Relative Toxicity of Six Insecticides to *Cycloneda sanguinea* and *Harmonia axyridis* (Coleoptera: Coccinellidae)¹

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Abstract Two ladybeetles, Cycloneda sanguinea (L.) and Harmonia axyridis Pallas, were exposed to leaf residues and topical applications of six insecticide formulations commonly used in citrus production in Florida. Exposure of larvae to leaf residues of chlorpyrifos corresponding to 1/100th the recommended field rate caused 55 and 73 percent mortality to larvae of C. sanguinea and H. axyridis, respectively. Cycloneda sanguinea was more sensitive than H. axyridis to all other materials tested. Both fenpropathrin and ethion plus petroleum oil caused significant mortality of larvae of both species as a leaf residue at the field rate and of C. sanguinea larvae at 1/10th the field rate. Sublethal doses of ethion delayed larval development in both species. Imidacloprid was toxic to larvae of both species as a leaf residue at the recommended rate and at 1/10th the recommended rate following topical application. Topical application of imidacloprid at the recommended rate killed 84.6% of adult C. sanguinea, whereas H. axyridis adults were unaffected. Adult beetles survived topical applications of chlorpyrifos, ethion + petroleum oil, and fenpropathrin at concentrations that were lethal to larvae. Esteem® (Valent USA Corp., Walnut Creek, CA) and Spinosad® (Dow Agrosciences, Indianapolis, IN) were relatively benign but did cause 38% and 28% mortality, respectively, of C. sanguinea larvae in topical assays. Fenpropathrin and Spinosad demonstrated repellency to adults of C. sanguinea, but not to adults of H. axyridis. The generally greater sensitivity of C. sanguinea to insecticides may represent a selective disadvantage for this native species in the citrus ecosystem relative the introduced H. axyridis.

Key Words *Cycloneda sanguinea, Harmonia axyridis*, insecticides, leaf residue bioassays, repellency, topical bioassays, toxicity

The ladybeetles, *Cycloneda sanguinea* L. and *Harmonia axyridis* Pallas, are important general predators of citrus pests in Florida. Both are biological control agents of the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Michaud 1999) and the Asian citrus psyllid, *Diaphorina citri* Kuwayama, (Michaud 2000a), two introduced pests that lack effective parasitoids in Florida. Although primarily aphidophagous, both beetle species are generalist feeders known to consume a wide range of soft-bodied insects including soft scales (Sousa and Perez 1977), armored scale crawlers (van Brussel and Bhola 1970, de Crouzel et al. 1979, MacClure 1983), eggs of Lepidoptera (Kim and Noh 1968, McDaniel and Sterling 1979), whitefly (Link et al. 1980) and even mites (Lucas et al. 1997). While *C. sanguinea* is an indigenous species, *H. axyridis* is nonindigenous, having originated in Southeast Asia. However, it is unclear whether its

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establishment in the United States was the result of intentional releases (McClure 1986) or accidental introductions (Day et al. 1995).

Organophosphate insecticides are currently the mainstay of the chemical arsenal to control insect pests in Florida citrus, particularly in groves with production destined for fresh market or gift fruit operations. For example, 10, 7 and 5% of orange acreage received at least one treatment of chlorpyrifos in the years 1993, 1995 and 1997, respectively (Florida Agricultural Statistics Service 1998); the corresponding percentages for grapefruit acreage were 12, 1, and 6%. Over the same period, ethion was applied on 53, 17 and 15% of orange acreage, and 48, 40 and 33% of grapefruit acreage. The use of these organophosphate compounds is scheduled to be phased out by legislation proposed by the EPA under the Food Quality Protection Act (Environmental Protection Agency 1999). The research reported here compares the relative toxicity of two organophosphates to C. sanguinea and H. axyridis with that of some newer insecticides recently labeled for use in citrus. Toxicity to C. sanguinea and H. axyridis larvae was determined using both topical applications and time exposures to leaf residues under controlled laboratory conditions. Adults of both species were subjected to topical applications of insecticides at concentrations that were toxic to their larvae. The behavioral responses of adults to residues of the materials on filter disks were observed in separate assays.

Materials and Methods

Insects. Cycloneda sanguinea and H. axyridis larvae were reared on a diet of frozen Ephestia spp. eggs, bee pollen, water and a liquid diet formulation (Entomos Inc., Gainesville, FL) in individual plastic Petri dishes $(5.5 \times 1.0 \text{ cm})$. The water and liquid diet were encapsulated in polymer beads that permitted both larvae and adults access to the contents, while maintaining a low relative humidity in the dishes. Adult beetles of both species were maintained in 1-L ventilated glass Mason[®] jars (50 to 90/jar) filled with strips of shredded wax paper for their first 9 to 12 d of adult life. The jars were kept in a greenhouse maintained at $24 \pm 2^{\circ}$ C, 60 $\pm 5^{\circ}$ RH, with natural lighting and provisioned with water (on a wick), the liquid diet presented in stretched Parafilm® (American National Can, Greenwich, CT) domes, bee pollen, and frozen Ephestia spp. eqgs. Adult females were transferred to individual plastic Petri dishes for oviposition. Ovipositing females were provided with water beads, diet beads, frozen Ephestia eggs, and bee pollen as necessary. Eggs were harvested daily and held in an incubator at 24°C, 60 ± 5% RH under fluorescent light (P:S – 16:8). These hatched approximately 3.5 ± 0.5 d later under these conditions. Larvae were provided with Ephestia eggs for their first 24 h of life and were used for experiments on the morning of the second day when they were 24 ± 6 h old.

Insecticides. Six insecticidal formulations, representing five different chemical classes, were selected for testing against both coccinellid species. These were chlorpyrifos (Lorsban[®] 4E, Dow Agrosciences, Indianapolis, IN 46268), Esteem[®] (0.86 EC, Valent USA Corporation, 1333 N. California Blvd., Walnut Creek, CA), Ethion (4 Miscible (FMC Corporation, Agr. Chem. Group, 1735 Market St., Philadelphia, PA) in combination with 1% petroleum oil (Sunspray[®] 9E, Sunoco Inc. (R & M), Ten Penn Center, 1801 Market St., Philadelphia, PA), fenpropathrin (Danitol[®] 2.4 EC, Valent USA Corp.), imidacloprid (Provado[®] 2.4 EC flowable, Bayer Corporation, 100 Bayer Road, Building 4, Pittsburgh, PA), and Spinosad[®] (2 EC, Dow Agroscience). The concentrations tested approximated field rates as recommended either on the product

label, or in the Florida Citrus Pest Management Guide (Knapp 2000). In all cases it was assumed that field applications would be made in 935 L of water per ha. Because materials are typically applied on citrus trees in volumes ranging from 935 to 2800 L per ha, depending on available equipment and the nature of tank mixes, the initial concentrations tested in these experiments represented the 'high-end' of the concentration range that beetles would likely be exposed to under field conditions.

Topical assays. Insecticides were tested at various concentrations, beginning with a concentration corresponding to the recommended field rate obtained from the product label, assuming dilution of the specified vol or wt of material in 935 L of water. When the field rate of an insecticide caused mortality that was significant when corrected for control mortality, lower concentrations were tested. When the field rate of an insecticide did not result in significant mortality, it was tested again at double the concentration. Coccinellid larvae 24 (± 6) h-old were placed in groups of eight into plastic Petri dishes $(5.5 \times 1.0 \text{ cm})$. Larvae (n = 16 replicates, 1 larva/replicate) in control groups were sprayed with 1 ml of distilled water in a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth Herts, UK); larvae (n = 16 replicates, 1 larva/replicate) in treatment groups were sprayed with 1 ml aqueous solution of the test material. Larvae were then individually transferred to clean plastic Petri dishes (5.5 \times 1.0 cm) and provisioned with *Ephestia* eggs, water beads, diet beads and bee pollen. Larvae were checked for mortality at 4 h, 24 h, and daily thereafter until emergence of adults. Larvae were provided with fresh food every 3 d until pupation or death. Dates of pupation and adult emergence were recorded for each replicate. Mortality data were corrected using Abbott's formula (Abbott 1925) and analyzed using a Chi-square Goodness-of-fit test, and data for larval development time were analyzed by one-way ANOVA (SPSS 1998). For materials demonstrating significant toxicity to larvae, additional topical assays were performed with adult beetles (4 to 6 wks old) that were identical in every way to the larval assays with the exception that beetles were monitored for mortality for only 48 h.

Leaf residue assays. Freshly-picked grapefruit leaves were washed in a dilute solution of Chlorox[®] bleach, rinsed in distilled water, and blotted dry on paper towels. Leaf disks (3 cm diam) were punched from the leaves using a sharpened piece of steel pipe. Leaf disks in a treatment series (n = 16 replicates, 1 larva/replicate) were sprayed with 1 ml of aqueous solution of the test material in the Potter Precision Spray Tower. Leaf disks in control series (n = 16 replicates, 1 larval/replicate) were sprayed with 1 ml distilled water. Both treated and control leaf disks were placed into numbered plastic Petri dishes (3.5 × 1.0 cm). Approximately 2 to 3 mg of Ephestia eggs was placed in the center of each disk as a food source and to entice larvae to walk over the treated surface of the leaf. Single, $24 (\pm 6)$ h-old coccinellid larvae were then transferred to each dish. Larvae were transferred to clean plastic Petri dishes ($5.5 \times$ 1.0 cm) after 24 h exposure to residues and provisioned with Ephestia eggs, bee pollen, diet beads and water beads. All replicates were checked for mortality daily. Larvae were provided with fresh food every 3 d until pupation or death. Dates of pupation and adult emergence were recorded for each larva. Mortality data were analyzed using a Chi-square Goodness-of-fit test, and data for larval developmental time were analyzed by one-way ANOVA (SPSS 1998). Additional topical assays were performed on adult beetles with materials demonstrating toxicity to larvae. These trials were identical in every way to the larval assays with the exception that adult beetles were monitored for mortality for a period of 48 h.

Repellency assays. Repellency of the test materials against adults was evaluated using beetles from stock colonies. Each trial employed 20 beetles, one per replicate, and each beetle was used only once. Circular filter papers (9.0 cm diam) were divided in half by a pencil line. The treatment half of each filter disk was sprayed with 1 ml aqueous solution of the test material in the Potter Precision Spray Tower while the other (control) half was covered with a half piece of filter disk and remained untreated. Filter disks were then placed individually into plastic Petri dishes $(9.0 \times 1 \text{ cm})$, with the walls coated with Fluon® (Australian Entomological Supplies Ltd., PO Box 250, Bangalow, NSW 2479, Australia) applied to force the insects to remain on the paper surface. One-half the dishes had treated surfaces oriented on the left, the other half, on the right, to control for any possible directional bias. Adult beetles were then transferred to the dishes, one per dish, and observed repeatedly at intervals of approximately 15 min, for a total of 12 observations. The experiments were conducted on a laboratory bench under soft-white fluorescent light at an ambient temperature of 24 (±1)°C. Only beetles that were resting on either the treated or untreated side of the filter disks were tallied for their position during each observation. Beetles that were moving or that straddled the boundary were excluded from tallies during a particular observation. Following each observation, beetles were gently disturbed with a sable hair brush to cause them to change position prior to the subsequent observation. The data were analyzed using a Chi-square Goodness-of-fit test that assumed equal distribution of valid observations between treated and untreated portions of the filter papers in the absence of any treatment effect.

Results and Discussion

The most toxic compound was chlorpyrifos. Larvae of both beetle species incurred significant mortality (P < 0.001) when exposed to leaf residues corresponding to 1/10th and 1/100th the recommended field rate (Fig. 1A). However, 100% of adult beetles of both species (n = 16) survived topical applications of 0.045% chlorpyrifos, a concentration corresponding to 1/10th the recommended field rate.

Fenpropathrin also was highly toxic as a leaf residue, causing 100% mortality to larvae of both species at the recommended field concentration. In similar trials, Sun et al. (1999) also found fenpropathrin highly toxic to *H. axyridis*. Olszak (1999) reported toxicity of leaf residues of fenpropathrin to *Adalia bipunctata* (L.) larvae. However, *C. sanguinea* larvae experienced significant mortality following exposure to leaf residues of 1/10th the recommended concentration, whereas *H. axyridis* larvae did not (Fig. 2A). Topical applications of fenpropathrin at 1/100th the recommended concentration were tolerated by larvae of both species, although larvae of *H. axyridis* surviving exposure to fenpropathrin experienced accelerated larval development relative to control larvae (Table 1). Adult beetles were again more tolerant than larvae; 93.8% of *C. sanguinea* (n = 16) and 100% of *H. axyridis* (n = 16) survived topical applications of o.005% fenpropathrin (1/10 the field rate). Fenpropathrin is also marketed as an acaricide, and use of this product against citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), could potentially have negative impact on coccinellid populations, particularly those of *C. sanguinea*.

Ethion plus petroleum oil, as either a leaf residue or topically applied, was toxic at the recommended rate to *H. axyridis* larvae, and at 1/10th the recommended rate to *C. sanguinea* larvae (Fig. 1C). *Cycloneda sanguinea* (Table 2) and *H. axyridis* (Table 1) larvae that survived exposure to ethion + oil exhibited significantly retarded devel



Fig. 1. Percent mortality (corrected using Abbott's formula) of coccinellid larvae (n = 16 in all trials; solid bars = *C. sanguinea*; hatched bars = *H. axyridis*) exposed to different concentrations of insecticides as leaf residues and in topical applications: (a) chlorpyrifos, (b) Esteem, (c) Ethion + petroleum oil. The calculated field rates assume application in 935 L water per ha. Mortality was compared between treatments and controls with a Chi-Square Goodness-of-fit test (*, P < 0.05; **P < 0.01; ***, P < 0.001).



Fig. 2. Percent mortality (corrected using Abbott's formula) of coccinellid larvae (n = 16 in all trials; solid bars = *C. sanguinea*; hatched bars, = *H. axyridis*) exposed to different concentrations of insecticides as leaf residues and in topical applications: (a) fenpropathrin, (b) imidacloprid, (c) Spinosad. The calculated field rates assume application in 935 L water per ha. Mortality was compared between treatments and controls with a Chi-Square Goodness-of-fit test (*, *P* < 0.05; ***P* < 0.01; ***, *P* < 0.001).</p>

	<i>H. axyridis</i> Mean developmental time (±SEM)					
Product	% a.i.	Control	Treatment	F d.f.		Р
Chlorpyrifos	0.0045	14.8 ± 0.26	15.5 ± 0.50	1.512	1, 17	0.236
Esteem®	0.0013	13.0 ± 0.43	12.7 ± 0.25	0.441	1, 26	0.512
	0.0026	12.1 ± 0.14	12.3 ± 0.18	0.145	1, 27	0.706
Ethion + oil	0.0003/0.10	15.6 ± 0.52	16.2 ± 0.50	0.622	1, 26	0.438
	0.003/1.0	13.5 ± 0.13	14.6 ± 0.39	10.276	1, 24	0.004
Fenpropathrin	0.0005	14.5 ± 0.26	13.9 ± 0.03	11.431	1, 19	0.003
	0.005	14.0 ± 0.33	14.8 ± 0.29	3.232	1, 20	0.087
Imidacloprid	0.0005	14.6 ± 0.16	15.0 ± 0.33	1.385	1, 24	0.251
	0.005	15.9 ± 0.47	18.7 ± 1.50	3.871	1, 19	0.064
Spinosad®	0.05	15.0 ± 0.22	15.5 ± 0.29	1.660	1, 26	0.209
·	0.10	14.9 ± 0.32	15.8 ± 0.19	5.051	1, 28	0.033

Table 1. Mean (±SEM) larval developmental times in days for *H. axyridis* larvae exposed to leaf residues of various insecticides for 24 h*

* Treatments were compared with a one-way ANOVA.

opment relative to their control counterparts. The delayed development of larvae surviving exposure to ethion is an additional negative impact of this material on coccinellid populations.

Imidacloprid was toxic to larvae of both species as a leaf residue applied at the recommended rate, and at 1/10th the recommended rate in topical applications (Fig. 2B). One hundred percent of adult *H. axyridis* survived a topical application of 0.005% imidacloprid, but *C. sanguinea* adults experienced 84.6% mortality (Chi-square = 58.22, P < 0.001). Viggiani et al. (1998) reported imidacloprid to be highly toxic to *Rodolia cardenalis* (Mulsant).

Only Esteem and Spinosad caused no significant mortality of larvae of either species as leaf residues, whether applied at a concentration corresponding to the field rate, or double the field rate (Figs. 1B, 2C, respectively), although topical applications of both materials caused some mortality of *C. sanguinea*. Adults of both species receiving topical applications of Esteem and Spinosad at double the recommended field rate had 100% survival (n = 16 in both cases). Differential susceptibility of life stages to pesticides is well recognized and adults of both coccinellid species appeared less sensitive than larvae to all compounds tested.

Both fenpropathrin and Spinosad demonstrated repellency to adults of *C. san-guinea*, but were not repellent to *H. axyridis* adults (Table 3). Fenpropathrin is claimed by the producer to have repellent activity toward pests. Repellency of beneficial species could be an added bonus, provided untreated refuges are available for them. Adults of *H. axyridis* were not repelled by any material; on the contrary, they were more often observed resting on portions of filter paper treated with Esteem[®] than on control surfaces (Table 3). The combination of ethion plus oil arrested the movement of *C. sanguinea* adults. Smith and Krischik (1999) noted that adult *Coleomegilla maculata* (Degeer) experienced reduced mobility on imidacloprid-treated sunflower plants. Similarly, Vincent et al. (2000) found that contact with imidacloprid-treated

		<i>C. sanguinea</i> Mean developmental time (±SEM)				
Product	% a.i.	Control	Treatment	F	d.f.	Ρ
Chlorpyrifos	0.0045	11.4 ± 0.37	12.5 ± 1.21	0.751	1, 11	0.405
Esteem®	0.0013	11.7 ± 1.00	10.8 ± 0.26	1.120	1, 16	0.306
	0.0026	11.2 ± 0.44	10.7 ± 0.30	0.647	1, 21	0.430
Ethion + oil	0.0003/0.10 0.003/1.0	11.6 ± 0.29 **	12.6 ± 0.27	6.062 —	1, 25	0.021
Fenpropathrin	0.0005	9.6 ± 0.24	9.6 ± 0.19	0.300	1, 21	0.865
	0.005	10.0 ± 0.20	12.0 ± 2.00	2.687	1, 16	0.121
Imidacloprid	0.0005	11.3 ± 0.30	11.0 ± 0.33	0.454	1, 16	0.510
	0.005	**	—			—
Spinosad®	0.05	11.3 ± 0.66	11.4 ± 0.63	0.034	1, 18	0.855
	0.10	11.9 ± 0.74	13.8 ± 0.92	2.409	1, 14	0.143

Table 2. Mean (\pm SEM) larval developmental times in days for *C. sanguinea* larvae exposed to leaf residues of various insecticides for 24 h^{*}

* Treatments were compared with a one-way ANOVA.

** Too few larvae survived to provide a meaningful comparison.

glass plates by *H. axyridis* third instars caused them to spend more time on the plates, but this was not the case for the adult beetles observed in these trials. Although the consequences of such responses in the field are open to interpretation, the arrested movement of *C. sanguinea* observed in response to surfaces treated with Ethion plus oil could lead to extended exposure to residues of these materials and increased mortality. However, it should be noted that this bioassay was designed to test the contact repellency of materials, rather than their attractiveness *per se*, and that arrested movement over surfaces treated with these materials does not imply that beetles would orient toward such materials from any distance.

It should be noted that many coccinellids readily consume prey items that do not support their development or reproduction (Michaud 2000b) and that diet can influence susceptibility to insecticides. In particular, Kalushkov (1999) found that adults of *A. bipunctata* fed unsuitable prey became increasingly susceptible to pyrethroid insecticides, including fenpropathrin. The standardized diet employed in these experiments yields excellent survival of both species and may, therefore, underestimate the sensitivity of these species under field conditions when diet may be less optimal. Although both Esteem and Spinosad appear potentially compatible with biological control by coccinellids, further tests are needed to evaluate possible effects of chronic exposure to sub-lethal doses on adult reproductive performance. For example, Smith and Krischik (1999) found that exposure to imidacloprid caused delayed onset of oviposition in females of *C. maculata*.

Cycloneda sanguinea was the most abundant aphidophagous coccinellid in Florida citrus state-wide prior to 1998 (Michaud 2000b). However, recent observations suggest that the abundance of this species in citrus is declining, while that of *H. axyridis* is increasing. The present work reveals that larvae of *C. sanguinea* are more sensitive than those of *H. axyridis* to five of the six materials tested, and equally

20) observed on treated versus control areas of	art*
able 3. Mean (±SEM) numbers of <i>C. sanguinea</i> and <i>H. axyridis</i> adults (n =	Petri dish arenas over a series of 12 observations made 15 min ap

		C. sanguine	a			H. axyridis		
Compound	Control	Treatment	X²	ط	Control	Treatment	×	٩
Chlorpyrifos	9.4 ± 0.34	9.1 ± 0.23	0.01	ns	8.4 ± 0.69	8.1 ± 0.58	0.93	ns
Fenpropathrin	10.3 ± 0.82	4.3 ± 0.26	15.71	<0.001	7.8 ± 0.65	10.3 ± 0.68	2.06	ns
Esteem®	9.1 ± 0.66	8.0 ± 0.33	0.35	su	6.2 ± 0.32	10.0 ± 0.51	5.45	<0.05
Ethion + oil	4.4 ± 0.47	12.8 ± 0.51	24.27	<0.001	6.6 ± 0.69	8.7 ± 0.47	1.71	su
Imidacloprid	7.2 ± 0.38	9.3 ± 0.31	1.71	su	8.3 ± 0.66	9.2 ± 0.49	0.29	ns
Spinosad®	11.3 ± 0.68	7.3 ± 0.48	5.19	<0.05	6.7 ± 0.66	7.6 ± 0.60	0.35	su

* Observations were compared using a Chi-square, Goodness-of-fit test.

susceptible to the sixth. Similarly, Mizell and Schiffhauer (1989) found that *C. sanguinea* was very sensitive to a range of pesticides that included organophosphates and pyrethroids. Consequently, the use of such insecticides to protect citrus seedlings in nurseries, or producing trees in established groves, would be expected to accelerate the displacement of the native *C. sanguinea* by the invasive *H. axyridis*.

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