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Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae)

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Abstract

Use of selective insecticides, such as spinosad and indoxacarb, that are more toxic to lepidopteran pests than to *Harmonia axyridis* (Pallas), could facilitate conservation of this predator in sweet corn integrated pest management (IPM). We examined the effects of spinosad and indoxacarb on survival, development, and reproduction of *H. axyridis* by spraying first instars and adult females. Treatments for the first instar assay were spinosad at 10, 25, and 50% of the field rate (FR), indoxacarb at 10% FR, and water (untreated check). We recorded survival of each life stage, developmental time to adults, and adult weight. Treatments for the adult female assay were spinosad at 50 and 100% FR, indoxacarb at 50% FR, and water (control). Each day, we recorded female survival and reproductive capacity. Indoxacarb decreased survival of first instars and adults, extended the developmental time for first instars to become adults, and reduced the fecundity of *H. axyridis* females. Spinosad decreased survival of first instars, extended the time for first instars to become adults, decreased weight gain, and reduced the fertility of *H. axyridis* females. Our results suggest that spinosad and indoxacarb may reduce *H. axyridis* population growth by affecting its survival, development, and reproduction. We also conclude that indoxacarb, when applied at 10% FR, has more lethal and sublethal effects on *H. axyridis* than spinosad applied at 10, 25 or 50% FR. The importance of sublethal effects of insecticides, as well as acute toxicity, in toxicological studies with natural enemies is discussed within the context of biological control and IPM.
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Keywords: *Harmonia axyridis*; Conservation biological control; Sublethal effects; Insecticides; Toxicity

1. Introduction

Sweet corn, throughout much of the US is intensively managed with insecticides for the European corn borer, *Ostrinia nubilalis* (Hübner), and the corn earworm, *Helicoverpa zea* (Boddie) (Bartels and Hutchison, 1995; Hutchison et al., 2004). The extensive use of insecticides in crop systems, however, may cause resurgence of the primary pest, replacement by secondary pest populations (Elzen, 2001), environmental contamination (Frank et al., 1990), effects on nontarget organisms (Croft, 1990), and development of pest resistance (Brattsten et al., 1986). Therefore, alternatives to chemically intensive pest management are necessary. In light of this necessity, the incorporation of biological control offered by existing natural enemies into integrated pest management (IPM) programs in sweet corn could reduce insecticide applications, thus benefiting growers, consumers, and the environment. The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), is a common predator in sweet corn (Musser and Shelton, 2003a; Wold et al., 2001), preying on *O. nubilalis* eggs (Hoogendoorn and Heimpel, 2002; Musser and Shelton, 2003b) and on a secondary pest, the corn leaf aphid, *Rhopalosiphum maidis* Fitch (Hoogendoorn and Heimpel, 2004). Conservation of

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this predator may enhance biological control of these pests in sweet corn.

The relatively new insecticides, spinosad and indoxacarb, are effective against several lepidopteran pests (Burkness et al., 2003; Wing et al., 2000). Since the primary pests in sweet corn are lepidopterans (i.e., *O. nubilalis* and *H. zea*), use of these insecticides on this crop is expected to increase. Spinosad is a macrocyclic lactone that causes involuntary muscle contractions, tremors, and eventually paralysis of treated insects (Salgado, 1998). Indoxacarb is an oxadiazine that makes treated insects stop feeding and go into mild convulsions or permanent paralysis (Tillman et al., 2001; Wing et al., 2000).

Spinosad and indoxacarb have shown relatively low toxicity to natural enemies (Michaud and Grant, 2003; Sparks et al., 2001) and have therefore been designated as reduced-risk insecticides (Organic Materials Review Institute, 2002). Most studies that showed the toxicity of these insecticides to natural enemies, however, used acute mortality as the primary criterion for susceptibility (Galvan et al., 2005; Michaud and Grant, 2003; Miles and Dutton, 2000; Tillman and Mulrooney, 2000). Acute toxicity tests, however, detect only lethal effects of insecticides (Stark and Banks, 2003; Stark et al., 1997). Sublethal effects of insecticides that show low toxicity to natural enemies and leave many survivors after treatment cannot be fully evaluated using acute toxicity approaches (Wennergren and Stark, 2000). For example, 80% of adult parasitic wasps, *Catolaccus grandis* (Burks), survived after exposure to spinosad (Elzen et al., 2000). However, no *C. grandis* pupae developed from *Anthonomus grandis grandis* Boheman second instars treated with spinosad at only 1/4 of the rate that resulted in 80% adult *C. grandis* survival (Elzen et al., 2000).

Research on sublethal effects seeks to discover the negative, nonlethal impacts of insecticides on various life history parameters that may affect population dynamics (e.g., Stark and Banks, 2003). Insecticides have been shown to have effects on sex ratio and time to adult emergence (Vinson, 1974), fecundity and development of the female ovipositor (Lawrence, 1981), egg hatch (Stark et al., 1992), weight gain and locomotory behavior (Vincent et al., 2000), pre-oviposition period and mutations in offspring (Stark and Banks, 2003), and feeding behavior (Singh et al., 2004). By ignoring these sublethal effects, toxicological studies that evaluate only lethal effects may underestimate the negative impacts of insecticides on natural enemy populations.

Few studies have examined the sublethal effects of insecticides on Coccinellidae (e.g., Grafton-Cardwell and Gu, 2003; Liu and Stansly, 2004; Mani et al., 1997). For *H. axyridis*, various insecticides have been shown to have sublethal effects on weight gain (methoxyfenozide) (Carton et al., 2003), mobility (imidacloprid and λ-cyhalothrin) (Provost et al., 2003; Vincent et al., 2000), and fecundity (imidacloprid) (Weissenberger et al., 1997). Because of the paucity of information on sublethal effects of spinosad and indoxacarb on *H. axyridis* and the likely increase in the use of these chemistries in agriculture, there is a need for examination of their sublethal effects. The objective of this research was to evaluate the impacts of spinosad and indoxacarb on survival, development, and reproductive capacity of *H. axyridis*.

2. Materials and methods

2.1. Insects

Harmonia axyridis were obtained from a laboratory colony founded from adults collected during October 2003 at the Rosemount Research and Outreach Center, University of Minnesota, Rosemount, MN. Following collection, beetles were held in 1.96 L plastic dishes with ~200 beetles per dish, and maintained at 10 ± 1 °C and a photoperiod of 16:8 (L:D) h. Prior to experimentation, the dishes containing beetles were warmed to 25 ± 1 °C with a photoperiod of 16:8 (L:D) h (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, and pea aphids, *Acyrthosiphon pisum* (Harris), a diet made from freeze-dried drone honey bee, *Apis mellifera* L., pupae (Okada and Matsuka, 1973), 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, the 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 of the F_1 generation were placed individually into separate plastic petri dishes (60 × 15 mm), and were reared to the desired developmental stages (i.e., first instars or adults) on a diet of freeze-dried drone *A. mellifera* pupae.

2.2. First instar assay

The study was conducted as a randomized complete block design for first instar *H. axyridis*. The experiment consisted of five treatments and three replications through time, with 20–25 individuals per replication. Treatments used in this study included: spinosad (SpinTor 2SC, Dow AgroSciences LLC) at 10% of the field rate (FR) [0.011 kg AI/ha (0.0094 lb AI/ac)]; spinosad at 25% FR [0.0275 kg AI/ha (0.0235 lb AI/ac)]; spinosad at 50% FR [0.055 kg AI/ha (0.047 lb AI/ac)]; indoxacarb (Avaunt WG, E.I. du Pont de Nemours and Company) at 10% FR [0.0062 kg AI/ha (0.0055 lb AI/ac)]; and an untreated check (i.e., water). Partial rates for spinosad (10, 25, and 50% FR) and indoxacarb (10% FR) were used because field rates

were highly toxic to first instars in previous study (Galvan et al., 2005). Treatments were applied using a motorized spray chamber with a single XR-Teejet 8002 flat fan nozzle, as in Galvan et al. (2005). The sprayer was calibrated to deliver the equivalent of 233.87 L/ha (25 gal/ac) at 242.32 kPa (35 psi). For treatment application, 20–25 larvae (24 ± 6 h after emergence) were placed into plastic petri dish bottoms (150 × 15 mm), which were then placed into the spray chamber. After larvae in the petri dish bottoms were treated, they were removed from the spray chamber, covered, and allowed to dry for 1 h before individuals were transferred to separate untreated plastic petri dishes (60 × 15 mm) lined with 55 mm filter paper disks, and held at the aforementioned environmental conditions. Larvae were provided an ad libitum supply of freeze-dried drone *A. mellifera* pupae, and water as described above. Survival and developmental stage of each individual was checked every 24 h from treatment to adult emergence. Instars were distinguished based on stage specific descriptions provided by Koch (2003). For larvae and adults, survival was defined as the ability to crawl when stimulated with a fine camel-hair brush. Larvae considered dead were maintained and monitored for possible recovery until the end of the experiment. However, no larvae recovered after being classified as dead. For pupae, survival was based on the ability to molt to the next stage. If an adult did not emerge in 10 days, pupa was considered dead. Adults were weighed within 24 ± 6 h after emergence.

Mean proportionate survival of each instar, pupae, and adults for each replication were transformed by arcsine square root (Southwood and Henderson, 2000). Mean developmental time for each stage for each replication was square root transformed. Transformed proportions and means were analyzed across sample periods using repeated measures analysis of variance with a first order autoregressive covariance structure (Proc mixed; SAS Institute, 2000) (Galvan et al., 2005; Koch et al., 2005). The analysis of variance model included main effects for stage, treatment, and the interaction of the main effects. If the main effect for treatment (across sample periods) was significant ($P < 0.05$), then differences among levels were tested for using Bonferroni adjusted contrasts of the least squares means for each pairwise combination of levels. Results of this analysis, presented throughout the text, refer to differences among treatments across sample periods.

Weights of *H. axyridis* adults were averaged within replications for each treatment. Adult weights were analyzed using analysis of variance ($P < 0.05$) (Proc ANOVA; SAS Institute, 2000).

2.3. Female adult assay

After emergence, 25 females and 25 males were allowed to mate for 14 days in a 1.96-L plastic dish. The adults were provided an ad libitum supply of live *A. glycines* and *A. pisum*, and freeze-dried drone *A. mellifera* pupae, and water as described above. Females were then separated from males and used in the assays.

The study was conducted as a randomized complete block design for adult female *H. axyridis*. The experiment consisted of four treatments and three replications through time, with 15–20 mated females per replication. Treatments used in this study included: spinosad at 50% FR [0.055 kg AI/ha (0.047 lb AI/ac)]; spinosad at 100% FR [0.11 kg AI/ha (0.094 lb AI/ac)]; indoxacarb at 50% FR [0.031 kg AI/ha (0.0275 lb AI/ac)]; and an untreated check (i.e., water). Partial rates for spinosad (50% FR) and indoxacarb (10% FR) were used because field rates were highly toxic to adults in a previous study (Galvan et al., 2005). Treatments were applied as described for the first instar assay. After females in the petri dish bottoms were treated, they were removed from the spray table, covered, and allowed to dry for 1 h before individuals were transferred to separate untreated plastic petri dishes (60 × 15 mm) lined with 55 mm filter paper disks. The females were provided an ad libitum supply of live *A. glycines* and *A. pisum*, and freeze-dried drone *A. mellifera* pupae, and water as described above. The petri dishes containing females were daily checked for oviposition and survival at 15:00 h for 30 days. If eggs were found, the females were removed and transferred to new petri dishes (60 × 15 mm) provisioned with food and water. Eggs were maintained at 25 ± 1 °C and a photoperiod of 16:8 (L:D) h for 14 days or until egg hatch. Each day, the number of live females, females laying eggs, eggs laid per female and hatched eggs was recorded. The terms fecundity (number of eggs laid per female) and fertility (proportionate egg hatch) used by other authors (i.e., Medina et al., 2004; Stark et al., 1992) will be employed here. Female survival was defined as the ability to crawl when stimulated with a fine camel-hair brush. Fertility was defined as the ability of neonates to emerge and exit their chorion.

The proportion of females surviving, females laying eggs, egg hatch, and the number of eggs laid per female over 30 days were averaged within replications for each treatment. The proportions were transformed by arcsine square root (Southwood and Henderson, 2000). Transformed proportions and the means of eggs laid per female were analyzed using ANOVA. Proportions and means were separated using the protected Fisher's least significant difference (LSD) test ($P < 0.05$) (SAS Institute, 2000).

3. Results

3.1. First instar

Tested rates of spinosad (10, 25 or 50% FR) and indoxacarb (10% FR) showed lethal and sublethal effects to *H. axyridis* larvae, with a dose response observed across

rates of spinosad (Fig. 1; Tables 1 and 2). Survival in all insecticide treatments (42 ± 2 to 88 ± 5%) was significantly lower than in the untreated check (96 ± 4%) (Fig. 1; Tables 1 and 2). Survival of *H. axyridis* treated with spinosad at 10 or 25% FR was significantly higher than that of beetles treated with spinosad at 50% FR or indoxacarb at 10% FR (Fig. 1; Tables 1 and 2). Survival of *H. axyridis* treated with indoxacarb at 10% FR was the lowest among all treatments (Fig. 1; Tables 1 and 2). All insecticide treatments resulted in a rapid decline in survival by the end of first instar (Fig. 1). Survival remained relatively constant through the other life stages (Fig. 1). Tested rates of spinosad (10, 25 or 50% FR) and indoxacarb (10% FR) increased the developmental time of *H. axyridis* compared with untreated individuals (Tables 1 and 2). Developmental time of *H. axyridis* treated with spinosad at 25 or 50% FR, or indoxacarb at 10% FR was significantly longer (17.96 ± 0.14 to 18.29 ± 0.36 days) than the untreated check (17.14 ± 0.35 days) (Tables 1 and 2). Developmental time of *H. axyridis* treated with spinosad at 10% FR did not differ significantly from the untreated check (Tables 1 and 2). There was a trend for decreasing *H. axyridis* weight with increasing rates of spinosad ($F = 3.37$; $df = 4, 2$; $P = 0.0674$) (Table 2).

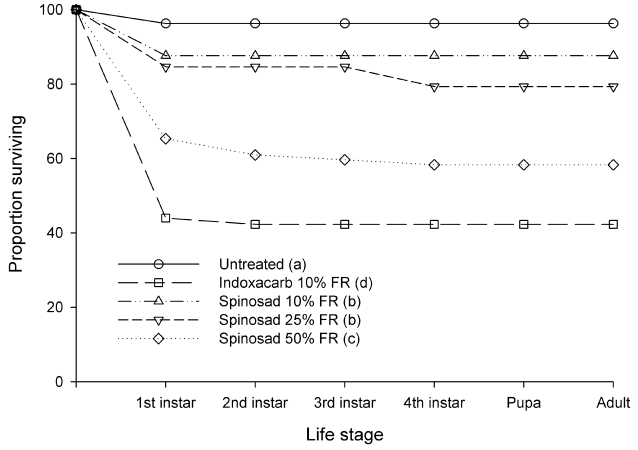

Fig. 1. Survival of *H. axyridis* after first instars (24 h ± 6 old) were treated with spinosad at 10, 25 or 50% of field rate (FR) or indoxacarb at 10% FR. Treatments in the legend followed by the same letters in parentheses do not differ significantly ($P < 0.05$), repeated measures analysis of variance and Bonferroni adjusted contrasts.

Table 1
Repeated measures analysis of variance for survival and developmental time of *H. axyridis* after first instars were treated with spinosad (10, 25 or 50% FR) and indoxacarb (10% FR)

Source	*df*	*F*	*P*
Survival			
Stage	6, 70	35.62	<0.0001
Treatment	4, 70	92.52	<0.0001
Stage × Treatment	24, 70	2.64	0.0009
Developmental time			
Stage	4, 50	1011.13	<0.0001
Treatment	4, 50	15.83	<0.0001
Stage × Treatment	16, 50	3.14	0.0010

Table 2
Lethal and sublethal effects of indoxacarb and spinosad on weight, developmental time, and survival of newly emerged *H. axyridis* adults after they were sprayed as first instars (age: 24 ± 6 h)

Treatment	Mean (±SEM) survival (proportion)[a,b]	Mean (±SEM) developmental time (day)[a]	Mean (±SEM) weight (mg)[c]
Untreated	0.96 ± 0.04a	17.14 ± 0.35a	29.69 ± 2.86a
Indoxacarb 10% FR[d]	0.42 ± 0.02d	17.96 ± 0.14b	27.61 ± 3.78a
Spinosad 10% FR	0.88 ± 0.05b	17.83 ± 0.03ab	27.84 ± 3.43a
Spinosad 25% FR	0.79 ± 0.06b	18.29 ± 0.36bc	26.68 ± 3.57a
Spinosad 50% FR	0.58 ± 0.03c	18.86 ± 0.12c	25.31 ± 2.06a

[a] Means were analyzed using repeated measures analysis of variance and Bonferroni adjusted contrasts; means followed by the same letter do not differ significantly ($P < 0.05$).
[b] Mean survival of adults was determined 1 day after emergence.
[c] Means were analyzed using analysis of variance; means followed by the same letter do not differ significantly ($P < 0.05$).
[d] FR, field rate.

3.2. Female adults

Indoxacarb and spinosad affected different life history parameters for adult female *H. axyridis* (Table 3). Indoxacarb at 50% FR reduced survival and fecundity of *H. axyridis* females (Table 3). The proportion of females laying eggs, however, was not statistically different among treatments ($F = 3.59$; $df = 3, 2$; $P = 0.0857$) (Table 3). Spinosad at 50 or 100% FR reduced fertility of *H. axyridis* females (Table 3). The relative production of female offspring showed that indoxacarb at 50% FR caused the greatest reduction in production of female offspring, and was followed by spinosad at 100% FR and spinosad at 50% FR (Table 3). The effects of spinosad in reducing fertility lasted for 2 weeks, after which the females appeared to recover from any insecticidal effects (Fig. 2).

4. Discussion

The International Organization of Biological Control (IOBC) suggests a tiered approach to evaluate the potential effects of insecticides on natural enemies with laboratory studies followed by semi-field and field tests (Hassan, 1998). According to the IOBC, once an insecticide is tested in the laboratory and shows no toxicity to natural enemies, no further semi-field or field studies are needed (Hassan, 1998). Nonetheless, as our data indicate, this approach ignores potential sublethal effects that insecticides may have on natural enemies. For example, we observed that spinosad did not affect the survival of *H. axyridis* adults, but did reduce female fertility.

Spinosad and indoxacarb have shown high toxicity to lepidopteran pests (Burkness et al., 2003; Wing et al., 2000)

Table 3
Lethal and sublethal effects of indoxacarb and spinosad on *H. axyridis* females that were treated 14 days after adult emergence

Treatment	Mean (±SEM)				
	Proportion surviving[a]	Proportion of females laying eggs[a]	Number of eggs laid per female (fecundity)[a]	Proportion of egg hatch (fertility)[a]	Relative production of female offspring[b]
Untreated	1.00 ± 0.00a	0.57 ± 0.01a	864.69 ± 16.10a	0.99 ± 0.00a	1.00
Indoxacarb 50% FR[c]	0.60 ± 0.15b	0.37 ± 0.05a	639.27 ± 9.03b	0.95 ± 0.02ab	0.28
Spinosad 50% FR	1.00 ± 0.00a	0.56 ± 0.03a	889.16 ± 63.12a	0.85 ± 0.09bc	0.87
Spinosad 100% FR	1.00 ± 0.00a	0.59 ± 0.07a	849.20 ± 38.64a	0.75 ± 0.08c	0.77
P	0.0032	0.0857	0.0328	0.0071	
F	15.27	3.59	5.82	11.27	
df	3, 2	3, 2	3, 2	3, 2	

[a] Means followed by same letters do not differ significantly ($P < 0.05$); protected least significant difference test (LSD).
[b] This number was calculated as the relativized product of the proportion of females surviving, proportion of females laying eggs, number of eggs laid per female, proportion of eggs hatching, and 0.5 for a 1:1 sex ratio (Heimpel and Lundgren, 2000).
[c] FR, field rate.

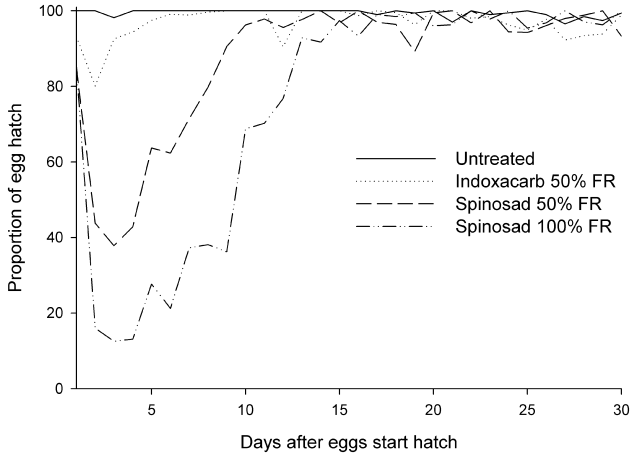

Fig. 2. Fertility (proportionate egg hatch) of *H. axyridis* after mated females (14 days after adult emergence) were treated with spinosad and indoxacarb (FR, field rate).

and low acute toxicity to natural enemies (Galvan et al., 2005; Michaud and Grant, 2003; Tillman and Mulrooney, 2000). Our results, however, showed that both spinosad and indoxacarb, even at reduced rates, have negative impacts on important life history parameters of *H. axyridis*.

Indoxacarb caused a decrease in survival of first instars and adults, extended the time for first instars to become adults, and reduced the fecundity of *H. axyridis* females. We are aware of only one study that evaluated the sublethal effects of indoxacarb on *H. axyridis*. Contrary to our results, Michaud and Grant (2003) reported that indoxacarb did not extend the developmental time of *H. axyridis*. However, we cannot explain the contradictory results found by both studies because the methods differ in each study and the data reported by Michaud and Grant (2003) are not shown.

Spinosad is classified as an environmentally and toxicologically reduced-risk insecticide by the United States Environmental Protection Agency (EPA, 1997), and it is used in practical IPM as a biorational insecticide (Williams et al., 2003). However, our results showed that spinosad decreased survival of first instars, extended the time from first instar to adult, decreased weight gain, and reduced female fertility. We are unaware of any other studies reporting sublethal effects of spinosad on *H. axyridis* or on any other coccinellid. Most insect predators, such as *Chrysoperla carnea* (Stephens) (Medina et al., 2003; Viñuela et al., 2001) and *Orius insidiosus* (Say) (Elzen, 2001) did not show changes in reproductive and developmental time parameters following exposure to spinosad. However, Medina et al. (2003) observed that spinosad shortened the life span of *C. carnea*, and Yoo and Kim (2000) observed a reduction in the fecundity of predatory mites. By contrast, several parasitic wasps show some sensitivity to spinosad (Williams et al., 2003). For example, Schneider et al. (2004) reported decreased adult emergence and longevity of the endoparasitoid, *Hyposoter didymator* (Thunberg), treated with spinosad, and 14 out of 15 studies reported some sort of sublethal effects on parasitoids following treatment with spinosad (reviewed by Williams et al., 2003).

Explanations regarding differential susceptibility among life stages, and how insecticides affect the development of *H. axyridis* are unclear. Early life stages of natural enemies are known to be more susceptible to insecticides than the later life stages (e.g., Youn et al., 2003). Differential susceptibility may occur because of changes in enzyme activity and target-site sensitivity (Cho et al., 2002). Extended developmental time of treated individuals may be partially explained by reduced food uptake as a consequence of insecticidal effects (e.g., paralysis) in the earlier instars. This decline in feeding could also contribute to a decrease in weight gain.

Specific reasons why spinosad and indoxacarb reduced the reproductive capacity of *H. axyridis* are unknown. Grosch and Hoffman (1973) suggested that the reduction in fecundity of parasitic wasps treated with insecticides could be related to decreased food uptake, disturbed somatic physiology, or cytotoxic destruction of potential

eggs. Electron microscopy studies showed that follicles of *C. carnea* females treated with azadirachtin were smaller than those of untreated females (Medina et al., 2004).

This study showed that spinosad and indoxacarb have lethal and sublethal effects on *H. axyridis*, which may affect population dynamics in the field via reduction in survival and reproduction. Although spinosad reduced fertility for only 2 weeks, this reduction may decrease *H. axyridis* population densities and, as a result, its capacity to control pests. For example, aphids are abundant in corn fields for only four to six weeks (Schellhorn and Andow, 1999) and a decrease of *H. axyridis* fertility for 2 weeks may limit the ability of this predator to maintain adequate predator–prey ratios necessary to suppress aphid populations. Both insecticides extended the developmental time from first instar to adult, which may delay the beginning of the reproductive period and, consequently, affect the timing and population size of next generation. However, because indoxacarb also decreased fecundity and showed greater acute toxicity than spinosad, the negative impacts of this insecticide on the overall reproductive capacity of *H. axyridis* were greater than those of spinosad.

Even though both insecticides conferred lethal and sublethal effects on *H. axyridis*, they likely will provide more benefits to an IPM program in sweet corn than conventional insecticides that have shown high acute toxicity within the Coccinellidae (e.g., Galvan et al., 2005; Michaud and Grant, 2003; Musser and Shelton, 2003a). Our results also emphasize the limitations of toxicological studies based solely on acute toxicity. Further work is needed to evaluate the toxicity of spinosad and indoxacarb using other routes of exposure such as leaf residues and treated prey.

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