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Toxicity of the insect growth regulator novaluron to the non-target predatory bug *Podisus maculiventris* (Heteroptera: Pentatomidae)

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Abstract

Novaluron is a novel insect growth regulator that exhibits potent insecticidal activity against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). The predatory bug *Podisus maculiventris* (Say) is a natural enemy of the potato beetle found throughout North America, and is widely available commercially. Anticipating the use of novaluron in *L. decemlineata* management, we conducted laboratory experiments to determine the susceptibility of *P. maculiventris* to novaluron. Although there was a 2- to 3-day delay in the onset of toxic effects, second instars were susceptible by direct contact and through exposure to potato foliage treated with field rates. When *P. maculiventris* eggs were dipped in field rate novaluron solutions, there was no significant effect on percent hatch, but there was subsequently a sharp decrease in the ability of hatched nymphs to molt. Similarly, fifth instars actively preyed on *L. decemlineata* larvae dipped in field rate novaluron solutions, but were thereafter unable to molt into adults. Female *P. maculiventris* adults caged with *L. decemlineata* larvae and novaluron treated potato plants had reduced longevity compared to those caged with untreated potato plants. Further, oviposition and hatch of eggs from adults on novaluron treated plants was significantly reduced. Although novaluron has demonstrated selectivity in favor of beneficial insects in other studies, these results suggest that *P. maculiventris* would be adversely affected.

Keywords: *Leptinotarsa decemlineata*; *Podisus maculiventris*; Novaluron; Insect growth regulator; Non-target toxicity; Sublethal toxicity; Biological control

1. Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is the most important defoliator of potato in North America and is considered one of the most significant agricultural pests worldwide. Heavy reliance on chemical insecticides to manage *L. decemlineata* has resulted in rapid development of resistance to virtually all insecticides used against it (Bishop and Grafius, 1996; Hare, 1990; Mota-Sanchez et al., 2005). In an attempt to reduce reliance on broad-spectrum insecticides and to manage the resistance problem, extensive research has been dedicated to integrating biological control into *L. decemlineata* management. Stinkbugs (Heteroptera: Pentatomidae) are the most specialized *L. decemlineata* predators (Cloutier et al., 2002). The most common pentatomid species attacking *L. decemlineata* are the two-spotted stinkbug, *Perillus bioculatus* (F.), and the spined soldier bug, *Podisus maculiventris* (Say) (Casagrande, 1987). *P. bioculatus* appears to be a *L. decemlineata* specialist, suggesting it would be a preferred candidate for inundative releases, where impacts on non-target species would be of concern (Cloutier et al., 2002; Hough-Goldstein, 1996). However, laboratory and field studies have found *P. maculiventris* to be as effective in controlling *L. decemlineata* populations (Biever and Chauvin, 1992; Hough-Goldstein and McPherson, 1996), and while

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commercial mass production of *P. bioculatus* has been problematic, *P. maculiventris* is available from many biological control suppliers. Thus, the latter is currently a more viable option for augmentative control of *L. decemlineata*.

Although stinkbug augmentation has successfully controlled *L. decemlineata* in field studies (Biever and Chauvin, 1992; Cloutier and Bauduin, 1995; Cloutier and Jean, 1998; Hough-Goldstein and McPherson, 1996), sole reliance on biological control as a management tactic has usually failed to provide effective control of the pest, especially at high population densities (Ferro, 1994; Hare, 1990). Nonetheless, growers could gain significant benefit from predators for control of *L. decemlineata* if they were not eliminated by frequent broad-spectrum insecticide use (Cloutier et al., 2002), suggesting that biorational, selective insecticides could be key in increasing the role of biological control in *L. decemlineata* management. Indeed, potato insect pest management programs that have adopted selective insecticides within conventional potato production have achieved high yields and low pest densities equivalent to conventional fields treated with broad-spectrum insecticides, with the added advantage of conserving high predator densities (Hilbeck et al., 1998; Koss et al., 2005; Reed et al., 2001). In addition, biorational compounds have modes of action different from those of broad-spectrum insecticides, making them useful tools in resistance management programs.

Novaluron (1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxy-ethoxy)phenyl]-3-(2,6- difluorobenzoyl)urea) is a novel benzoylphenyl urea insect growth regulator that exhibits potent insecticidal activity against several important foliage feeding insect pests (Cutler et al., 2005a; Ishaaya et al., 2002, 2001; Malinowski and Pawinska, 1992). By inhibiting chitin formation, novaluron selectively targets larval insect stages, thus minimizing impacts on adults of non-target species and giving it potential in integrated pest management (IPM) programs. It had no effect on phytoseiid mite field populations (Ishaaya et al., 2001), mortality and development of the soil-dwelling predatory mite, *Stratiolaelaps scimitus* (Womersley) (Cabrera et al., 2005), and greenhouse populations and percent parasitism of the parasitoid *Encarsia formosa* Gahan (Ishaaya et al., 2002). It was found to have to no acute toxicity to rove beetles, *Atheta coriaria* Kraatz (S. Jandricic, personal communication), and bumble bees, *Bombus impatiens* Cresson or leafcutter bees, *Megachile rotundata* (F.) (A. King, personal communication). Novaluron is currently registered for use against *L. decemlineata* in the US (trade name Rimon), and is undergoing registration in Canada. It demonstrated potent activity against *L. decemlineata* larvae in the laboratory (Cutler et al., 2005a) and provided excellent prolonged control in field experiments (Malinowski and Pawinska, 1992). However, the toxicity of novaluron to natural enemies of *L. decemlineata* has not been evaluated, meaning the IPM potential of novaluron in *L. decemlineata* management is unclear.

A number of researchers have studied the impact of pesticides on stinkbug predators (De Clercq et al., 1995; De Cock et al., 1996; Hough-Goldstein and Keil, 1991; Mohaghegh et al., 2000; Tillman and Mullinix, 2004; Vandekerkhove and De Clercq, 2004; Wilkinson et al., 1979; Yu, 1988). Anticipating the use of novaluron in future *L. decemlineata* management, we investigated the susceptibility of *P. maculiventris* to novaluron. Tests were conducted exposing *P. maculiventris* nymphs and eggs to novaluron through direct contact. As *P. maculiventris* is an omnivore, further experiments were conducted exposing bugs to novaluron treated *L. decemlineata* larvae and potato foliage. For comparison, nymphs and eggs also were exposed to imidacloprid, a neonicotinoid insecticide used widely in *L. decemlineata* management in North America. Finally, an experiment was done to determine if adult *P. maculiventris* fed novaluron treated potato foliage and *L. decemlineata* larvae experienced sublethal effects on lifespan and reproduction. The viability of progeny from these adults was also assessed.

2. Materials and methods

2.1. Insects

Podisus maculiventris adults were obtained from Dr. Christine Noronha, Crops and Livestock Research Center, Agriculture and Agri-Food Canada, Charlottetown, Prince Edward Island, Canada. The colony was founded in 2002 from adults purchased from the Bug Factory (Nanoose Bay, British Columbia, Canada), and maintained in insecticide-free environment. In this study, *P. maculiventris* were reared on mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (25 ± 1 °C, 16:8 (L:D), 65 ± 5% RH). Cotton wicks soaked with tap water were provided to all stages as a moisture source. *L. decemlineata* used in experiments were from an insecticide-susceptible strain reared for over 60 generations on potato foliage (27 ± 1 °C, 16:8 (L:D), 65 ± 5% RH) at the Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada in London, Ontario.

2.2. Nymph direct contact exposure

Technical grade novaluron (96.0% purity, Makhteshim-Agan of North America, Raleigh, NC) and imidacloprid (97.9% purity, Bayer CropScience Canada, Calgary, AB) were dissolved in acetone + olive oil (19 + 1 by volume) to produce concentrations ranging from 1 to 1000 ppm. Controls consisted of insects treated with the acetone + olive oil solvent only. Insecticide was applied to the insects with a Potter spray tower using methods similar to those described by Harris et al. (1962). Second instars were immobilized by placing them in a 6 °C fridge for 30 min prior to exposure to insecticides. Groups of 10 second instars were subsequently placed dorsal surface up on filter paper in glass petri dishes (100×15 mm), placed in the spray tower, and sprayed with 5 ml of insecticide solution. Treated insects were transferred to

disposable petri dishes (90 × 15 mm) containing five mealworms, a water-soaked cotton wick (30 mm) and potato foliage, and were maintained at 25 ± 1 °C, 16:8 (L:D), and 65 ± 5% RH. Mealworms, water and foliage were replenished as needed (usually every second day), and mortality was recorded after five days. There were at least four replicates per concentration and each assay was repeated a minimum of two times, providing a sample size of at least 80 insects per concentration. Seven concentrations producing approximately 5–95% mortality were used for each insecticide. Concentrations lethal to 50% (LC_{50}) and 95% (LC_{95}) of the nymphs, confidence limits (CL), and slopes were determined by probit analysis (SAS Institute, 2001).

2.3. Nymph exposure to treated foliage

The proposed Canadian label for novaluron (Rimon 10EC, 100 g AI/L, Makhteshim-Agan of North America, Raleigh, NC), recommends an application rate of 220–878 ml/ha for *L. decemlineata* control (R. Everich, personal communication). At a spray volume of 350 L/ha—traditionally felt to be the minimum volume for effective potato canopy coverage in Ontario (OMAF, 2004)—the concentration of novaluron in spray solutions at an application rate of 250 ml/ha would be approximately: 250 ml/ha × 1 L/1000 ml × 100 g AI/L × 1 ha/350 L × 1 L/1000 g × 1,000,000 ppm = 71 ppm. A spray solution applied at 875 ml/ha would have a novaluron concentration of approximately 250 ppm. The Canadian label for imidacloprid (Admire 240F, 240 g AI/L, Bayer CropScience Canada, Calgary, AB), recommends applications of 200 ml/ha for *L. decemlineata* control, giving an imidacloprid concentration of approximately 137 ppm in a spray solution applied at a volume of 350 L/ha. Therefore, Rimon 10EC and Admire 240F were each suspended in deionized water to give stock solutions of 1000 ppm. Dilutions were subsequently made to produce 71 and 250 ppm novaluron solutions, representing "low-" and "high-rate" field applications, respectively. A 137 ppm imidacloprid solution was prepared representing the typical field application rate applied for *L. decemlineata* control.

Four centimeter diameter discs were cut from potato leaves with a stainless steel cork borer. Discs were dipped in deionized water or insecticide solution for approximately 6 s and placed on a wire rack until dry. The dry discs were then placed individually in sterile Gelman 47 mm microbiological dishes, each containing a 42.5 mm diameter filter paper. Five *P. maculiventris* second instars were placed on to each leaf disc and each dish was covered. There were 10 replicates per treatment. The dishes were arranged in a randomized complete block design and transferred to a holding room (25 ± 1 °C, 16:8 (L:D), 65 ± 5% RH). Insects were allowed to feed on potato foliage for 48 h and then were transferred to clean plastic petri dishes (50 × 15 mm) containing untreated potato foliage, a cotton wick saturated with deionized water, and two mealworms. Mortality was recorded after 2 (i.e., when insects were transferred to clean foliage), 4, 6, and 8 days, and foliage and water were replenished as needed. Percent mortality data were arcsine transformed (Zar, 1996) and analyzed for treatment and time effects over the whole experiment using repeated measures ANOVA. Means were separated between treatments on separate days using the Tukey test ($\alpha = 0.05$) (SAS Institute, 1997). Back-transformed data are presented in the results.

2.4. Nymph food chain exposure through treated *L. decemlineata* larvae

Third instar *L. decemlineata* were individually dipped in deionized water, 71, or 250 ppm novaluron for 4 s, placed dorsal side up on glass petri plates (100 × 15 mm) and air dried for 1 h. Novaluron solutions were prepared as described Section 2.3. For each treatment, three third instar *L. decemlineata* were placed in a plastic petri dish (50 × 15 mm) containing a water-soaked cotton wick and potato foliage. A fifth instar *P. maculiventris* nymph held without food for 24 h was then added to each dish. Dishes were covered, moved to a holding room (25 ± 1 °C, 16:8 (L:D), 65 ± 5% RH), and monitored daily. After the initial three *L. decemlineata* larvae were eaten, untreated larvae were added to each dish ad lib. Fresh potato foliage and water were replenished as needed. Each day the number of consumed larvae and dead *P. maculiventris* were recorded, and dead larvae were removed. If *P. maculiventris* fifth instars molted, the time to molt (days after initiation of the experiment) was recorded. Adults were provided water and *L. decemlineata* larvae as needed and monitored for up to 5 days after molt, but larval consumption during this stadium was neither recorded nor used in analyses. The experiment was arranged in a randomized complete design with eight replicates per treatment. The effect of novaluron treated *L. decemlineata* third instars on *P. maculiventris* nymph predation (time to consume the initial 3 larvae and total larvae consumed) was analyzed by ANOVA. Data were square root transformed before analysis using the equation $X' = \sqrt{X + 0.5}$ (Zar, 1996), as a Shapiro–Wilk test found the data to be non-normal (SAS Institute, 1997). The effect of consumption of novaluron treated *L. decemlineata* larvae on *P. maculiventris* nymph molting ability was determined by contingency table analysis (SAS Institute, 1997). Back-transformed data are presented in the results.

2.5. Ovicidal activity

Podisus maculiventris egg masses (mean = 15.8 eggs per egg mass; range = 9–29 eggs per egg mass) were clipped from paper towel pieces that served as oviposition sites for caged adults. Egg masses were individually dipped in deionized water, 71 ppm novaluron, 250 ppm novaluron, or 137 ppm imidacloprid for 4 s, individually placed in sterile Gelman 47 mm microbiological dishes, and transferred to a holding room (25 ± 1 °C, 16:8 (L:D), 65 ± 5% RH).

Solutions were prepared as described in Section 2.3. On hatching, egg masses were placed on a cotton wick saturated with deionized water. Two days later neonates were counted and percent hatch was determined. Insects were held until they molted into second instars or died. The experiment was arranged in a randomized complete design with six replicates per treatment. Percent hatch and molt of neonates, and time to hatch was compared among treatments by ANOVA with means separation by the Tukey test ($\alpha = 0.05$) (SAS Institute, 1997). Percent hatch and molt data were arcsine transformed before analysis (Zar, 1996). Time to hatch data were square root transformed before analysis, as described in Section 2.4, as a Shapiro–Wilk test found these data to be non-normal (SAS Institute, 1997). Back-transformed data are presented.

2.6. Effects on adult *P. maculiventris*

Effects of novaluron on *P. maculiventris* adults feeding on novaluron treated potato foliage were determined. Potato plants were grown in an insecticide-free greenhouse in 100 mm diameter pots containing Pro-Mix potting soil. Plants (20–30 cm high) were removed from the greenhouse and treated with insecticide outside. Insecticides were applied in water at a rate of 900 L/ha using a hand-held, CO_2 pressurized, R&D plot sprayer fitted with a single D-4 orifice disc and a #25 swirl plate. Rimon 10EC was applied at rates of 25 g AI/ha (low-rate) or 850 g AI/ha (high-rate) in water. Plants were allowed to air dry and were then transferred to a holding room (25 ± 1 °C, 16:8 (L:D), $65 \pm 5\%$ RH). Adult *P. maculiventris* were collected from rearing cages the same day plants were treated with insecticide. Groups of approximately 20 newly emerged adults were added to cages containing untreated potato plants, low-rate plants, or high-rate plants, each containing approximately 25 third/fourth instar *L. decemlineata*. Adults fed on *L. decemlineata* larvae and potato plants for 3–4 days before male and females copulated. Mating pairs were then moved to separate oviposition cages (one pair per cage) containing two control plants, two low-rate plants, or two high-rate plants. There were five replicates (5 mating pairs) per treatment. Three third/fourth instar *L. decemlineata* were added to each cage and replenished as needed. Oviposition cages consisted of potted plants placed in plastic trays containing sand, covered with an open-bottomed cage ($30 \times 30 \times 40$ cm high) screened with 500 μm black mesh. Numbers of egg masses laid and eggs per egg mass were recorded daily for up to 21 days. Newly deposited egg masses were clipped from plants or removed from the side of the cages, placed individually in 50 mm diameter plastic petri dishes containing a cotton wick (20 mm) saturated in distilled water, and were maintained in the same holding room as the caged plants. Hatch of each egg mass was monitored daily and first instars were held in the petri dishes until they molted or died. If a *P. maculiventris* adult died, the date of death and sex was recorded. The surviving adult was left to feed and oviposit (if a female) until it died, up to 21 days after initiation of the experiment. As well, qualitative observations on adult behavior were made.

Differences in longevity of male and female *P. maculiventris* adults were compared among treatments by ANOVA. The number of egg masses laid per female, the number of eggs per egg mass, percent hatch of eggs, and percent molt of nymphs from eggs were analyzed for treatment and time effects over the whole experiment using a general linear model (SAS Institute, 1997). Multiple comparisons where conducted by the Tukey test ($\alpha = 0.05$). Back-transformed data are presented in the results.

3. Results

3.1. Nymph direct contact exposure

Novaluron was toxic to *P. maculiventris* nymphs by direct contact; application of a solution containing only 18.7 ppm was needed to kill 50% of second instars (Table 1). It was generally less toxic to second instars than imidacloprid by direct contact. The difference in toxicity between these compounds was significant at the LC_{50}, as indicated by the lack of overlap of the 95% CL, but not at the LC_{95} (Table 1).

3.2. Nymph exposure to treated foliage

There was a significant effect of treatment ($F = 15.28$; $df = 3$; $P < 0.0001$) and time ($F = 113.06$; $df = 3$; $P < 0.0001$) on *P. maculiventris* nymph mortality during exposure to treated potato foliage (Fig. 1). A significant treatment–time interaction was also found ($F = 11.20$; $df = 9$; $P < 0.0001$). Although there was no treatment effect after 2 days ($F = 2.82$; $df = 3$; $P = 0.052$—marginally insignificant at $\alpha = 0.05$), a significant increase in nymph mortality due to treatment was found after 4 ($F = 3.15$; $df = 3$; $P = 0.037$), 6 ($F = 30.22$; $df = 3$; $P < 0.0001$), and 8 ($F = 41.21$; $df = 3$; $P < 0.0001$) days. After 4 days, nymph mortality was highest on the imidacloprid treated foliage, although the difference was not significant compared to the control or high-rate novaluron treatment ($\alpha = 0.05$). After 6 and 8 days, however, nymphs exposed to both high- and low-rate novaluron treated potato foliage had significantly higher mortality than the other treatments. Second instars in the novaluron treatments usually molted, but died as third instars. These third instars displayed symptoms of sublethal intoxication, including lethargy and ataxia, and often were unable to shed their exuvia. All nymphs

Table 1
Direct contact toxicity of novaluron and imidacloprid to *P. maculiventris* second instars 5 days after treatment

Insecticide	*n*	Slope (± SEM)	χ^2	LC_{50} (95% CL) (ppm)	LC_{95} (95% CL) (ppm)
Novaluron	676	1.07 (0.24)	0.71	18.7 (9.8–27.4)	65.0 (28.6–779.2)
Imidacloprid	868	1.32 (0.28)	2.49	5.0 (3.2–6.8)	86.9 (25.7–1544.2)

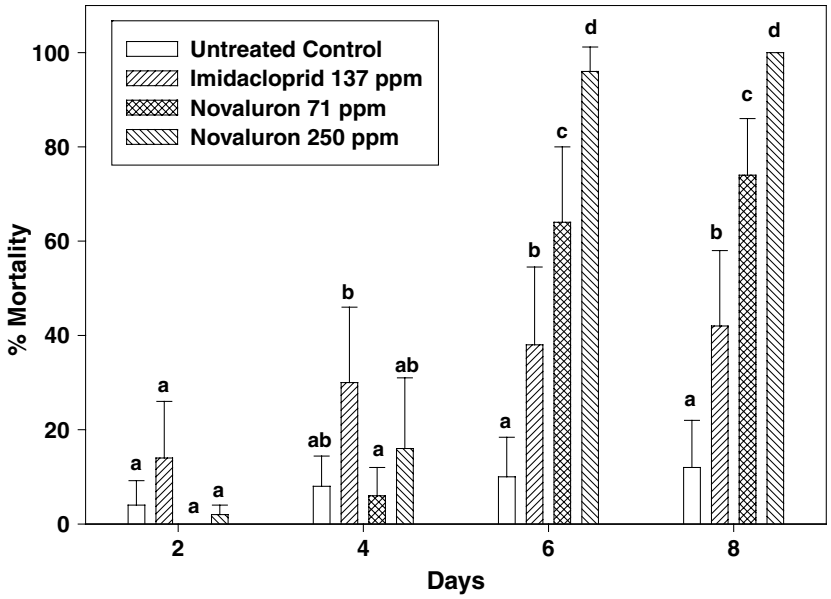

Fig. 1. Mortality (± 95% CL) of *P. maculiventris* second instars after exposure to untreated, imidacloprid (Admire 240F), or novaluron (Rimon 10EC) treated foliage. Nymphs were exposed to treated foliage for 48 h and thereafter transferred to clean containers with untreated foliage, water, and prey (*Tenebrio molitor*) for the remainder of the experiment. Percent data were arcsine transformed for analysis; back-transformed data are presented. Bars with different letters above them, for a given day, are significantly different ($P \leqslant 0.05$, Tukey test).

exposed to high-rate novaluron treated foliage died by the end of the experiment, while about 75% died on the low-rate foliage (Fig. 1). Although about 60% of second instars from the imidacloprid treatment molted and remained alive after 8 days, survivors displayed symptoms of sublethal intoxication similar to those observed in the novaluron treatment. In all treatments, nymphs were observed feeding on plant juices, especially early in the experiment.

3.3. Nymph food chain exposure through treated L. decemlineata larvae

Fifth instar *P. maculiventris* that were held without food for 24 h readily preyed on *L. decemlineata* larvae. There was no significant difference in time taken to eat the initial three larvae, whether they were untreated or dipped in high- or low-rate novaluron solutions ($F = 1.40$; $df = 2$; $P = 0.28$). There was a marginally significant effect of treatment on total number of larvae eaten by *P. maculiventris* nymphs during the fifth stadium ($F = 3.478$; $df = 2$; $P = 0.049$), although there was no significant difference in the duration of this stadium ($F = 2.518$; $df = 2$; $P = 0.10$) (Table 2). In addition, there was a major reduction in molting ability of fifth instars after feeding on *L. decemlineata* larvae treated with novaluron ($\chi^2 = 25.73$; $df = 2$; $P < 0.0001$) (Table 2). While 100% of nymphs that fed on untreated *L. decemlineata* larvae molted and lived to the end of the experiment, no adult emergence was observed in nymphs that fed on low-rate novaluron treated larvae, and only 1 nymph exposed to the high-rate novaluron treated larvae molted. The emerged *P. maculiventris* adult from the high-rate treatment displayed symptoms of sublethal intoxication, including lethargy, ataxia, incomplete wing formation, and abstention from feeding and drinking, and died 4 days after molt. In most cases, nymphs exposed to novaluron treated *L. decemlineata* larvae died before ecdysis, although insects died during ecdysis in a few replicates. Fifth instars that fed on untreated *L. decemlineata* larvae molted after approximately 4 days, resulting in adults with normal morphology and behavior.

3.4. Ovicidal activity

When eggs were dipped in novaluron or imidacloprid solutions, there was no significant effect on days to hatch after treatment ($F = 2.46$; $df = 3$; $P = 0.092$) or on the percent hatch of treated eggs ($F = 1.73$; $df = 3$; $P = 0.193$). However, the ability of first instars from treated eggs to molt

Table 2
Effects of novaluron on fifth instar *P. maculiventris* through exposure to treated third instar *L. decemlineata*

Treatment	Days in fifth stadium (± SEM)[A]	Days to consume 3 initial *L. decemlineata* larvae (± SEM)	Total *L. decemlineata* larvae consumed (± SEM)	Mean % molt (±95% CL)[B]
Control	4.4 (0.5) a	1.6 (0.2) a	2.9 (0.7) a	100 (0) a
Novaluron, 71 ppm	5.9 (0.5) a	1.4 (0.2) a	4.0 (0.3) ab	0 (0) c
Novaluron, 250 ppm	6.9 (1.2) a	1.5 (0.3) a	5.9 (1.3) b	12.5 (6.7) b

[A] Means within columns followed by the same letter are not significantly different ($P \leqslant 0.05$, Tukey test).
[B] Percent data were arcsine transformed before analysis. Back-transformed data are presented.

Table 3
Effect of novaluron and imidacloprid on *P. maculiventris* egg hatch and subsequent molt of first instars (N1)

Treatment	Mean days to hatch (± SEM)[A]	Mean % hatch (± 95% CL)[B]	Mean % N1 molt (± 95% CL)
Control	3.3 (0.4) a	94.1 (7.2) a	100 (0) a
Novaluron, 71 ppm	3.0 (0.8) a	79.3 (25.5) a	6.7 (13.2) b
Novaluron, 250 ppm	4.3 (0.5) a	89.9 (6.8) a	0 (0) c
Imidacloprid, 137 ppm	4.8 (0.4) a	68.7 (23.4) a	6.8 (6.8) b

[A] Means within columns followed by the same letter are not significantly different ($P \leqslant 0.05$, Tukey test).
[B] Percent data were arcsine transformed before analysis. Back-transformed data are presented.

was significantly reduced ($F = 105.88$; $df = 3$; $P < 0.0001$) in the treatments (Table 3). While first instars that emerged from novaluron treated eggs generally appeared healthy and survived up to ecdysis, there was no molt of nymphs from the high-rate solution and less than 10% molt of nymphs from the low-rate solution (Table 3). Many of the first instars from imidacloprid treated eggs exhibited sublethal signs of poisoning such as reduced mobility and feeding, and died within 24 h of hatch.

3.5. Effects on adult *P. maculiventris*

Adult *P. maculiventris* were observed feeding on plant juices and *L. decemlineata* throughout the experiment. *L. decemlineata* larvae often were able to feed on potato foliage over 24 h before being attacked by *P. maculiventris* adults. While there was no difference in longevity of *P. maculiventris* males on treated or untreated plants ($F = 2.06$; $df = 2$; $P = 0.17$), we did find a treatment effect on female longevity ($F = 6.34$; $df = 2$; $P = 0.019$) (Table 4). Although three females in control cages lived the full 21 days of the experiment, 2 from control cages were lost (escaped from cages and not recovered) on days 12 and 14. When the experiment was terminated at 21 days, 6 of 8 adults were still alive in the controls (2 females were lost), while only 2 of 19 adults remained alive in the novaluron treatments (1 female was lost).

Oviposition and hatch of eggs from adults on novaluron treated plants was significantly reduced (Table 4). Fewer egg masses were oviposited by females in the novaluron treatments ($F = 4.69$; $df = 2$; $P = 0.010$), and there was a general decrease in oviposition of egg masses over time in each of the treatments (control: $F = 3.18$; $df = 18$; $P = 0.0002$, low-rate novaluron: $F = 3.78$; $df = 18$; $P < 0.0001$, high-rate novaluron: $F = 3.00$; $df = 18$; $P = 0.0004$). A total of 41, 30, and 22 egg masses were oviposited by *P. maculiventris* females in the control, low-rate, and high-rate novaluron treatments, respectively. There was no treatment-time interaction ($F = 1.13$; $df = 36$; $P = 0.30$) effect on egg mass oviposition. There also was a significant decrease in the number of eggs per egg mass ($F = 4.17$; $df = 2$; $P = 0.019$) in the novaluron treatments

Table 4
Effect of feeding on novaluron treated potato foliage and *L. decemlineata* larvae on adult *P. maculiventris* longevity, egg production, egg hatch, and subsequent molt of emerged 1st instar nymphs (N1)[A]

Biological parameter	Treatment mean (± SEM)[B]		
	Untreated	25 g AI/ha	85 g AI/ha
Female longevity (days)	21.0 (0) a	14.8 (1.4) b	18.3 (1.1) ab
Male longevity (days)	19.8 (1.0) a	15.8 (2.0) a	16.0 (1.7) a
Egg masses/day[C]	0.4 (0.06) a	0.3 (0.07) ab	0.2 (0.05) b
Eggs/egg mass[C]	16.1 (1.3) a	13.2 (3.0) ab	10.9 (1.9) b
% Hatch[D,E]	75.7 (10.6) a	10.8 (9.5) b	9.1 (10.8) b
% Molt of hatched N1[D,E]	92.9 (1.7) a	12.2 (4.5) b	0.0 (0.0) c

[A] Upon emergence, virgin adult male and female *P. maculiventris* were placed on potato plants that were treated with water or novaluron with a hand-held sprayer ($n = 5$ per treatment). *L. decemlineata* larvae were added to cages ad lib. Adults fed and oviposited for up to 21 day after initiation of the experiment.
[B] Means within each row followed by the same letter are not significantly different ($P \leqslant 0.05$; Tukey test).
[C] Data were square root transformed for analysis. Back-transformed data are presented.
[D] Data were arcsine transformed for analysis. Back-transformed data are presented.
[E] Mean percent hatch and successful molt over 21 days.

(Table 4). While the number of eggs per egg mass did not change as the experiment progressed in the control ($F = 1.86$; $df = 13$; $P = 0.08$) or high-rate novaluron treatment ($F = 1.40$; $df = 9$; $P = 0.28$), a significant fluctuation over time in the number of eggs per egg mass was found in the low-rate novaluron treatment ($F = 3.70$; $df = 8$; $P = 0.008$). Over the whole experiment, females on untreated foliage laid 656 eggs, while females on low- and high-rate novaluron treated foliage laid 390 and 242 eggs, respectively. Although eggs were laid by *P. maculiventris* females in all treatments, there was a marked decrease in the hatch of eggs ($F = 153.45$; $df = 2$; $P < 0.0001$) and subsequent molt of nymphs in the novaluron treatments. Only about 10% of eggs hatched in the novaluron treatments and none of the first instars molted from the high-rate treatment (Table 4).

Several behavioral observations indicated *P. maculiventris* adults in the novaluron treatments experienced effects of sublethal intoxication. Adults on treated plants sometimes displayed reduced walking ability, poor coordination, and ataxia. They sometimes fell off plants, remaining motionless on their backs. Only after probing with a needle or forceps was it clear that these adults were alive. Although we did not record the number of *L. decemlineata* larvae preyed on by adults throughout the experiment, qualitatively we did not notice any differences between the control and novaluron treatments in the number of larvae consumed.

4. Discussion

Podisus maculiventris is endemic throughout North America. A polyphagous predator of over 90 insect species (McPherson, 1982), its importance as a natural enemy is well recognized (Coll and Ruberson, 1998; Schaefer and

Panizzi, 2000). Despite its potential for biological control of *L. decemlineata*, it is unlikely that *P. maculiventris* could consistently regulate this pest on its own, meaning exposure to insecticides would often occur. Heavy reliance on insecticides has, paradoxically, both exacerbated *L. decemlineata* insecticide resistance and has hindered optimal utilization of natural enemies. Therefore, increased use of compounds that could concurrently reduce pest densities, maintain high natural enemy populations, and curb resistance development would be particularly welcome in *L. decemlineata* management.

Although novaluron is generally considered a selective, reduced risk insecticide, our results indicate that all three *P. maculiventris* life stages would be susceptible to novaluron through several routes of exposure. The direct contact LC_{50} of novaluron for *P. maculiventris* second instars was 18.7 ppm, a concentration that falls within the range of toxicity of broad-spectrum insecticides reported by Yu (1988) and Hough-Goldstein and Keil (1991), albeit novaluron was less toxic than several of these compounds. Novaluron had similar direct contact toxicity to *L. decemlineata* second instars ($LC_{50} = 27$ ppm) (Cutler et al., 2005a). Considering that solutions of approximately 71–250 ppm (250–875 ml/ha) novaluron would be applied to control *L. decemlineata*, the direct contact selectivity for *P. maculiventris* nymphs would be negligible. In contrast, *Bacillus thuringiensis* var. *san diego* and cryolite were found to be practically non-toxic to *P. bioculatus* nymphs by topical exposure (Hough-Goldstein and Keil, 1991), as were topical applications of diflubenzuron, the first developed benzoylphenyl urea, to *P. maculiventris* (De Clercq et al., 1995). The selectivity of diflubenzuron is partly due to its tendency to remain in the cuticular waxes following direct contact (Retnakaran and Wright, 1987). Unfortunately, the increased direct contact efficacy of novaluron against several pest species (Cutler et al., 2005a; Ishaaya et al., 1998) may result in decreased selectivity in favor of beneficial species. The imidacloprid direct contact LC_{50} we found for *P. maculiventris* second instars was 13-fold lower than that measured by De Cock et al. (1996) for fifth instars. This discrepancy may reflect a greater susceptibility of younger instars to imidacloprid, intra-specific variation, or may simply be due to differing experimental methods. As with *P. maculiventris*, Cutler et al. (2005a) found that *L. decemlineata* larvae were more susceptible to imidacloprid than novaluron.

Podisus maculiventris second instars were highly susceptible to novaluron by exposure to treated foliage; 100% of nymphs died when exposed to foliage treated with the high recommended rate of novaluron, while about 75% died when exposed to the lower rate. The delay in the onset of novaluron toxicity was expected as it acts by inhibiting chitin synthesis, which occurs predominantly during molt. In addition to contact with treated foliage, the activity of novaluron in this experiment was probably due to ingestion since *P. maculiventris* is an omnivore requiring water and nutrients from plant juices, in addition to animal protein, for optimal growth and development (Ruberson et al., 1986). Its susceptibility to novaluron treated potato foliage therefore highlights an additional challenge for the integration of omnivorous predators and insecticides in pest management programs. Not only must compounds of interest exhibit no residual toxicity to the natural enemy, but exposure also must be minimized through ingestion of plant material. Although not systemic, foliar applied novaluron does have trans-laminar activity (Ishaaya et al., 2002) and its biological activity may persist as long as 5 weeks after application (Cutler et al., 2005b). Thus, *P. maculiventris* nymphs could be exposed to lethal concentrations of novaluron on plant foliage for extended periods during searches for prey and colonization (or recolonization) of treated fields.

The response of *P. maculiventris* to other insecticides through residual contact and ingestion of treated water or plant juices has been variable. Wilkinson et al. (1979) found adults and nymphs to be highly susceptible to organophosphorus insecticides, but tolerant to the pyrethroids, fenvalerate, and permethrin through residual contact. Diflubenzuron was harmless to *P. maculiventris* nymphs by residual contact but was highly toxic when ingested via drinking water (De Clercq et al., 1995), as was imidacloprid (De Cock et al., 1996). Nymphs exposed to encapsulated λ-cyhalothrin by ingestion and residual contact quickly recovered after knock-down (Vandekerkhove and De Clercq, 2004). Hough-Goldstein and Keil (1991) reported that *P. bioculatus* nymphs were highly susceptible to esfenvalarate, oxamyl, and endosulfan through exposure to treated potato foliage, but were unaffected by exposure to *B. thuringiensis* and cryolite treated foliage.

Although *P. maculiventris* fifth instars readily preyed on *L. decemlineata* larvae dipped in field-rate novaluron solutions, adult emergence was almost completely suppressed. In some cases this resulted in prolongation of the fifth instar stadium and greater total predation during that stage in the novaluron treatments. Similarly, fifth instars fed pyriproxyfen treated *Spodoptera exigua* (Hübner) larvae suffered high mortality at molt (De Clercq et al., 1995). Adults and nymphs also were susceptible to prey treated with organophosphorus or neonicotinoid insecticides (Tillman and Mullinix, 2004). Hough-Goldstein and Keil (1991) found that *P. bioculatus* third instars were highly susceptible to endosulfan, and moderately so to esfenvalarate and oxamyl, but tolerant of *L. decemlineata* larvae treated with *B. thuringiensis* and cryolite.

Few studies have investigated ovicidal activity of insecticides on heteropteran eggs. We found that eggs dipped in field-rates of novaluron resulted in no reduction in percent hatch or adverse effects in behavior of first instars, but molt of emerging first instars was greatly diminished. Results with imidacloprid were comparable. In contrast, Hough-Goldstein and Keil (1991) found that applications of esfenvalarate, oxamyl, endosulfan, *B. thuringiensis* or cryolite to *P. bioculatus* eggs had no impact on hatch or mortality of hatched nymphs. Eggs of several pest species are susceptible to novaluron applications (Cutler et al., 2005a; Ishaaya et al., 2002, 1996).

Emphasis on sublethal toxicological studies in entomology has increased in recent years. Insects that survive insecticide application may still suffer sublethal intoxication resulting in reduced longevity, developmental rates, numbers of offspring, and body weights. Behavioral effects such as reduced sexual competitiveness and ability to find food or resources also could occur (Starks and Banks, 2003). We found that longevity of *P. maculiventris* females was reduced by dual exposure to novaluron treated potato plants and larvae. Some control females were lost during the experiment, which reduced the sample size and variance of the control, producing a statistically significant result. Nonetheless, at the end of the experiment most remaining adults were alive in the controls (6 of 8), while few remained in the novaluron treatments (2 of 19). Although we ended the experiment at 21 days due to reduced quality of experimental plants, it is likely that adults in the controls could have survived well beyond this point since those used in our rearing procedures routinely survive over 2 months. The observations that *P. maculiventris* adults in the novaluron treatments were often ataxic or immobilized on their backs for prolonged periods provide further evidence of sublethal intoxication. Similar effects were observed in *L. decemlineata* adults after prolonged exposure to novaluron treated potato foliage (Cutler et al., 2005a).

Reduced fecundity and egg viability in adult *L. decemlineata* exposed to novaluron treated potato foliage was previously reported (Cutler et al., 2005a; Malinowski and Pawinska, 1992). These effects also occurred in *P. maculiventris* adults placed on novaluron treated potato foliage with *L. decemlineata* larvae. Adults in treatments laid only 35–60% of the eggs laid by those on untreated foliage. These reduced reproductive outputs may have been due to effects on reproductive systems, suboptimal nutrition due to reduced feeding, altered mating behavior as a result of sublethal intoxication, or a combination of factors. More importantly, however, egg hatch and molt of emerged nymphs in the novaluron treatments was almost completely arrested. The experimental design employed is probably a worst case scenario as *P. maculiventris* populations in the field would likely have access to novaluron-free refugia. Additionally, rainfall and UV radiation would undoubtedly reduce duration of the biological activity of novaluron in the field compared to our laboratory set-up. Nonetheless, foliar applied novaluron has very persistent biological activity under field conditions (Cutler et al., 2005b; Ishaaya et al., 1998, 2002, 2001), suggesting long-term adverse effects on *P. maculiventris* adults could occur under field conditions. In contrast to these results, diflubenzuron and pyriproxyfen (De Clercq et al., 1995), diafenthiuron and imidacloprid (De Cock et al., 1996), and deltamethrin (Mohaghegh et al., 2000) had no effect on *P. maculiventris* oviposition. However, whereas the present study exposed adults for 21 days to plants treated with field rates of insecticide and prey that fed upon the treated foliage, in two of these studies *P. maculiventris* was exposed to sublethal doses of insecticide (De Clercq et al., 1995; De Cock et al., 1996), and in all three studies exposure time was shorter.

Use of selective insecticides is an effective strategy for conservation biological control, as well as natural enemy augmentations. Novaluron has demonstrated good selectivity against several beneficial insects making it a valuable tool in several IPM programs. However, our results indicate that all *P. maculiventris* life stages are susceptible to novaluron through various routes of exposure. Although there are numerous factors to consider when extrapolating laboratory data to the field, results of the present laboratory experiments suggest that applications of novaluron against pests like *L. decemlineata* would probably have little selectivity in favor of *P. maculiventris* populations.

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