

Effects of Systemic Imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae)

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ABSTRACT The coccinellid predator *Coleomegilla maculata* (DeGeer) is found throughout the central and eastern United States and is a potential biological control agent for interiorscapes. Currently, the systemic insecticide imidacloprid is widely used in interiorscape and landscape integrated pest management. Effects of imidacloprid on the fitness and behavior of *C. maculata* were examined by confining groups of adults with inflorescences of treated sunflower, *Helianthus annuus* L. 'Big Smile'; chrysanthemum, *Chrysanthemum morifolium* Ramat. 'Pelee'; and dandelion, *Taraxacum officinale* Wiggers. Confinement with inflorescences from imidacloprid-treated plants significantly decreased the general mobility of *C. maculata* in each plant system. The magnitude of the reduction in mobility varied with plant species. In the sunflower bioassay, survivorship was lower for beetles exposed to imidacloprid at the label rate and twice the label rate (38.3 ± 6.60 and $20.0 \pm 6.71\%$ survival, respectively) than for beetles confined to untreated controls ($97.5 \pm 2.50\%$ survival). Also, in the sunflower bioassay, beetles exposed to the label rate and twice the label rate of imidacloprid took longer to produce their 1st eggs (9.78 ± 2.01 and 14.00 ± 2.10 d after bioassay respectively) than beetles from untreated controls (2.56 ± 0.50 d after bioassay). The results reported here indicate that the use of imidacloprid may not be compatible with the coccinellid predator *C. maculata*.

KEY WORDS *Coleomegilla maculata*, imidacloprid, nontarget effects, landscape integrated pest management, systemic insecticides

CONSERVATION OF NATURAL enemies is an important component of modern integrated pest management (IPM) (Hilbeck et al. 1998a). In urban ecosystems, many pest populations are maintained at low densities by indigenous natural enemies (Flanders 1986, Raupp et al. 1992, Cobb 1997). Pest outbreaks after pesticide applications illustrate the importance of conserving natural enemies in urban landscapes (Merritt et al. 1983, Raupp et al. 1992, Hanks and Denno 1993, Terry et al. 1993). Pesticides that are compatible with natural enemies can be effective tools for urban IPM (Raupp et al. 1992).

Imidacloprid, a chloronicotinyl insecticide, was introduced to landscape pest management in the early 1990s (Sclar et al. 1998). Its systemic activity, low mammalian toxicity, and effectiveness against a wide range of pests make it an attractive choice for a variety of plant protection programs, including landscape pest management (Mullins 1993). It is effective against many pests, including aphids, scale insects, whiteflies, some Coleoptera, and some Lepidoptera (Mullins 1993). Besides its widespread use in landscapes, imidacloprid is commonly used in interiorscapes because its long-term effectiveness makes frequent applications unnecessary, its systemic activity makes its use safe in public areas, and it is effective against the most common interiorscape pests (West 1998). It has a mode of action similar to that of nicotine, functioning as an agonist on the nicotinic acetylcholine receptor

in the postsynaptic membranes (Boyd and Boethel 1998). Imidacloprid slowly degrades in the insect and the agonistic nature causes substantial disorder in the insect's nervous system.

Effects of imidacloprid on several nontarget insects have been described. Sclar et al. (1998) showed that the predator *Orius tristicolor* (White) suffered higher mortality when confined with imidacloprid-treated foliage than when confined with untreated foliage. Mizell and Sconyers (1992) found significant mortality for several insect species, including the predator *Hippodamia convergens* (Guérin-Méneville) after exposure to imidacloprid residues. Boyd and Boethel (1998) found that *Geocoris punctipes* (Say) adults were as susceptible to the foliar residue of imidacloprid as they were to the residues of methyl parathion (organophosphate) and permethrin (pyrethroid) 24 h after application.

Soil application of granular imidacloprid is the most common application method in interiorscapes. This application may be less harmful to nontarget insects than the foliar spray because it is less likely to come in direct contact with the nontarget insect (Mizell and Sconyers 1992). However, many natural enemies supplement their diet by feeding on plant material, either by feeding directly on the plant as with *O. tristicolor* (Sclar et al. 1998) or by feeding on plant pollen or nectar in the case of some predatory beetles and parasitic wasps (Hagen 1986). If the insecticide is trans-

located to flowers, then these insects may ingest imidacloprid. Additionally, systemic pesticides can in some cases be translocated to the surface of the plant or enter a vapor stage and come in direct contact with insects on the plant (Croft 1990). Any of these modes of contact could affect beneficial insects associated with imidacloprid-treated plants.

Coleomegilla maculata (DeGeer) was selected as the test organism for this study for 2 reasons. First, it is a potential biological control agent for interiorscapes, which currently release *H. convergens* for aphid control. *C. maculata*, unlike *H. convergens*, is a facultative pollen feeder; laboratory studies have shown that it is able to complete its life cycle on pollen alone (Hodek and Honek 1997). During periods of low prey density, populations of the biological control agent could be maintained on pollen from flowering plants in the interiorscape. Second, *C. maculata* has a broad geographic range in the central and eastern United States (Gordon 1985) and is commonly found in many urban and agricultural systems.

This research examined nontarget effects of the systemic insecticide imidacloprid on *C. maculata*. The effects of imidacloprid on beetle behavior and fitness were measured after confining the beetles with inflorescences of imidacloprid-treated plants. Behavioral parameters measured were walk rate and flip time. Several life history parameters including survivorship, days to 1st oviposition, and daily egg production were measured.

Materials and Methods

Plant Systems. The effects of imidacloprid on *C. maculata* were examined in 3 species of Asteraceae (Compositae). Sunflower, *Helianthus annuus* L. 'Big smile', and chrysanthemum, *Chrysanthemum morifolium* Ramat. 'Pelee', are common ornamental plants in urban landscapes and interiorscapes. Dandelion, *Taraxacum officinale* Wiggers, was selected because observations from the field indicate that *C. maculata* visit the plants early in the season to feed on pollen. For turfgrass pest control, granular imidacloprid is mixed with fertilizer and applied widely in the urban landscape. Consequently, imidacloprid may be translocated to the flowers of dandelions and affect *C. maculata*.

Study Organisms. *C. maculata* for the sunflower and chrysanthemum bioassays were reared by IPM Biologicals (Eagan, MN). Beetles used in the dandelion bioassay were reared from a laboratory colony at the University of Minnesota, St. Paul. Before the bioassays, males and females for each bioassay were kept together and mating was frequent. Beetles were maintained on diet (described below) and kept under a photoperiod of 16:8 (L:D) h, 25 ± 1°C, and 55–75% RH. Sunflowers, chrysanthemums, and dandelion flowers matured at different rates, which resulted in the initiation of bioassays at different times. Consequently, beetles were of slightly different ages at the onset of the bioassays. Within each plant system, however, beetle age and colony source was consistent.

Beetles used in the sunflower bioassay were 28–30 d old, in the chrysanthemum bioassay 14–16 d old, and 17–20 d old in the dandelion bioassay.

Treatments. Four treatments were used. For the 2 experimental treatments, potted plants were treated with Marathon 1% granular (1% [AI] imidacloprid) (Olympic Horticultural, Mainland, PA) at the maximum labeled rate appropriate for the pot size (1×), and twice that label rate (2×). Individual dandelions and chrysanthemums were grown in 15.2-cm pots; each pot received 20 mg (AI) (1×) or 40 mg (AI) (2×). Sunflowers, which were larger plants and grown in 25.4-cm pots, received 27 mg (AI) (1×) or 54 mg (AI) (2×). The control treatments in each bioassay consisted of untreated plants. A starvation treatment was included to determine the effects of 7 d without access to pollen or nectar. If beetles confined to flower heads were not feeding on pollen then no differences among treatments should be found when compared with the starvation treatment. Beetles were confined to the experimental arenas (described below) for 7 d. For the beetles confined to the flower heads of the untreated and treated plants, only pollen and nectar produced by the flower head was available as food. A wetted cotton wick was included in each experimental arena and rewetted daily.

Imidacloprid was applied to sunflowers and chrysanthemums when the flowers were in their early bud stage. Imidacloprid was therefore present in the soil and available to the plants while they were developing flowers. We began the sunflower bioassay 25 d after application, when the sunflower inflorescences were open and producing pollen. For the chrysanthemums, the bioassay began 36 d after application of imidacloprid when their inflorescences were open and producing pollen. Dandelions began continuously producing flowers ≈7 mo after planting seeds. At this time, all open and developing flower heads were removed and imidacloprid was applied to the pots. The experiment began 10 d after application when new flowers were in full bloom. During the 10-d postapplication and prebioassay period, unused flower heads were removed daily and discarded.

Bioassay Designs. In the sunflower bioassay, 105 plants per treatment and 4 beetles per plant were used. For the chrysanthemum bioassay, 11 plants per treatment and 4 beetles per plant were used. In these 2 bioassays, beetles were confined using a bag (28 by 33 cm) made of no-see-um cloth (Balson Hercules Group, New York) that was placed over the flower head and secured at the bottom with a rubber band. In the dandelion bioassay, mason jars (355 ml) were set up in the laboratory, each containing 4 beetles. A small section of no-see-um cloth was placed over the mouth of each jar and secured with a rubber band. Two blocks of 5 jars per treatment were set up on subsequent days for a total of 10 jars per treatment. Dandelion flower heads were cut from plants in the greenhouse, placed in glass scintillation vials with water, and put in the mason jars, 1 per jar. Dandelion flower heads stay open for 24–48 h. Closed flower

heads were removed from the jars and replaced with open flower heads every other day.

Behavioral Parameters Measured. After 7 d of confinement in the experimental arenas, the behavioral parameters walk rate and flip time were measured. Walk rate was measured by placing each beetle on a piece of multipurpose office paper (75 g/m²). As the beetle walked, its path was followed and traced with a pencil and the time recorded. The length of the path was measured using a string and converted to a walking rate of cm/s. Time spent following an individual beetle ranged from 1.31 to 30 s. If a beetle did not move in 30 s it was given a score of 0. A 2nd behavioral parameter, flip time, was included in the study to examine another possible reduction in general mobility caused by exposure to imidacloprid-treated plants. The behavioral parameter flip time was selected as a way of testing the ability of a beetle to right itself after dropping from an untreated or imidacloprid-treated plant. Flip time was measured by placing each beetle dorsal side down on a piece of Fisher brand medium-porosity filter paper (Fairlawn, NJ). Time until the beetle turned itself upright was recorded. If a beetle was unable to right itself in 30 s it was given a score of 31. Beetles that had died during the first 7 d of the bioassay were not included in the analysis of behavioral parameters.

Fitness Parameters Measured. After the behavioral parameters were measured, the beetles were placed individually in plastic petri dishes (60 by 50 mm) and kept in laboratory incubators. They were maintained under a photoperiod of 16:8 (L:D) h, 25 ± 1°C, and 55–75% RH. Survivorship, days to 1st oviposition (ovipositing beetles only), and daily egg production (ovipositing beetles only) were monitored for 30 d. Each petri dish contained a 0.5-ml centrifuge tube filled with distilled water and a cotton string for a wick. Tubes were refilled with water as needed. Each day, beetles were fed a small cube of "diet 7" as described by Atallah and Newsom (1966). This diet was modified by omitting tegosept, aureomycin, and potassium hydroxide.

Statistical Analysis. All behavioral and fitness parameters from the sunflower and chrysanthemum bioassays were compared using one-way analyses of variance (ANOVAs). Data for flip times from the sunflower bioassay were log transformed, data for days to 1st oviposition from the sunflower bioassay were square root transformed, and data for flip times from the chrysanthemum bioassay were transformed to inverse of the square root to equalize variance. Reported means are untransformed. In the dandelion bioassay, survivorship, days to 1st oviposition, and daily egg production for beetles were analyzed using one-way ANOVAs testing for treatment effects. Flip time and walk rate for beetles in the dandelion bioassay were analyzed using a two-way ANOVA testing for treatment and day effects. Data for flip times from the dandelion bioassay were transformed to the inverse of the square root to equalize variance. Reported means are untransformed. Means for all variables were separated using the Ryan-Einot-Gabriel-Welsh multiple

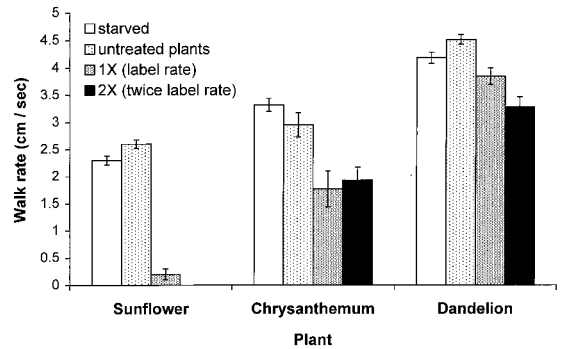


Fig. 1. Mean ± SE walking rates for *C. maculata* after 7 d of confinement to imidacloprid-treated plants, untreated plants, or starvation arenas. See Table 1 for ANOVA results.

range test option, means statement, PROC GLM, SAS, SAS Institute 1998), which controls for type I experiment wise error.

Results

Exposure to imidacloprid affected beetles in all plant systems tested. General mobility was significantly reduced, indicated by a decreased walk rate (Fig. 1; Table 1) and increased flip time (Fig. 2; Table 1), in the imidacloprid treatments in all 3 plant systems. The magnitude of the differences, however, varied with plant system. In sunflower, behavioral effects were very strong with only 1 of 19 beetles in the 2× treatment scoring a walking rate and 0 of 19 successfully flipping (Table 1). Differences were highly significant in both walking rate ($P < 0.001$) and flip time ($P < 0.001$). Because no beetles moved in the 2× treatment, the assumption of constant variance was violated. When this treatment was removed from the analysis, a significant treatment effect was still observed in both walking rate ($F = 250.6$; $df = 2, 27$; $P < 0.001$) and flip time ($F = 111.5$; $df = 2, 27$; $P < 0.001$). For beetles in the chrysanthemum and dandelion bioassays, all beetles in the 2× and 1× treatments successfully flipped and walked, but their general mobility was reduced. In the chrysanthemum bioassay, both walking rate ($P < 0.001$) and flip time ($P < 0.001$) showed significant treatment effects. In the dandelion bioassay, walking rate ($P < 0.001$) and flip time ($P < 0.001$) showed significant treatment effects.

A significant treatment effect on *C. maculata* survivorship was observed in the sunflower bioassay ($P < 0.001$). There was no statistically significant difference between survivorship for beetles from the untreated plants (97.5%) and the starvation treatment (85.5%). However, survivorship for beetles confined to the flower heads of the 1× (38.3%) and 2× (20.0%) treated sunflowers was significantly lower than controls (Fig. 3; Table 1). No treatment effect on survivorship was found in either the chrysanthemum bioassay ($P = 0.20$) or the dandelion bioassay ($P = 0.40$).

A significant treatment effect was observed in days to 1st oviposition in the sunflower bioassay ($P <$

Table 1. Effects of imidacloprid on *C. maculata* behavior and fitness in 3 plant systems

Plant system	Treatment	Mean ± SE				
		Walk rate, cm/s	Flip time, s	Survivorship, %	No. days to 1st oviposition	No. beetles that laid eggs
Sunflower	Starved	2.30 ± 0.08a	2.27 ± 0.23a	85.5 ± 5.50a	6.95 ± 0.66b	19
	Untreated	2.60 ± 0.08a	3.62 ± 0.85a	97.5 ± 2.50a	2.56 ± 0.50a	16
	1X	0.20 ± 0.10b	28.04 ± 1.63b	38.3 ± 6.60b	9.78 ± 2.01b	9
	2X	0b	31b	20.0 ± 6.71b	14.00 ± 2.10c	4
	F	F = 362.9	F = 274.2	F = 46.1	F = 15.1	
	P	P < 0.001	P < 0.001	P < 0.001	P < 0.001	
Chrysanthemum	Starved	3.32 ± 0.12a	1.41 ± 0.14a	97.72 ± 2.27a	10.61 ± 1.66a	23
	Untreated	2.95 ± 0.22a	2.44 ± 0.67a	100a	10.64 ± 1.66a	22
	1X	1.77 ± 0.33b	9.69 ± 2.52b	90.9 ± 5.08a	11.42 ± 1.75a	19
	2X	1.93 ± 0.24b	6.34 ± 1.55b	93.18 ± 3.52a	8.86 ± 1.73a	22
	F	F = 9.8	F = 10.8	F = 1.6	F = 0.4	
	P	P < 0.001	P < 0.001	P = 0.20	P = 0.76	
Dandelion	Starved	4.18 ± 0.11ab	0.99 ± 0.07a	100	13.56 ± 1.38a	16
	Untreated	4.52 ± 0.10a	1.13 ± 0.13a	100	15.05 ± 1.98a	17
	1X	3.85 ± 0.16b	2.02 ± 0.24b	100	15.88 ± 1.42a	16
	2X	3.28 ± 0.19c	4.51 ± 1.34b	97.5 ± 2.5	10.85 ± 0.86a	20
	F	F = 16.7	F = 9.9	F = 1.0	F = 2.6	
	P	P < 0.001	P < 0.001	P = 0.40	P = 0.063	

Means within plant system and column followed by the same letter are not significantly different ($P = 0.05$); Ryan-Einot-Gabriel-Welsch multiple F test.

0.001). Beetles from the 2× treatment had the longest delay in egg production (14.0 d). Days to 1st egg production in the 1× and the starvation treatments were not statistically different from one another (9.8 and 6.9 d, respectively). Beetles from untreated sunflower inflorescences had the shortest number of days to 1st oviposition (2.6 d) (Fig. 4; Table 1). No treatment effect on days to 1st oviposition was found in either the chrysanthemum bioassay ($P = 0.76$) or the dandelion bioassay ($P = 0.063$).

Discussion

Exposure to imidacloprid reduced the general mobility of *C. maculata* in all plant systems. The mode of action of imidacloprid, as an agonist in the insect's postsynaptic membrane, makes changes in behavior and general mobility possible. In a study on the effects

of systemic imidacloprid on the potato aphid, *Macrosiphum euphoribae* (Thomas), the walking rate of the aphid was significantly reduced at 3 of the 4 concentrations tested (Boiteau and Osborn 1997). A reduction in general mobility can affect the prey-finding abilities of natural enemies (Croft 1990), or it can expose natural enemies to mortality factors such as predation and desiccation (Ffrench-Constant and Vickerman 1985). The decrease in general mobility observed in the current study indicates that imidacloprid-treated plants may diminish searching behavior and prey consumption by *C. maculata*.

The decrease in beetle mobility after exposure to imidacloprid was much greater in the sunflower bioassay than in either the chrysanthemum or dandelion

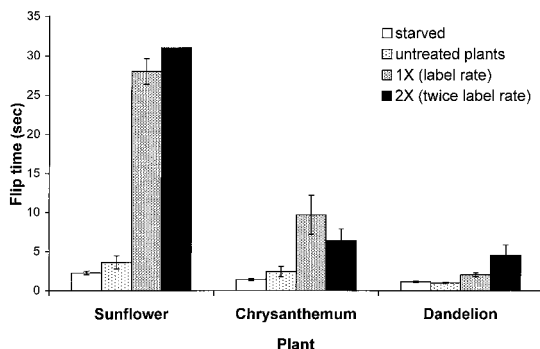


Fig. 2. Mean ± SE flip times for *C. maculata* after 7 d of confinement to imidacloprid-treated plants, untreated plants, or starvation arenas. See Table 1 for ANOVA results.

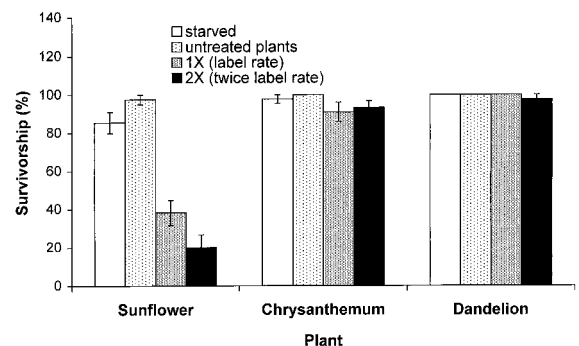


Fig. 3. Mean ± SE percent survivorship of *C. maculata* at the end of the experiments. These beetles were confined to imidacloprid-treated plants, untreated plants, or starvation arenas for 7 d, then kept individually in petri dishes and fed artificial diet daily for the subsequent 30 d. See Table 1 for ANOVA results.

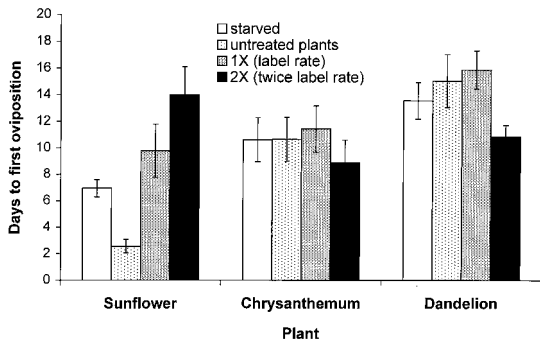


Fig. 4. Mean \pm SE days to first oviposition for *C. maculata*. These beetles were confined to imidacloprid-treated plants, untreated plants, or starvation arenas for 7 d, then kept individually in petri dishes and fed artificial diet daily for the subsequent 30 d. Day of first oviposition was recorded for each ovipositing beetle beginning with the first day in petri dishes. See Table 1 for ANOVA results.

bioassay. This difference in magnitude of the treatment effect among the plant systems could be the result of a differing amount of imidacloprid present in the pollen or plant, or it could be the result of preferential feeding by the beetles on sunflower pollen. If, through preferential feeding, beetles in the sunflower bioassay received a larger dose, we would expect the magnitude of the effects to be greater. Despite the differences in magnitude, the effects of imidacloprid on general mobility are significant in each plant system.

Survivorship was significantly reduced by exposure to imidacloprid only in the sunflower bioassay (Fig. 3). Similarly, oviposition was significantly delayed by exposure to imidacloprid only in the sunflower system (Fig. 4). These data are based on a small sample; 9 surviving beetles laid eggs in the 1 \times treatment and only 4 beetles produced eggs in the 2 \times treatment (Table 1). Beetles in the sunflower bioassay could have received a higher dose of imidacloprid because of higher levels present in the plant, or because of preferential feeding on sunflower pollen or nectar. Additionally, beetles used in the sunflower bioassay were slightly older. This could influence both their survivorship and fecundity. However, the 3 plant systems revealed the same pattern of effects on *C. maculata* behavior, which is the critical result for this study.

There are several possible mechanisms for the effects of imidacloprid on the behavior of *C. maculata*. The beetles may have been exposed to the imidacloprid by consuming it through the pollen or nectar, by tarsal contact with the leaf or petal surface, or by contact with a vapor layer around the plant. In the case of the dandelion bioassay, no foliage was included in the mason jars and only flower heads were brought from the greenhouse. Therefore, any observed treatment effects in the dandelion bioassay are the result of contact with the flower head. This implies that the imidacloprid was translocated to the flower head and affected beetles either through ingestion, contact with

the flower parts, or contact with a vapor layer around the flower parts.

Days to 1st oviposition was shorter for beetles in the 2 \times treatment than for any other treatment in both the chrysanthemum and the dandelion bioassays (Fig. 4). Although this trend was not statistically significant, it appeared in 2 plant systems. Several researchers have shown increases in fecundity after pesticide exposure, indicating the complex nature of pesticide effects (Croft 1990).

Days to 1st oviposition (Fig. 4) varied widely among the 3 plant systems. Beetles from untreated sunflowers produced eggs 2.6 d after bioassay, whereas beetles in the untreated chrysanthemums (10.6 d after bioassay) and the untreated dandelions (15.0 d after bioassay) had a longer delay. Differences in pollen quality could have a significant impact on preoviposition time or egg production rate (Hodek and Honek 1997). Furthermore, the difference in beetle ages and colony sources could account for the differences in fecundity.

Systemic insecticides and plant-expressed toxins that behave like systemics are commonly thought to have minimal impact on nontarget organisms. Genetically engineered crops, containing a gene from *Bacillus thuringiensis* variety *kurstaki* (Berliner) that encodes for the expression of an insecticidal toxin specific to Lepidoptera, are currently available in the United States and their use is likely to increase (Ostlie et al. 1997, Hilbeck et al. 1998a). Several studies demonstrate the minimal nontarget effects of this plant-expressed toxin on beneficial insects. *Chrysoperla carnea* Stephens, *Coleomegilla maculata*, and *Orius insidiosus* (Say) had no changes in mortality or development time after eating pollen from corn, *Zea mays* L., expressing the *B. thuringiensis* variety *kurstaki* gene (Pilcher et al. 1997). *H. convergens* larvae and adults were unaffected by exposure to *Myzus persicae* (Sulzer), green peach aphid, reared on potatoes, *Solanum tuberosum* L., expressing the *B. thuringiensis* variety *tenebrionis* gene, which is the *B. thuringiensis* variety specific to beetles (Dogan et al. 1996).

Other studies, however, illustrate the potential for plant-expressed toxins to affect nontarget organisms. Hilbeck et al. (1998a) found that *C. carnea* larvae, reared on *B. thuringiensis* variety *kurstaki*-fed *Ostrinia nubilalis* (Hübner) larvae, suffered higher mortality and longer developmental time than *C. carnea* reared on *B. thuringiensis*-free larvae (Hilbeck et al. 1998a). Also, *B. thuringiensis* variety *kurstaki* incorporated into artificial diet led to higher mortality and slower development time for *C. carnea*, indicating *B. thuringiensis* has a direct toxic effect on the predator (Hilbeck et al. 1998b). Losey et al. (1999) demonstrated the direct toxic and subtle effects of pollen from corn expressing the *B. thuringiensis* toxin. Larvae of *Danaus plexippus* (L.) reared on milkweed leaves, *Asclepias curassavica* L., dusted with pollen from corn expressing the toxin ate less, grew more slowly, and suffered higher mortality than larvae given pollen from *B. thuringiensis*-free corn or larvae given no pollen.

Imidacloprid, a chloronicotinyl, works on a wider range of taxa than the more specific varieties of *B.*

thuringiensis. Consequently, nontarget effects of imidacloprid may occur in a broad range of insect taxa. Sclar et al. (1998) showed nontarget effects of imidacloprid on the heteropteran *O. tristicolor* (White). Wallner et al. (1999) found that *Phacelia tanacetifolia* Bentham excrete 3–10 ppb of imidicloprid in the nectar after seed treatment with Gaucho ([AI] imidacloprid). This concentration, however, had no effect on behavior or survivorship of the honey bee, *Apis mellifera* L. When sucrose containing imidacloprid at concentrations >20 ppb was fed to honey bees, it caused a reduction in foraging behavior and induced changes in the dancing behavior that discouraged bees that were not fed imidacloprid-treated sucrose from foraging (Kirchner 1999). Our study confirms the potential for systemic imidacloprid to have nontarget effects on the behavior and fitness of a common predator, *C. maculata*. Widespread use of imidacloprid in many urban systems may reduce the general mobility and number of *C. maculata* present in these systems. These effects could in turn increase pest populations by reducing or eliminating predation by the commonly found *C. maculata*.

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