms were removed from the spray chamber and allowed to dry for 1 h before 40 aphids were transferred to clean plastic Petri dishes (60×15 mm) each containing one *H. axyridis* third instar, or 80 aphids with one *H. axyridis* adult.

Water or food, other than the aphids in the treatments with treated prey, was not provided for any treatment in the first 24 h post-treatment. After 24 h, live individuals from all treatments were transferred to clean plastic Petri dishes (60×15 mm) lined with 55 mm filter paper disks. In these new Petri dishes, an *ad libitum* supply of freeze-dried *A. mellifera* drone pupae and water were provided as described above. Mortality of larvae or adults was recorded 2 and 7 days after treatment, with mortality defined as immobility of the insects upon stimulation with a fine camel-hair brush.

2.3 Data analysis

Logistic regression (PROC LOGISTIC)[23] was used to analyze the relationship between treatments and mortality of *H. axyridis* at 2 and 7 days after treatment. Logistic regression was chosen because the response variable was dichotomous (i.e., dead or live beetles) and the errors were binomially distributed.[24] Forward stepwise logistic regression was used to calculate the significance ($P < 0.05$) of the treatment variables – main effects (i.e., spray and route of exposure) and their interaction. The general equation for logit probability functions for *H. axyridis* mortality

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was

$$P(x) = \frac{e^{(\alpha+\beta x)}}{1 + e^{(\alpha+\beta x)}}$$

where α is the intercept, β is the slope, x is the parameter and e (approximately equal to 2.71828) is the base for natural logarithms. The intercept for each model was the log odds of mortality that occurred when third instars or adults were treated with water via topical application. The parameter β_1 is the increment in log odds for indoxacarb 100% FR, β_2 is that for indoxacarb 50% FR, β_3 is that for spinosad 100% FR, β_4 is that for insecticide residues and β_5 is that for treated prey. The intercept and the parameters were estimated using maximum likelihood methods. Therefore, the intercept and the parameters are maximum likelihood estimates (MLE), a value that maximizes the likelihood of the effects that the intercept and each parameter have over *H. axyridis* mortality. If the MLE of the intercept or parameters are significant ($P < 0.05$), they significantly account for mortality of *H. axyridis* and will be included in the model. Each parameter either pools the mortality across all routes of exposure for a given insecticide or pools the mortality across all insecticides for a given route of exposure. For example, indoxacarb 100% FR pools the effects of this treatment across topical application, insecticide residues and treated prey. Once the intercept and parameters of the model are determined, then the comparisons between each parameter and the reference parameters are determined by estimating the odds ratio of each comparison. Odds ratio estimates represent the odds of *H. axyridis* being killed by a parameter (e.g., indoxacarb 100% FR) compared with the reference parameter (e.g., water). Therefore, the effects of indoxacarb 100% FR, indoxacarb 50% FR, and spinosad 100% FR on *H. axyridis* mortality were compared with the effects of water. Similarly, the effects of insecticide residues and treated prey were compared with the effects of topical application. Finally, contrasts were calculated using exponential estimates for pairwise combinations of insecticides and routes of exposure that were not compared using the odds ratio. The models were fitted by using incremental effects parameterization.[24–26]

3 RESULTS

3.1 Third instars

Indoxacarb at both rates was insecticidal to *H. axyridis* larvae via all three routes of exposure 2 days after treatment (Table 1, Fig. 1A). Spinosad at 100% FR was insecticidal to *H. axyridis* larvae (Table 1). Figure 1A shows that spinosad 100% FR, via insecticide residues, was insecticidal to third instars 2 days after treatment, but it was not so via topical application and treated prey. The logistic regression model of proportional mortality of *H. axyridis* third instars 2 days after treatment included the intercept, indoxacarb 100% FR, indoxacarb 50% FR, spinosad 100% FR and insecticide residues ($P < 0.05$) (Table 1, Fig. 1A). The two-way interaction (spray × route) was not significant (stepwise selection: $\chi^2 = 3.99$, DF = 6, $P = 0.68$). Indoxacarb 100% FR, indoxacarb 50% FR or spinosad 100% FR increased the odds of larval mortality when compared with water, although the spinosad results fell just short of the $P = 0.05$ significance level (Table 2). For example, *H. axyridis* exposed to indoxacarb 100% FR had 128 times higher odds of dying than *H. axyridis* exposed to water. Insecticide residues also increased the odds of larval mortality when compared with topical application (Table 2). When contrasts were used to verify the significance of pairwise comparisons with all insecticides, indoxacarb at 100% FR, followed by indoxacarb at 50% FR, showed the most insecticidal activity to third instars (Table 3). In addition, contrasts showed that larval mortality was significantly higher when exposed to insecticide residues than when exposed to treated prey (Table 3).

Indoxacarb at both rates was insecticidal to *H. axyridis* larvae via the three routes of exposure 7 days after treatment (Table 1, Fig. 1B). Spinosad, however, was not insecticidal to *H. axyridis* larvae (Table 1). The logistic regression model of proportional mortality of *H. axyridis* third instars 7 days after treatment included the intercept, indoxacarb 100% FR, indoxacarb 50% FR and insecticide residues ($P < 0.05$) (Table 1, Fig. 1B). The two-way interaction (spray × route) was not significant (stepwise selection: $\chi^2 = 9.87$, DF = 6, $P = 0.13$). Indoxacarb 100% FR or indoxacarb 50% FR increased the odds of larval mortality when compared with water (Table 2). Insecticide residues increased the odds of larval mortality when compared with topical application (Table 2). When contrasts were used to verify the significance of pairwise comparisons with all insecticides, indoxacarb 100% FR or indoxacarb 50% FR was more insecticidal to larvae than spinosad 100% FR (Table 3). There was no significant difference in mortality between larvae exposed to insecticide residues or treated prey (Table 3).

3.2 Adults

At 2 and 7 days after treatment, indoxacarb at both rates was insecticidal to *H. axyridis* adults via all three routes of exposure, while spinosad was not insecticidal (Table 1, Figs 1C and D). The logistic regression model of the proportion of *H. axyridis* adults dead 2 and 7 days after treatment included the intercept, indoxacarb 100% FR, indoxacarb 50% FR, insecticide residues and treated prey ($P < 0.05$) (Table 1, Figs 1C and D). The two-way interaction (spray × route) was not significant 2 days after treatment (stepwise selection: $\chi^2 = 7.15$, DF = 6, $P = 0.31$) nor 7 days after treatment (stepwise selection: $\chi^2 = 6.10$, DF = 6, $P = 0.41$). Indoxacarb 100% FR or indoxacarb 50% FR increased the odds of adult mortality when compared with water (Table 2). Insecticide residues or treated prey increased the

Table 1. Maximum likelihood estimates (MLEs)[a] from logistic regression, with water and topical application as the reference parameters, of the proportional mortality of *Harmonia axyridis* third instars and adults 2 and 7 days after insecticide treatment in the laboratory

Parameter	MLE	SE	Wald χ^2	P[b]
		Third instars 2 days after treatment[c]		
Intercept	−3.77	0.69	29.65	<0.0001
Indoxacarb 100% FR	4.85	0.72	45.51	<0.0001
Indoxacarb 50% FR	3.90	0.69	32.25	<0.0001
Spinosad 100% FR	1.35	0.69	3.85	0.0497
Insecticide residues	1.64	0.49	11.00	0.0009
Treated prey	−0.54	0.45	1.43	0.2311
		Third instars 7 days after treatment[d]		
Intercept	−4.88	1.42	11.86	0.0006
Indoxacarb 100% FR	7.78	1.76	19.56	<0.0001
Indoxacarb 50% FR	6.52	1.44	20.33	<0.0001
Spinosad 100% FR	2.19	1.15	3.61	0.0576
Insecticide residues	2.47	1.14	4.73	0.0297
Treated prey	0.60	1.04	0.33	0.5635
		Adults 2 days after treatment[e]		
Intercept	−7.15	1.75	16.73	<0.0001
Indoxacarb 100% FR	7.27	1.76	17.14	<0.0001
Indoxacarb 50% FR	5.81	1.67	12.16	0.0005
Spinosad 100% FR	0.95	1.66	0.33	0.5647
Insecticide residues	4.11	1.18	12.06	0.0005
Treated prey	2.21	0.80	7.68	0.0056
		Adults 7 days after treatment[f]		
Intercept	−6.41	1.62	15.61	<0.0001
Indoxacarb 100% FR	7.37	1.65	19.97	<0.0001
Indoxacarb 50% FR	6.38	1.59	16.15	<0.0001
Spinosad 100% FR	1.55	1.46	1.13	0.2883
Insecticide residues	3.37	1.12	9.12	0.0025
Treated prey	1.54	0.75	4.22	0.0400

[a] MLE is a value that maximizes the likelihood of effects that the intercept and each parameter have on *H. axyridis* mortality.
[b] If the MLEs of the intercept or parameters are significant ($P < 0.05$), they will be included in the model.
[c] Hosmer–Lemeshow goodness of fit test: $P = 0.7109$. The model does not provide an adequate fit to the data if $P < 0.05$. Maximum rescaled $R^2 = 0.81$.
[d] Hosmer–Lemeshow goodness of fit test: $P = 0.81$. Maximum rescaled $R^2 = 0.81$.
[e] Hosmer–Lemeshow goodness of fit test: $P = 0.95$. Maximum rescaled $R^2 = 0.63$.
[f] Hosmer–Lemeshow goodness of fit test: $P = 0.45$. Maximum rescaled $R^2 = 0.82$.

odds of adult mortality when compared with topical application (Table 2). When contrasts were used to verify the significance of pairwise comparisons with all insecticides, indoxacarb 100% FR or indoxacarb 50% FR was more insecticidal to adults than spinosad 100% FR (Table 3). There was no significant difference in mortality between adults exposed to insecticide residues or treated prey (Table 3).

4 DISCUSSION

Harmonia axyridis is a dominant generalist predator in sweetcorn in the Midwestern US. In this system, *H. axyridis* has been documented preying on several sweetcorn pests such as the European corn borer, *Ostrinia nubilalis* Hübner,[27,28] and the corn leaf aphid, *Rhopalosiphum maidis* Fitch.[29] Conservation of this predator using selective insecticides could improve the compatibility of biological control within a sweetcorn IPM program.

In general, the toxicities of indoxacarb and spinosad to *H. axyridis* have proven to be less than those of conventional insecticides.[9] However, as the present results suggest, these insecticides were not entirely harmless to *H. axyridis* (Fig. 1). These results corroborate previous studies[9,20] where indoxacarb and spinosad caused lethal and sublethal effects on *H. axyridis*. In addition, the present results showed that indoxacarb was more insecticidal to larvae and adults than spinosad, which was insecticidal to the larval stage but not to the adult stage. Indoxacarb has also been shown to be insecticidal to other predators such as *Podisus maculiventris* (Say).[13] Spinosad, however, has shown insecticidal activity against parasitoids,[30] while only a few studies have documented harmful insecticidal effects on predators.[31,32]

Indoxacarb and spinosad are relatively new insecticides that have shown low toxicity to natural enemies of insect pests in sweetcorn.[33] The selectivity of these insecticides is attributed to changes in the feeding behavior of predators[18] and to their unique mode of action and bioactivation.[16,34] However, as the present results suggest, indoxacarb and to a lesser extent spinosad are not completely safe to *H. axyridis*,

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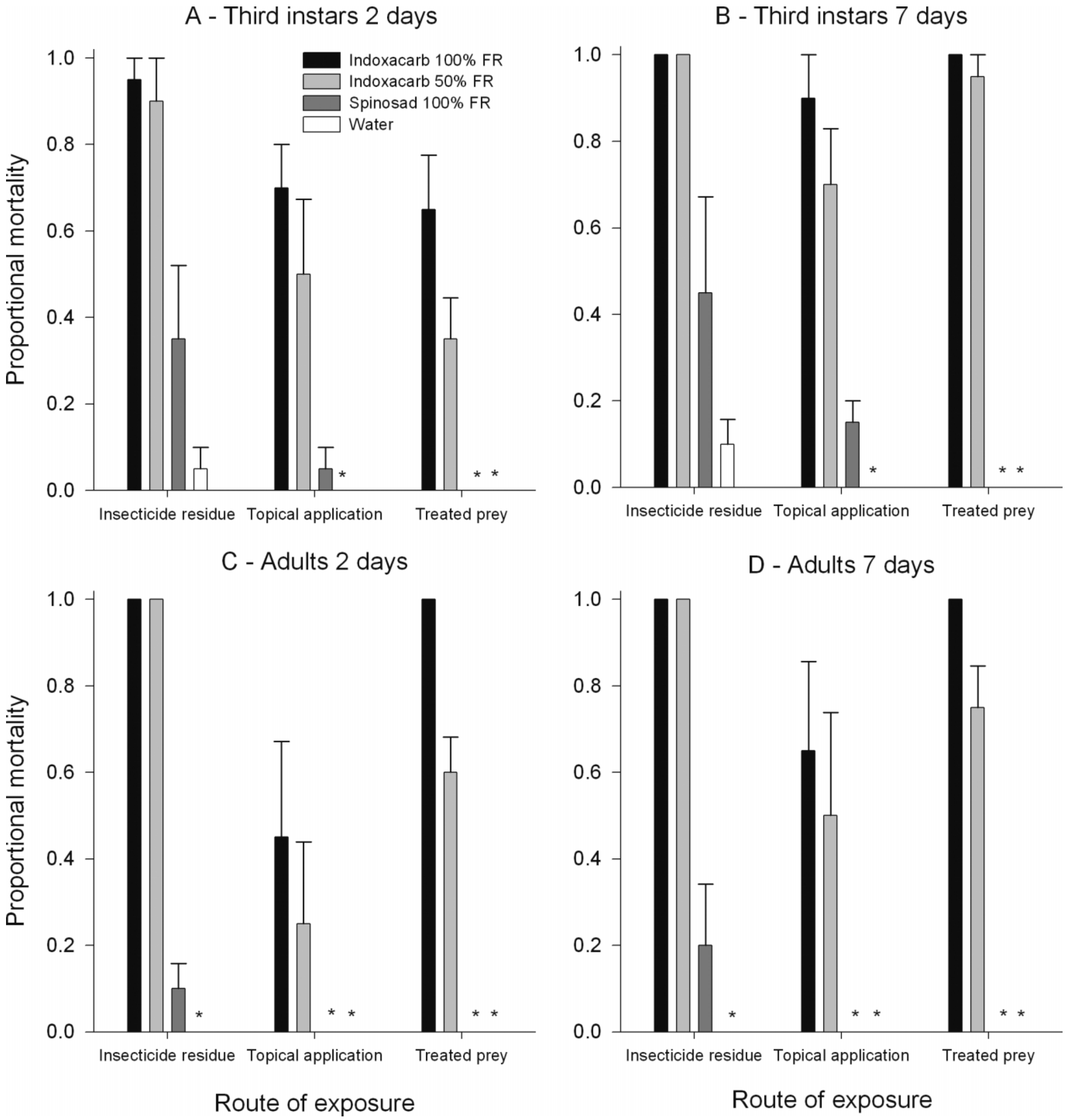

Figure 1. Proportional mortality (±SE) of *Harmonia axyridis* after third instars and adults were exposed to indoxacarb at 100 or 50% of field rate (FR), or spinosad at 100% FR or water via insecticide residues, topical application and treated prey (* denotes that no mortality was observed).

showing insecticidal activity to third instars and adults of *H. axyridis* (indoxacarb) or only to third instars (spinosad).

The results of the present study indicate that the toxicity of indoxacarb and spinosad to *H. axyridis* may vary with route of exposure. Larvae of this predator were more susceptible to both insecticides when in contact with residues than when exposed to topical application. Adults, however, were more susceptible to both insecticides when in contact with residues and treated prey than when exposed to topical application. In a recent review,[30] authors concluded that spinosad was more insecticidal to predators when exposed to residues than when predators were directly sprayed or fed with treated prey. In addition, spinosad-treated surfaces caused the highest mortality to lacewing adults compared with those that were directly sprayed or fed treated prey.[35] In contrast, spinosad was more insecticidal to the lady beetles *Cycloneda sanguinea* (L.) and *H. axyridis*[36] and to the big-eyed bug *Geocoris punctipes* (Say)[18] when topically applied as opposed to exposure to residues. Previous studies have shown that indoxacarb was more insecticidal to *G. punctipes*[18] and *P. maculiventris*[13] via oral toxicity than via residues. However, in both studies, predators were in contact with indoxacarb-treated prey for a

Table 2. Odds ratio estimates[a] from logistic regression comparing the reference parameters water and topical application with insecticides and other routes of exposure, respectively, for the proportional mortality of *Harmonia axyridis* third instars and adults 2 and 7 days after insecticide treatment in the laboratory

Effect	Estimate[b]	95% Wald confidence limits[c]
	Third instars 2 days after treatment	
Indoxacarb 100% FR versus water	127.93	31.25–523.73
Indoxacarb 50% FR versus water	49.28	12.84–189.19
Spinosad 100% FR versus water	3.86	1.00–14.88
Insecticide residues versus topical application	5.14	1.95–13.54
Treated prey versus topical application	0.58	0.24–1.41
	Third instars 7 days after treatment	
Indoxacarb 100% FR versus water	>999.99	76.23 to >999.99
Indoxacarb 50% FR versus water	675.23	39.77 to >999.99
Spinosad 100% FR versus water	8.94	0.93–85.68
Insecticide residues versus topical application	11.81	1.27–109.38
Treated prey versus topical application	1.83	0.24–14.12
	Adults 2 days after treatment	
Indoxacarb 100% FR versus water	>999.99	46.05 to >999.99
Indoxacarb 50% FR versus water	333.41	12.74 to >999.99
Spinosad 100% FR versus water	2.60	0.10–67.06
Insecticide residues versus topical application	60.98	5.99–620.77
Treated prey versus topical application	9.14	1.91–43.73
	Adults 7 days after treatment	
Indoxacarb 100% FR versus water	>999.99	62.53 to >999.99
Indoxacarb 50% FR versus water	592.59	26.33 to >999.99
Spinosad 100% FR versus water	4.73	0.27–83.38
Insecticide residues versus topical application	29.04	3.26–258.44
Treated prey versus topical application	4.68	1.07–20.48

[a] Odds ratio estimates represent the odds of *H. axyridis* being killed by a parameter (e.g., indoxacarb 100% FR) compared with the reference parameter (e.g., water).
[b] Indicates how many times more the odds are for *H. axyridis* to die when exposed to a parameter (e.g., indoxacarb 100% FR) than to the reference parameter (e.g., water).
[c] If the confidence interval contains 1, then the two effects were not significantly different at the $\alpha = 0.05$ level.

longer period of time, compared with the exposure to residues. In the present study, *H. axyridis* was in contact with residues and treated prey for the same amount of time. In addition to the differences in time of insecticide exposure, variability in insecticidal susceptibility among different orders, species and populations of insects[37,38] may also explain the higher insecticidal activity of indoxacarb against *G. punctipes* and *P. maculiventris* via oral toxicity than via residues. These differences may be attributed to mechanisms of detoxification and to routes of insecticide penetration that may be unique in an individual of particular order, species or population.

It is not completely understood why *H. axyridis* is more susceptible when exposed to insecticide residues or treated prey than when topically sprayed. The insecticidal activity of indoxacarb when exposed orally is probably related to its mode of bioactivation. When orally applied, indoxacarb is more rapidly bioactivated to its sodium channel blocking *N*-decarbomethoxylated metabolite than when topically applied.[18] The relatively higher insecticidal activity of indoxacarb and spinosad via residues may be explained by the insecticide dose or time of insecticide exposure. Individuals may be exposed to a greater amount of insecticide active ingredient via residues than via treated prey or topical application. In the present study, when *H. axyridis* was exposed via residues, the insecticides were applied to the inside of the top and bottom of Petri dishes (60×15 mm). The treated area that insects were exposed to was $\approx 5600\,mm^2$. Since the spray chamber was calibrated to deliver $233.9\,L\,ha^{-1}$ ($2.339 \times 10^{-4}\,mL\,mm^{-2}$), each Petri dish (top and bottom) received 1.30 mL of spray solution (water and insecticide). By contrast, when *H. axyridis* was exposed via treated prey, the insecticides covered the surface area of 40 aphids ($\approx 20\,mm^2$) or 280 times less active ingredient than insecticide residues for third-instar *H. axyridis*, and 80 aphids ($\approx 40\,mm^2$) or 140 times less active ingredient than insecticide residues for adult *H. axyridis*. This amounts to 280 and 140 times less potential exposure via treated prey compared with residues for third instars and adults respectively. When *H. axyridis* was exposed via topical application, the insecticides covered the surface area of the third instar or adult ($\approx 36\,mm^2$), resulting in 155 times less potential exposure by topical application than via insecticide residues. Therefore, the amount of active ingredient to which an individual was potentially exposed in the residue treatments was considerably greater than that in the treated prey or topical application treatments.

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Table 3. Contrasts of the proportional mortality of *Harmonia axyridis* third instars and adults 2 and 7 days after insecticide treatment in the laboratory. Contrasts were calculated using exponential estimates for pairwise combinations of insecticides and routes of exposure that were not compared using the odds ratio

Contrast	DF	Wald χ^2	*P*
Third instars 2 days after treatment			
Indoxacarb 100% FR versus indoxacarb 50% FR	1	4.59	0.0322
Indoxacarb 100% FR versus spinosad 100% FR	1	38.60	<0.0001
Indoxacarb 50% FR versus spinosad 100% FR	1	23.46	<0.0001
Insecticide residues versus treated prey	1	18.16	<0.0001
Third instars 7 days after treatment			
Indoxacarb 100% FR versus indoxacarb 50% FR	1	0.79	0.3752
Indoxacarb 100% FR versus spinosad 100% FR	1	14.61	0.0001
Indoxacarb 50% FR versus spinosad 100% FR	1	16.47	<0.0001
Insecticide residues versus treated prey	1	3.18	0.0745
Adults 2 days after treatment			
Indoxacarb 100% FR versus indoxacarb 50% FR	1	3.56	0.0593
Indoxacarb 100% FR versus spinosad 100% FR	1	20.59	<0.0001
Indoxacarb 50% FR versus spinosad 100% FR	1	14.46	0.0001
Insecticide residues versus treated prey	1	2.87	0.0905
Adults 7 days after treatment			
Indoxacarb 100% FR versus indoxacarb 50% FR	1	1.81	0.1781
Indoxacarb 100% FR versus spinosad 100% FR	1	24.01	<0.0001
Indoxacarb 50% FR versus spinosad 100% FR	1	19.19	<0.0001
Insecticide residues versus treated prey	1	2.87	0.0900

The duration of insecticide exposure could also explain the relatively higher insecticidal activity of indoxacarb and spinosad via residues compared with the other routes. *Harmonia axyridis* that were exposed to insecticides via residues were held in treated Petri dishes for 24 h, while beetles exposed to insecticides via topical application were in contact with insecticides during the time of insecticide application only, i.e., 20–30 s. In addition, the metal mesh that covered the Petri dishes during topical application could have intercepted some insecticide and therefore reduced the final dose received by the insects.

This study showed that, under laboratory conditions, indoxacarb is insecticidal to adults and larvae of *H. axyridis* and that spinosad is not as insecticidal as indoxacarb to this predator. In a sweetcorn IPM program, indoxacarb could potentially impact *H. axyridis* populations and decrease the contribution offered by this predator to control pests. Therefore, to obtain the best results of integrating chemical and biological controls in sweetcorn, spinosad may be preferred over indoxacarb when *H. axyridis* is the most abundant natural enemy. This study also showed that route of exposure alters insecticide toxicity to *H. axyridis*. The present findings suggest that the assessment of the toxicity of selective insecticides, such as indoxacarb and spinosad, should be determined via all routes of exposure likely to occur in the field, because the toxicity can vary significantly among routes of exposure. Future studies should concentrate on explaining the reasons for differential susceptibility of *H. axyridis* when exposed to the same insecticide via different routes of exposure. Results of these studies should improve the understanding of the toxicodynamics of the insecticides in insects, and thereby could be used to enhance the conservation of natural enemies through ecological and physiological selectivity.

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