Toxicity of Commonly Used Insecticides in Sweet Corn and Soybean to Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae)

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ABSTRACT Use of insecticides with low toxicity to natural enemies is an important component of conservation biological control. In this study, we evaluated the toxicity of insecticides used in sweet corn, *Zea mays* L., and soybean, *Glycine max* (L.) Merr., to the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), under laboratory and field conditions. Field experiments conducted in sweet corn in 2003 and 2004 and in soybean in 2003, showed that *H. axyridis* was the most abundant predator. In sweet corn, densities of *H. axyridis* larvae in plots treated with spinosad or indoxacarb were generally higher than in plots treated with chlorpyrifos, carbaryl, bifenthrin, and λ-cyhalothrin. In soybean, densities of *H. axyridis* larvae in plots treated with chlorpyrifos were higher than in plots treated with λ-cyhalothrin. Laboratory experiments were conducted to evaluate the acute toxicity of insecticides to eggs, first and third instars, pupae, and adults. Spinosad, followed by indoxacarb, were the least toxic insecticides for all life stages of *H. axyridis*. Conventional insecticides showed high toxicity to *H. axyridis* when applied at field rates under laboratory conditions. Overall, first instars were most susceptible to the insecticides tested, followed by third instars and adults, eggs, and pupae. Our results suggest that spinosad, and to a lesser extent indoxacarb, offer reduced toxicity to *H. axyridis* and would be beneficial for conservation biological control in agricultural systems where *H. axyridis* is abundant.

KEY WORDS *Harmonia axyridis*, conservation biological control, sweet corn, soybean, topical assay

CONSERVATION BIOLOGICAL CONTROL IS the use of human-induced environmental modifications to benefit natural enemies (Obrycki and Kring 1998). The adverse impact of insecticides on natural enemies can be mitigated through choice of insecticide, dosage, or timing of insecticide application (Obrycki and Kring 1998). Biological control and selective insecticides have proven to be compatible tactics in integrated pest management (IPM) programs (Giles and Obrycki 1997). Integrating biological control with selective insecticides also can minimize the likelihood of pest resurgence (Johnson and Tabashnik 1999) and possibly reduce the number of insecticide applications (Hutchison et al. 2004b).

Sweet corn, *Zea mays* L., and soybean, *Glycine max* (L.) Merr., in Minnesota could benefit from the use of conservation biological control. Minnesota is a leading state in the United States for sweet corn production, with 53,419 ha (132,000 acres) grown for processing and 4,047 ha (10,000 acres) grown for fresh market (Hutchison and O'Rourke 2003). Minnesota sweet corn 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. 2004a). *O. nubilalis* typically oviposits on the underside of leaves within the ear zone (Mason et al. 1996). *H. zea* oviposits primarily on fresh corn silks (Flood et al. 1995). After eggs hatch, larvae of both species tend to move into the ear tip (Flood et al. 1995). Once larvae of either species enter the ear, the efficacy of management tactics, including insecticides, is minimal. The narrow time window for effective insecticide application, coupled with the relatively short residual activity of many of the commonly used pyrethroids (Rinkleff et al. 1995), motivates many growers to use an intensive, calendar-based spray program.

Soybean is the most widely grown crop in Minnesota, averaging 2.9 million ha (7.1 million acres) harvested annually over the 2001 and 2002 growing seasons (Hunst and Howse 2003). Despite the large amount of land devoted to soybean, this crop has historically received little insecticidal input (Hunst and Howse 2003). However, after the arrival of the soybean aphid, *Aphis glycines* Matsumara, many soybean fields have been treated with insecticide (Landis et al. 2003). *A. glycines* has potential to cause yield reductions through direct impacts on plant physiology (Macedo et al. 2003) and as a vector of viral pathogens to soybean (Clark and Perry 2002). If insecticide ap-

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plications become necessary on a wide scale, large amounts of active ingredient would be applied over the vast area devoted to soybean.

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), has become a dominant predator in both sweet corn and soybean agroecosystems (Wold et al. 2001, Musser and Shelton 2003a, Rutledge et al. 2004). This coccinellid has been documented feeding on a diversity of prey (e.g., Aphididae, Psyllidae, Coccoidea, and eggs and larvae Lepidoptera and Coleoptera) and was repeatedly introduced to North America as a classical biological control agent (Koch 2003). *H. axyridis* has proven effective at suppressing populations of the target pests such as the pecan aphid complex (Mizell 2003). The biological control offered by *H. axyridis* has "spilled over" from pecan groves into various other systems. In sweet corn, *H. axyridis* has been documented preying on *O. nubilalis* eggs (Hoogendoorn and Heimpel 2002, Musser and Shelton 2003b) and an occasional pest, the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hoogendoorn and Heimpel 2004). In soybean, *H. axyridis* seems to contribute to suppression of the soybean aphid (Fox et al. 2004, Rutledge et al. 2004). One step toward incorporating *H. axyridis* biological control into IPM programs in sweet corn and soybean is to assess its compatibility with commonly used insecticides. The objective of this study was to evaluate the toxicity of selected insecticides used in sweet corn and soybean to *H. axyridis* under field and laboratory conditions, with the purpose of generating IPM guidelines for natural enemy conservation.

Materials and Methods

Field Experiments. Field experiments were conducted in 2003 and 2004 at the Rosemount Outreach and Extension Center, University of Minnesota, Rosemount, MN. For the sweet corn experiments, 'Code-40′ seed (Green Giant, General Mills, Inc., Minneapolis, MN) was planted on 20 May 2003 and 18 May 2004. Seed was planted 2.54 cm (1 in.) in depth with 76.20-cm (30-in.) row spacing at a rate of 59,800 seeds per ha (24,200 seeds per acre) into sandy loam soil. A randomized complete block design was used with four replications and eight treatments in 2003, and five treatments in 2004. Each plot was eight rows by 7.62 m (25 feet). A single fallow skip row separated plots within blocks, and a 3.05-m (10-foot) fallow alley separated blocks. Standard production practices were followed (Foster et al. 2003), including applications of the herbicides metolachlor (Dual II Magnum, Syngenta Crop Protection, Inc., Greensboro, NC) at 1.79 kg (AI)/ha (1.6 lb [AI]/acre) and a marketed combination of primisulurfon and dicamba (North Star, Syngenta Crop Protection, Inc.) at 0.35 kg product/ha (5 oz product/acre).

The insecticides tested in these experiments were applied as in a typical *O. nubilalis* management program, with the first application occurring at ≈5% silk and two subsequent sprays occurring at approximately 1-wk intervals (Hutchison et al. 2004a). In 2003, insecticides were applied on 28 July and 4 and 11 August. In 2004, insecticides were applied on 3, 11, and 17 August. In 2003, treatments included chlorpyrifos (Lorsban 4E, Dow AgroSciences LLC, Indianapolis, IN) at 0.73 kg (AI)/ha (0.65 lb [AI]/acre); carbaryl (Sevin XLR Plus, Bayer CropScience, Kansas City, MO) at 1.96 kg (AI)/ha (1.75 lb [AI]/acre); bifenthrin (Capture 2 EC, FMC Corporation, Philadelphia, PA) at 0.045 kg (AI)/ha (0.040 lb [AI]/acre); λ-cyhalothrin (Warrior CS, Syngenta Crop Protection, Inc.) at 0.028 kg (AI)/ha (0.025 lb [AI]/acre); spinosad (SpinTor 2 SC, Dow AgroSciences LLC) at 0.11 kg (AI)/ha (0.094 lb [AI]/acre); spinosad (Entrust 80 WP, Dow AgroSciences LLC) at 0.11 kg (AI)/ha (0.094 lb [AI]/acre); indoxacarb (Avaunt WG, E.I. du Pont de Nemours and Company, Wilmington, DE) at 0.062 kg (AI)/ha (0.055 lb [AI]/acre); and an untreated check. In 2004, insecticide treatments included: carbaryl (Sevin XLR Plus, Bayer CropScience) at 1.96 kg (AI)/ha (1.75 lb [AI]/acre); bifenthrin (Capture 2 EC, FMC Corporation) at 0.045 kg (AI)/ha (0.040 lb [AI]/acre); spinosad (SpinTor 2 SC, Dow AgroSciences LLC) at 0.11 kg (AI)/ha (0.094 lb [AI]/acre); indoxacarb (Avaunt WG, E.I. du Pont de Nemours and Company) at 0.062 kg (AI)/ha (0.055 lb [AI]/acre); and an untreated check.

Insecticides were applied using a CO_2-pressurized backpack sprayer with a 3.05-m (10-foot) boom with six nozzles (XR-Teejet 8002 flat fan, with no screen). The sprayer was calibrated to deliver 233.87 liters/ha (25 gal/acre) at 242.32 kPa (35 psi). The boom was held overhead to apply the insecticides over the top of the plants. Plots were sampled for generalist predators after each insecticide application (sample dates: 30 July and 7 and 13 August 2003; and 5, 13, and 20 August 2004). On each sample date, 10 randomly selected plants from the middle six rows of each plot were sampled using visual whole-plant inspection (Wold et al. 2001). Adult and immature stages of the various generalist predators were identified using a diagnostic guide (Schellhorn 2003) and voucher specimens in the Insect Museum (Department of Entomology, University of Minnesota). Counts of generalist predators were recorded for each plant.

For the soybean experiment, plots were established in a commercial soybean field double-cropped after peas. In 2003, plots were setup in the southwestern corner of a 26-ha (64.2-acre) field of 'AgroPro 1080 RR' soybean that was planted on 24 June. Seed was planted 3.81 cm (1.50 in.) in depth into sandy loam soil. A randomized complete block design was used with four replications and three treatments. Each plot was eight rows by 7.62 m (25 feet). One row separated plots within blocks, and 3.05 m (10 feet) separated blocks. Plots were maintained using standard production practices, including an application of glyphosate (Honcho, Monsanto Company, St. Louis, MO) at 2.34 liters/ha (32 oz/acre) on 1 August for weed control. The insecticides tested in this experiment were applied to mimic an *A. glycines* management program (i.e., sprays triggered by an action threshold of ≈250 aphids per plant; Hodgson 2004). All insecticide treatments were applied on 24 July. Treatments included: chlorpyrifos (Lorsban 4E, Dow AgroSciences LLC) at 0.73 kg (AI)/ha (0.65 lb [AI]/acre); λ-cyhalothrin (Warrior CS, Syngenta Crop Protection, Inc.) at 0.028 kg (AI)/ha (0.025 lb [AI]/acre); and an untreated check. Insecticides were applied using a CO_2-pressurized back pack sprayer as described above. Plots were sampled on 25 July and 1, 7, and 13 August. On each sample date, randomly selected plants from the middle six rows of each plot were sampled using visual whole-plant inspection. Ten plants were inspected in each plot on the first sample date, and 20 plants were inspected in each plot on subsequent sample dates. Generalist predators were identified and recorded as described above.

For all field experiments, counts of *H. axyridis* were averaged within plots for each sample date, giving an average density per plant for each plot on each sample date. The averaged densities were then transformed using log x + 0.00001. Transformed predator densities were analyzed across sample dates using repeated measures analysis of variance (ANOVA) with a first order autoregressive covariance structure (PROC MIXED, SAS Institute 2000). The ANOVA model included main effects for date, treatment, and the interaction of the main effects. If the main effect for treatment (across sample dates) was significant ($P < 0.05$), then differences among levels were tested 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 dates.

Laboratory Experiment. *H. 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). After collection, beetles were held in 1.96-liter plastic dishes with ≈200 beetles per dish and maintained at 10 ± 1°C and a photoperiod of 16:8 (L:D) h. Before experimentation, the dishes containing beetles were warmed to 25 ± 1°C, and the beetles were allowed to mate for 14 d. Adult female *H. axyridis* were then maintained individually in plastic petri dishes (60 by 15 mm) lined with 55-mm filter paper disks, and held at 25 ± 1°C and a photoperiod of 16:8 (L:D) h. The females were provided ad libitum supply of live *A. glycines*, 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. 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 by 15 mm) provisioned with food and water. Eggs were maintained at 25 ± 1°C and a photoperiod of 16:8 (L:D) h. After egg hatch and dispersal (i.e., ≈1 d after hatching), larvae of the F_1 generation were placed individually into separate plastic petri dishes (60 by 15 mm) and reared to the desired developmental stages at 25 ± 1°C and a photoperiod of 16:8 (L:D) h on a diet of freeze-dried *A. mellifera* drone pupae.

The experiment was conducted as separate randomized complete block designs for eggs, first and third instars, pupae, and adults of *H. axyridis.* The experiment for each stage consisted of seven treatments and three replications over time, with 15 individuals per replication for larval, pupal, and adult stages. For eggs, individual clusters laid on wax paper were considered replications (i.e., one cluster equals one replication). Pupae for use in the experiment were carefully removed from the substrate using a knife.

Treatments used in this experiment included chlorpyrifos (Lorsban 4E, Dow AgroSciences LLC) at 0.73 kg (AI)/ha (0.65 lb [AI]/acre); carbaryl (Sevin XLR Plus, Bayer CropScience) at 1.96 kg (AI)/ha (1.75 lb [AI]/acre); bifenthrin (Capture 2 EC, FMC Corporation) at 0.045 kg (AI)/ha (0.040 lb [AI]/acre); λ-cyhalothrin (Warrior CS, Syngenta Crop Protection, Inc.) at 0.028 kg (AI)/ha (0.025 lb [AI]/acre); spinosad (SpinTor 2 SC, Dow AgroSciences LLC) at 0.11 kg (AI)/ha (0.094 lb [AI]/acre); indoxacarb (Avaunt WG, E.I. du Pont de Nemours and Company) at 0.062 kg (AI)/ha (0.055 lb [AI]/acre); and an untreated check (i.e., water). Treatments were applied using a motorized spray chamber with a single XR-Teejet 8002 flat fan nozzle, as in Katovich et al. (1996). The sprayer was calibrated to deliver the equivalent of 233.87 liters/ha (25 gal/acre) at 242.32 kPa (35 psi). For treatment application, one cluster of eggs or 15 individuals for larval, pupal or adult stages were placed into plastic petri dish bottoms (150 by 15 mm) and then placed into the spray chamber. Eggs were 24 ± 6 h old at treatment, and first and third instars were sprayed 24 ± 1 h after molting. Pupae and adults were sprayed 48 ± 12 h after molting. After treatment, the petri dish bottoms were removed from the spray chamber, covered, and allowed to dry for 1 h before individuals were transferred to clean plastic petri dishes (60 by 15 mm) lined with 55-mm filter paper disks, and held at the aforementioned environmental conditions. Water was provided to all stages as described above. Larvae and adults were provided ad libitum supply of freeze-dried *A. mellifera* drone pupae. Survival of larvae and adults was determined at 0.33, 1, 2, 3, and 5 d and was defined as the ability to crawl when stimulated with a fine camel's-hair brush. For eggs and pupae, survival was based on the ability to molt to the next life stage. Percentage of egg hatch was recorded at 24, 32, 40, and 48 h after treatment. Percentage of adult emergence was recorded every 24 h for 5 d after treatment.

Data for survival of larvae and adults, egg hatch, and adult emergence were transformed using arcsine square root because some observations were <0.2 and >0.8 (Southwood and Henderson 2000). Transformed proportions were analyzed across sample periods using repeated measures ANOVA with a first order autoregressive covariance structure (PROC MIXED, SAS Institute 2000). The ANOVA model included main effects for time, treatment, and the interaction of the main effects. If the main effect for treatment

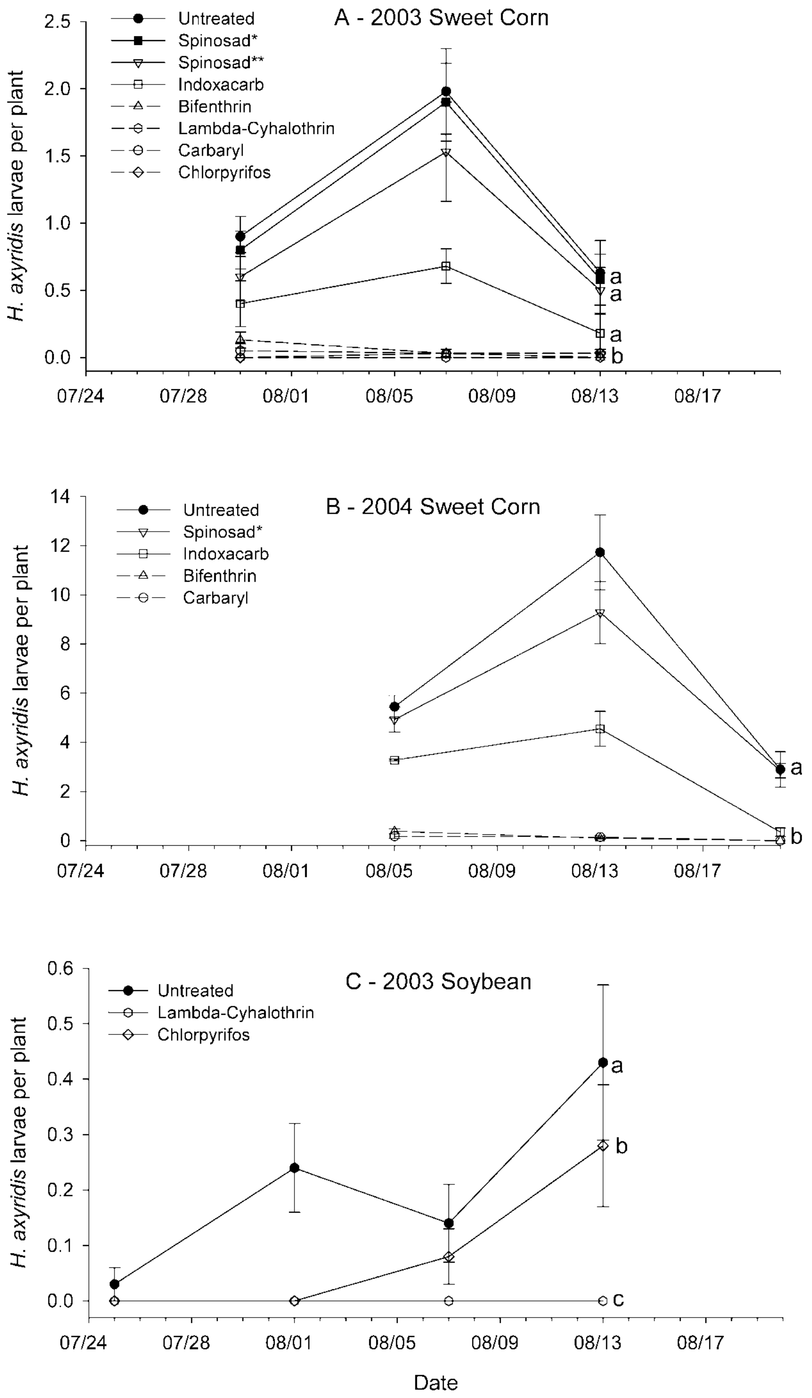

Fig. 1. Abundance of *H. axyridis* larvae in plots of sweet corn and soybean treated with insecticides in 2003 and 2004. Plots were treated on 28 July and 4 and 11 August 2003, and 3, 11, and 17 August 2004 for sweet corn, and on 24 July 2003 for soybean. Lines and symbols in the figure represent the mean combined counts of second, third, and fourth instar *H. axyridis* for each treatment. Individual lines or groups of overlapping lines followed by the same letter do not differ significantly ($P <$ 0.05) from each other through time, repeated measures ANOVA and Bonferroni adjusted contrasts. * Entrust and ** SpinTor.

(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.

Table 1. Repeated measures analysis of variance table for abundance of *H. axyridis* larvae in plots of sweet corn and soybean treated with insecticides in 2003 and 2004

Source	df	*F*	*P*
2003 Sweet corn[a]			
Date	2, 72	2.62	0.0794
Treatments	7, 72	36.12	<0.0001
Date × treatments	14, 72	1.55	0.1157
2004 Sweet corn[b]			
Date	2, 45	20.96	<0.0001
Treatments	4, 45	28.55	<0.0001
Date × treatments	8, 45	2.98	0.0091
2003 Soybean[c]			
Date	3, 36	6.65	0.0011
Treatments	2, 36	25.47	<0.0001
Date × treatments	6, 36	3.19	0.0129

[a] Residual covariance = 1.5101.
[b] Residual covariance = 1.2687.
[c] Residual covariance = 1.6389.

Results

Field Experiments. Coccinellids comprised 95.8, 99.4, and 97.5% of total predaceous life stages (i.e., larvae/nymphs and adults) among generalist predators (i.e., Coccinellidae, Nabidae, Anthocoridae, and Chrysopidae) observed across all plots and sample dates for sweet corn in 2003 and 2004 and soybean in 2003, respectively. Among coccinellids, *H. axyridis* (second–fourth instars and adults) was consistently the most abundant species (76.4% for sweet corn in 2003, 72.7% for 2004, and 48.0% for soybean in 2003). However, *H. axyridis* abundance may have been underestimated because first instars were not included in counts due to difficulties in identifying them to species. Other coccinellids included *Coleomegilla maculata* (De Geer), *Coccinella septempunctata* L., *Hippodamia tredecimpunctata tibialis* (Say), *Hippodamia convergens* Guérin-Méneville, and *Cycloneda munda* (Say).

In 2003, the density of *H. axyridis* larvae in sweet corn plots treated with spinosad products or indoxacarb did not differ significantly from untreated plots (Fig. 1A; Table 1). Densities of *H. axyridis* larvae in plots treated with chlorpyrifos, carbaryl, bifenthrin, or λ-cyhalothrin did not differ significantly from each other and were significantly lower than in spinosad, indoxacarb, or untreated plots (Fig. 1A; Table 1).

In 2004, the density of *H. axyridis* larvae in sweet corn plots treated with spinosad did not differ significantly from untreated plots (Fig. 1B; Table 1). In general, fewer *H. axyridis* larvae were found in plots treated with indoxacarb, carbaryl, or bifenthrin than in plots treated with spinosad or untreated plots (Fig. 1B; Table 1).

In 2003, densities of *H. axyridis* larvae in soybean plots treated with λ-cyhalothrin were significantly lower than in plots treated with chlorpyrifos or untreated plots (Fig. 1C; Table 1). Densities of *H. axyridis* larvae were significantly lower in plots treated with chlorpyrifos compared with untreated plots (Fig. 1C; Table 1).

Laboratory Experiment. Survival in the untreated check was >95% in all experiments; therefore, an adjustment for control mortality (Abbott 1925) was not used (Figs. 2 and 3). Survival of first instars was significantly lower in all insecticide treatments than in the untreated check (Fig. 2A; Table 2). Survival of first instars treated with spinosad was significantly higher than that of those treated with indoxacarb, chlorpyrifos, carbaryl, bifenthrin, or λ-cyhalothrin (Fig. 2A; Table 2). Survival of third instars and adults treated with spinosad did not differ significantly from the untreated check (Fig. 2A and B; Table 2). Survival of third instars and adults treated with indoxacarb was significantly lower than spinosad or untreated check (Figs. 2A and 2B; Table 2). Survival of third instars and adults treated with carbaryl, bifenthrin, chlorpyrifos, or λ-cyhalothrin was zero (Figs. 2A and 2A; Table 2).

Egg hatch in spinosad or indoxacarb treatments did not differ significantly from the untreated eggs (Fig. 3A; Table 3). Egg hatch in chlorpyrifos was significantly lower than in spinosad, indoxacarb, or untreated eggs (Fig. 3A; Table 3). However, all larvae that emerged from eggs treated with chlorpyrifos died soon after emergence. Thus, although chlorpyrifos showed low ovicidal activity, it was highly toxic to neonates. Egg hatch in carbaryl, bifenthrin, or λ-cyhalothrin treatments was zero (Fig. 3A; Table 3). Adult emergence from pupae treated with spinosad, indoxacarb, or carbaryl did not differ significantly from the untreated pupae (Fig. 3B; Table 3). Adult emergence from pupae treated with bifenthrin was significantly lower than that of untreated pupae and pupae treated with spinosad, indoxacarb, or carbaryl (Fig. 3B; Table 3). However, ≈40% of the adults that emerged from pupae treated with carbaryl or bifenthrin died soon after emergence. Adult emergence from pupae treated with chlorpyrifos or λ-cyhalothrin was zero (Fig. 3B; Table 3).

Discussion

H. axyridis was the most abundant coccinellid in sweet corn and soybean fields in our studies. This exotic species has become a dominant coccinellid in many arboreal habitats (Michaud 2002a) and row crops (Rutledge et al. 2004). Factors potentially contributing to the rapid rise to dominance of *H. axyridis* over native species include high fecundity and generalist feeding behavior (Michaud 2002a), intraguild predation (Cottrell 2004, Sato and Dixon 2004), and tolerance to entomopathogens (Cottrell and Shapiro-Ilan 2003), fungicides (Michaud 2001), acaricides (Michaud 2002b), and insecticides (Michaud 2002c). With the likely permanence of the establishment of *H. axyridis*, biological control of sweet corn and soybean pests could benefit from conservation of this dominant predator.

Chemical and biological control are both important for management of insect pests in sweet corn and soybean. For years, conventional insecticides such as carbaryl, chlorpyrifos, bifenthrin, and λ-cyhalothrin have been used in these systems, but they also may

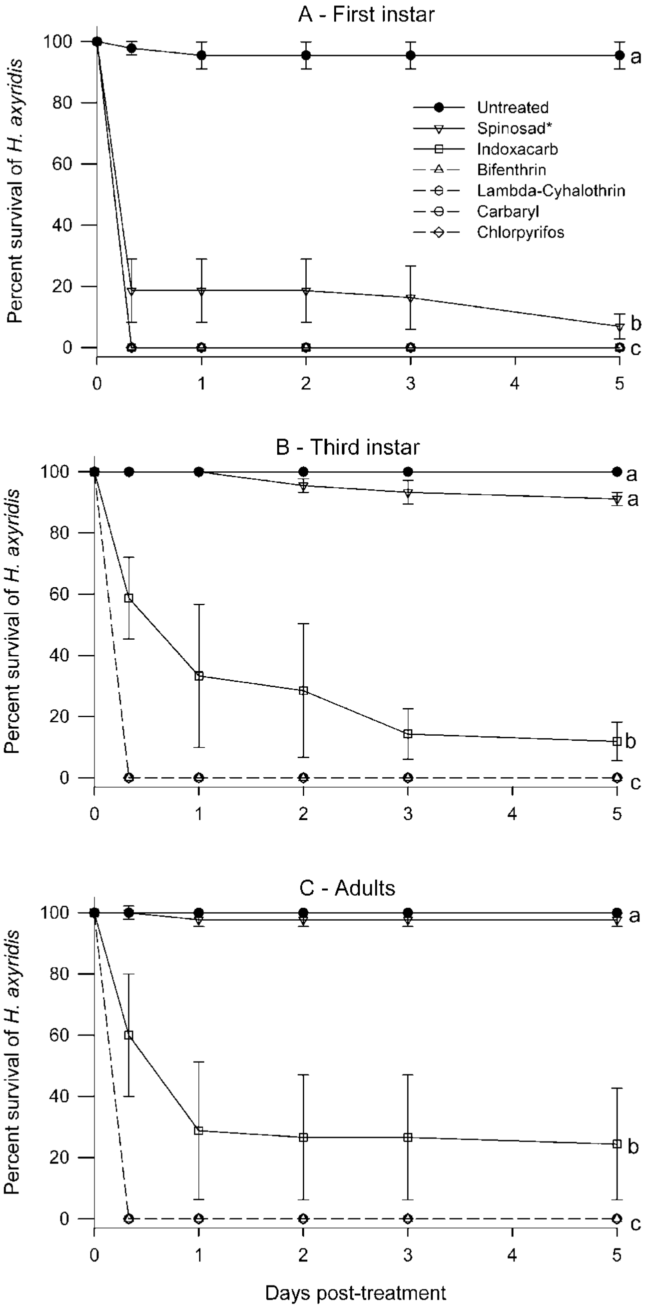

Fig. 2. Effects of insecticides on percentage survival of *H. axyridis* larvae and adults. Lines and symbols in the figure represent the mean percentage of survival for each treatment. Individual lines or groups of overlapping lines followed by the same letter do not differ significantly ($P < 0.05$) from each other through time, repeated measures ANOVA and Bonferroni adjusted contrasts. * SpinTor.

contribute to reductions in natural enemy populations. In the past decade, new insecticides with unique modes of action have shown high toxicity to insect pest populations, while being relatively nontoxic to natural enemies. New chemistries, such as spinosad and indoxacarb, may prove essential to the integration of chemical and biological control in IPM programs.

In our study, densities of *H. axyridis* were higher in plots treated with spinosad or indoxacarb than in plots treated with conventional insecticides. The relatively low toxicities of spinosad and indoxacarb are in agreement with recent research on *H. axyridis.* In New York, Musser and Shelton (2003a) also found greater densities of predators, including *H. axyridis,* in sweet

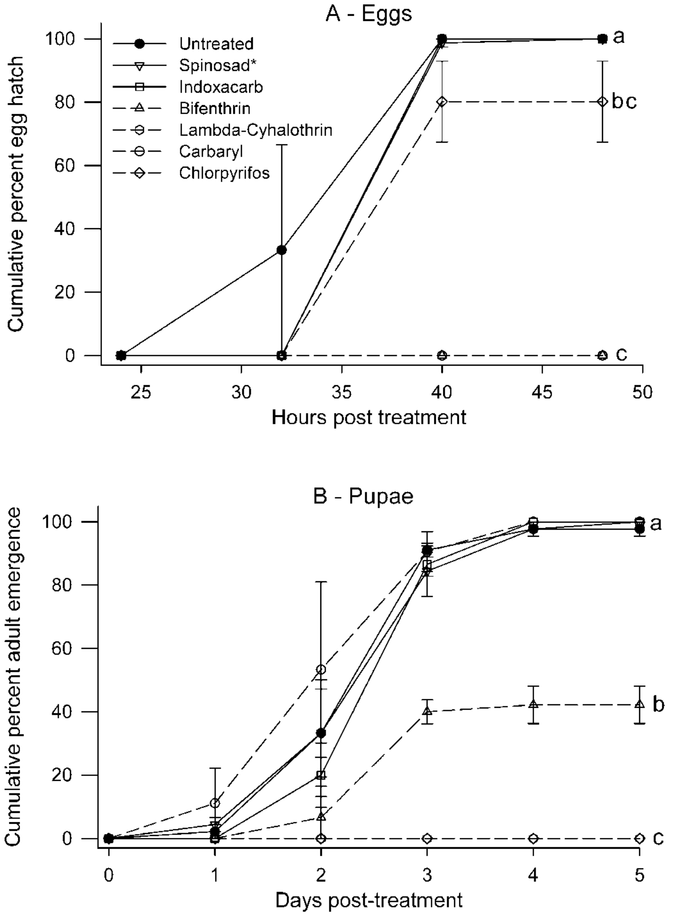

Fig. 3. Effects of insecticides on percentage of egg hatch and adult emergence of *H. axyridis*. Lines and symbols in the figure represent the mean percentage egg hatch or adult emergence for each treatment. Individual lines or groups of overlapping lines followed by the same letter do not differ significantly ($P < 0.05$) from each other through time, repeated measures ANOVA and Bonferroni adjusted contrasts. * SpinTor.

corn plots treated with spinosad or indoxacarb than in plots treated with λ-cyhalothrin. More specifically, we found greater densities of *H. axyridis* in plots treated with spinosad than in plots treated with indoxacarb. This result corroborates those of Ochou and Martin (2003) where more coccinellids were found in areas treated with spinosad than in areas treated with indoxacarb.

Under laboratory conditions, spinosad was the least toxic insecticide to *H. axyridis*. In previous studies, spinosad also showed low toxicity to *H. axyridis* (Michaud 2002c) as well as to *H. convergens* and *C. septempunctata* (Miles and Dutton 2000). Similar results were found by Tillman and Mulrooney (2000) showing low toxicity of spinosad in topical applications to adults of *H. convergens* and in leaf residue assays to *H. convergens, C. maculata, Geocoris punctipes* (Say), and the parasitic wasps *Cardiochiles nigriceps* Viereck and *Cotesia marginiventris* (Cresson). Nonetheless, the same authors observed high mortality of the parasitic wasps, *C. nigriceps, C. marginiventris,* and *Bracon mellitor* Say, and the predatory bug, *G. punctipes*, after topical application. These results suggested that the toxicity of spinosad may vary by route of exposure for a particular species. Spinosad also showed high toxicity to second instars of predatory thrips *Scolothrips takahashii* Priesner and lady beetle *Stethorus japonicus* Kamiya (Mori and Gotoh 2001), and sublethal effects to adults of predatory mites and lacewings (Williams et al. 2003).

Spinosyn A and spinosyn D, the two active components of spinosad, are produced by the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao (Sparks et al. 2001). The combination of a novel molecule and the mode of action of spinosad have resulted in an effective insecticide against several insect pests (Salgado 1998, Salgado et al. 1998), including lepidopteran pests in Minnesota sweet corn (Burkness et al. 2003). Although the toxicity of spinosad to insect pests has been similar or greater than pyrethroids, carbamates, and organophosphates (Sparks et al. 2001), spinosad has provided more selectivity to predators compared with the conventional insecticides. Reasons for this selectivity have not yet been deter-

Table 2. Repeated measures analysis of variance table for percentage survival of *H. axyridis* larvae and adults treated with insecticides in the laboratory

Source	df	*F*	*P*
First instars[a]			
Time	5, 84	374.46	<0.0001
Treatments	6, 84	227.56	<0.0001
Time × treatments	30, 84	9.2	<0.0001
Third instars[b]			
Time	5, 84	206.20	<0.0001
Treatments	6, 84	319.68	<0.0001
Time × treatments	30, 84	13.91	<0.0001
Adults[c]			
Time	5, 84	127.80	<0.0001
Treatments	6, 84	214.47	<0.0001
Time × treatments	30, 84	9.21	<0.0001

[a] Residual covariance = 0.01619.
[b] Residual covariance = 0.01967.
[c] Residual covariance = 0.03059.

mined. However, because spinosad is more toxic through ingestion than through contact (Organic Materials Review Institute 2002), natural enemies that do not feed on tissues of treated plants may be protected from this insecticide (Michaud 2003).

Indoxacarb was the second least harmful insecticide to *H. axyridis* under laboratory and field conditions. Indoxacarb has a novel mode of action that blocks voltage-gated sodium channels and is very active against several lepidopteran pests (Wing et al. 2000). Previous studies also have documented low toxicity to coccinellids, lacewings, predatory bugs, and parasitic wasps (Michaud and Grant 2003). Tillman et al. (2001) suggested that the selectivity of indoxacarb to predators may be associated with changes in the feeding behavior of predators. Because indoxacarb has to be ingested by insects to be bioactivated (Tillman et al. 2001), it was highly toxic to the predator *Podisus maculiventris* (Say) after feeding on treated-food (Tillman and Mullinix 2004). However, it was harmless when the predator exposure was limited to contact with residues (Tillman and Mullinix 2004).

Carbaryl, chlorpyrifos, bifenthrin, and λ-cyhalothrin were highly toxic to *H. axyridis.* Given the broad-spectrum activity of these insecticides, this finding is not surprising, and they do not usually contribute to biological control. Previous studies have demonstrated the high toxicity of conventional insecticides to several species of Coccinellidae (Michaud 2002c, Michaud and Grant 2003). In soybean where spinosad and indoxacarb are not used, we did find differences in the toxicity of conventional insecticides, with higher densities of *H. axyridis* larvae in plots treated with chlorpyrifos than with λ-cyhalothrin. One laboratory study also revealed toxicity differences among conventional insecticides, with higher adult emergence and survival from pupae treated with carbaryl than from pupae treated with chlorpyrifos, bifenthrin, or λ-cyhalothrin.

Table 3. Repeated measures analysis of variance table for percentage of egg hatch and adult emergence of *H. axyridis* treated with insecticides in the laboratory

Source	df	*F*	*P*
Eggs[a]			
Time	3, 56	115.31	<0.0001
Treatments	6, 56	54.07	<0.0001
Time × treatments	18, 56	15.28	<0.0001
Pupae[b]			
Time	5, 84	132.01	<0.0001
Treatments	6, 84	89.62	<0.0001
Time × treatments	30, 84	10.57	<0.0001

[a] Residual covariance = 0.03921.
[b] Residual covariance = 0.03237.

Life stages of *H. axyridis* showed different susceptibility to all insecticides. First instars were the most susceptible, followed by third instars and adults, eggs, and then pupae. In a previous study, *H. axyridis* first instars were also the most susceptible and pupae were the most tolerant to the insecticides acetamiprid, etofenprox, imidacloprid, and thiamethoxam (Youn et al. 2003). Differences in susceptibility among life stages may occur because of changes in enzyme activity and target-site sensitivity (Cho et al. 2002).

This study provides a comprehensive examination of the acute toxicity of commonly used agricultural insecticides to various stages of *H. axyridis.* Overall, results of both the laboratory and field studies indicated that spinosad was least toxic, and indoxacarb moderately toxic to *H. axyridis* compared with conventional insecticides. Use of these insecticides would likely contribute to successful conservation biological control in crops where coccinellids are the most common natural enemies. Spinosad and indoxacarb were selective with regard to acute toxicity, but further work is needed to evaluate the residual toxicity of these insecticide and their potential sublethal effects.

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