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Acute and sublethal toxicity of novaluron, a novel chitin synthesis inhibitor, to *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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Abstract: The acute and sublethal toxicities of novaluron, a novel chitin synthesis inhibitor, to a laboratory-reared insecticide-susceptible strain of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), were determined. Novaluron exhibited excellent residual (120 h LC_{50} = 0.42 mg litre^{-1}) and good direct contact (120 h LC_{50} = 27 mg litre^{-1}) activity against second-instar larvae (L2). Hatch of eggs exposed by direct contact to novaluron solutions ≥100 mg litre^{-1} was significantly reduced, as was the ability of emerged first-instar larvae to moult. L2 from eggs exposed to ≥100 mg litre^{-1} novaluron weighed significantly less ($P < 0.0001$) than those from untreated eggs. However, L2 from eggs treated with 1 mg litre^{-1} novaluron weighed significantly more ($P \leq 0.05$) than those from untreated eggs, suggesting novaluron can have a hormetic effect on *L decemlineata* larval development. *Leptinotarsa decemlineata* mating pairs fed foliage treated with novaluron at 25 or 75 g AI ha^{-1} produced approximately 25% fewer egg masses and eggs per mass. Hatch of eggs on treated foliage was almost completely suppressed, and longevity of male beetles was reduced by approximately 50% when fed foliage treated with novaluron at 75 g AI ha^{-1}.

Keywords: novaluron; Colorado potato beetle; *Leptinotarsa decemlineata*; sublethal toxicity; ovicidal; hormesis

1 INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most important insect defoliator of potato worldwide. Left uncontrolled, *L decemlineata* populations, particularly late-instar larvae, can quickly defoliate entire potato fields and sharply reduce yields.[1,2] Limited utility of alternative control methods has meant heavy reliance on chemicals to manage *L decemlineata*, resulting in rapid development of insecticide resistance and exhaustion of all insecticide options in many potato-growing regions. The beetle has evolved resistance to at least 41 different active ingredients worldwide[3] and in Canada, resistance to organochlorine,[4–6] carbamate, organophosphorus and pyrethroid insecticides[6–8] has developed. Most Canadian potato growers currently depend solely on the chloronicotinyl insecticide imidacloprid to manage *L decemlineata*. Inevitably, increased LC_{50} values and decreased mortality of second-instar larvae to a diagnostic dose have developed in Canadian *L decemlineata* populations, suggesting that widespread resistance to imidacloprid will soon develop if an insecticide with a novel mode of action is not incorporated into management programs.[9,10]

Better management of *L decemlineata* could be achieved by implementing Integrated Pest Management (IPM) programmes, which curb resistance development by decreasing reliance on insecticides. An integral component of IPM is the use of selective insecticides that allow non-target, beneficial parasites, predators, and pollinators to survive insecticide treatment.[11] Unfortunately, most insecticides available for *L decemlineata* control are non-selective, impeding development of effective IPM. Research assessing the potential of novel, IPM-compatible insecticides to control *L decemlineata* is essential if consistent, long-term, economical management is to be achieved.

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Novaluron is a novel benzoylphenyl urea insecticide that exhibits potent insecticidal activity against several important foliage feeding insect pests.[12–15] By inhibiting chitin formation, it selectively targets larval insect stages that actively synthesize chitin and seldom impacts adults of non-target beneficial species.[14,15] Malinowski and Pawinska[16] reported that novaluron provided long-term control of *L decemlineata* larvae by ingestion and contact, and reduced egg viability in adults. However, we have found no other published studies investigating its potential against *L decemlineata*. Laboratory tests were therefore done to determine the acute toxicity of novaluron to various life stages of the pest. For larvae, the toxicity of novaluron was compared with that of other insecticides commonly used to control *L decemlineata* in Canada. In addition, experiments were conducted to determine if adults fed novaluron-treated potato foliage experienced sublethal effects on lifespan, oviposition rates, fertility and behavior.

2 MATERIALS AND METHODS

2.1 Insects

An insecticide-susceptible *L decemlineata* strain reared for over 50 generations on potato foliage [27 (±1) °C; 16:8 h light:dark photoperiod; 65 (±5)% RH] at the Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada in London, Ontario, was used in all experiments. Rearing methods and conditions were similar to those used by Harris and Svec.[4] Five life stage categories were evaluated in experiments: egg, first-third instars and adult. Larval instars were distinguished by measuring head capsule width: first instar (L1), 0.6–0.7 mm; second instar (L2), 0.9–1.1 mm; third instar (L3), 1.3–1.7 mm.[17] Larvae used in experiments were 24–48 h post-moult for each instar and egg masses were 24–48 h old. Adult *L decemlineata* used in sublethal experiments were less than 24 h old at the experiment's initiation.

2.2 Residual bioassays

Novaluron 100 g litre^{-1} EC (Rimon 10EC®, Makhteshim-Agan of North America, Raleigh, NC), imidacloprid 240 g litre^{-1} F (Admire 240F®, Bayer CropScience Canada Inc., Calgary, AB), spinosad 480 g litre^{-1} SC (Tracer 480SC®, Dow AgroSciences Canada Inc., Calgary, AB) and lambda-cyhalothrin 120 g litre^{-1} EC (Matador 120EC®, Syngenta Crop Protection Canada Inc., Guelph, ON) were each suspended in reverse-osmosis water (RO-water) to give stock dispersions of 1000 mg AI litre^{-1}. Dilutions were subsequently made to give a range of concentrations from 0.001–10.0 mg AI litre^{-1}.

A leaf dip bioassay method was modified from Hilton *et al*.[18] Trifoliate potato leaves of similar size were cut from plants grown in an insecticide-free greenhouse and then brought to the laboratory for bioassay. Leaves were immersed in the desired insecticide concentration for approximately 6 s and were placed on wire racks until dry. The petiole of each leaf was inserted into a floral water pick (Sproule Enterprises Ltd, Mississauga, ON) containing RO-water to maintain freshness, which was then inserted into the bottom of a polystyrene cup in an upright position. Five L1, L2 or L3 were placed on to the foliage. Each cup was covered with a glass Petri dish lid and transferred to a holding room [27 (±1) °C, 24:0 h light:dark photoperiod, 65 (±5)% RH]. After 48 h, surviving larvae from each cup were transferred to clean waxed paper cups, given untreated potato foliage, covered with a glass Petri lid and returned to the holding room. Larval mortality was recorded 24, 48, 72, 96 and 120 h after treatment. Larvae were recorded as dead if they were unresponsive to gentle probing with a needle.

For each insecticide, at least three bioassays × four replicates per bioassay × 5 larvae per replicate were conducted, per concentration. A minimum of eight concentrations producing 5–95% mortality was used to generate regression lines. Concentration–mortality regression lines were generated for each insecticide by probit analysis.[19] Concentrations lethal to 50% (LC_{50}) and 95% (LC_{95}) of *L decemlineata*, confidence limits (CL) and slopes were determined. Differences between LC values were considered significant if the 95% CL did not overlap ($P \leq 0.05$).

2.3 Contact bioassays

The contact toxicity of novaluron was assessed on eggs and L2. Technical grade novaluron (Rimon Technical®, 96.0% purity, Makhteshim-Agan of North America, Raleigh, NC) was dissolved in acetone + olive oil (19 + 1 by volume) to produce concentrations ranging from 1 to 1000 mg litre^{-1}. 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*.[20] Groups of 10 L2 were placed dorsal surface up on filter paper in glass Petri dishes (90 mm in diameter), placed in the spray tower and sprayed with insecticide solution (5 ml). Treated insects were transferred to clean waxed paper cups and were supplied with fresh untreated potato foliage. There were at least three bioassays × two replicates per bioassay × 10 larvae per replicate conducted per concentration. Data analysis, holding conditions and mortality assessments were as described in Section 2.2.

In bioassays with egg masses, potato leaves containing *L decemlineata* egg masses (mean = 29.7 (±2.9) eggs per egg mass; range = 11–61 eggs per egg mass) were removed from adult oviposition cages. Excess foliage around each egg mass was removed with scissors. Egg masses were placed in plastic Petri dishes (90 mm diameter) lined with a piece of Whatman No. 1 filter paper and maintained in a holding room as described in Section 2.2 for 24 h before bioassay. After 24 h, individual egg masses in glass Petri dishes were treated with solvent only,

1, 10, 100 or 1000 mg litre^{-1} novaluron solution in the spray tower, as described earlier. Treated egg masses were individually transferred to sterile plastic Petri dishes (25 mm diameter) lined with Whatman No. 1 filter paper and returned to the holding room. There were six replicates per treatment. Percentage hatch, measured as number of emerged larvae per initial number of eggs, was recorded daily. On hatching, L1 were provided untreated potato foliage daily. Six days after treatment, numbers of live L2 and dead larvae were recorded. Live L2 were weighed with a Sartorius BP 61S digital scale (precision 0.1 mg). Percentage hatch, number of live L2 and L2 weights were analyzed by analysis of the variance (ANOVA) and means were separated using the Tukey test.[21] Percent data were arcsine transformed before the analysis.[22] Data were back-transformed for presentation in text, tables or figures.

2.4 Sublethal effects on adult *Leptinotarsa decemlineata*

Sublethal effects on *L decemlineata* adults feeding on novaluron-treated potato foliage were determined. Potato plants were grown in an insecticide-free greenhouse in pots (10 cm diameter) containing Pro-Mix® potting soil. Plants (30–40 cm high) were removed from the greenhouse and treated outside. Insecticides were applied in water at a rate of 900 litres ha^{-1} using a hand-held, carbon dioxide pressurized, R&D plot sprayer fitted with a single D-4 orifice disc and a #25 swirl plate. Novaluron 100 g litre^{-1} EC was applied at rates of 25 g AI ha^{-1} (low-rate) or 75 g AI ha^{-1} (high-rate) in RO-water. Plants were left to air dry and were then transferred to a holding room [22 (±1) °C; 16:8 h light:dark photoperiod; 65 (±5)% RH].

Adult *L decemlineata* were collected from pupation/emergence cages the same day that plants were treated with insecticide. Approximately 20 newly emerged adults were added to each feeding cage containing untreated potato plants, low-rate plants or high-rate plants. The beetles were allowed to feed until male and female beetles initiated copulation. Mating pairs were then moved to separate oviposition cages (one pair per cage) containing three control plants, three low-rate plants or three high-rate plants. Oviposition cages consisted of potted plants placed in plastic trays containing sand covered with an open-bottomed cage (30 × 30 × 40 cm high) screened with black mesh (500 μm). To compensate for foliage eaten by the adults and to retain plant integrity, the three plants in each cage were replaced after 14 days. Surrogate plants were treated, as described earlier, on the day of exchange. Plants were watered with tap water throughout the experiment, as needed. Number of egg masses laid and eggs per mass were recorded daily for 45 days. Newly deposited egg masses were clipped from plants and placed individually in plastic Petri dishes (2.0 cm diameter), which were maintained in the same holding room as the caged plants. Percentage hatch of each egg mass was recorded daily. Emerged L1 were provided with untreated potato foliage daily. Six days after treatment, numbers of live L2 and dead larvae were recorded. If an adult beetle died, the date of death and sex was recorded. The surviving beetle was left to feed and oviposit (if a female) until it died, or up to 45 days after initiation of the experiment. Dissection or deductive reasoning confirmed the sex of dead beetles, ie if the living beetle in the mated pair continued to lay eggs the dead beetle was known to be a male. Qualitative observations on adult behavior, such as feeding rates and locomotion, were made as well.

ANOVA was used to determine the effect of novaluron treatments on mean adult longevity, oviposition (number of egg masses per day, number of eggs per mass, total number of eggs), percentage egg hatch and larval development after emergence from egg masses. Linear regressions were performed to determine changes in oviposition rates and percentage egg hatch and successful moult of emerged L1 over time.[21] Percentage data were arcsine transformed[22] before the analysis, and means were separated using the Tukey test. Arcsine transformed data were back-transformed after analysis for presentation in text, tables or figures.

3 RESULTS AND DISCUSSION

3.1 Residual bioassays

In addition to biological control and biotechnology research, present-day agriculture requires continued development of biorational insecticides that selectively target pest species while sparing non-target organisms. The widespread development of insect resistance to conventional insecticides provides further incentive to study alternatives and more ecologically sound compounds in IPM programs.[23] However, a lack of selective insecticides has impeded development of IPM for *Leptinotarsa decemlineata*. Previous research indicates that growers could rely significantly on generalist predators to control *L decemlineata* if they were not eliminated by frequent use of broad-spectrum insecticides.[24] This suggests that selective compounds such as novaluron, which has minimal effect on natural enemies,[14,15] could play a key role in the development of practical IPM for *L decemlineata*.

Novaluron was highly toxic to *L decemlineata* larvae in residual bioassays. Residual activity is due to ingestion of and, probably to a lesser extent, body contact with treated foliage. At 120 h after initiation of the bioassay, concentrations of 0.69, 0.42 and 1.32 mg litre^{-1} killed 50% of exposed L1, L2 and L3, respectively, while 1.56, 1.68 and 4.18 mg litre^{-1} killed 95% (Table 1). Others have reported excellent residual activity against larval stages of the lepidopteran pests *Spodoptera littoralis* (Boisduval), *Spodoptera exigua* (Hübner) and *Helicoverpa armigera* (Hübner).[12,13] Over all time intervals, lambda-cyhalothrin was most toxic to L2,

Table 1. Acute residual toxicity of novaluron to first, second and third-instar Colorado potato beetle, *Leptinotarsa decemlineata*, larvae after 48, 72, 96 and 120 h[a]

Instar[b]	Time (h)	Slope (±SEM)	χ^2	LC_{50} (mg litre^{-1}) (95% CL)	LC_{95} (mg litre^{-1}) (95% CL)
First	48	3.61 (±0.76)	189.33	1.47 (1.11–2.18)	4.19 (2.65–12.92)
(1546)	72	3.36 (±0.64)	145.06	1.11 (0.86–1.50)	3.44 (2.28–8.65)
	96	4.47 (±1.33)	52.36	0.86 (0.46–1.81)	2.06 (1.22–3.94)
	120	4.62 (±0.96)	67.73	0.69 (0.54–0.80)	1.56 (1.22–2.77)
Second	48	3.77 (±0.69)	60.84	1.45 (1.20–1.90)	3.97 (2.71–9.35)
(1190)	72	3.48 (±0.60)	56.94	1.10 (0.90–1.37)	3.28 (2.30–6.96)
	96	2.49 (±0.31)	35.62	0.61 (0.49–0.73)	2.78 (2.00–4.77)
	120	2.72 (±0.27)	26.87	0.42 (0.34–0.49)	1.68 (1.33–2.35)
Third	48	3.61 (±0.96)	59.17	2.53 (1.92–4.11)	7.24 (4.33–69.93)
(1079)	72	3.59 (±0.33)	10.97	1.53 (1.36–1.70)	4.40 (3.63–5.89)
	96	3.28 (±0.43)	22.26	1.43 (1.17–1.68)	4.52 (3.41–7.55)
	120	3.28 (±0.37)	14.43	1.32 (1.09–1.52)	4.18 (3.31–6.14)

[a] Larvae were exposed to treated foliage for 48 h and then transferred to untreated foliage for the remainder of the bioassay.
[b] *n* (number of larvae tested) in parentheses.

Table 2. Acute residual toxicity of novaluron, imidacloprid, lambda-cyhalothrin and spinosad to second-instar Colorado potato beetle, *Leptinotarsa decemlineata,* larvae after 24, 48, 72, 96 and 120 h[a]

Insecticide[b]	Time (h)	Slope (±SEM)	χ^2	LC_{50} (mg litre^{-1}) (95% CL)	LC_{95} (mg litre^{-1}) (95% CL)
Novaluron	24[c]	—	—	—	—
(1190)[c]	48	3.77 (±0.69)	60.84	1.45 (1.20–1.90)	3.97 (2.71–9.35)
	72	3.48 (±0.60)	56.94	1.10 (0.90–1.37)	3.28 (2.30–6.96)
	96	2.49 (±0.31)	35.62	0.61 (0.49–0.73)	2.78 (2.00–4.77)
	120	2.72 (±0.27)	26.87	0.42 (0.34–0.49)	1.68 (1.33–2.35)
Imidacloprid	24	2.61 (±0.43)	1.91	0.25 (0.21–0.30)	1.08 (0.76–2.09)
(925)	48	2.66 (±0.50)	10.80	0.15 (0.09–0.26)	0.64 (0.34–1.80)
	72	2.20 (±0.25)	5.46	0.15 (0.13–0.18)	0.84 (0.59–1.41)
	96	2.23 (±0.25)	4.12	0.13 (0.11–0.15)	0.69 (0.50–1.12)
	120	1.92 (±0.24)	3.93	0.11 (0.09–0.13)	0.76 (0.58–1.63)
Lambda-cyhalothrin	24	1.74 (±0.80)	14.10	0.059 (0.045–0.073)	0.52 (0.40–0.71)
(725)	48	1.94 (±0.31)	3.02	0.032 (0.023–0.045)	0.23 (0.14–0.54)
	72	1.94 (±0.29)	1.41	0.040 (0.032–0.052)	0.28 (0.17–0.73)
	96	1.99 (±0.29)	1.15	0.033 (0.026–0.041)	0.22 (0.14–0.51)
	120	1.64 (±0.30)	1.26	0.031 (0.023–0.042)	0.31 (0.16–1.17)
Spinosad	24	2.49 (±0.84)	20.75	0.17 (0.10–0.25)	0.77 (0.58–9.96)
(962)	48	2.74 (±0.97)	27.64	0.13 (0.061–0.20)	0.51 (0.36–2.47)
	72	2.80 (±0.75)	16.83	0.098 (0.012–0.21)	0.38 (0.18–266.13)
	96	3.48 (±0.79)	14.71	0.098 (0.050–0.14)	0.29 (0.17–3.84)
	120	3.36 (±0.77)	14.18	0.094 (0.045–0.13)	0.29 (0.18–4.01)

[a] Larvae were exposed to treated foliage for the first 48 h and transferred to untreated foliage for the 72–120 h interval.
[b] *n* (number of larvae tested) in parentheses.
[c] No mortality at 24 h for novaluron.

followed by spinosad, imidacloprid and novaluron. After 120 h, the novaluron LC_{50} was approximately 1/14 that of lambda-cyhalothrin, 1/5 that of spinosad and 1/4 that of imidacloprid (Table 2). Although lambda-cyhalothrin was significantly more potent than novaluron, an examination of the 120-h LC_{95} values shows overlap of the 95% confidence limit estimates of spinosad, imidacloprid and novaluron, suggesting that differences in the potency of these compounds are probably minimal (Table 2). Furthermore, the slopes of the novaluron probit lines were in most cases higher than those of the other compounds at each time interval. Only in the case of spinosad at 96 and 120 h were the slopes greater than those of novaluron (Table 2). As the dose-response function slope is negatively correlated with variability in susceptibility of a population to a compound, the relatively high slope in response to novaluron indicates a high degree of homogeneity of toxicological response, whereas the lower slope in response to cyhalothrin indicates a comparatively greater range of susceptibilities within the tested population. Hypothetically, variable kinetics of compound partitioning to the leaf surface could partly explain differences in L2 response to residual exposures of novaluron, imidacloprid and spinosad. While the water solubilities of spinosad (89 000 µg litre^{-1}, spinosyn A; 500 µg litre^{-1}, spinosyn D)[25] and imidacloprid (610 000 µg litre^{-1})[25] are high, it is low for novaluron (3 µg litre^{-1}).[25] Thus, equal concentrations of insecticide solution used in the

leaf-dip bioassay do not necessarily result in the transfer of equal concentrations to the leaf surface for insect residual exposure.

Relative to the other tested insecticides, novaluron was slower to act. After 24 h exposure to novaluron treated foliage, no larval mortality was recorded at any concentration. In contrast, 24-h lethal effects were recorded with larvae fed foliage treated with lambda-cyhalothrin, spinosad or imidacloprid (Table 2). This was expected, as novaluron is bioactive specifically during periods of chitin synthesis, such as moulting or replacement of the peritrophic membrane. In contrast, bioactivity of the conventional insecticides does not depend on developmental processes in insects. After 48 h of feeding on novaluron-treated foliage, however, larvae from each instar died (Table 1). In addition, as time progressed, lower concentrations of novaluron were lethal. For each instar, LC_{50} values after 120 h were significantly less than those at 48 h, and for L1 and L2, the 120-h LC_{50} was significantly lower than the 72-h LC_{50} (Table 1). Third-instar larvae were generally less susceptible to ingested novaluron than L1 and L2, although overlap of LC_{50} 95% CL with those of L1 and L2 at specific time intervals indicates that the differences were not always significant (Table 1). Second-instar larvae were significantly more susceptible to novaluron than L1 after 120 h. It was expected that L1would be more susceptible, since larval instars generally become more tolerant as they develop into larger stadia.[18] The larger L2 probably ingested more foliage and therefore received a higher dose of novaluron.

3.2 Contact bioassays

Novaluron exhibited good direct contact activity against L2. Only 27 mg litre^{-1} was needed to kill 50% of the L2 after 120 h, while 252 mg litre^{-1} killed 95% of the larvae after 120 h (Table 3). However, LC_{50} and LC_{95} values for the contact bioassays were, respectively, 64- to 89-fold and 135- to 250-fold higher than those of the residual bioassays, depending on exposure time. Insecticide penetration of insect cuticle is commonly related to the $\log P_{ow}$ ($\log_{10}$ of the octanol–water partition coefficient), a function of overall hydrophobicity of the molecule. Compounds with high $\log P_{ow}$ tend to dissolve and remain within an insect cuticle.[26] Indeed, the high $\log P_{ow}$ (4.3)[25] and low water solubility (3 µg litre^{-1})[25] of novaluron suggest that, following a topical application, it would be pharmacokinetically favorable for the molecule to remain in the cuticular waxes, thus reducing the amount of compound reaching the target site. Although slopes of the regression lines from the contact bioassays were generally lower than those of the residual bioassay, the only statistically significant difference ($\alpha = 0.05$) was between the 48-h regression lines (data not shown). Ishaaya *et al*[13] reported excellent control of greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) with novaluron through a combination of contact and residual activity. In addition, potency against *T. vaporariorum* increased when the formulation included an ingredient that facilitated penetration of the cuticle. Contact activity of diflubenzuron, the first benzoylphenyl urea discovered, varies among species. Some insects, eg *S. littoralis*, are more susceptible by contact than ingestion, while others, eg *Pieris brassicae* L, completely tolerate topical applications.[27]

Novaluron also was effective against *L decemlineata* eggs by direct contact. Concentration had a significant effect on egg hatch ($F = 3.71$; $df = 4$; $P = 0.0074$). Percentage hatch of egg masses exposed to 100 mg litre^{-1} was significantly lower than that of egg masses exposed to solvent only or to 1 mg litre^{-1} ($P \leq 0.05$) (Fig 1). Differences between mean percentage hatch of egg masses exposed to 10 or 1000 mg litre^{-1} of novaluron and of those exposed to solvent only were not statistically significant ($P \leq 0.05$) (Fig 1). Since, even in the presence of fresh foliage, neonates that emerged first from eggs often cannibalized adjacent

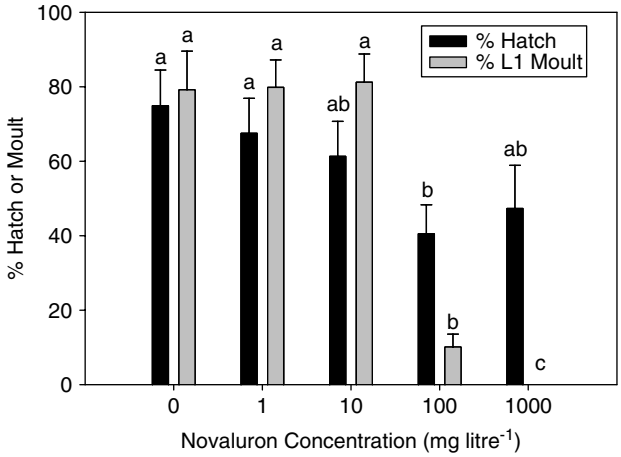

Figure 1. Percentage successful hatch (% hatch) and moult of first-instar larvae (% L1 moult) (±95% CL) of Colorado potato beetle, *Leptinotarsa decemlineata*, egg masses after direct contact treatment with novaluron in a Potter spray tower. Bars of the same colour with the same letter above them are not significantly different ($P \leq 0.05$; Tukey test).

Table 3. Direct contact toxicity of novaluron to second-instar Colorado potato beetle, *Leptinotarsa decemlineata*, larvae ($n = 785$) 48, 72, 96 and 120 h after treatment; larvae were provided untreated foliage after treatment

Time (h)	Slope (±SEM)	χ^2	LC_{50} (mg litre^{-1}) (95% CL)	LC_{95} (mg litre^{-1}) (95% CL)
48	1.76 (±0.26)	0.87	115.6 (86.1–160.8)	950.0 (550.7–2792.6)
72	1.98 (±0.31)	0.27	71.5 (52.5–94.7)	482.9 (297.8–1135.3)
96	1.94 (±0.44)	4.00	54.1 (32.0–73.3)	383.0 (224.7–1404.4)
120	1.70 (±0.26)	1.14	27.2 (17.537.4)	251.5 (157.8–557.1)

eggs before they were able to hatch, percentage egg hatch was only about 75% in egg masses exposed to solvent only. No novaluron treatment reduced egg hatch below 40% (Fig 1).

The concentration of novaluron applied to the egg masses had a significant impact on the ability of emerged L1 to moult successfully ($F = 93.69$; $df = 4$; $P < 0.0001$). While no statistically significant difference in moulting success was found at concentrations of 10 mg litre^{-1} or lower, moulting success fell sharply following application of 100 or 1000 mg litre^{-1} novaluron ($P \leq 0.05$) (Fig 1). No L1 emerging from egg masses exposed to 1000 mg litre^{-1} novaluron moulted into L2. Further, weights of L2 6 days after treatment differed significantly depending on the novaluron concentration to which egg masses had been exposed ($F = 251.88$; $df = 4$; $P < 0.0001$). Although L2 from egg masses treated with 10–1000 mg litre^{-1} weighed significantly less than those from the control ($P \leq 0.05$), L2 from egg masses treated with 1 mg litre^{-1} novaluron weighed *more* ($P \leq 0.05$) than control L2 (Fig 2). An examination of the 95% CL of L2 weights (mg) from control egg masses (2.34–2.63) and those treated with 1 mg litre^{-1} novaluron (2.92–3.18) indicates that this difference was far from being only marginally significant. This suggests that low concentrations of novaluron can have a hormetic effect (stimulatory effect of a toxicant at low concentrations) on larval development of *L decemlineata*. The phenomenon of hormetic dose-response is widely documented in the toxicological literature (eg Calabrese and Baldwin[28]), and hormetic responses by insects to low doses of insecticides have been reported. Ramachandran *et al*[29] found that low doses of azadirachtin increased survival, larval weight and rate of development of the red flour beetle *Tribolium castaneum* (Herbst). Similar effects of permethrin on the stinkbug *Podisus distinctus* (Stål)[30] and of several insecticides on the house cricket *Acheta domesticus* L[31] have been documented. This is the first report of an insecticide having a hormetic effect on *L decemlineata* development, and is also the first report of benzoylphenyl urea induced hormesis in an insect.

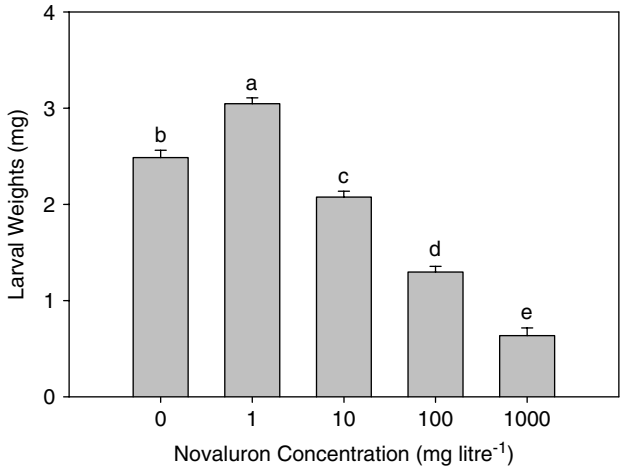

Figure 2. Weights (±SEM) of second-instar Colorado potato beetle, *Leptinotarsa decemlineata*, larvae 6 days after treatment of eggs by direct contact with novaluron in a Potter spray tower. Bars with the same letter above them are not significantly different ($P \leq 0.05$; Tukey test).

3.3 Sublethal effects on adult *Leptinotarsa decemlineata*

Most toxicology studies focus on survival/mortality. However, individuals that survive toxicant exposure may still sustain significant injury, which may be manifested as reduced longevity, developmental rates, fertility or fecundity, or changes in behavior such as feeding, searching and oviposition.[32] This study included an investigation of the sublethal effects of novaluron on adult *L decemlineata*. While no difference between treatments was observed in female longevity ($F = 0.51$; $df = 2$; $P = 0.61$), male beetles on potato foliage treated with the high-rate novaluron lived approximately half as long as those on untreated or low-rate novaluron treated foliage ($F = 7.50$; $df = 2$; $P = 0.0077$) (Table 4).

Table 4. Effect of feeding on novaluron-treated foliage on adult Colorado potato beetle, *Leptinotarsa decemlineata,* egg production, longevity, egg hatch and subsequent moult of emerged first-instar larvae (L1)[a]

	Treatment mean (±SEM)		
Biological parameter	Untreated	25 g AI ha^{-1}	75 g AI ha^{-1}
Female longevity (days)	43.2 (±2.8) a[b]	37.6 (±5.6) a	40.0 (±2.7) a
Male longevity (days)	38.4 (±5.8) a	42.2 (±2.4) a	20.2 (±4.0) b
Total eggs female^{-1}	641.2 (±79.8) a	356.4 (±96.9) a	311.2 (±115.4) a
Eggs day^{-1}	12.7 (±0.9) a	7.4 (±0.8) b	6.3 (±0.7) b
Egg masses day^{-1}	0.8 (±0.06) a	0.6 (±0.06) b	0.5 (±0.05) b
Eggs egg mass^{-1}	21.3 (±0.1) a	15.8 (±1.2) b	17.0 (±1.2) b
% Hatch[c,d]	69.1 (±5.9) a	16.9 (±6.1) b	1.6 (±2.3) c
% Moult of hatched L1[c,d]	92.9 (±2.5) a	12.2 (±9.0) b	0.0 c

[a] Upon emergence from pupation cages, virgin adult male and female *L decemlineata* were placed on potato plants that were treated with water or novaluron with a hand-held sprayer ($n = 5$ per treatment). Beetles fed and oviposited for up to 45 days after initiation of the experiment.
[b] Means within each row followed by the same letter are not significantly different ($P \leq 0.05$; Tukey test).
[c] Percent data were arcsine transformed for analysis. Back-transformed means and 95% CL are presented.
[d] Mean percentage hatch and successful moult over 45 days.

Several behavioral observations indicated that adult beetles that had fed on treated foliage experienced effects of sublethal intoxication. Adults on treated plants often had reduced walking ability and poor coordination. They fell off the plants more frequently, and often remained motionless on the bottom of the cages for up to an hour at a time. If on their backs beetles frequently had their legs extended outward, which is usually a sign of death. Only after probing with a needle was it clear that these adults were still alive. Slight convulsions also were observed in some beetles as they lay on their backs. In addition, over the first 7 days of the experiment there generally was less defoliation of treated than untreated plants. These observations may have resulted from an effect of novaluron on the integrity and/or development of the peritrophic membrane of the adult *L decemlineata*. This membrane protects the midgut from digestive enzymes, pathogenic organisms and harmful chemicals.[33] Chitin is constantly being synthesized in the peritrophic membrane during active stages of the insects life cycle.[34] Diflubenzuron has suppressed synthesis of chitin during peritrophic membrane formation in the migratory locust *Locusta migratoria* L,[35] the blowfly *Calliphora erythrocephala* (Meigen)[36] and the cabbage armyworm *Mamestra brassicae* L.[37]

Malinowski and Pawinska[16] observed reduced egg viability in adults on novaluron-treated potato foliage. In the present study, oviposition rates were affected when adult *L decemlineata* fed on novaluron-treated potato plants. Over the whole experiment, adults that fed on plants treated with low-rate or high-rate of novaluron laid 42% and 50% fewer eggs per day ($F = 15.53$; $df = 2$; $P < 0.0001$), 28% and 29% fewer egg masses per day ($F = 6.69$; $df = 2$; $P < 0.0001$), and 26% and 20% fewer eggs per egg mass than adults that fed on untreated foliage ($F = 6.30$; $df = 2$; $P = 0.0021$), respectively (Table 4). For each treatment, there were significant decreases in the mean number of egg masses (untreated: $F = 23.69$; $df = 1$; $P < 0.0001$; low-rate novaluron: $F = 8.16$; $df = 1$; $P = 0.0048$; high-rate novaluron: $F = 25.43$; $df = 1$; $P < 0.0001$) and total number of eggs (untreated: $F = 35.55$; $df = 1$; $P < 0.0001$; low-rate novaluron: $F = 7.74$; $df = 1$; $P = 0.006$; high-rate novaluron: $F = 25.81$; $df = 1$; $P < 0.0001$) laid per female per day (Fig 3). The maximum number of eggs laid in 1 day for each treatment was 48 [9 days after treatment (DAT)] on untreated foliage, 51 (8 DAT) on foliage treated with the low-rate novaluron, and 43 (8 DAT) on foliage treated with high-rate novaluron. Differences in oviposition rates on untreated and treated plants may have been due to an effect of novaluron on adult fecundity, sub-optimal nutrition due to reduced feeding, or altered behavior/mating as a result of sublethal intoxication. All three factors may have acted together to reduce reproductive outputs. Although females fed potato foliage treated with low- and high-rate novaluron oviposited only 55% and 49%, respectively, as many eggs as females on untreated foliage, there was no significant difference among treatments in the total number of eggs laid per female ($F = 3.26$; $df = 2$; $P = 0.074$) (Table 4). The lack of statistical significance was due to the high variance amongst the replicates.

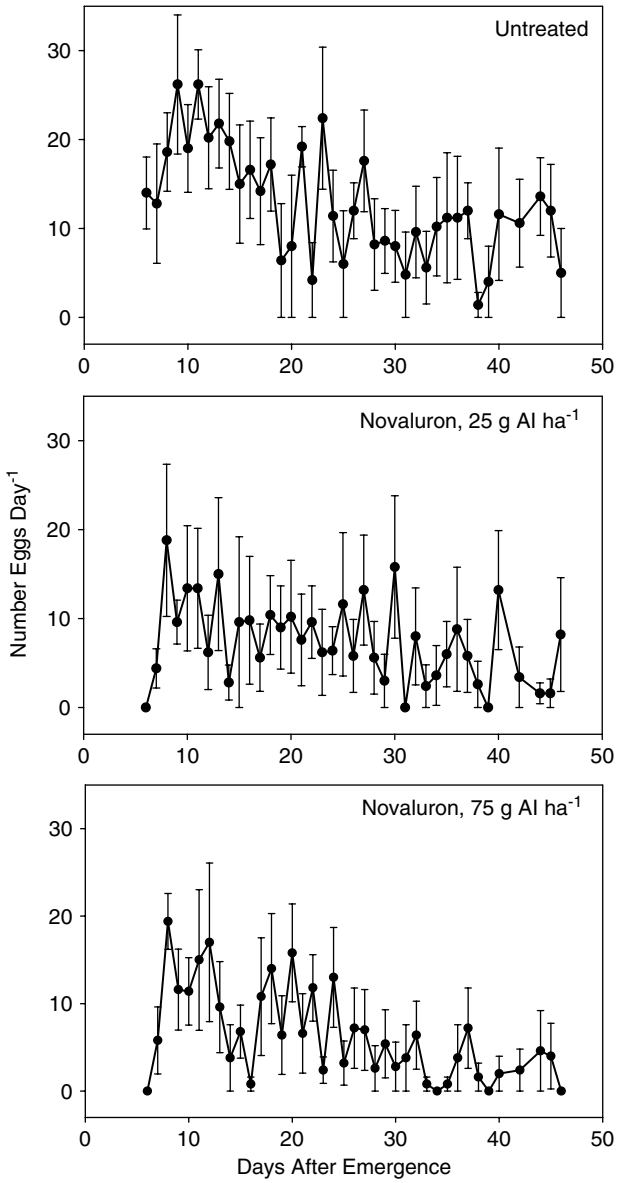

Figure 3. Mean (±SEM) daily Colorado potato beetle, *Leptinotarsa decemlineata*, oviposition (number of eggs per female) during feeding on untreated or novaluron-treated potato foliage.

Neonates that emerged from eggs first often cannibalized adjacent eggs in the mass before they were able to hatch, even in the presence of fresh foliage. Mean percentage egg hatch on untreated foliage was, therefore, only 70% (Table 4). Nonetheless, egg hatch on novaluron-treated foliage was greatly reduced compared with that on untreated foliage ($F = 30.50$; $df = 2$; $P < 0.0001$) (Table 4). Microscopic observation of embryos inside the eggs laid on treated foliage indicated that the embryos developed normally. Mandibles, setae, and legs of the unhatched larvae were clearly visible. Grosscurt[38] also reported normal

development of housefly larvae in unhatched eggs from adult females treated with diflubenzuron. Electron microscopic observation revealed that *L decemlineata* embryos from females fed diflubenzuron had altered cuticle formation, with the normal lamellate cuticle being replaced by an amorphous cuticular region with globular structures.[27] Similar effects on embryos from *L decemlineata* adults fed novaluron probably occurred in the present study. While no change in percentage hatch over time was observed in eggs from the untreated ($F = 3.29$; $df = 38$; $P = 0.072$) and high-rate novaluron treatment ($F = 0.029$; $df = 38$; $P = 0.86$), significant increase in hatch occurred in eggs from low-rate novaluron-treated plants ($F = 11.75$; $df = 38$; $P = 0.0009$). It is possible that, at the low application rate, novaluron residues degraded over time to levels insufficient to cause ovicidal activity after ingestion by *L decemlineata* adults. There also was a strong effect of novaluron treatment on the ability of hatched L1 to moult into L2 ($F = 57.63$; $df = 2$; $P < 0.0001$) (Table 4). Percentage moult did not change over the duration of the experiment for the novaluron treatments, but it did decrease near the end of the experiment in the control ($F = 5.33$; $df = 38$; $P = 0.022$) (data not shown).

Malinowski and Pawinska[16] previously found that a single novaluron treatment was able to suppress *L decemlineata* population densities below economic threshold levels throughout a whole season by stomach and contact larvicidal action, reduced egg viability and antifeedant effects. We also have found that under high *L decemlineata* pressure, Rimon 10EC applications provide excellent foliar protection (Cutler GC, unpublished). Although it is difficult to extrapolate laboratory data to a field situation, our results suggest that growers applying novaluron in the field could expect excellent control of *L decemlineata* larvae by ingestion of treated foliage, but limited control by contact. The proposed Canadian label for Rimon 10EC recommends an application rate of 220–878 ml ha^{-1} (Everich R, 2004, pers. comm.). It also is felt that application of a minimum of 350 litres ha^{-1} is needed for effective coverage of a potato canopy.[39] At this volume, the concentration of novaluron in the spray solution when applied at 878 ml ha^{-1} would be: 878 ml ha^{-1} × 0.001 litre × 100 g AI litre^{-1} × 1/350 ha litre^{-1} × 1/1000 litre g^{-1} × 1 000 000 mg litre^{-1} = 250.86 mg litre^{-1}. Considering that the residual LC_{95} for L2 was 1.68–3.97 mg litre^{-1}, L2 would be ingesting concentrations approximately 63 times the LC_{95}. In contrast, the direct contact LC_{95} was 250–950 mg litre^{-1}, suggesting that many L2 would be exposed to sublethal direct contact concentrations of novaluron, even when applied at the highest recommended rate. Our data also indicate that adults feeding on foliage treated with novaluron would lay fewer eggs, with a high percentage being unviable, supporting the observations of Malinowski and Pawinska.[16] Ovicidal activity and arrested moult of emerged L1 has been observed in field trials when Rimon 10EC was applied at a rate of 500 ml ha^{-1} (Cutler GC, unpublished). Although successful moult of L1 emerging from egg masses sprayed with novaluron concentrations ≥ 100 mg litre^{-1} was greatly reduced, *L decemlineata* in the field usually oviposit on the underside of leaves where egg masses often escape direct contact with insecticides.

4 CONCLUSIONS

These results indicate that novaluron exhibits excellent insecticidal activity against *L decemlineata* larvae through ingestion and can greatly reduce fecundity and egg hatch when adults feed on novaluron-treated foliage. In addition to selective toxicity favoring survival of non-target beneficial insects, *L decemlineata* populations in most potato growing regions have had limited exposure to chitin synthesis inhibitors, making cross-resistance with conventional compounds unlikely. Novaluron could thus be a valuable tool in effective IPM programs against *L decemlineata*.

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