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Comparing Effects of Insecticides on Two Green Lacewings Species, *Chrysoperla johnsoni* and *Chrysoperla carnea* (Neuroptera: Chrysopidae)

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ABSTRACT This study compared lethal and sublethal effects of five insecticides, chlorantraniliprole, cyantraniliprole, spinetoram, novaluron, and lambda-cyhalothrin, on adult and second instars of two green lacewing species, *Chrysoperla carnea* (Stephens) and *Chrysoperla johnsoni* Henry, Wells and Pupedis (Neuroptera: Chrysopidae) in the laboratory. Formulated pesticides were tested using concentrations equivalent to the high label rate dissolved in 378.5 liters of water. Novaluron and lambda-cyhalothrin were toxic to larvae and no treated larvae survived to the adult stage. Larva to adult survival was reduced in chlorantraniliprole, cyantraniliprole, and spinetoram treatments. Larva to adult developmental time and sex ratio were not different among the treatments within a species. Chlorantraniliprole, cyantraniliprole, spinetoram, and lambda-cyhalothrin treatments were highly toxic to adults of both species. *C. johnsoni* females had lower fecundity than *C. carnea* females in the control. Fecundity of females was similar in the control and novaluron treatment within each species. However, fertility and egg viability were negatively impacted for both species when females were treated with novaluron. *C. carnea* females had higher fertility and egg viability than *C. johnsoni* females in the control. Adults of both species had similar longevity in the control and novaluron treatment and adult longevity was not gender specific. All insecticides tested were toxic to *C. johnsoni* and *C. carnea* either at the immature or adult stage or both. Results of this study demonstrate a similarity between *C. johnsoni* and *C. carnea* for pesticide toxicity irrespective of their varied geographical distributions.

KEY WORDS biological control, generalist predator, green lacewing, reduced-risk insecticides, OP-replacement insecticides

Most western United States orchard integrated pest management (IPM) programs have historically relied heavily on organophosphorus (OP) insecticides from their introduction in the late 1950 until mid-1990s (Jones et al. 2009). Enactment of Food Quality Protection Act of 1996 has resulted in the modification of use patterns and removal (or pending removal) of many OP insecticides that had previously been widely used (US EPA 1996, Jones et al. 2010). A result of the loss of OP insecticides is that pest management systems, including those used in orchard crops, have begun transitioning to newer pesticide chemistries with novel modes of action and lower mammalian toxicities (Whalon et al. 1999, Agnello et al. 2009, Kim et al. 2011).

Although these newer insecticides are safer for humans and the environment, some have harmful effects against arthropod natural enemies in agricultural systems, thus, potentially causing secondary pest outbreaks (Kim et al. 2006, Crampton et al. 2010). These insecticides are not always compatible with the natural enemies that help manage secondary pests. Available information on how these newer reduced-risk and OP-replacement insecticides affect the natural enemies beyond their lethal levels is sparse (US EPA 1997, Kim et al. 2006). Pesticide compatibility with biological control agents is a major concern to IPM practitioners and it is important to have knowledge about the activity of insecticides toward pests, nontarget insects, and the environment (Stark et al. 2004). Hence, understanding the impact of pesticides on beneficial arthropods is necessary to successfully integrate biological control into agro-ecosystems (Croft 1990).

Green lacewings (Neuroptera: Chrysopidae) are important predators of insect pests and mites of apples, pears, and walnuts in the United States (Westigard et al. 1986, Mansfield and Mills 2002, Horton et al. 2009, Jones et al. 2011). Lacewings are considered as important natural enemies of several pests of economic importance because of their polyphagous feeding habits (New 1975, Principi and Canard 1984, Stelzl and Devetak 1999). Chrysopids are generalist predators that feed on small arthropod pests and their eggs,

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including aphids, leafhoppers, lepidopteran pests, and mites (Ridgway and Murphy 1984, Borror et al. 1992, Senior and McEwen 2001). Larval lacewings fulfill many of the requirements of an effective biological control agent and are voracious active predators with excellent search capacity (Bond 1980). Efficacy of lacewings for biological control has been well recognized for >250 yr (Senior and McEwen 2001). Insecticides used to control arthropod pests in tree fruit orchards can affect green lacewings (Lehnert 2012).

In this study, we used two important green lacewing species to investigate the effects of reduced-risk and OP-replacement insecticides on these natural enemies. The European species, *Chrysoperla carnea* (Stephens) is considered an important green lacewings species and has frequently been used as a model insect to study beneficial arthropods (Henry et al. 2001). It has been mass-reared and released in croplands all over the world including North America (Henry et al. 2001). *Chrysoperla johnsoni* Henry, Wells and Pupedis is a North American green lacewing species with similar appearance to *C. carnea* (Henry et al. 1993). It is one of the common green lacewing species found in tree fruit orchards in the western United States including Oregon, Washington, Idaho, California, and Arizona (Henry 1993).

Our objective was to study the effects of reduced-risk and OP-replacement insecticides on *C. carnea* and *C. johnsoni* while comparing the model green lacewing species to our local species using an assay with multiple routes of pesticide exposure. In contrast to tests defined by the International Organization for Biological Control (IOBC) to study pesticide effects to natural enemies in the laboratory using dried residues of fresh pesticide deposits (Hassan 1985, Vogt et al. 2000), our assay combined contact, residual, and oral routes of exposure that makes it more robust than a single route of exposure assay.

Materials and Methods

***C. johnsoni* and *C. carnea* Colony Rearing.** Colonies of *C. johnsoni* and *C. carnea* were maintained at 23°C, 50–60% relative humidity (RH), and a photoperiod of 16:8 (L: D) h in the laboratory. The *C. johnsoni* colony was initiated with field collected (summer 2010 and 2011) larvae from pear orchards in Hood River, OR. The *C. carnea* colony was initiated with commercially purchased second instar larval stages (BioBest, Leamington, ON, Canada). Adults of each species were reared in an open-top glass aquarium (26 by 30 by 50 cm) (Aqua Culture [size 10], Wal-Mart, Bentonville, AR) with a wire mesh lid (http://www.glasscages.com). To facilitate egg laying, the opening at the top of the aquarium was covered with a piece of cheese cloth (56 by 92 cm) (#90, 17.3 by 14.2 threads/cm, http://www.onlinefabricstore.net) and secured with the wire mesh screen top. Artificial diet consisting of eggs, honey, wheat germ, brewer's yeast, condensed milk, and water was prepared in the laboratory and used to feed the adults (Vogt et al. 2000). A thin layer of the adult diet was applied to the nonabsorbent side of a piece of Benchkote surface protector paper (12 by 24 cm) (Fisherbrand, Fisher, Pittsburgh, PA) and attached to the interior wall of the adult cage using masking tape (Cinta Adhesiva [Premium], Ace Brand Hardware Corp., Brook, IL). Water was provided by using a 15-cm medium cotton wick (Ref. No. 201208, Richmond Dental, Charlotte, NC) inserted through the lid of a clear plastic vial (5-cm diameter, 196 ml) with water. Adults were provided with new food, water, and cheese cloth cover three times a week. Each week, 60 eggs (≈24 h old) were collected and reared individually to adults as described below to maintain the colony.

Immature green lacewings are cannibalistic (Duelli 1981, Rojht 2009), thus, each egg was reared individually in a covered 28-ml translucent plastic portion cup (Georgia Pacific Dixie, Atlanta, GA). Before use, a thin layer (0.5 cm) of fluon (Insect-a-Slip) (#2871B, Bio Quip Products, Inc., Rancho Dominguez, CA) was applied to the upper interior of the portion cup to prevent larva from crawling into the lid and getting crushed while opening. To avoid static electricity that impacted larval transfer and when opening the lid for feeding, cardboard placement, and monitoring their development, the exterior wall of the cup was wiped with a cloth sprayed with a compound that eliminated static electricity (Static Guard, Alberto-Culver USA Inc., Melrose Park, IL). For larval rearing, newly laid eggs (<24 h old) were collected from the cheese cloth used in the adult cage for egg laying. The cheese cloth was placed on top of the adult cage 24 h before the egg collection and then removed when it was time to collect eggs. Eggs were individually plucked by their stalks using fine forceps and placed singly in portion cups and secured with lids. A small amount (≈0.2–0.3 g) of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (purchased from Beneficial Insectary, Redding, CA, and stored in a freezer at −6°C) was placed in each cup as food for emerging larva. The cups were arranged in batches of 30 in cup-trays (# 9040, Bio-Serv, Frenchtown, NJ). When the larva developed into a third instar, a piece of corrugated cardboard (1.5 by 1.5 cm) was provided as a substrate for pupation. Food was replaced two times a week until the formation of pupa. Emerged adults were released to the adult colony.

Insects. This bioassay was conducted using 0–1 d old second instar and 1–2 d old adult (male and female) *C. johnsoni* and *C. carnea.* Eggs (<24 h old) collected to rear experimental insects (second instars and adults) were obtained from a new batch of adults. These adults, produced from eggs collected from the colony, were reared in a separate adult cage for egg collection. Second larval stage was identified by one shed exuviae attached to the cup wall. Newly emerged adults were sexed (Vogt et al. 2000) and placed in gender specific adult cages. Both larvae and adults were fed as described in the previous section.

Insecticides. The following five insecticides listed with their maximum label rates were tested as formulated material: chlorantraniliprole (Altacor 35 WG), DuPont Crop Protection, Wilmington, DE) 110.4 g

active ingredient (AI)/ha, cyantraniliprole (Exirel 100SE), DuPont Crop Protection (Wilmington, DE) 149.9 g (AI)/ha, spinetoram (Delegate 25 WG, Dow Agro Sciences LLC, Indianapolis, IN) 122.6 g (AI)/ha, novaluron (Rimon 0.83 EC, Chemtura AgroSolutions, Middlebury, CT) 363.4 g (AI)/ha, and lambda-cyhalothrin (Warrior II CS, Syngenta LLC Inc., Greensboro, NC) 46.6 g (AI)/ha. Distilled water was used as the control treatment. Each pesticide was tested using concentrations that were equivalent to the maximum label rate dissolved in 378.5 liters of water.

Bioassay–Lethal Effects. Custom made glass arenas consisting of a glass cylinder (Wheaton Glass Warehouse, Millville, NJ) standing on a glass plate (Cincinnati Gasket, Cincinnati, OH). Adult bioassay arenas consisted of 7.5 cm dia × 6 cm tall × 3.2 mm thick glass cylinders place upright on a 9 by 9 cm and 2.3 mm thick glass plate. Larval arena dimensions were: 4.4 cm dia × 6 cm tall × 2.3 mm thick glass cylinders placed on a 6 by 6 cm and 2.3 mm thick glass plate. To hold each cylinder upright on the glass plate, four aluminum strips (1 cm wide × 3 cm long × 1.5 mm thick and bent to 90° angle) were glued to the side of the lower exterior cylinder wall corresponding to four corners of the plate with hot glue before use. Small binder clips were used to hold the metal strips to the glass plate floor. All organisms in adult and larval bioassays were exposed to treatment compounds via contact exposure, residual exposure, and ingestion exposure methods, thus we measured toxicity using a worst-case cumulative exposure scenario. Insects (contact exposure), arenas and cheese cloth lids (residual exposure), and *E. kuehniella* eggs and adult diet (ingestion exposure) were treated as below.

Cheese cloth lids and *E. kuehniella* eggs were treated by drenching them in 100 ml of each treatment for 30 s and then air-dried. A Potter spray tower (Burkard Scientific, Uxbridge, United Kingdom) (103 kPa, intermediate nozzle) was used to treat the glass cylinders and plates, insects, and adult diet using 2 ml of solution for each application. Glass cylinders and plates were treated separately. After treatment, they were removed from the spray tower after a 5 s settling time, air-dried for 30 min and then assembled. The adult food was treated after it was applied as a thin layer to the bottom exterior surface of a 9 cm-dia glass petri dish. Larvae and adults were treated in a 9 cm-dia glass petri dish as a group of three larvae or a single pair of adult male and female per replicate per treatment, respectively (larvae; $n = 15$ [5 replicates] and adults: $n = 24$ [12 replicates].

Treated insects were then transferred with a soft brush to the glass arenas. Each larva was reared individually in a treated glass arena because of their cannibalistic nature. Larvae were provided with treated *E. kuehniella* eggs (≈0.2–0.3 g). Paired adults, one male and female per arena, were transferred to arenas and covered with cheese cloth lids secured with rubber bands. Adult diet was provided to treated adults as a thin layer placed on a piece of Benchkote paper described above (2.5 by 5.5 cm) and attached to the treated arena wall with a piece of reusable adhesive putty (DAP Bluestik, DAP products Inc., Baltimore, MD). All adults were provisioned with distilled water using a small (38 mm dia) piece of water-soaked cotton roll (Richmond Dental, Charlotte, NC) placed in a clear capless disposable micro-centrifuge tube (1.7 ml) (Cat. no. 20170-575), (VWR International, LLC, Radnor, PA) attached to the bottom of the arena with a piece of putty. All arenas were placed in an environmental growth chamber (Percival I-36LLVLC8, Percival Scientific Inc., Perry, NC) at 23°C, 60% RH, and a photoperiod of 16:8 (L: D) h. Adult or immature *C. johnsoni* and *C. carnea* were assessed for mortality daily for 10 d after treatment (DAT). Untreated *E. kuehniella* eggs (≈0.2–0.3 g) and fresh adult food were provided to all surviving larvae and adults, respectively, 72 h after treatment. Treated larvae were reared until the emergence of adults. Surviving treated adults were assessed for sublethal effects as described below.

Bioassay–Sublethal Effects-Larva to Adult Developmental Time, Survival, and Sex Ratio. Larvae that survived treatments were reared until they emerged as adults and their developmental time was determined. Gender of the emerged adults was determined using the methods described above by Vogt et al. (2000). Adult sex ratio was calculated as the percentage of females ([females/(males + females)] * 100). All surviving larvae were provided with *E. kuehniella* eggs as mentioned above.

Bioassay–Sublethal Effects-Adult Male and Female Longevity, Fecundity, Fertility, and Egg Viability. Surviving adults were reared and provided with food and water three times a week until they died. Cheese cloth lids from the adult arenas were collected every other day to evaluate the number of eggs each female laid and subsequent egg hatch for 20 d. To assess egg viability, a circular area (≈8 cm dia) was cut from the cheese cloth lid that covered the top of the arena and placed into individual 9 cm-dia glass petri dishes. The piece of cut cheese cloth was examined under a microscope to count the number of eggs laid. A small amount (≈0.2–0.3 g) of *E. kuehniella* eggs was added to each petri dish as food for emerging larvae. The petri dish was covered with its glass lid and secured with two rubber bands. All petri dishes were placed in the environmental growth chamber indicated above and were monitored daily for egg hatch and the number of larvae that emerged were counted and removed. *E. kuehniella* eggs (≈0.2–0.3 g) were provided as food for newly emerging larvae two times a week.

Statistical Analyses. A completely randomized experimental design (CRD) was used for both larvae and adult experiments. A one-way analysis of variance (ANOVA) was performed (PROC MIXED) for mortality, developmental time, survival, sex ratio, fecundity, fertility, and egg viability. A two-way ANOVA was performed for adult longevity by gender (SAS Institute 1999).

Means were compared at $P \leq 0.05$ significance level for all experiments (LSMEANS) (SAS Institute 1999). Proportion of mortality, survival, and sex ratio were arcsine-square root transformed before ANOVA to stabilize variances (Zar 1984).

Table 1. Mortality (%) (mean ± SEM) of second instar *Chrysoperla carnea* and *Chrysoperla johnsoni* treated with different insecticides or water (control) 1, 2, and 10 d after treatment (DAT)

Treatment	Max. label rate/ha	Mg AI/ liter	Mortality (%)[a]					
			1 DAT		2 DAT		10 DAT	
			C. carnea	*C. johnsoni*	*C. carnea*	*C. johnsoni*	*C. carnea*	*C. johnsoni*
Control	N/A	N/A	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d	6.7 ± 6.7e	6.7 ± 6.7e
Chlorantraniliprole	315.2 g	117.9	0.0 ± 0.0c	0.0 ± 0.0c	6.7 ± 6.7d	0.0 ± 0.0d	26.7 ± 6.7de	26.7 ± 6.7de
Cyantraniliprole	1.5L	160.2	0.0 ± 0.0c	0.0 ± 0.0c	6.7 ± 6.7d	13.3 ± 13.3d	26.7 ± 6.7de	26.7 ± 16.3de
Novaluron	3.7L	388.5	0.0 ± 0.0c	13.3 ± 8.2bc	33.3 ± 18.3bc	86.7 ± 8.2a	100.0 ± 0.0a	100.0 ± 0.0a
Spinetoram	490.4 g	131.1	0.0 ± 0.0c	0.0 ± 0.0c	20.0 ± 8.2cd	6.7 ± 6.7d	46.7 ± 17.0cd	40.0 ± 16.3de
Lambda-cyhalothrin	187.1 ml	49.9	0.0 ± 0.0c	20.0 ± 13.3ab	20.0 ± 13.3cd	20.0 ± 13.3cd	73.3 ± 6.7bc	66.7 ± 18.3b

[a] Means within each DAT for *C. carnea* and *C. johnsoni* followed by the same letters are not significantly different at $P > 0.05$ (Least Square Means [LSMEANS] Test).

Insect Identification and Species Verification. Insect identification and species verification of *C. johnsoni* and *C. carnea* was provided by C. S. Henry (University of Connecticut, Department of Ecology and Evolutionary Biology).

Voucher Specimens. Voucher specimens of *C. johnsoni* and *C. carnea* were deposited in the entomology insect collection at Oregon State University, Mid-Columbia Agricultural Research and Extension Center (Hood River, OR).

Results

Bioassay–Lethal Effects-Larvae. All *C. carnea* larvae and most of the *C. johnsoni* were alive at 1 DAT (Table 1). Mortality was observed in *C. johnsoni* larvae treated with novaluron or lambda-cyhalothrin 1 DAT (Table 1). *C. johnsoni* larvae treated with lambda-cyhalothrin had significant mortality at 1 DAT ($F = 2.11$; df = 11, 44; $P = 0.0393$). Novaluron caused the highest larval mortality 2 DAT in both species ($F = 6.74$; df = 11, 44; $P = 0.0001$) and all novaluron-treated larvae were dead 10 DAT. There was no significant larval mortality in all other treatments at 2 DAT in both species. Lambda-cyhalothrin caused significant mortality to both species and spinetoram to *C. carnea* by 10 DAT ($F = 12.19$; df = 11, 44; $P = 0.0001$). There was no statistically significant mortality of larvae treated with chlorantraniliprole or cyantraniliprole treatments by 10 DAT.

Bioassay–Lethal Effects-Adults. Adult *C. johnsoni* and *C. carnea* treated with either cyantraniliprole or lambda-cyhalothrin had higher mortality at 1 and 2 DAT and none survived by 10 DAT (1 DAT: $F = 42.08$, df = 11, 121, $P = 0.0001$; 2 DAT: $F = 6.74$, df = 11, 44, $P = 0.0001$; 10 DAT: $F = 6.74$, df = 11, 44, $P = 0.0001$) (Table 2). Significantly higher mortality was observed for adult *C. johnsoni* treated with spinetoram at 1 and 2 DAT (1 DAT: $F = 42.08$, df = 11, 121, $P = 0.0001$; 2 DAT: $F = 41.39$, df. = 11, 44, $P = 0.0001$). Most of the adult *C. johnsoni* and *C. carnea* treated with spinetoram died by 10 DAT. Although chlorantraniliprole treated adults had a lower number of deaths compared with most other treatments by 2 DAT, none survived by 10 DAT in either species. There was no significant mortality of adults in the control or those treated with novaluron in either species.

Bioassay–Sublethal Effects-Larva to Adult Survival. Larva to adult survival was lower in all insecticide treatments compared with the number of larvae that survived to adults in the control for both *C. johnsoni* and *C. carnea* ($F = 16.68$; df = 11, 44; $P = 0.0001$) (Table 3). None of the larvae of either species survived to adult in the novaluron or lambda-cyhalothrin treatments.

Bioassay–Sublethal Effects-Larva to Adult Developmental Time. There was no difference in larva to adult developmental time among the treatments within a species (Table 3). There were no differences between treatments in larva to adult development time for *C. johnsoni* larvae; however, those treated with either chlorantraniliprole or cyantraniliprole had a longer larva to adult developmental time compared with *C. carnea* larvae ($F = 3.31$; df = 7, 26; $P = 0.0119$).

Table 2. Mortality (%) (mean ± SEM) of adult *Chrysoperla carnea* and *Chrysoperla johnsoni* treated with different insecticides or water (control) 1, 2, and 10 DAT

Treatment	Max. label rate/ha	Mg AI/ liter	Mortality (%)[a]					
			1 DAT		2 DAT		10 DAT	
			C. carnea	*C. johnsoni*	*C. carnea*	*C. johnsoni*	*C. carnea*	*C. johnsoni*
Control	N/A	N/A	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0c	0.0 ± 0.0c
Chlorantraniliprole	315.2 g	117.9	16.7 ± 9.4de	25.0 ± 7.5de	29.2 ± 9.6d	25.0 ± 7.5de	100.0 ± 0.0a	100.0 ± 0.0a
Cyantraniliprole	1.5L	160.2	83.3 ± 9.4b	83.3 ± 7.1b	83.3 ± 9.4b	83.3 ± 7.1b	100.0 ± 0.0a	100.0 ± 0.0a
Novaluron	3.7L	388.5	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	4.2 ± 4.2c	4.2 ± 4.2c
Spinetoram	490.4 g	131.1	25.0 ± 7.5de	50.0 ± 12.3c	58.3 ± 5.6c	70.8 ± 9.6b	100.0 ± 0.0a	91.7 ± 5.6b
Lambda-cyhalothrin	187.1 ml	49.9	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a

[a] Means within each DAT for *C. carnea* and *C. johnsoni* followed by the same letters are not significantly different at $P > 0.05$ (LSMEANS Test).

Table 3. Larva to adult survival (%), developmental time (d), and adult sex ratio (%) (mean ± SEM) of *Chrysoperla carnea* and *Chrysoperla johnsoni* treated as second instars with different insecticides or water (control)

Treatment	Max. label rate/ha	Mg AI/liter	Survival (%)[a] larva to adult		Developmental time (d)[a] larva to adult		Adult sex ratio (%)[a,b]	
			C. carnea	*C. johnsoni*	*C. carnea*	*C. johnsoni*	*C. carnea*	*C. johnsoni*
Control	N/A	N/A	86.7 ± 8.2a	80.0 ± 8.2a	17.9 ± 0.4c	19.0 ± 0.0bc	53.4 ± 6.4a	50.0 ± 22.4a
Chlorantraniliprole	315.2 g	117.9	20.0 ± 8.2cde	26.7 ± 6.7cde	18.5 ± 0.2c	19.9 ± 0.6ab	56.6 ± 11.3a	53.4 ± 3.4a
Cyantraniliprole	1.5L	160.2	6.7 ± 6.7de	13.3 ± 8.2de	18.4 ± 0.2c	19.8 ± 0.8ab	66.8 ± 9.1a	53.4 ± 22.6a
Novaluron	3.7L	388.5	0.0 ± 0.0e	0.0 ± 0.0e	—	—	—	—
Spinetoram	490.4 g	131.1	33.3 ± 10.5bcd	40.0 ± 6.7bcd	17.9 ± 0.1c	18.9 ± 0.1bc	50.0 ± 20.4a	50.0 ± 22.4a
Lambda-cyhalothrin	187.1 ml	49.9	0.0 ± 0.0e	0.0 ± 0.0e	—	—	—	—

[a] Means within each fitness parameter for *C. carnea* and *C. johnsoni* followed by the same letters are not significantly different at $P > 0.05$ (LSMEANS Test).
[b] Adult sex ratio (adults emerged from treated larvae) calculated as the percentage of females = ([females/(males + females)] * 100).

Bioassay–Sublethal Effects-Adult Sex Ratio of Adults Emerged from Treated Larvae. There were no differences among the treatments for the sex ratio of adults that emerged from *C. johnsoni* and *C. carnea* larvae (Table 3). The sex ratio was ≈1:1 ratio of females to males ($F = 0.12$; df = 7, 27; $P = 0.9963$).

Bioassay–Sublethal Effects-Adult Longevity. Adult longevity was not different between males and females within a treatment and species (Table 4). Longevity of adults treated with novaluron was similar to the longevity of adults in the control. Adults treated with chlorantraniliprole, cyantraniliprole, spinetoram, or lambda-cyhalothrin treated had shorter longevity compared with the adults in the control or novaluron treatment ($F = 22.07$; df = 23, 223; $P = 0.0001$).

Bioassay–Sublethal Effects-Fecundity. No adult females survived the chlorantraniliprole, cyantraniliprole, spinetoram, and lambda-cyhalothrin treatments preventing the assessment of fecundity. Within the control and novaluron treatment, fecundity of *C. carnea* was higher than the fecundity of *C. johnsoni* ($F = 3.74$; df = 3, 22; $P = 0.0259$) (Table 5). There was no difference in fecundity within each species for females treated with either novaluron or the control.

Bioassay–Sublethal Effects-Fertility. Female fertility was higher for *C. carnea* than *C. johnsoni* in the control ($F = 36.44$; df = 3, 22; $P = 0.0001$) (Table 5). Significantly higher fertility was observed in both *C. johnsoni* and *C. carnea* females in the control compared with the fertility of females treated with novaluron ($F = 36.44$; df = 3, 22; $P = 0.0001$). Despite the large number of eggs laid by the females treated with novaluron, only a negligible number from either species hatched.

Bioassay–Sublethal Effects-Egg Viability. Egg viability was significantly higher for C. *carnea* than for *C. johnsoni* in the control ($F = 359.82$; df = 3, 22; $P = 0.0001$). Egg viability was lower in novaluron treatments for both species compared with egg viability in the controls, ($F = 359.82$; df = 3, 22; $P = 0.0001$) (Table 5). Egg viability 1% or less for both species when females were treated with novaluron.

Discussion

We demonstrated that the five insecticides evaluated in this laboratory study negatively affected *C. johnsoni* and *C. carnea* using a robust multiple route of exposure assay. Most of these insecticides had direct lethal effects and caused significant mortality to *C. johnsoni* and *C. carnea* larvae and adults. Some insecticides had sublethal effects on larval to adult developmental time and survival and adult female fecundity, fertility, and egg viability. Although most of the insecticides tested had only lethal activity, novaluron exhibited both direct and indirect negative effects on both lacewing species. As the management of primary tree fruit pests moves away from traditional broad spectrum OP insecticides to newer reduced-risk and OP-replacement insecticides, the potential impact of these newer products on the stability of IPM is an important concern.

Although the European green lacewing *C. carnea* was a commercially purchased and laboratory-reared

Table 4. Longevity (d) (mean ± SEM) of adult *Chrysoperla carnea* and *Chrysoperla johnsoni* treated with different insecticides or water (control)

Treatment	Max. label rate/ha	Mg AI/liter	Adult longevity (d)[a]			
			C. carnea		*C. johnsoni*	
			Male	Female	Male	Female
Control	N/A	N/A	43.0 ± 10.1bc	48.3 ± 8.4bc	46.0 ± 8.8bc	45.6 ± 9.1bc
Chlorantraniliprole	315.2 g	117.9	3.6 ± 0.7d	5.5 ± 0.6d	3.8 ± 0.7d	2.9 ± 0.7d
Cyantraniliprole	1.5L	160.2	2.3 ± 0.1d	2.4 ± 0.4d	1.3 ± 0.2d	1.3 ± 0.2d
Novaluron	3.7L	388.5	54.8 ± 18.0ab	46.8 ± 5.0bc	46.0 ± 11.8bc	38.0 ± 7.5c
Spinetoram	490.4 g	131.1	1.6 ± 0.2d	4.4 ± 0.5d	1.7 ± 0.4d	3.3 ± 0.8d
Lambda-cyhalothrin	187.1 ml	49.9	1.0 ± 0.0d	1.0 ± 0.0d	1.0 ± 0.0d	1.0 ± 0.0d

[a] Means between species and gender followed by the same letters are not significantly different at $P > 0.05$ (LSMEANS Test).

Table 5. Fecundity, fertility, and egg viability (%) (mean ± SEM) of adult *Chrysoperla carnea* and *Chrysoperla johnsoni* treated with different insecticides or water (control)

Treatment	Max. label rate/ha	Mg AI/ liter	Fecundity[a,b]		Fertility[a,c]		Egg viability (%)[a,d]	
			C. carnea	*C. johnsoni*	*C. carnea*	*C. johnsoni*	*C. carnea*	*C. johnsoni*
Control	N/A	N/A	694.4 ± 70.8ab	442.8 ± 92.9c	472.2 ± 47.1a	284.2 ± 59.9b	68.3 ± 1.1a	59.7 ± 3.8b
Chlorantraniliprole	315.2 g	117.9	—	—	—	—	—	—
Cyantraniliprole	1.5L	160.2	—	—	—	—	—	—
Novaluron	3.7L	388.5	737.5 ± 70.8a	492.0 ± 64.0bc	4.8 ± 0.8c	2.5 ± 0.7c	0.8 ± 0.2c	0.6 ± 0.2c
Spinetoram	490.4 g	131.1	—	—	—	—	—	—
Lambda-cyhalothrin	187.1 ml	49.9	—	—	—	—	—	—

[a] Means within each fitness parameter for *C. carnea* and *C. johnsoni* followed by the same letters are not significantly different at $P > 0.05$ (LSMEANS Test).
[b] Fecundity = avg. total no. of eggs laid per 20 d period.
[c] Fertility = avg. total no. of eggs hatched.
[d] Egg viability (%) = [(Fertility/Fecundity)*100].

species, it showed sensitivity to these insecticides in a similar manner to the field-collected and laboratory-reared western United States species, *C. johnsoni*. This demonstrates the toxicity of these chemicals to two green lacewing species irrespective of their varied geological distribution and further confirms *C. carnea*'s suitability as a model insect for predicting pesticide impacts on similar species. We speculate that the higher fecundity observed for *C. carnea* was a result of its long-term selection of high reproductive females in commercially reared insect colonies when compared with recently field collected and laboratory-reared *C. johnsoni*.

The lethal and sublethal effects of novaluron on lacewings were significant in this study. Novaluron killed all *C. johnsoni* and *C. carnea* larvae but none of the adults. This allowed us to observe novaluron-treated female reproductive performance including egg production, fertility, and viability. Those adult *C. carnea* and *C. johnsoni* females that survived being treated with novaluron laid a large number of healthy-looking eggs which appeared similar to eggs laid by females in the control. However, only a negligible number of these eggs were fertile. Thus, expanding this experiment to investigate sublethal effects including egg viability has given us valuable information on this insecticide's effects beyond acute toxicity.

In addition to mortality and reduced fecundity, exposure to pesticide toxicants may result in simultaneous manifestation of multiple sublethal effects such as shortened life span, mutations in offspring, weight loss and changes in fertility rates, behavior, preoviposition time, developmental rates, and sex ratio in insects (Stark and Banks 2003, Stark et al. 2004). The insecticides and assay method we used in this study resulted in high treatment mortality that limited the evaluation of sublethal effects. We did not observe any significant difference in adult longevity by gender between *C. carnea* and *C. johnsoni* in either the control or novaluron treatment. In contrast, the evaluation of larva to adult developmental time, survival, and sex ratio of emerged adults could not be completed because no novaluron-treated larvae survived.

According to Croft (1990) and Banken and Stark (1998), mortality or sublethal effects of pesticides occur through three routes: that is, direct contact with the insecticide, residual uptake from treated surfaces and food chain uptake through the consumption of prey or host plants containing the pesticide. Acute toxicity assays using only topical application may not be predictive of impacts of pesticides in the field (Stark et al. 1995) because beneficial organisms may receive pesticide exposure from multiple routes (Longley and Stark 1996). We incorporated all three routes of pesticide intake into our lacewing studies. The IOBC has developed standard protocols for the analysis of the impact of pesticides on nontarget organisms (Hassan 1985, Vogt et al. 1998, Vogt 2000). According to IOBC standards used to test acute toxicity of pesticides, individual test organisms of uniform age are either exposed to dried residue on treated surfaces or directly sprayed and moved to a clean surface and monitored for mortality or reduction in predation or parasitism in the laboratory. By using a method that incorporates multiple routes of pesticide exposure, we used a more robust method to test insecticide toxicity on biological control agents.

Demographic toxicological analysis or the life table response experiment (LTRE) is another approach used in testing side effects of pesticides on natural enemies (Stark et al. 2004). The advantage of this approach is that a total measure of the effect is determined which incorporates lethal and sublethal effects into one end point, the intrinsic rate of natural increase (r) (Stark et al. 2004). Although not evaluated in this experiment, we conducted a similar study to estimate the intrinsic rate of natural increase (r) of *C. carnea* when exposed to these same insecticides at the maximum field rate and $1/10^{th}$ of that amount. Results from that study demonstrated a negative r for *C. carnea* when treated with chlorantraniliprole, cyantraniliprole, or lambda-cyhalothrin and 76.5 and 88.6% reduction in the r when lacewings were treated with novaluron or spinetoram, respectively (unpublished data). Information from that other study combined with the results from this current study demonstrates the potential toxic effects of certain reduced-risk and OP-replacement insecticides to insect predators.

In response to the FQPA 1996, the EPA has established classification for two new insecticide groups, reduced-risk and OP- replacement insecticides (US EPA 1996, 1997). The EPA defined reduced-risk insecticides as those that exhibit lower environmental impact and reduced toxicity to humans, mammals and other wildlife, while reducing the risk of ground water contamination (US EPA 2006). According to an EPA statement that the use of reduced-risk insecticides could improve conservation of natural enemies and therefore contribute to the success of IPM programs (US EPA 2006). In this study, we demonstrated that the OP-replacement insect growth regulator (IGR) insecticide novaluron did not exhibit characteristics to support it as safe material to green lacewings under laboratory conditions. The same conclusion can be made for the three reduced-risk insecticides chlorantraniliprole, cyantraniliprole, and spinetoram. It is important to note that results from laboratory assays conducted on glass surfaces need to be verified in the field because residue levels on plant surfaces in the field will likely be different under various environmental conditions.

In summary, results from this laboratory study demonstrate the potential that several reduced-risk and OP-replacement insecticides can negatively affect green lacewings. In general, we observed similar results between one of our native species (*C. johnsoni*) and *C. carnea*, our model test insect. Based upon these laboratory results, there is a possibility that use of some of these products in the field may disrupt pest management programs and cause secondary pest outbreaks and additional pesticide use. Using our robust assay with multiple routes of exposure and studying these chemicals beyond their acute toxicities has provided an opportunity to find hidden toxic properties of these insecticides to natural enemies which otherwise might have been overlooked. Information obtained from this study can be used to develop strategies to safely use these products in tree fruit orchards and similar cropping systems.

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