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Effect of soil application of imidacloprid on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), used for biological control in greenhouse

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Abstract

In the greenhouse, survival of adult green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), was reduced after feeding on flowers from plants treated with a soil application of imidacloprid (Marathon 1% G, label rate and twice label rate). Percent survival for *C. carnea* at 10 d was statistically different between treatments and controls: 79% for untreated flowers, 14% for label rate, and 6% for twice label rate. Trembling was observed in imidacloprid treatments, but not controls. A cold anthrone test showed that *C. carnea* were feeding equally from all treatments, confirming that lacewings were not starving to avoid feeding on treated flowers. In support of these data, a previous study in this system using residue analysis demonstrated that soil-applied imidacloprid was translocated to flower nectar. Consequently, plants treated with imidacloprid for the control of greenhouse pests will reduce populations of *C. carnea* and lower their efficacy as biological control agents.
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Keywords: Greenhouse IPM; Imidacloprid; Systemic insecticide; *Chrysoperla carnea*; Chrysopidae; Neuroptera

1. Introduction

Integrated pest management (IPM) depends on multifaceted control measures. Consequently, it is important to know the potential side effects of using insecticides with augmentative and conservation biological control. Sales and use of neonicotinoid insecticides soar above organophosphates, carbamates, and pyrethroids for pest control (Matsuda et al., 2001). One of these, imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine, is a systemic neonicotinoid insecticide applied to the soil to control many sucking and chewing insects. Imidacloprid acts upon the nervous system, causing blockage of postsynaptic acetylcholine receptors (Ware, 2000). Because of the systemic mode of action and low toxicity to humans, imidacloprid has become a popular insecticide worldwide for use in ornamentals, field crops, and vegetables (Ishaaya and Horowitz, 1998; Matsuda et al., 2001; Nauen and Denholm, 2005). Imidacloprid is registered in approximately 120 countries and is used on over 140 different agricultural crops (Buffin, 2005). It is marketed under many names depending on concentration and how it is administered. Trade names include Gaucho, Provado, Admire, Marathon, Merit, Imicide, Confidor, Intercept, Winner, Premier, and Premise.

The Koppert Biological System website is an important resource for IPM that lists compatibility between natural enemies and insecticides (Koppert, 2005). The website considers the use of foliar-applied imidacloprid harmful to beneficial insects, such as the green lacewing *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae); however, soil drenches of imidacloprid are listed as compatible with *C. carnea*. This recommendation is supported in a review of insecticides with novel modes of action (Ishaaya and Horowitz, 1998).

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According to the National Union of French Beekeepers (Apiservices, 2005) and Commissioners and Beekeepers of the European Union (Bonizzoni et al., 2006) imidacloprid should not be used due to its non-target effects on honey bees. Gaucho was banned in France from 1999 to 2006 (Anonymous, 2005). Some research has supported the claim by bee keepers of "mad bee disease". In the field, imidacloprid used as a seed treatment, (Gaucho, 40.7% AI, Bayer CropScience, Research Triangle Park, NC) is linked to alteration in foraging, recruitment, and mortality of honey bee, *Apis mellifera* (L.). In a review paper that used published data to estimate the exposure of honey bees to imidacloprid, it was found that honey bees were exposed to lethal and sublethal doses in fields that regularly used imidacloprid (Rortais et al., 2005). When honey bees were given 24 ppb imidacloprid in artificial nectar, they experienced decreased foraging activity (60%) and decreased activity in the hive (Decourtye et al., 2004). In corn, seed treatments of Gaucho resulted in 2.1 ppb imidaclopird in pollen (Bonmatin et al., 2005). In sunflowers, Gaucho when applied as a seed treatment resulted in 13 ppb in pollen (Laurent and Rathahoa, 2003).

Effects of foliar sprays of imidacloprid are documented. Bumble bees, *Bombus terrestris* (L.), showed 30% mortality in 24 h after imidacloprid was sprayed on cucumber plants (Incerti et al., 2003). After foliar applications of imidacloprid in cotton, the wasp parasitoid, *Microplitis croceipes* (Cresson), when fed on nectar, had 25% decrease in longevity and 77% reduction in host finding (Stapel et al., 2000). Survival for fifth instar nymphs of spined soldier bug, *Podisus maculiventris* (Say), was 50% when exposed topically to 70 ppb imidacloprid (De Cock et al., 1996).

For greenhouse, there are limited data on the effects of soil applications of imidacloprid on nectar feeding by biological control agents. *Bombus terricola occidentalis* (Greene) showed 96% mortality after foraging on tomato flowers from plants treated with a soil application of imidacloprid (Merit 75W, 75% AI, Bayer Environmental Science, Research Triangle Park, NC) (Al-Jabr, 1999). Mortality of the minute pirate bug, *Orius tristicolor* (White), increased when it pierced marigold leaves, *Tagetes erecta* (L.), treated with a soil drench of imidacloprid (Marathon 1% G, 1% AI, Olympic Horticultural Products, Mainland, PA) (Sclar et al., 1998). A soil drench of imidacloprid (Marathon 1% G) was translocated to flowers of sunflowers and caused 38% mortality in the pink lady beetle, *Coleomegilla maculata* (DeGeer), when confined to flowers for food (Smith and Krischik, 1999). The soil or irrigation applied formulation of imidacloprid, Intercept 60 WP (60% AI, Bayer CropScience, Calgary, Canada), states on the label that the product may result in adverse affects on pollinators and other beneficials in the greenhouse (Bayer, 2004).

The objective of this experiment was to investigate the effect of soil applications of imidacloprid on adult *C. carnea* used for biological control in greenhouse, since few studies evaluate the effects of soil-applied imidacloprid on nectar feeders. *C. carnea* larvae are generalist predators that attack eggs and soft-bodied insects, such as aphids, spider mites, whiteflies, and thrips. After feeding on insects, larvae turn into adults that feed on nectar and lay eggs. We evaluated whether adult *C. carnea* had reduced survival when fed flowers from buckwheat, *Fagopyrum esculentum* (Moench), and Mexican milkweed, *Asclepias curassavica* (L.), treated with soil-applied imidacloprid.

2. Materials and methods

2.1. Insect systems

Three thousand adult *C. carnea* were obtained from Rincon–Vitova Insectaries (Ventura, CA) 1 week before the start of each of three replicate experiments. This permitted *C. carnea* to feed before the experiment started and habituate to the food array and cages. Adult *C. carnea* arrived in paper containers and were distributed among nine mesh cages (30 cm × 30 cm × 30 cm) (BioQuip, Rancho Dominquez, CA) for the first replicate and twelve mesh cages for the second and third replicates. Upon release, cages were supplied with three 35 × 10 mm petri dishes smeared with commercial *C. carnea* diet (Rincon–Vitova), four tubes with water (Aquatube, Syndicate Sales, Kokomo, IN), four tubes with 20% honey–water, and two tubes with untreated flowers of *F. esculentum* and *A. curassavica*. The cages were cleaned and provisioned daily until the start of the experiment.

2.2. Plant systems

Fagopyrum esculentum and *A. curassavica* were used as nectar plants. *F. esculentum* is commonly used in organic production to provide shelter and nectar for beneficial insects, such as lady beetles, *Hippodamia convergens* (Guérin-Ménéville), minute pirate bugs, *Orius insidiousus* (Say), and adult *C. carnea* (Plotkin, 2005). *Asclepias curassavica* has small, open flowers comprising an umbel which produces large amounts of sucrose-rich nectar that attract beneficial insects (Wyatt and Broyles, 1994). Plants were grown in the greenhouse under 16:8 photoperiod at 20 °C daytime and 16 °C nighttime temperatures. Plugs of *A. curassavica* plants were obtained from North Creek Nurseries (Landenberg, PA) and seeds of *F. esculentum* were obtained from Johnny's Select Seeds (Winslow, ME). Multiple plants were grown in 15-cm pots containing Scott's Metro-Mix soilless media and 10 g Osmocote (Scotts Sierra Horticultural Products Company, Marysville, OH). Plants were fertilized weekly with a dilute drench of Peters 20-20-20 Fertilizer (Allentown, PA). At time of flowering, imidacloprid granules (Marathon 1% G) were applied to the soil surface (6 g, 1x, label rate and 12 g, 2x, twice label rate) at 3 weeks prior to feeding. The application rate was determined by extrapolation of soil volume for the recommended label rate of a 4-inch pot in which our preliminary studies were performed.

2.3. Bioassays of adults: Survival and trembling at 1 d and 10 d

The experiment consisted of three experiments replicated sequentially. At the start of the experiment, honey–water tubes and artificial diet were removed. Replicate One had three cages per treatment, 112 *C. carnea* adults per cage, four flowers each *F. esculentum* and *A. curassavica* for a *C. carnea*/flower ratio of 14/1; Replicate Two had four cages per treatment, 135 *C. carnea* adults per cage, six flowers of *F. esculentum* and two flowers of *A. curassavica* for a *C. carnea*/flower ratio of 17/1; and Replicate Three had four cages per treatment, 177 *C. carnea* adults per cage, nine flowers of *F. esculentum* and two flowers of *A. curassavica*, for a *C. carnea*/flower ratio of 15/1.

Each day surviving and trembling *C. carnea* were counted. In all three replicate experiments, behavioral changes, such as trembling, were observed in only 1x and 2x treatments. Trembling in *C. carnea* was expressed as an inability to fly or turn over when placed on their backs. Survival and trembling for each replicate was analyzed by ANOVA. If the Levene's test for homogeneity was significant, then data were transformed with a Cox-box transformation. If the assumptions of homogeneity with transformation could not be achieved, a Welch test was used (SAS, 2005). The three replicate experiments were analyzed as a randomized complete block (RCB) for treatment and block effects using PROC GLM (SAS, 2004).

2.4. Cold anthrone test

A cold anthrone test was performed to determine the presence of fructose sugars from nectar within the guts of *C. carnea* after feeding on flowers for 24 h (Van Handel, 1967). The anthrone reagent was prepared by adding 380 ml of sulfuric acid to 150-ml deionized water and 0.75 g of anthrone was stirred over an ice bath. *C. carnea* from C, 1x, and 2x treatments and a starved treatment were frozen, crushed, and 5 ml of anthrone reagent was pipetted onto them. After 1 h, a dark green color indicated a positive reaction, confirming the presence of fructose in nectar. Data were analyzed by a Chi-square (χ^2) test.

3. Results

3.1. Bioassays of adults: Survival and trembling at 1 d and 10 d

Chrysoperla carnea that fed on flowers from treated plants showed significantly reduced survival over time in all three replicate experiments (Fig. 1). At 1 d, the percent survival for 1x was 95% (all replicates: 95%, 93%, and 98%), for 2x was 92% (95%, 84%, 96%) and for control was 97% (95%, 97%, and 100%) (Fig. 2). Control treatments were significantly different from 2x treatments ($F = 12.72$; $df = 2, 23$; $P = 0.0002$), replicate experiments were significantly different ($F = 20.45$; $df = 2, 23$; $P < 0.0001$), and there was significant treatment by replicate interactions ($F = 6.76$; $df = 2, 23$; $P = 0.0009$). When analyzed separately, replicate experiments one and three showed no significant difference among control and flowers from treated plants, while in replicate two, control treatments had significantly higher survival than 2x treatments (replicate one: $F = 0.21$; $df = 2, 5$; $P = 0.817$; replicate two: $F = 10.24$; $df = 2, 9$; $P = 0.015$; replicate three: $F = 3.88$; $df = 2, 9$; $P = 0.061$).

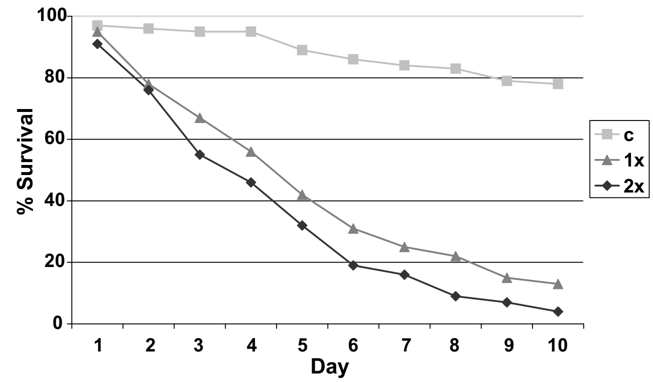

Fig. 1. Survival through time of adult *C. carnea.*

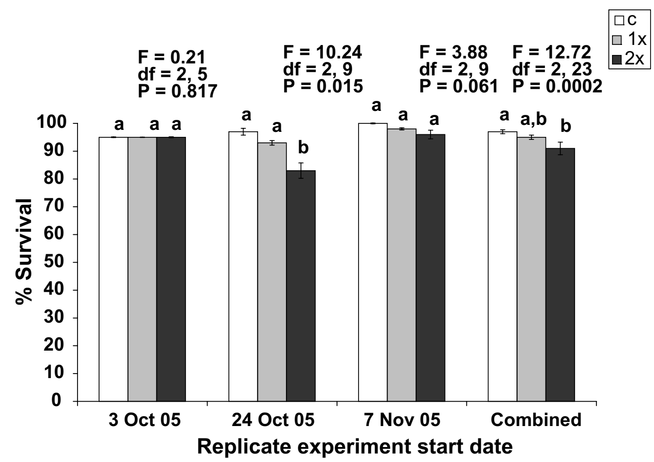

Fig. 2. Bioassays: Survival at 1 d of adult *C. carnea.*

At 10 d, the percent survival for 1x was 14% (all replicates: 24%, 13%, and 4%), for 2x was 6% (13%, 2%, and 2%), and for control was 79% (86%, 70%, and 82%) (Fig. 3). Control treatments were significantly different from 1x and 2x treatments ($F = 456.9$; $df = 2, 24$; $P < 0.0001$), replicates were significantly different ($F = 9.2$; $df = 2, 24$; $P = 0.0011$), and there was significant treatment by replicate interaction ($F = 3.39$; $df = 4, 24$; $P = 0.0246$). When analyzed separately, control treatments had significantly higher survival than 1x and 2x treatments in all three replicate experiments (replicate one: $F = 83.3$; $df = 2, 5$; $P = 0.0051$; replicate two: $F = 1512.$; $df = 2, 9$; $P < 0.0001$; replicate three: $F = 195.5$; $df = 2, 9$; $P < 0.0001$).

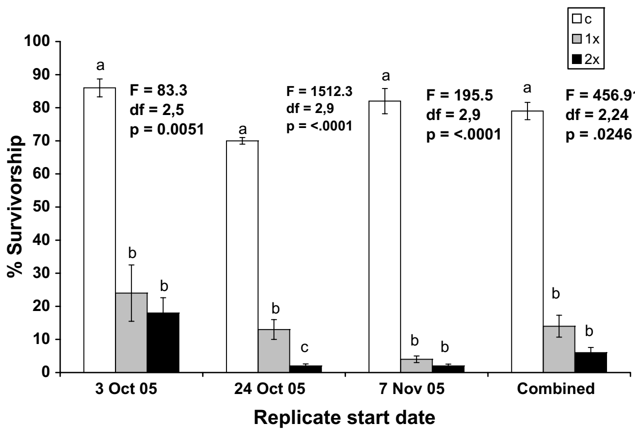

Fig. 3. Bioassays: Survival at 10 d of adult *C. carnea.*

Chrysoperla carnea that fed on flowers from treated plants showed significantly higher trembling in all three replicate experiments (Fig. 4). At 6 d, the percent trembling for 1x was 28% (all replicates: 50%, 22%, and 13%), for 2x was 27% (40%, 26%, and 15%) and for control was 0.7% (0%, 2%, and 0%) (Fig. 5). The results show that control treatments were significantly different from 1x and 2x treatments ($F = 16.4$; $df = 2, 24$; $P < 0.0001$), replicates were

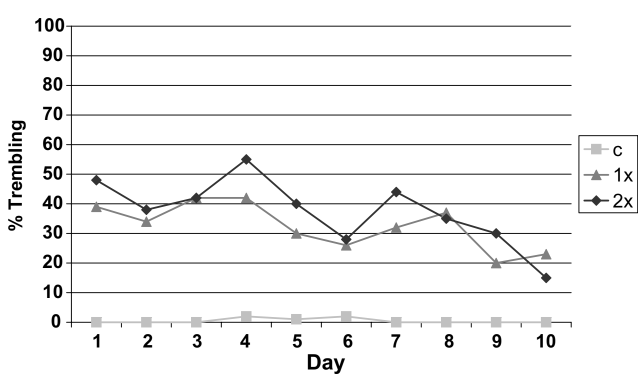

Fig. 4. Trembling through time of adult *C. carnea.*

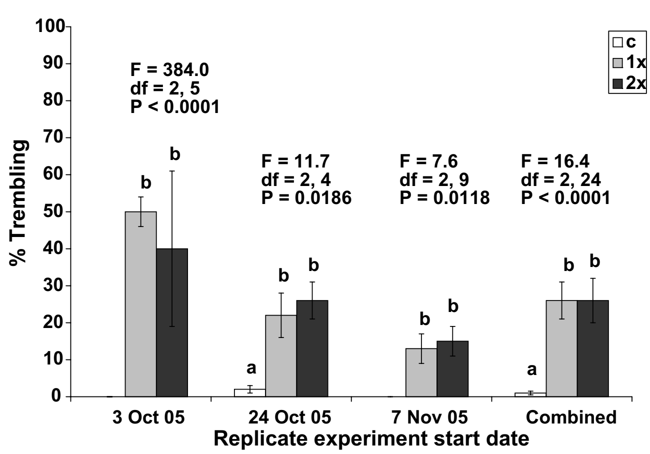

Fig. 5. Bioassays: Trembling at 6 d of adult *C. carnea.*

significantly different ($F = 7.1$; $df = 2, 24$; $P < 0.0038$), and there was not significant treatment by replicate interaction ($F = 2.2$; $df = 2, 2\ 4$; $P = 0.1025$). When analyzed separately, control treatments had significantly lower trembling than 1x and 2x treatments in all three replicate experiments (replicate one: $F = 384.0$; $df = 2, 5$; $P < 0.0001$; replicate two: $F = 11.7$; $df = 2, 4$; $P = 0.0186$; replicate three: $F = 7.6$; $df = 2, 9$; $P = 0.0118$).

3.2. Cold anthrone test

A cold anthrone test showed that *C. carnea* fed equally from all treatments, confirming that *C. carnea* did not starve to avoid feeding on treated flowers (Table 1). The number tested positive for sugars for 1x was 72% (80% and 63%), for 2x was 82% (87% and 77%), and for controls was 78% (73% and 83%). The starved population tested negative for sugars.

4. Discussion

For greenhouse biocontrol, adult *C. carnea* are good dispersers and will lay eggs near pests (Williams, 1999), while larvae purchased from insectaries are randomly released from paper containers. Providing a nectar source containing sugar and protein will increase fecundity and extend adult *C. carnea* survival (Gurr et al., 2004). However, reducing their numbers in the greenhouse or field, or disrupting their mating will result in less larvae and consequently less predation with potential increases in pest densities (Medina et al., 2004).

Greenhouse growers depend on both biological and chemical control for pest management, and need to use compatible control strategies. This study demonstrated that soil application of imidacloprid was translocated to flowers and decreased survival of adult *C. carnea* when they fed on nectar. The cold anthrone test demonstrated that *C. carnea* fed on flowers from treated plants and did not die from starvation. In addition, *C. carnea* fed equally on flowers from treated and untreated plants, indicating that imidacloprid did not exhibit anti-feedant properties. *C. carnea* suffered from toxic effects, as exhibited by trembling, inability to fly, and inability to right themselves when placed upside down. This abnormal behavior was also found in a study on spined soldier bug, *P. maculiventris*, when exposed to imidacloprid in drinking water (De Cock et al., 1996). Residue analysis of *F. esculentum* flowers confirms the translocation of imidacloprid to nectar. Imidacloprid concentration was 0 ppm in untreated flowers, 15 ppb in 1x flowers, and 29 ppb in 2x flowers (Krischik et al., 2007).

We could find few studies that evaluated the effects of soil-applied imidacloprid on beneficial insects in the greenhouse. *B. occidentalis* mortality was increased 96% after foraging on tomato flowers from plants treated with a soil application of imidacloprid (Al-Jabr, 1999). Mortality of *O. tristicolor* increased when it pierced leaves of *T. erecta* treated with a

Table 1
Cold anthrone test for sugars in nectar that were present in lacewing guts after 24 h of exposure to flowers from imidacloprid-treated plants and control

Treatment	Replicate One % (*n*/30)	Replicate Two % (*n*/30)	Combined % (*n*/60)
Label rate, 1x	80 (24)	63 (19)	72 (43)
Label rate, 2x	87 (26)	77 (23)	82 (49)
Untreated	73 (22)	83 (25)	78 (47)
Starved	0 (0)	0 (0)	0 (0)
Chi-square (χ^2) for all treatments	60.60, $df = 3$, $P < 0.0001$	52.64, $df = 3$, $P < 0.0001$	110.91, $df = 3$, $P < 0.0001$
Chi-square (χ^2) for treatments w/o starved	1.65, $df = 2$, $P = 0.4386$	3.23, $df = 2$, $P = 0.1985$	1.76, $df = 2$, $P = 0.4150$

Positive response indicates presence of sugars and nectar feeding.

soil drench of imidacloprid (Sclar et al., 1998). A soil drench of imidacloprid was translocated to flowers of sunflowers and *C. maculata* experienced 38% mortality after feeding on flowers (Smith and Krischik, 1999). The formulation of imidacloprid, Intercept 60 WP (60% AI, Bayer CropScience, Calgary, Canada), states on the label that the product may result in adverse affects on bees, predators, and parasitoids in the greenhouse (Bayer, 2004).

A few studies document the effects of imidacloprid sprays on beneficials. *B. terrestris* experienced 30% mortality when foraging on cucumbers previously sprayed with imidacloprid (Incerti et al., 2003). In a spray chamber bioassay, 83.3% of adult *C. carnea* died in 24 h when exposed to imidacloprid (Elzen et al., 1998). *C. carnea* adults had 60% shorter longevity after feeding on sugar water conatining10 ppm imidacloprid, while larvae had 60% mortality after consuming eggs sprayed with 10 ppm imidacloprid (Kumar and Santharam, 1999).

Due to its systemic mode of action and soil application method, many growers think imidacloprid conserves beneficials. This is the first study that showed a soil application of imidacloprid is toxic to *C. carnea* adults when nectar feeding. However, effects of soil applications of systemic insecticides on other beneficials that nectar feed merits research attention.

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