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Research

Efficacy of Chlorantraniliprole Seed Treatments Against Armyworm (*Mythimna unipuncta* [Lepidoptera: Noctuidae]) Larvae on Corn (*Zea mays*)

G. E. Carscallen,[1] S. V. Kher,[2] and M. L. Evenden[1,3,]

[1]Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, [2]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5 and [3]Corresponding author, e-mail: mevenden@ualberta.ca

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Abstract

Mythimna unipuncta (Haworth) (Lepidoptera: Noctuidae) is an important insect pest of corn (*Zea mays* L.) in North America and can cause severe damage during outbreaks. Insecticides are the main control method; however, development of insecticide resistance poses management challenges and necessitates the use of novel insecticides. A synthetic insecticide, chlorantraniliprole, belonging to the anthranilic diamides, targets insect ryanodine receptors and is a potential alternative to conventional insecticides for management of *M. unipuncta*. We determined the efficacy of seed treatment with chlorantraniliprole alone compared with a positive control consisting of a neonicitinoid seed treatment of thiamethoxam and combinations of both compounds against *M. unipuncta* larvae in laboratory bioassays. Bioassays were conducted on two different growth stages of *M. unipuncta* larvae (instars 3 and 5) and two plant growth stages (V1 and V2 corn) in clip cages and whole plant experiments. Larval mortality, head capsule width, and feeding injury were measured. The chlorantraniliprole seed treatment alone or in combination with thimethoxam at different doses affected survival of *M. unipuncta* larvae. In all bioassays except one, larval mortality occurred earlier when a combination of chlorantraniliprole and thimethoxam seed treatment was used when compared with a thimethoxam alone seed treatment. *Mythimna unipuncta* larvae developed faster on the untreated control corn plants compared with corn with insecticide seed treatments. Foliar injury was low in both chlorantraniliprole alone and chlorantraniliprole + thimethoxam treatments compared with the control. Chlorantraniliprole thus offers potential alternative to conventionally used insecticides in the management of *M. unipuncta* in corn.

Key words: Rynaxypyr, anthranilic, diamide, Noctuidae, ryanodine

The armyworm, *Mythimna unipuncta* (Haworth, 1809) (Lepidoptera: Noctuidae), is a polyphagous pest of various crops in North America (Gavloski and Meers 2011), with a global distribution (Guppy 1961, McNeil 2011, CABI 2012). In North America, the pest occurs from southern Canada to the southern United States (Guppy 1961). Larvae of *M. unipuncta* prefer to feed on cultivated cereal and forage crops, including oat (*Avena sativa* L.), wheat (*Triticum aestivum*. L.), barley (*Hordeum vulgare* L.), and corn (*Zea mays* L.) (Guppy 1961, Metcalf and Metcalf 1993). In corn, larvae feed on leaves and strip them to mid-ribs (Schaafsma et al. 2007). Localized populations reach outbreak levels every 5–20 yr throughout northeastern North America (Guppy 1961, Beirne 1971). Sporadic outbreaks have resulted in 50–100% defoliation in corn in the United States and Canada (Guppy 1961, Iowa State University 2004) with total estimated yield losses of approximately $2 million (McDonough et al. 1980).

Infestations of *M. unipuncta* in Canada are the result of adult moths migrating from overwintering sites in the subtropical and tropical regions of North America (Gavloski and Meers 2011). Females lay an average of 966 eggs in multiple clusters on plant leaves over a period of 1 wk (Guppy 1961). Eggs hatch in three to 8 d in the field at a temperature range of 18–23°C, and larvae develop through six larval instars (Guppy 1961, Cook et al. 2004). A prepupal period of 3–10 d is followed by pupation in silken cells for approximately 24 d in the field at 17°C (Guppy 1961). Two to three generations occur in a growing season (Gavloski and Meers 2011). In seedling corn, the presence of two or more larvae per seedling and >10% injury warrant insecticide applications, while the economic threshold level for corn past the six-leaf stage is 50% or more injury within the cropping area (Manitoba Agriculture 2016).

Many different methods of managing *M. unipuncta* have been examined (Medeiros et al. 2000, Akhtar et al. 2008)

including chemical, biological, and semiochemical-based management (McDonough et al. 1980, Medeiros et al. 2000, Costamagnaet al. 2004, Akhtar et al. 2008). In nonoutbreak years, *M. unipuncta* does not cause economic damage as populations are controlled by natural enemies (Laub and Luna 1992). Sporadic outbreaks, however, can cause significant damage and management of these populations relies on insecticidal intervention (British Columbia Ministry of Agriculture 2017). Overuse of insecticides leading to development of resistance (Schaafsma et al. 2007) may influence *M. unipuncta* management, as there is evidence for lack of efficacy of pyrethroids (Willson and Stinner 1994) and several botanical insecticides (Akhtar et al. 2008) against this species. The expression of Mon810 in hybrid corn, which produces the Cry1A (b) toxin was initially highly effective against *M. unipuncta* (Schaafsma et al. 2007), but some populations have since developed resistance to Mon810 (García et al. 2015). *Mythimna unipuncta* larvae fed Bt-corn eliminate the crystal toxin at a high rate by retaining it in the peritrophic membrane (Pérez-Hedo et al. 2012, Pérez-Hedo et al. 2013). The resistance trait in some *M. unipuncta* is autosomal and partially dominant (González-Cabrera et al. 2013). Despite the secondary pest status of *M. unipuncta*, it has the potential to evolve resistance to pest management practices and is thereby a risk to agricultural production.

Management of *M. unipuncta* requires pesticides with novel biochemical mechanisms (Lahm et al. 2007). Insect calcium channels can be targeted through a binding action to ryanodine receptor channels (RyRs) that causes muscle contraction and death (Cordova et al. 2006). Calcium channels play an important role in arthropod muscle cell function through effects on contraction and cell signaling (Selby et al. 2017). Ryanodine receptors are a complex of intracellular calcium-gated channels that regulate the release of intracellular calcium for optimal muscle function (Selby et al. 2017, Cordova et al. 2006). The synthetic insecticide, DPX-E2Y45 (chlorantraniliprole, Rynaxypyr), targets insect RyRs and affects calcium channel functioning (Lahm et al. 2007). This new class of insecticides, the anthranilic diamides, is a potential alternative to neurotoxic insecticides (Lahm et al. 2007). Chlorantraniliprole has low toxicity to mammals (Lahm et al. 2007) and nontarget arthropods (Martinou et al. 2014). Chlorantraniliprole has been effectively used against several lepidopteran pests such as tobacco budworm (*Heliothis virescens*) (Fabricius) (Lepidoptera: Noctuidae), fall armyworm (*Spodoptera frugiperda*) (Smith), bollworm (*Helicoverpa zea*) (Bodie), and codling moth (*Cydia pomonella*) (L.) (Lepidoptera: Tortricidae) (Temple et al. 2009, Pluciennik 2012). It is also effective against the variegated cutworm (*Peridroma saucia*) (Lepidoptera: Noctuidae) (Hübner) in vegetable crops (Scott-Dupree 2007), and the western bean cutworm (*Striacosta albicosta*) (Smith) (Lepidoptera: Noctuidae) in common bean (*Phaseolus vulgaris* L.) (Goudis et al. 2016). Limited published information is available on the efficacy of chlorantraniliprole on *M. unipuncta* in Canada, where it has recently been registered for the seed treatment of corn to manage pests including wireworms, cutworms, and seed corn maggot (Health Canada 2016). In this context, our investigation aimed to determine the efficacy of seed treatment with chlorantraniliprole against *M. unipuncta* larvae compared with a positive control consisting of a neonicitinoid seed treatment of thiamethoxam (Cruiser, Syngenta Inc., Basel, Switzerland) and an untreated control. We measured the effects of seed treatment on time to larval mortality, larval development (head capsule width), and feeding injury of *M. unipuncta*.

Materials and Methods

Host Plants

Corn plants (cultivar: P0987XR; nontransgenic) were grown in plastic pots (12.7 cm diameter) using Sunshine Mix No. 2 potting mixture (Ingredients: Canadian sphagnum peat moss, coarse perlite, gypsum, and dolomitic limestone, Sun Grow Horticulture Canada Ltd., Vancouver, BC). Pots were seeded with two corn seeds, but plants were later thinned to one per pot so that a single plant was considered a replicate. Plants in the first-leaf (V1) and second-leaf (V2) collar stage were used in bioassays (Table 1). Plant stage was measured using the leaf collar method (Abendroth et al. 2011) and the first round leaf was not counted.

Plants were fertilized with 200 ml of 10-52-10 (NPK) fertilizer (0.48 g/1,000 ml of water) and 20-8-20 (NPK) fertilizer (2 g/1,000 ml). Fertilizer was applied twice in the second week of plant growth, once before and once after thinning for the V1 plants. A subsequent fertilization treatment was applied on approximately Day 18 for larger V2 plants.

Insect Colony

The *M. unipuncta* colony originated from a laboratory colony at the University of Western Ontario, London, ON, Canada. At the University of Alberta, larvae were reared on a pinto bean-based diet (Shorey and Hale 1965) at 24°C under a 16:8 (L:D) h light:dark regime until use in the bioassay. Larval instars 3 and 5 were used in experiments.

Table 1. Seed treatment and dose (µg) of active ingredient per seed used in feeding bioassays

Treatment	Treatment name	Insecticide (dose, µg)	Fungicide (dose, UAT)
1	Chlorantraniliprole 250 µg	DPX-E2Y45[a] (250)	Maxim Quattro[b](64)
2	Chlorantraniliprole 500 µg	DPX-E2Y45 (500)	Maxim Quattro (64)
3	Chlorantraniliprole 750 µg	DPX-E2Y45 (750)	Maxim Quattro (64)
4	Chlorantraniliprole 250 µg +thiamethoxam 250 µg	DPX-E2Y45 (250), Cruiser[c] (250)	Maxim Quattro (64)
5	Chlorantraniliprole 500 µg +thiamethoxam 250 µg	DPX-E2Y45 (500), Cruiser (250)	Maxim Quattro (64)
6	Chlorantraniliprole 500 µg +thiamethoxam 250 µg + proprietary polymer	DPX-E2Y45 (500), Cruiser (250), Polymer L323B	Maxim Quattro (64)
7	Thiamethoxam	Cruiser (250)	Maxim Quattro (64)
8	Control	Control (no insecticide)	Maxim Quattro (64)

[a]Active ingredient = Rynaxypyr.
[b]Active ingredient = Fludioxonil, Metalaxyl- M and S isomer, Azoxystrobin, and Thiabendazole.
[c]Active ingredient = Thiamethoxam.

Insecticide Treatments

Four feeding bioassays were conducted from July 2013 to February 2014 to test the efficacy of chlorantraniliprole (DPX-E2Y45) seed treatments of corn on growth and mortality of *M. unipuncta*. In all experiments, seven insecticide seed treatments were tested and compared with an untreated control treatment with fungicide alone (Maxim Quattro, Syngenta) (Table 1). The seed treatments included three doses of chlorantraniliprole alone, three dose combinations of chlorantraniliprole + thimethoxam, a positive control consisting thiamethoxam alone, and an untreated control of fungicide alone. Pre-treated seed was obtained from DuPont Canada.

Clip Cage Experiments

Three separate feeding bioassays were conducted using cylindrical clip cages between July and September 2013. Experiments took place in a growth room maintained at 16:8 (L:D) h photo regime at 24°C. Corn plants were arranged randomly on a table beneath growth lights in the chamber. Two different larval stages of *M. unipuncta* (instar 3 and 5) were used in separate bioassays. The cages consisted of mesh-lined hollow tubes constructed from film canisters (5 cm × 2 cm [height by diameter]) that had sponge-foam edging to provide a seal when tied to the plants with string attached at the top and the base of the cage.

Bioassay 1: V1 Corn and Third-Instar Larvae

The first bioassay tested the effect of insecticide-treated corn plants at the first-leaf (V1) stage on third-instar *M. unipuncta* larvae in the cage. A clip cage was attached to the third leaf of a corn plant and three *M. unipuncta* larvae were set inside the cage. All seed treatments were arranged in a completely randomized design on a greenhouse bench. Clip cages were checked once a day throughout larval development or until death. A dead larva was defined as one that did not move when stimulated with a paintbrush. Dead larvae were preserved in 70% ethanol and head capsule width was measured using a dissecting scope fitted with a micrometer to the nearest tenth of a millimeter at 10× magnification.

Bioassay 2: V2 Corn and Third-Instar Larvae

The second bioassay tested the effect of insecticide-treated corn plants at the second-leaf (V2) stage on third-instar *M. unipuncta* larvae in the cage. A clip cage was attached to the third leaf and three *M. unipuncta* larvae were set inside the cage. All seed treatments were arranged in a completely randomized design on a greenhouse bench as in Bioassay 1. Clip cages were checked once a day throughout larval development or until death. Dead larvae were preserved in and measured as in Bioassay 1.

Bioassay 3: V2 Corn and Fifth-Instar Larvae

The third bioassay tested the effect of insecticide-treated corn plants at the second-leaf (V2) stage on fifth-instar *M. unipuncta* larvae. A single fifth-instar larva was placed inside each clip cage attached to the third leaf of treated corn plants. Treatments were arranged as in Bioassay 1. Clip cages were checked and larvae were preserved and measured as in Bioassay 1.

Bioassay 4: V1 Corn, Whole Plant, and Third-Instar Larvae

Bioassay 4 tested the effect of the insecticide treatments on larval growth and mortality when larvae fed on the entire plant. Third-instar larvae were exposed to whole plants at the V1 stage. Plants (n = 10 per treatment) were grown in 15.24 cm diameter pots for this bioassay. Two larvae were placed at the base of each plant on a corrugated plastic (Home Depot, Edmonton, Canada) insert positioned on top of the growth medium and the entire plant was enclosed in a mesh sleeve. All enclosures were checked daily until larvae died or pupated. Days until death were recorded. Head capsules of dead larvae preserved in 70% ethanol were measured to the nearest tenth of a millimeter at 10× magnification.

After the removal of larvae from plants, all leaves (with and without feeding injury) from each plant were digitally scanned (ImageJ 1.34s, United States National Institute of Health). A leaf injury index on a five-point scale was used to measure feeding injury: 0 (0–10% of leaf area removed by feeding); 1 (11–20%); 2 (21–30%); 3 (31–50%); 4 (51–70%); and 5 (71–100%). All scanned leaf images visually assessed to assign an injury score.

Statistical Analyses

The data for larval mortality, head-capsule sizes, and mean feeding injury on corn plants subjected to different insecticidal seed treatments were analyzed using SAS (SAS Institute 2008c). We tested whether the data satisfied assumptions of normality and variance homogeneity using Shapiro–Wilk and Kolmogorov–Smirnov test and Leven's test, respectively. When the data did not conform to these assumptions, appropriate distributions were fitted.

For data on time to larval mortality from Bioassay 1, a Kruskal–Wallis test was used due to a lack of fit to the Gaussian distribution and lack of model convergence using other distributions. The data on head capsule size for bioassay 1 was analyzed using PROC MIXED as the data conformed to assumptions (SAS Institute 2008c). Seed treatment was a fixed effect while larvae within clip cage and date were treated as random effects. Means were compared using a Tukey's post hoc test.

For Bioassay 2, larval mortality among treatments was compared using Generalized Estimating Equations (GEEs) with a Poisson distribution using PROC GLIMMIX (SAS Institute 2008b), while head-capsule widths were compared using PROC MIXED, as described earlier.

For Bioassay 3, time to death of larvae among treatments was compared using GEEs with a Poisson distribution using PROC GENMOD (SAS Institute 2008a), while the head-capsule sizes were compared using PROC MIXED.

For Bioassay 4, the data on time to larval mortality was analyzed by fitting GEEs using PROC GENMOD with seed treatment as the fixed effect. Mean comparisons were conducted using a Tukey's test. The data on head-capsule width were compared using PROC MIXED, while the data on feeding injury (expressed in terms of mean feeding scores) were compared using chi-square test (PROC FREQUENCY; SAS Institute 2013). For the feeding injury data in Bioassay 4, GEEs were fitted to the level of leaf consumption using PROC GENMOD (SAS Institute 2008a). A Poisson distribution was fitted to the data using DIST=P option of PROC GENMOD and LOG as the LINK function. Means were compared using Tukey's test.

Results

Bioassay 1: V1 Corn and Third-Instar Larvae

The time to death of third-instar larvae feeding on corn plants at the V1 stage differed significantly with insecticidal seed treatment (F = 9.37, df = 7, 142, P < 0.0001) (Fig. 1a). The average time to death ranged between 1.5 and 3 d. Third-instar larvae lived the

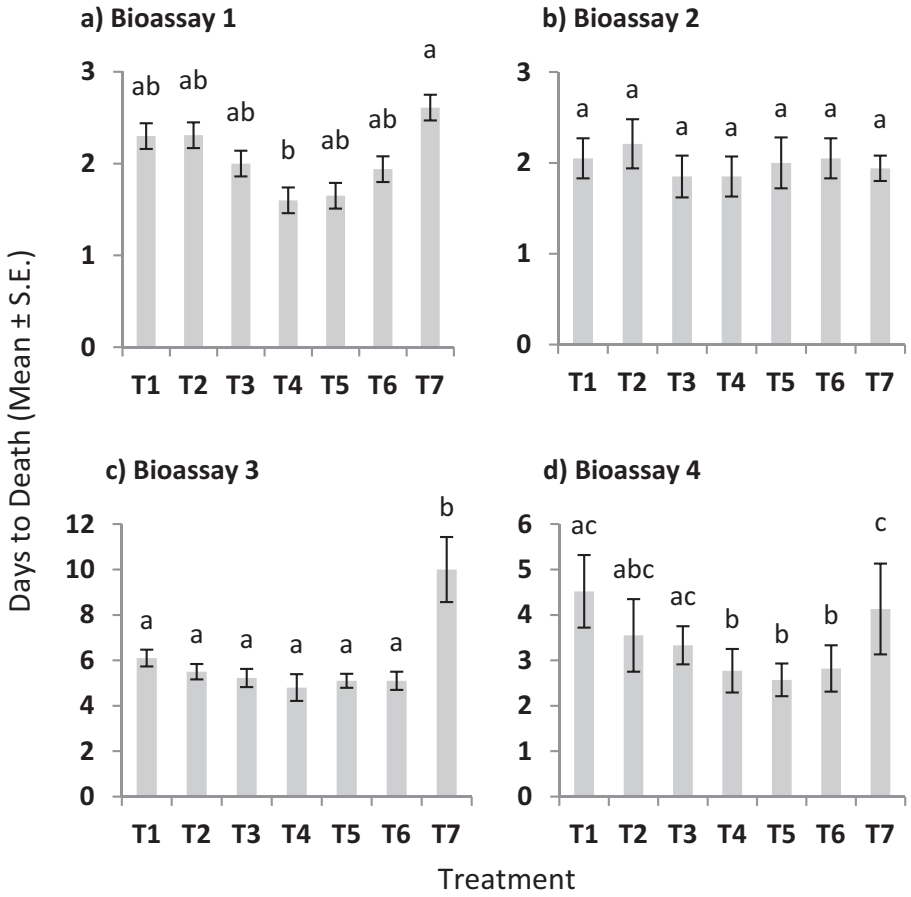

Fig. 1. Time (days) until death of third-instar *M. unipuncta* larvae after feeding on corn grown from the various insecticidal-treated seed in: (a) Bioassay 1, when third-instar larvae fed on V1 stage corn in clip cages (analyzed with a Kruskal–Wallis test); (b) Bioassay 2, when third-instar larvae fed on V2 stage corn in clip cages (analyzed with GEEs with a Poisson distribution using PROC GLIMMIX); (c) Bioassay 3, when fifth-instar larvae fed on V2 stage corn in clip cages (analyzed with GEEs with a Poisson distribution using PROC GENMOD); and (d) Bioassay 4 when third-instar larvae fed on whole plants of V1 stage corn (analyzed with GEE using PROC GENMOD and a Tukey's test). T1–T7 indicate the insecticidal seed treatments tested (Table 1). The non-insecticidal control treatment (T8) is not included in the analyses as most larvae were still alive at the end of the experiment. Different letters above the bars indicate statistical significance between groups.

longest on V1 plants with seeds treated with thimethoxam alone at a dose of 250 µg, which was significantly longer than larvae that fed on V1 plants with seeds treated with chlorantraniliprole 250 µg + thiamethoxam 250 µg. All of the other seed treatments resulted in a time to death that was intermediate between treatments 4 and 7 (Fig. 1a).

The width of larval head capsules differed among larvae that fed on V1 plants grown from the variously treated seeds (F = 9.37, df = 7, 142, P < 0.001). The head capsule size ranged between 0.9 mm and 1.16 mm, which indicates that the larvae died as third instars. Development proceeded the farthest for larvae in treatment 8 (control) that had a mean head capsule width of 1.16 ± 0.03 mm. Larvae were least developed at death if they fed on plants treated with chlorantraniliprole 250 µg + thiamethoxam 250 µg as the mean head capsule width was 0.88 ± 0.03 mm. The mean head capsule sizes did not differ significantly among larvae reared on plants in T1–T7, but larvae reared on treated plants were all smaller than those reared on the untreated control (Fig. 2a).

Bioassay 2: V2 Corn and Third-Instar Larvae

The mean time to death of third-instar *M. unipuncta* larvae fed on V2 corn plants did not differ significantly with insecticidal seed treatment (F = 0.87, df = 6, 109, P > 0.05) (Fig. 1b). The time to death ranged between 1 and 2 d for larvae on treated plants with a mean of 2.00 ± 0.09 d. Head capsule sizes of larvae at death differed among the treatments (F = 15.03, df = 7, 141, P < 0.001) (Fig. 2b). The head capsule width was largest for larvae in treatment 8 (control) that had a mean head capsule width of 1.34 ± 0.07 mm. Head capsule width was smallest for larvae that died after feeding on plants from chlorantraniliprole 250 µg treated seeds (mean head capsule width of 0.94 ± 0.07 mm). The head capsule width of larvae that died while feeding on plants from seed treatments 1 to 6 did not differ significantly, but were significantly smaller than those on treatment 8 plants (control). Seed treatment with thiamethoxam (250 µg) alone in treatment 7 resulted in larvae with larger head capsules at death than in treatment 1 (chlorantraniliprole 250 µg) or treatment 2 (chlorantraniliprole 500 µg) (Fig. 2b).

Bioassay 3: V2 Corn and Fifth-Instar Larvae

Fifth-instar larvae that fed on corn plants treated with different insecticidal seed treatments differed significantly in terms of time to death (χ^2 = 24.35, df = 6, P < 0.001) (Fig. 1c). Time to death was faster for larvae fed plants in treatments 1–6 (4.80 – 6.10 d) when compared with those fed plants from seeds treated with

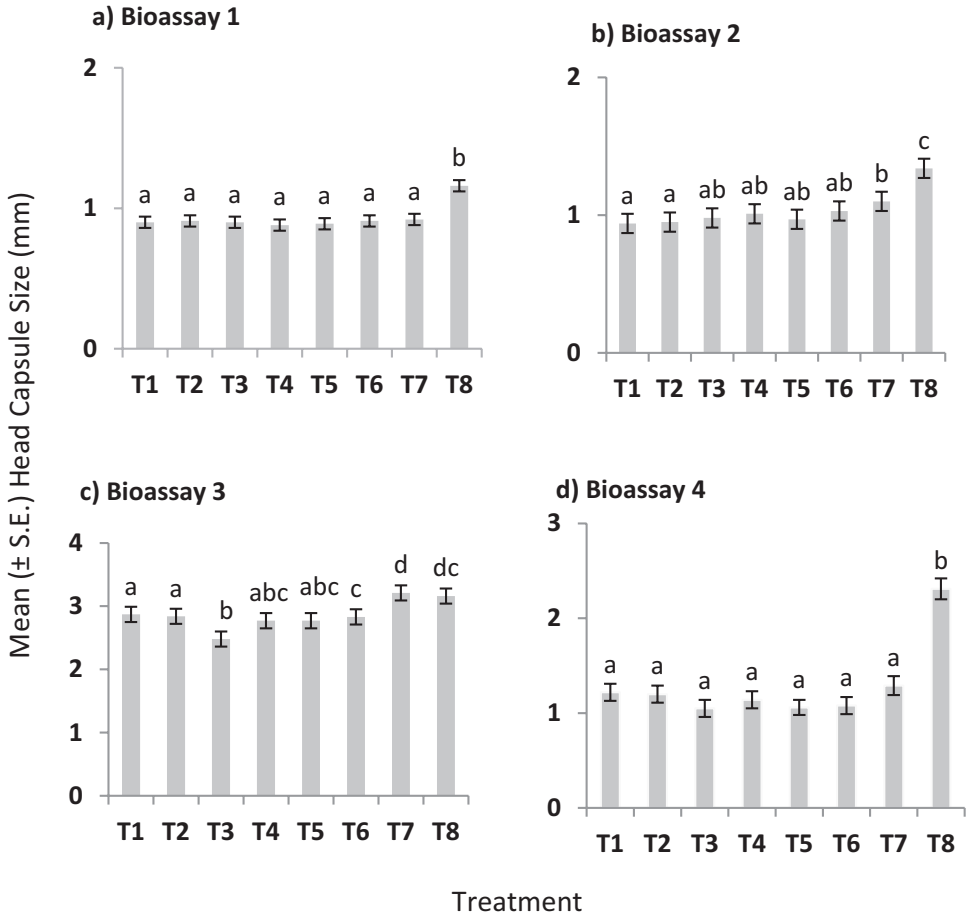

Fig. 2. Head capsule size (mm) of *M. unipuncta* larvae after feeding on corn grown from the various insecticidal-treated seed in: (a) Bioassay 1, when third-instar larvae fed on V1 stage corn in clip cages; (b) Bioassay 2, when third-instar larvae fed on V2 stage corn in clip cages; (c) Bioassay 3, when fifth-instar larvae fed on V2 stage corn in clip cages; and (d) Bioassay 4 when third-instar larvae fed on whole plants of V1 stage corn;. T1–T8 indicate the seed treatments tested (Table 1). The non-insecticidal control treatment (T8) is included in the analyses and compared with the head capsules of the other larvae. Different letters above the bars indicate statistical significance between groups. All bioassays were analyzed separately using PROC MIXED.

thiamethoxam alone in treatment 7 (dose = 250 µg) that lived a mean of 10.00 ± 1.43 d. Larval head capsule width at death differed by insecticidal seed treatment ($F = 3.10$, df = 7, 66, $P < 0.001$) (Fig. 2c). Head capsules were smallest (mean: 2.48 ± 0.12 mm) for larvae fed corn from seeds treated with the highest dose of chlorantraniliprole (750 µg, Treatment 3). Larvae fed on plants from treatment 3 seeds had significantly smaller head capsules than larvae fed plants from seeds treated with lower doses of chlorantraniliprole in treatments 1 (250 µg) and 2 (500 µg). Head capsules of larvae that fed on plants from seeds treated with the various doses of chlorantraniliprole alone in treatments 1–3, were significantly smaller than the head capsules of control larvae in treatment 8 that had a mean head capsule width of 3.16 ± 0.12 mm at death. Head capsule measurements of larvae that fed on plants from seeds treated with chlorantraniliprole in combination with thiamethoxan (treatments 4–6) or with thiamethoxan alone (treatment 7) were intermediate between control larvae and those fed plants from seeds treated with chlorantraniliprole alone at various doses (treatments 1–3) (Fig. 2c).

Bioassay 4: V1 Corn, Whole Plant, and Third-Instar Larvae

Time to death of third-instar larvae differed significantly by insecticidal seed treatment ($\chi^2 = 16.15$, df = 6, $P < 0.01$) when larvae were allowed to feed freely on an entire corn plant in Bioassay 4. The time to death ranged between 2 and 5 d with the shortest time reported on plants treated with chlorantraniliprole 500 µg + thiamethoxam 250 µg (mean 2.57 ± 0.36 d). Time to death was longest on plants from chlorantraniliprole 250 µg treatment (mean 4.52 ± 0.80 d). In the whole plant bioassay, seed treatment with both insecticides (treatments 4–6) resulted in faster death of larvae than those fed plants from seeds treated with the lowest dose of chlorantraniliprole (250 µg) or thiamethoxan alone (250 µg) in treatments 1 and 7, respectively (Fig. 1d).

Larval head capsule size was smaller for larvae reared on insecticide seed-treated plants in the whole plant bioassay when compared with the nontreated control (Fig. 2d) ($F = 13.31$, df = 7, 125, $P < 0.0001$). Head capsule size at death did not significantly differ among larvae reared on plants from the various seed treatments.

The injury caused by larval feeding in the whole plant assay was significantly and equally reduced by all the insecticidal seed treatments when compared with the control ($\chi^2 = 94.26$, df = 7, $P < 0.0001$) (Fig. 3). The lowest mean injury score occurred for plants treated with chlorantraniliprole 750 µg and chlorantraniliprole 500 µg + thiamethoxam 250µg (score = 0.1), which represented 11–20% injury to the foliage, while the highest was for treatment 8 (control) (score = 3.8), which represented 31–50% foliar injury.

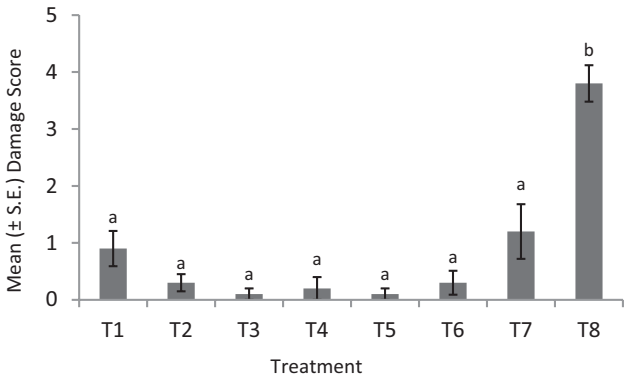

Fig. 3. Mean *M. unipuncta* larval injury score ratings of corn leaves of plants from the various insecticide seed treatments (Table 1). Leaves were assigned leaf injury scores ranging from 0 to 5: 0 = 0–10%, 1 = 11–20%, 2 = 21–30%, 3 = 31–50%, 4 = 51–70%, and 5 = 71–100%. Treatments were analyzed with a chi-square test using PROC FREQUENCY. Different letters above the bars indicate statistical significance between groups.

Discussion

Our results show that seed treatments with chlorantraniliprole alone or in combination with thiomethoxam at different doses affect developmental success of *M. unipuncta* larvae. In all clip cage bioassays, larval mortality occurred quickly (on average after 2 d of feeding in young larvae) on plants treated with a seed treatment of chlorantraniliprole and thimethoxam combined. Chlorantraniliprole causes rapid mortality in lepidopteran pests. Cabbage butterfly, *Pieris rapae* (L.) (Lepidoptera: Pieridae) larvae live on average 5 d after exposure to chlorantraniliprole (Su et al. 2017). Larvae of two key pests of cranberries, *Sparganothis sulfureana* and *Choristoneura parallela* (Lepidoptera: Tortricidae) die within 3 d of chlorantraniliprole application, and residual activity remains for 7 d post application (Rodriguez-Saona et al. 2016). Time to mortality in the current study was similar to that in previous studies in both chlorantraniliprole alone and chlorantraniliprole + thimethoxam treatments.

The thimethoxam alone seed treatment did not cause fast mortality of older larvae or affect larval development. A high dose of chlorantraniliprole alone was more effective against fifth instars than the combined treatments. Although not statistically significant, the head capsule size of larvae was numerically higher in chlorantraniliprole + thimethoxam treatment combinations, and thimethoxam seemed to decrease the effectiveness of chlorantraniliprole. Thimethoxam and chlorantraniliprole seed treatments can have differential effects on life stages of insects (Lanka et al. 2013, 2014). In the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), thimethoxam seed treatment is effective against adults but not the first-instar larvae, while chlorantraniliprole seed treatment affects survival of eggs and first instars of rice weevil but not adults (Lanka et al. 2013, Hamm et al. 2014). Thiamethoxam is also not effective against late-instar stages of white grubs, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Grewal et al. 2001). High rates of thiamethoxam seed treatment are required to manage populations of black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) in corn (Wilde et al. 2007); however, life-stage specific effects of thiamethoxam are not known. We speculate that the differential activity of thiamethoxam as reported in past studies (Lanka et al. 2013, 2014; Hamm et al. 2014) may have detracted from the activity of seed treatment combinations against *M. unipuncta*. This needs further examination and field validation.

Whole plant bioassays indicated that chlorantraniliprole + thimethoxam combinations caused quick mortality of third-instar larvae compared with various doses of chlorantraniliprole alone. Although a high dose of chlorantraniliprole alone affected development of third-instar larvae on whole corn plants, the dose–mortality relationships at various stages of plant growth and larval development need to be quantified (Lanka et al. 2013). Greater efficacy of combined seed treatment compared with standalone treatments in whole plant bioassays may be associated with quick mortality and systemic activity of the treatment combination. Systemic activity of thimethoxam declines with crop growth progression (Lanka et al. 2014). Some reports indicate chlorantraniliprole has longer systemic activity than other systemic insecticides (Ioriatti et al. 2009, Rodriguez-Saona et al. 2016). A single foliar application of chlorantraniliprole in grapes kills neonate larvae of the grapevine moth, *Lobesia botrana* (Denis & Schiffermuller) (Lepidoptera: Tortricidae) for 1 wk (Ioriatti et al. 2009). Duration of systemic activity in corn from seeds treated with chlorantraniliprole and thiamethoxam either alone or in treatment combination is not known, but our results indicate that a combined seed treatment is effective against *M. unipuncta* in whole plant bioassays. Products with short systemic activity may require multiple applications; which comes with monetary and environmental costs (Williamson et al. 2013). Reducing the number of applications of insecticides is one of the key challenges in the management of lepidopteran pests (Thrash et al. 2013). Corn seed treatment with chlorantraniliprole and thiamethoxam combinations may be beneficial to manage early *M. unipuncta* infestations of young instar larvae, due to quick mortality and potentially improved systemic activity (Thrash et al. 2013). A high dose of the combined treatment may be needed for protection from mature larvae when corn is at advanced growth stages, as the pest is multivoltine (Lanka et al. 2013).

Body size is an important life history trait that correlates with fitness in insects (Grunert et al. 2015). A change in larval development rate, as measured by head-capsule width, with insecticidal seed treatment may indicate negative effects on developmental physiology (Rodrigues et al. 2015a,b). Our bioassays indicate that *M. unipuncta* larvae develop faster and molt more frequently when fed the untreated control treatment compared with plants grown from seed treated with the various insecticide seed treatments. Larvae also attain comparatively greater head-capsule sizes when fed corn treated as seed with the thimethoxam alone treatment (250 µg) when compared with chlorantraniliprole alone treatments (treatment 1–250 µg, treatment 2–500 µg) on two leaf (V2) corn. Body size of mature larvae was affected by seed treatment of chlorantraniliprole at 750 µg. Similar developmental effects occur in the larval midge, *Chironomus riparius* Meigen (Diptera: Chironomidae), following exposure to chlorantraniliprole (Rodrigues et al. 2015a). Specific field examples on the effects of chlorantraniliprole on the development of cutworms or other lepidopteran pests are scarce, but our study indicates that the efficacy of chlorantraniliprole treatments varies with life stage of larval *M. unipuncta*.

Insecticide seed treatments were effective at reducing feeding injury by *M. unipuncta* when compared with the nontreated control plants. Overall, injury was low and comparable among all insecticidal treatments. Chlorantraniliprole application results in rapid cessation of feeding in lepidopteran pests that reduces feeding injury. Feeding stops within 30 min of exposure to treated plant material in the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), cabbage looper, *Trichoplusia ni* (Hubner), and diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Hannig et al. 2009). Feeding injury caused by *H. zea* and *T. ni* is

reduced by 90–99% in chlorantraniliprole-treated corn (Hannig et al. 2009). Our results indicate that chlorantraniliprole alone or in combination with thimethoxam caused early mortality, reduced feeding, and affected development in third-instar larvae in whole plant experiments. This is in line with the prior finding that chlorantraniliprole results in the cessation of feeding quickly and thus reduces injury (Hanning et al. 2009).

The current investigation was conducted under controlled settings to help understand chlorantraniliprole treatment efficacy on different larval stages of *M. unipuncta*. Further trials to test the efficacy of this insecticide under field conditions will be required to validate the findings. Nevertheless, our studies provide baseline information on the effects of chlorantraniliprole seed treatments on *M. unipuncta* larval development and mortality on corn. Chlorantraniliprole has been effectively used against insects that have developed resistance to neurotoxic insecticides (Hannig et al. 2009). In corn, a single application of chlorantraniliprole via seed treatment effectively controlled populations of the European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae) and the seed corn maggot, *Delia platura* (Meigen) (Diptera: Anthomyiidae) (Schmidt-Jeffris and Nault 2016). Chlorantraniliprole offers a potential option in insect pest management due to its novel mode of action, and safety to beneficial and nontarget organisms (Cordova et al. 2006, Hannig et al. 2009, Ioriatti et al. 2009, Schmidt-Jeffris and Nault 2016). Chlorantraniliprole does not affect survival and reproduction of seven species of parasitoids including *Trichrogramma* spp. (Hymenoptera: Trichogrammatidae) egg parasitoids, *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae), a common parasitoid of the diamondback moth, and *Aphidius rhopalosiphi* (DeStephani-Pérez) (Hymenoptera: Aphidiinae), an aphid-specific parasitoid (Brugger et al. 2010). Insecticides with new modes of action can be effective candidates for insecticide rotation programs and can be combined with natural enemy release programs (Tiwari and Stelinski 2013). This can help in the management of insecticide resistance in insect populations and improve the efficiency of integrated pest management programs. Chlorantraniliprole thus offers a potential alternative to conventionally used insecticides in the management of *M. unipuncta* in corn.

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References Cited

Abendroth, L. J., R. W. Elmore, M. J. Boyer, and S. K. Marlay. 2011. Corn growth and development. Iowa State University Extension, Ames, IA. (https://store.extension.iastate.edu/product/6065) (accessed 20 June 2017).

Akhtar, Y., Y. R. Yeoung, and M. B. Isman. 2008. Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*. Phytochem. Rev. 7: 77–88.

Beirne, B. P. 1971. Pest insects of annual crops in Canada. Mem. Entomol. Soc. Can. 78. pp.124.

British Columbia Ministry of Agriculture. 2017. True armyworm: information for B. C. Growers. (http://www.acrd.bc.ca/cms/wpattachments/wpID254atID2500.pdf) (accessed 25 April 2017).

Brugger, K. E., P. G. Cole, I. C. Newman, N. Parker, B. Scholz, P. Suvagia, G. Walker, and T. G. Hammond. 2010. Selectivity of chlorantraniliprole to parasitoid wasps. Pest Manag. Sci. 66: 1075–1081.

CABI. 2012. *Mythimna unipuncta* (rice armyworm).pdf. Invasive Species Compend. (http://www.cabi.org/isc/datasheet/45094) (accessed 27 June 2017).

Cook, K., S. Ratcliffe, M. Gray, and K. Steffey. 2004. Armyworm (*Pseudaletia unipuncta* Haworth) Insect fact sheet. Integr. Pest Manag. (http://extension.cropsciences.illinois.edu/fieldcrops/insects/armyworm/) (accessed 30 June 2017).

Cordova, D., E. A. Benner, M. D. Sacher, J. J. Rauh, J. S. Sopa, G. P. Lahm, T. P. Selby, T. M. Stevenson, L. Flexner, S. Gutteridge, et al. 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. Pestic. Biochem. Physiol. 84: 196–214.

Costamagna, A. C., F. D. Menalled, and D. A. Landis. 2004. Host density influences parasitism of the armyworm *Pseudaletia unipuncta* in agricultural landscapes. Basic Appl. Ecol. 5: 347–355.

García, M., F. Ortego, P. Hernández-Crespo, G. P. Farinós, and P. Castañera. 2015. Inheritance, fitness costs, incomplete resistance and feeding preferences in a laboratory-selected MON810-resistant strain of the true armyworm *Mythimna unipuncta*. Pest Manag. Sci. 71: 1631–1639.

Gavloski, J., and S. Meers. 2011. Arthropods of cereal crops in Canadian grasslands description of habitat, pp. 217–237. *In* K. D. Floate (ed.), Arthropods of Canadian grasslands (volume 2). Inhabitants of a changing landscape. Biological Survey of Canada, Ottawa, Ontario.

González-Cabrera, J., M. García, P. Hernández-Crespo, G. P. Farinós, F. Ortego, and P. Castañera. 2013. Resistance to Bt maize in *Mythimna unipuncta* (Lepidoptera: Noctuidae) is mediated by alteration in Cry1Ab protein activation. Insect Biochem. Mol. Biol. 43: 635–643.

Goudis, L. A., C. L. Trueman, T. S. Baute, R. H. Hallett, and C. L. Gillard. 2016. Insecticide efficacy and timing for control of western bean cutworm (Lepidoptera: Noctuidae) in dry and snap beans. J. Econ. Entomol. 109: 188–194.

Grewal, P. S., K. T. Power, and D. J. Shetlar. 2001. Neonicotinoid insecticides alter diapause behaviour and survival of overwintering white grubs (Coleoptera: Scarabaeidae). Pest Manag. Sci. 57: 852–857.

Grunert, L. W., J. W. Clarke, C. Ahuja, H. Eswaran, and H. F. Nijhout. 2015. A quantitative analysis of growth and size regulation in *Manduca sexta*: the physiological basis of variation in size and age at metamorphosis. PLoS One. 10: e0127988.

Guppy, J. C.1961. Life history and behaviour of the armyworm, *Pseudaletia unipuncta* (Haw.) (Lepidoptera: Noctuidae), in Eastern Ontario. Can. Entomol. 93: 1141–1153.

Hamm, J., S. Lanka, and M. Stout. 2014. Influence of rice seedling on efficacies of neonicotinoid and anthranilic diamide seed treatments against rice water weevil. Insects 5: 961–973.

Hannig, G. T., M. Ziegler, and P. G. Marçon. 2009. Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. Pest Manag. Sci. 65: 969–974.

Health Canada. 2016. Registration decision: Chlorantraniliprole. (http://publications.gc.ca/collections/collection_2016/sc-hc/H113-25-2016-17-eng.pdf) (accessed 3 July 2018).

Ioriatti, C., G. Anfora, G. Angeli, V. Mazzoni, and F. Trona. 2009. Effects of chlorantraniliprole on eggs and larvae of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae). Pest Manag. Sci. 65: 717–722.

Iowa State University. 2004. Armyworm defoliating young corn. (https://www.ipm.iastate.edu/ipm/icm/2004/6-14-2004/armyworm.html) (accessed 20 June 2017).

Lahm, G. P., T. M. Stevenson, T. P. Selby, J. H. Freudenberger, D. Cordova, L. Flexner, C. A. Bellin, C. M. Dubas, B. K. Smith, K. A. Hughes, et al. 2007. Rynaxypyr: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. Bioorg. Med. Chem. Lett. 17: 6274–6279.

Lanka, S. K., J. A. Ottea, J. A. Davis, A. B. Hernandez, and M. J. Stout. 2013. Systemic effects of thiamethoxam and chlorantraniliprole seed treatments on adult *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) in rice. Pest Manag. Sci. 69: 250–256.

Lanka, S. K., M. J. Stout, J. M. Beuzelin, and J. A. Ottea. 2014. Activity of chlorantraniliprole and thiamethoxam seed treatments on life stages of

the rice water weevil as affected by the distribution of insecticides in rice plants. Pest Manag. Sci. 70: 338–344.

Laub, C. A., and J. M. Luna. 1992. Winter cover crop suppression practices and natural enemies of armyworm (Lepidoptera: Noctuidae) in no-till corn. Environ. Entomol. 21: 41–49.

Manitoba Agriculture. 2016. Armyworms. (https://www.gov.mb.ca/agriculture/crops/insects/true-armyworm.html) (accessed 30 June 2017).

Martinou, A. F., N. Seraphides, and M. C. Stavrinides. 2014. Lethal and behavioral effects of pesticides on the insect predator *Macrolophus pygmaeus*. Chemosphere 96: 167–173.

McDonough, L. M., J. A. Kamm, B. A. Bierl-Leonhardt. 1980. Sex pheromone of the armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). J. Chem. Ecol. 6: 565–572.

McNeil, J. N. 2011. Studying the cost of migration: a comparison of *Pseudaletia unipuncta* populations from Canada and the Azores. Acoreana 7: 125–137.

Medeiros, J., J. S. Rosa, J. Tavares, and N. Simões. 2000. Susceptibility of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) to entomopathogenic nematodes (Rhabditida: steinernematidae and Heterorhabditidae) isolated in the Azores: effect of nematode strain and host age. J. Econ. Entomol. 93: 1403–1408.

Metcalf, R. L., and R. A. Metcalf. 1993. Destructive and useful insects: their habits and control. McGraw-Hill Inc., New York, NY.

Pérez-Hedo, M., C. López, R. Albajes, and M. Eizaguirre. 2012. Low susceptibility of non-target lepidopteran maize pests to the Bt protein Cry1Ab. Bull. Entomol. Res. 810: 1–7.

Pérez-Hedo, M., D. Reiter, C. López, and M. Eizaguirre. 2013. Processing of the maize Bt toxin in the gut of *Mythimna unipuncta* caterpillars. Entomol. Exp. Appl. 148: 56–64.

Pluciennik, Z. 2012. The modern insecticide (chlorantraniliprole) used to control codling moth (*Cydia pomonella* L.). J. Fruit Ornam. Plant Res. 20: 85–89.

Rodrigues, A. C., C. Gravato, C. Quintaneiro, C. Barata, A. M. Soares, and J. L. Pestana. 2015a. Sub-lethal toxicity of environmentally relevant concentrations of esfenvalerate to *Chironomus riparius*. Environ. Pollut. 207: 273–279.

Rodrigues, A. C., C. Gravato, C. Quintaneiro, O. Golovko, V. Žlábek, C. Barata, A. M. Soares, and J. L. Pestana. 2015b. Life history and biochemical effects of chlorantraniliprole on *Chironomus riparius*. Sci. Total Environ. 508: 506–513.

Rodriguez-Saona, C., A. C. Wanumen, J. Salamanca, R. Holdcraft, and V. Kyryczenko-Roth. 2016. Toxicity of insecticides on various life stages of two tortricid pests of cranberries and on a non-target predator. Insects 7: 1-20.

SAS Institute. 2008a. User's Guide. The genmod procedure, SAS/STAT® version 9.2. Cary, NC.

SAS Institute. 2008b. User's Guide. The glimmix procedure, SAS/STAT® version 9.2. Cary, NC.

SAS Institute. 2008c. User's Guide. The mixed procedure, SAS/STAT® version 9.2. Cary, NC.

SAS Institute. 2013. User's Guide. The frequency procedure, SAS/STAT® version 13.1. Cary, NC.

Schaafsma, A. W., M. L. Holmes, J. Whistlecraft, and S. A. Dudley. 2007. Effectiveness of three Bt corn events against feeding damage by the true armyworm (*Pseudaletia unipuncta* Haworth). Can. J. Plant Sci. 87: 599–603.

Schmidt-Jeffris, R. A., and B. A. Nault. 2016. Anthranilic diamide insecticides delivered via multiple approaches to control vegetable pests: a case study in snap bean. J. Econ. Entomol. 109: 2479–2488.

Scott-Dupree, C., R. Harris, E. Roddy, J. Leboeuf, and M. Moineddin. 2007. Population dynamics and susceptibility to insecticides of variegated cutworm attacking tomatoes in southwestern Ontario, Fresh Veg. Grow. Ontario. Guelph. (https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/7893/scottdupree2007_variegatecutwormtom.pdf?sequence=1&isAllowed=y)

Selby, T. P., G. P. Lahm, and T. M. Stevenson. 2017. A retrospective look at anthranilic diamide insecticides: discovery and lead optimization to chlorantraniliprole and cyantraniliprole. Pest Manag. Sci. 73: 658–665.

Shorey, H. H., and R. L. Hale. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. J. Ecol. Entomol. 58: 522–524.

Su, Q., H. Tong, J. Cheng, G. Zhang, C. Shi, C. Li, and W. Wang. 2017. Toxicity and efficacy of chlorantraniliprole on *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae) on cabbage. J. Agric. Sci. 9: 180.

Temple, J. H., P. L. Pommireddy, D. R. Cook, P. Marçon, and B. R. Leonard. 2009. Susceptibility of selected lepidopteran pests to Rynaxypyr®, a novel insecticide. J. Cotton Sci. 31: 23–31.

Thrash, B., J. J. Adanaczyk Jr., G. Lorenz, A. W. Scott, J. S. Armstrong, R. Pfannenstiel, and N. Taillon. 2013. Laboratory evaluations of lepidopteran-active soybean seed treatments on survivorship of fall armyworm (Lepidoptera: Noctuidae) larvae. Fla. Entomol. 96: 724–728.

Tiwari, S., and L. L. Stelinski. 2013. Effects of cyantraniliprole, a novel anthranilic diamide insecticide, against Asian citrus psyllid under laboratory and field conditions. Pest Manag. Sci. 69: 1066–1072.

Wilde, G., K. Roozeboom, A. Ahmad, M. Classen, B. Gordon, W. Heer, L. Maddux, V. Martin, P. Evans, K. Kofoid, et al. 2007. Seed treatment effects on early-season pests of corn and on corn growth and yield in absence of insect pests. J. Agric. Urban Entomol. 24: 177–193.

Williamson, R. C., P. J. Liesch, and G. R. Obear. 2013. Residual activity of chlorantraniliprole and other turfgrass insecticides against black cutworm (Lepidoptera: Noctuidae). Int. Turfgrass Soc. Res. J. 12: 1–4.

Willson, H. R., and B. R. Stinner. 1994. Recovery of field corn following insecticide treatment to arrest defoliation by *Pseudaletia unipuncta* (Haworth). J. Agric. Entomol. 11: 383–392.