Research Article

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Lethal and sublethal effects of chlorantraniliprole on three species of *Rhagoletis* fruit flies (Diptera: Tephritidae)

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Abstract

BACKGROUND: Chlorantraniliprole formulated as a 350 g kg^{-1} WG (Altacor 35WG) for management of apple maggot *Rhagoletis pomonella* (Walsh), blueberry maggot *R. mendax* Curran and cherry fruit fly *R. cingulata* (Loew) (Diptera: Tephritidae) was evaluated in laboratory assays and field trials.

RESULTS: A tarsal contact toxicity bioassay showed that a surface residue of 500 mg L^{-1} of chlorantraniliprole caused significantly higher mortality of male and female flies of all species compared with a control. Male apple maggot and blueberry maggot mortality was significantly higher than that for females, but there was similar mortality of male and female cherry fruit flies. An ingestion toxicity bioassay showed that 500 mg L^{-1} of chlorantraniliprole in diet caused significantly higher mortality of male and female flies of all species than the control, but there were no significant differences among the sexes. Delayed egglaying by females that had ingested chlorantraniliprole was found, but there were no significant sublethal effects on either the number of eggs laid or the egg hatch. Field trials with apple maggot and cherry fruit fly showed that protection of fruit by chlorantraniliprole was comparable with that of standard broad-spectrum insecticides.

CONCLUSIONS: The present data indicate that chlorantraniliprole has suppressant activity against *Rhagoletis* fruit flies, preventing fruit infestation primarily through direct lethal effects.

Keywords: anthranilic diamide; rynaxypyr; apple; blueberry; cherry; *pomonella*; *mendax*; *cingulata*

1 INTRODUCTION

Several species of Tephritid fruit flies of the genus *Rhagoletis* attack economically important fruit crops throughout the eastern United States. These include apple maggot, *R. pomonella* (Walsh), a major pest of apples, blueberry maggot, *R. mendax* Curran, which infests wild and cultivated blueberries, and cherry fruit fly, *R. cingulata* (Loew), a pest of sweet and tart cherries.[1–3] Apple maggot populations are high in feral apple trees, *Malus domestica* Borkh., and abandoned apple orchards, while blueberry maggot and cherry fruit fly use endemic host plants such as wild *Vaccinium* L. spp. and black cherry *Prunus serotina* Ehrh. respectively. *Rhagoletis* fruit flies are also quarantine pests for which there is zero tolerance in the food industry, requiring active management to enable sale and export of fruit. In spite of progress in monitoring,[4–7] and a better understanding of fly ecology,[3,8] growers still depend almost exclusively on applying insecticides to meet quality and export requirements.[9,10] Traditional control strategies for *Rhagoletis* fruit flies target female flies before they lay eggs into the fruit.[11,12] The larvae are the damaging life stage and the focus of grading standards, but they are difficult to control with insecticides, as they live in the pulp of the fruit. The need to control female flies before they insert eggs into the fruit is one of the main reasons that fruit growers maintain insecticide coverage throughout the period of adult fly activity. In recent years, the availability of effective insecticides, especially older organophosphates, has declined as a result of the implementation of the Food Quality Protection Act.[13] Moreover, the EPA's response to societal concerns about pesticide safety has been to increase availability of reduced-risk insecticide for pest management.

Chlorantraniliprole is a new insecticide in the anthranilic diamide class. It is a potent and selective activator of insect ryanodine receptors which are critical for muscle contraction.[14] Activation of the ryanodine receptors in insects affects calcium homeostasis by unregulated release of internal calcium in the cell, leading to feeding cessation, lethargy, muscle paralysis and ultimately death of the insect.[15] The high selectivity of chlorantraniliprole for insect over mammalian ryanodine receptors underlies its relatively low mammalian toxicity, with an acute oral LD_{50} of >5000 mg kg^{-1} in rats.[14] Chlorantraniliprole has been registered recently for use against larval Lepidopteran pests in several crops. Preliminary field trials in Michigan have shown moderate activity against *Rhagoletis* fruit flies.[16] To determine fully the potential value of chlorantraniliprole in the management of economically important *Rhagoletis*, and to determine the relative sensitivity of different species, it is necessary to understand the lethal and sublethal activity of this insecticide on adult flies.

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The objective of this study was to determine the efficacy of chlorantraniliprole formulated as a 350 g kg^{-1} WG on apple maggot, blueberry maggot and cherry fruit fly. Laboratory bioassays were conducted to quantify contact and ingestion toxicity, egglaying by females that had ingested chlorantraniliprole was measured and the proportion of eggs that hatched was determined. Small-plot field trials with chlorantraniliprole were also conducted to determine its performance in reducing fruit infestation by apple maggot and cherry fruit fly.

2 MATERIALS AND METHODS

2.1 Insects

Apple maggot, blueberry maggot and cherry fruit fly pupae were obtained from infested apples, blueberries and tart cherries respectively, collected in August 2006 in Fennville, Michigan. Pupae were stored at 4 °C for at least 3 months and brought to 25 °C as needed. After emergence, males and females were kept together in 30 × 30 × 30 cm screened plastic cages (Bioquip, Rancho Dominguez, CA). Flies were kept in the same laboratory where experiments were conducted, at 21–23 °C. Food consisted of yeast protein hydrolysate + sugar (1 + 3 by weight; Fisher Scientific, Pittsburgh, PA). Food and water were provided separately. Male and female flies used in the bioassays were on average 5 days old and ranged from 3 to 7 days.

2.2 Tarsal contact mortality

A series of bioassays was conducted to expose apple maggot, blueberry maggot and cherry fruit flies to chlorantraniliprole by tarsal contact using a surface exposure assay. In this experiment, varying concentrations of chlorantraniliprole 350 g kg^{-1} WG (Altacor 35WG; DuPont Crop Protection, Newark, DE) or a water control were applied to 0.95 L cylindrical polypropylene containers using a handheld sprayer delivering 1 mL of aqueous solution. With apple maggot, chlorantraniliprole was applied at 100 and 500 mg L^{-1}. The lower concentration (100 mg L^{-1}) is equivalent to the recommended field application rate. With blueberry maggot and cherry fruit fly, chlorantraniliprole was used at 500 mg L^{-1} only. Containers were dried in a fume hood for 30 min prior to fly introduction. Food consisting of yeast protein hydrolysate + sugar (1 + 3 by weight) spread on 4 cm^2 filter paper strips was provided in the containers. Water was provided separately, in a moist cotton pad. Food and water were at the bottom of the container on 4.7 cm diameter plastic lids and did not touch the treated surface.

With apple maggot, six male or female flies were placed in the container with one of the treatments, and each treatment was replicated 5 times for each sex. Fly mortality was measured daily up to 10 days after placement in the container. With blueberry maggot and cherry fruit fly, five male or female flies were placed in the container, and each treatment was replicated 6 times. Fly mortality was measured over time up to 4 days after placement in the container.

2.3 Ingestion mortality

This experiment measured mortality of apple maggot, blueberry maggot and cherry fruit flies exposed to chlorantraniliprole using a feeding assay method. A 500 mg L^{-1} dispersion of chlorantraniliprole was prepared using chlorantraniliprole 350 g kg^{-1} WG and a 400 g L^{-1} food solution in water. Food consisted of the same mixture of yeast protein hydrolysate and sugar as mentioned in Section 2.2. The untreated control consisted of 400 g L^{-1} food solution only. Filter paper was cut into 4 cm^2 strips, dipped into the insecticide and control solutions and let dry in a fume hood prior to introduction into the containers. The food on these strips was the only source of nutrition available to the flies. Water was provided separately. To minimize microbial growth, filter paper strips were replaced 5 days after the start of the experiment. Five individuals of each sex were placed in the container with one of the treatments. Each treatment was replicated 6 times for each species and sex. Mortality was measured daily up to 10 days after flies were introduced in the container.

2.4 Effects on fertility and fecundity

The effect of chlorantraniliprole on apple maggot fly fertility and fecundity was evaluated using virgin flies in a feeding and egglaying assay. Male and female apple maggot flies were separated at the day of emergence and kept in 30 × 30 × 30 cm plastic holding cages. A 400 g L^{-1} food solution containing 500 mg L^{-1} of chlorantraniliprole was prepared as described in Section 2.3. This solution was spread on 15 × 10 cm paper pads made of benchtop absorbent paper (Fisher Scientific, Pittsburgh, PA) and dried in a fume hood. Food pads containing chlorantraniliprole were placed in the cages holding three-day-old virgin male and female flies. Food had been removed from the cages holding the virgin flies 24 h prior to use in order to equalize their hunger status. The exposure to chlorantraniliprole lasted for 2 and 3 days in trials 1 and 2 respectively. The untreated control consisted of similar food pads without insecticide. After this period of exposure to chlorantraniliprole, groups of six treated flies in trial 1, or ten treated flies in trial 2, were introduced into 0.95 L containers together with the same number of untreated flies of the opposite sex. The treatments were: (1) exposed female with unexposed male (F + M−); (2) unexposed female with exposed male (F − M+); (3) unexposed female and male (F − M−). Dead flies were replaced with spare individuals of the cohort of flies that had been exposed to the same treatment.

In each container, flies were provided with a food strip, water and an egging device. Egging devices consisted of 3 cm diameter hemispheres made of 20 g L^{-1} of agar and 500 mL L^{-1} of commercial cherry juice (Juicy Juice, Nestlé, Glendale, CA) in water. The agar was made molten in a microwave oven and the solution was poured into 5 mL wells on a plastic mold. After cooling, the hemispheres were covered with stretched Parafilm. The egging devices were changed daily and the number of eggs per device was counted. To determine the percentage of eggs hatching, a sample of up to ten eggs was collected daily from each egging device and placed in petri dishes on moist filter paper. The proportion hatching was determined 5 days after eggs were placed in the petri dish. Trial 1 was conducted for 14 days, and trial 2 lasted 12 days.

2.5 Field trials

Small-plot field trials were conducted to determine the efficacy of chlorantraniliprole in preventing fruit infestation by apple maggot and cherry fruit fly. For apple maggot, chlorantraniliprole 350 g kg^{-1} WG applied at a rate of 73.6 g AI ha^{-1}, azinphos-methyl 500 g kg^{-1} WP (Guthion 50W; Bayer CropScience, Research Triangle Park, NC) applied at 1.1 kg AI ha^{-1}, imidacloprid 174 g L^{-1} SC (Provado 1.6F; Bayer CropScience) applied at 101.7 g AI ha^{-1} and an untreated control were compared. Treatments were sprayed onto apple trees of the cultivar Gala at the Trevor Nichols Research Complex in Fennville, Michigan, using an FMC 1029

airblast sprayer calibrated to deliver 935.4 L ha^{-1} at 4.0 km h^{-1}. Single-tree plots were arranged in a randomized complete block design with four replications. Tree spacing was 5.5 × 6.1 m, with at least one buffer tree and one buffer row separating all plots. Regular maintenance applications of fungicides, herbicides and insecticides were conducted in all plots prior to the field trial. Sprays were applied at 14 day intervals on 1 July, 15 July and 29 July 2005. Fruit infestation was determined on 12 August by collecting 35 L of randomly selected apples per replicate, which was approximately 200 fruit. The apples were brought to the laboratory and placed on racks over aluminum trays containing sand. Apple maggot larvae matured and emerged from the fruit, dropped into the tray and pupated. Five weeks later, on 16 September, pupae were collected by sifting the sand through a screen.

For cherry fruit fly, chlorantraniliprole 350 g kg^{-1} WG applied at 122.6 g AI ha^{-1}, imidacloprid 174 g L^{-1} SC (Provado 1.6F) applied at 101.7 g AI ha^{-1} and an untreated control were evaluated. Field solutions of imidacloprid contained the spreader Nu-Film 17 (Miller Chemical & Fertilizer Corporation, Hanover, PA) at 1.8 mL L^{-1}, and chlorantraniliprole contained 5 mL L^{-1} of the spreader MSO® Concentrate (Loveland Products, Greeley, CO) for better retention of the solution and coverage of the plants. Treatments were sprayed onto tart cherry trees of the cultivar Montmorency at the Northwest Michigan Horticulture Research Station, in Traverse City, Michigan, with an FMC 1229 airblast sprayer calibrated to deliver 561.2 L ha^{-1} at 4.8 km h^{-1}. Single-tree plots were arranged in a randomized complete block design with four replications. Regular maintenance applications of fungicides, herbicides and insecticides were conducted in all plots prior to the field trial. Two sprays of these treatments were applied on 13 June and 27 June 2007. Fruit infestation was determined on 12 July by collecting 10 kg of cherries per plot, approximately 2000 fruit, by shaking tree limbs with a Maibo pneumatic limb shaker (Maibo Mfg Inc., Italy). Cherries were collected on tarpaulins, weighed, placed on perforated containers lined with hardware cloth and transported to the laboratory for incubation. Cherry fruit fly pupae were collected 4 weeks later, using the same methods as described for apple maggot.

2.6 Data analyses

Mortality data for each species were analyzed using PROC MIXED of SAS[17] in a factorial design with sex and concentration as fixed factors and block as random. Analysis of variance using PROC MIXED was also conducted to determine differences among treatments in fertility and fecundity, with treatment as fixed and block as random factor. Data were square root or arcsine transformed, as appropriate, for uniformity of variance. Analysis of variance was conducted using PROC MIXED with apple and cherry infestation data, after square root transformation for uniformity of variance. In all comparisons, differences among treatment means were determined using the LSD test with $\alpha = 0.05$.

3 RESULTS

3.1 Tarsal contact mortality

After 10 days on a treated surface, there were significant differences in the mortality of apple maggot flies exposed by tarsal contact to 100 or 500 mg L^{-1} of chlorantraniliprole or a water control ($F = 28.1$; df = 2, 20; $P < 0.001$). The interaction between chlorantraniliprole concentration and fly sex was not significant ($F = 2.9$; df = 2, 20; $P = 0.08$). The mortality of apple maggot flies exposed to 500 mg L^{-1} of chlorantraniliprole was significantly higher than the mortality of flies exposed to 100 mg L^{-1} of chlorantraniliprole or a water control, and there were no significant differences between the mortality of flies exposed to 100 mg L^{-1} of chlorantraniliprole or a water control. The mortality of male apple maggot flies was significantly higher than the mortality of female apple maggot flies ($F = 20.0$; df = 1, 20; $P < 0.001$). At day 10, exposure of male apple maggot flies to a water control and to 100 and 500 mg L^{-1} of chlorantraniliprole resulted in 6.6 ± 4.0, 23.3 ± 4.0 and 66.6 ± 14.0% mortality (mean ± SE) respectively (Fig. 1, AM♂). With female apple maggot flies, the same treatments resulted in 3.3 ± 3.3, 0.0 ± 0.0 and 30.0 ± 9.7% mortality (mean ± SE) respectively (Fig. 1, AM♀).

After 4 days on a treated surface, there were significant differences in the mortality of blueberry maggot flies exposed by tarsal contact to a water control or 500 mg L^{-1} of chlorantraniliprole ($F = 10.1$; df = 1, 15; $P = 0.006$). There was no significant interaction between chlorantraniliprole concentration and fly sex ($F = 1.5$; df = 1, 15; $P = 0.24$). The mortality of blueberry maggot flies exposed to 500 mg L^{-1} of chlorantraniliprole was significantly higher than the mortality of flies exposed to a water control. There was also significantly higher mortality of male blueberry maggots than females ($F = 5.3$; df = 1, 15; $P = 0.04$). At day 4, exposure of male blueberry maggot to a water control or 500 mg L^{-1} of chlorantraniliprole resulted in 10.0 ± 10.0 and 46.6 ± 13.2% mortality (mean ± SE) respectively (Fig. 1, BM♂). With female blueberry maggot, exposure to a water control or 500 mg L^{-1} of chlorantraniliprole resulted in 0.0 ± 0.0 and 13.3 ± 6.6% mortality (mean ± SE) respectively (Fig. 1, BM♀).

There were significant differences in the mortality of cherry fruit flies exposed by tarsal contact to a water control or 500 mg L^{-1} of chlorantraniliprole ($F = 18.3$; df = 1, 15; $P < 0.001$). The interaction between chlorantraniliprole concentration and fly sex was not significant ($F = 3.6$; df = 1, 15; $P = 0.08$). Cherry fruit flies exposed to 500 mg L^{-1} of chlorantraniliprole showed significantly higher mortality than flies exposed to a water control, and there was no significant difference between the sexes ($F = 3.6$; df = 1, 15; $P = 0.08$). At day 4, contact of male blueberry maggot with a water control or 500 mg L^{-1} of chlorantraniliprole resulted in 0.0 ± 0.0 and 50.0 ± 13.4% mortality (mean ± SE) respectively (Fig. 1, CFF♂), whereas female flies showed 0.0 ± 0.0 and 16.6 ± 9.6% mortality (mean ± SE) respectively (Fig. 1, CFF♀).

3.2 Ingestion mortality

After 10 days enclosed with treated food, apple maggot flies exposed to 500 mg L^{-1} of chlorantraniliprole showed significantly higher mortality than untreated flies ($F = 77.5$; df = 1, 15; $P < 0.001$). Mortality differences between male and female flies were not significant ($F = 0.02$; df = 1, 15; $P = 0.88$), and neither was the interaction between chlorantraniliprole concentration and fly sex ($F = 0.63$; df = 1, 15; $P = 0.44$). Ten days of exposure of male apple maggot to control and 500 mg L^{-1} of chlorantraniliprole resulted in 16.6 ± 9.5 and 93.3 ± 6.6% mortality (mean ± SE) respectively (Fig. 2, AM♂). With female apple maggot, the same treatments resulted in 23.3 ± 9.5 and 90.0 ± 6.8% mortality (mean ± SE) respectively (Fig. 2, AM♀).

Blueberry maggot flies exposed to the control or 500 mg L^{-1} of chlorantraniliprole had significantly higher mortality than the control ($F = 14.2$; df = 1, 15; $P = 0.002$). There was no significant difference in mortality between the sexes ($F = 0.30$; df = 1, 15; $P = 0.60$), and no interaction between chlorantraniliprole concentration and fly sex ($F = 0.23$; df = 1, 15; $P = 0.64$). Ten

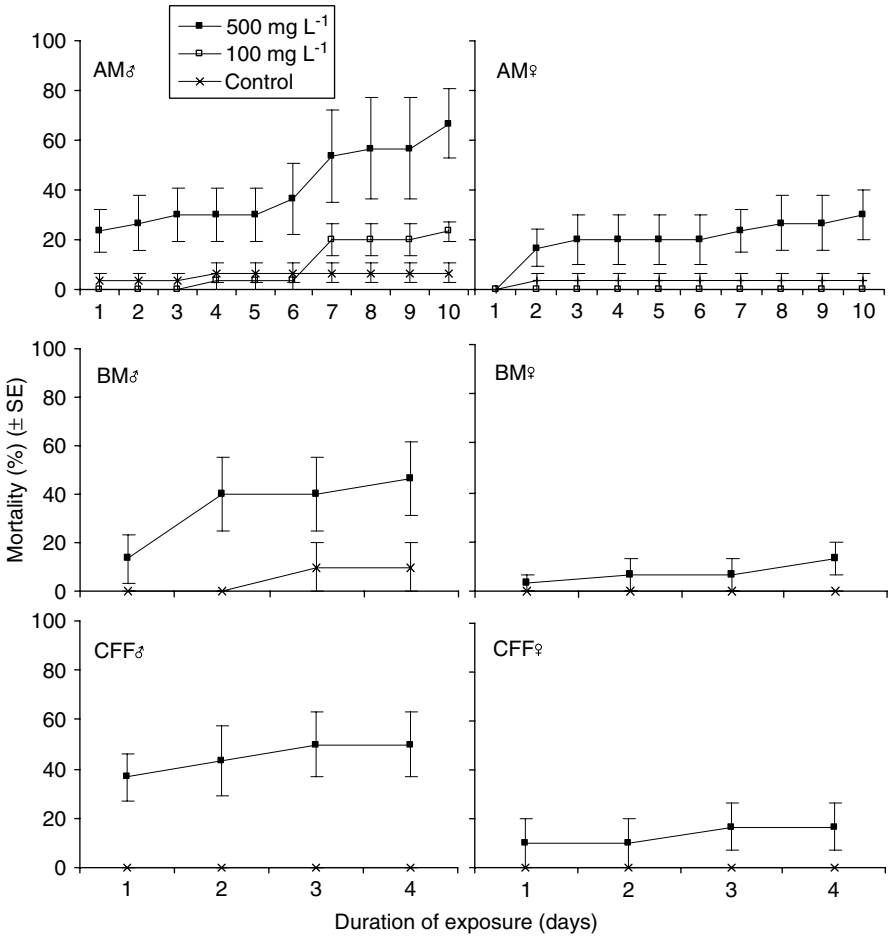

Figure 1. Mortality of *Rhagoletis* fruit flies in tarsal contact toxicity assays for apple maggot male (AM♂) and female (AM♀) exposed for 10 days to 100 or 500 mg L^{-1} of chlorantraniliprole and for blueberry maggot male (BM♂) and female (BM♀) and cherry fruit fly male (CFF♂) and female (CFF♀) exposed for 4 days to 500 mg L^{-1} of chlorantraniliprole.

days of exposure of male blueberry maggot to control and 500 mg L^{-1} of chlorantraniliprole resulted in 36.6 ± 10.8 and 93.3 ± 4.2% mortality (mean ± SE) respectively (Fig. 2, BM♂). With female flies, ingestion of the control and 500 mg L^{-1} of chlorantraniliprole resulted in 43.3 ± 19.6 and 80.0 ± 10.3% mortality (mean ± SE) respectively (Fig. 2, BM♀).

Cherry fruit fly mortality was significantly higher in 500 mg L^{-1} of chlorantraniliprole than in the control ($F = 312.4$; df = 1, 15; $P < 0.001$). There was no significant difference between fly sexes ($F = 0.06$; df = 1, 15; $P = 0.82$) and no significant interaction between chlorantraniliprole concentration and fly sex ($F = 0.06$; df = 1, 15; $P = 0.82$). Ten days of exposure of male cherry fruit fly to control and 500 mg L^{-1} of chlorantraniliprole resulted in 10.0 ± 6.8 and 100.0 ± 0.0% mortality (mean ± SE) respectively (Fig. 2, CFF♂). With females, ingestion of the control and 500 mg L^{-1} of chlorantraniliprole resulted in 6.6 ± 4.2 and 100.0 ± 0.0% mortality (mean ± SE) respectively (Fig. 2, CFF♀).

3.3 Effects on fertility and fecundity

In the first trial (Table 1) there were no significant differences among treatments in the number of eggs per container, number of eggs per day, number of eggs laid per day per fly ($F = 0.60$; df = 2, 10; $P = 0.57$) or in the percentage of eggs hatching ($F = 2.0$; df = 2, 10; $P = 0.19$). In the second trial (Table 1) there were also no significant differences among treatments in the number of eggs per container, number of eggs per day, number of eggs laid per day per fly ($F = 0.48$; df = 2, 10; $P = 0.63$) or in the percentage of eggs hatching ($F = 1.81$; df = 2, 10; $P = 0.21$). In both trials, however, egglaying in the treatment consisting of exposed females (F + M−) started later than in the other treatments (Table 2). These flies eventually recovered, and the average trial-long number of eggs laid was not significantly different among treatments (Table 1).

3.4 Field trials

Treatments varied significantly in the number of apple maggot larvae recovered from fruit ($F = 8.3$; df = 3, 9; $P = 0.006$). Infestation of apples from the plot treated with chlorantraniliprole was significantly lower than infestation of control apples, but similar to that of fruit treated with imidacloprid or azinphos-methyl (Table 3). For cherry fruit fly there were also significant differences among treatments in the number of larvae recovered from fruit ($F = 7.4$; df = 2, 6; $P = 0.02$). Cherries treated with chlorantraniliprole had significantly lower infestation than control fruit, and infestation was similar to that of fruit treated with imidacloprid (Table 3).

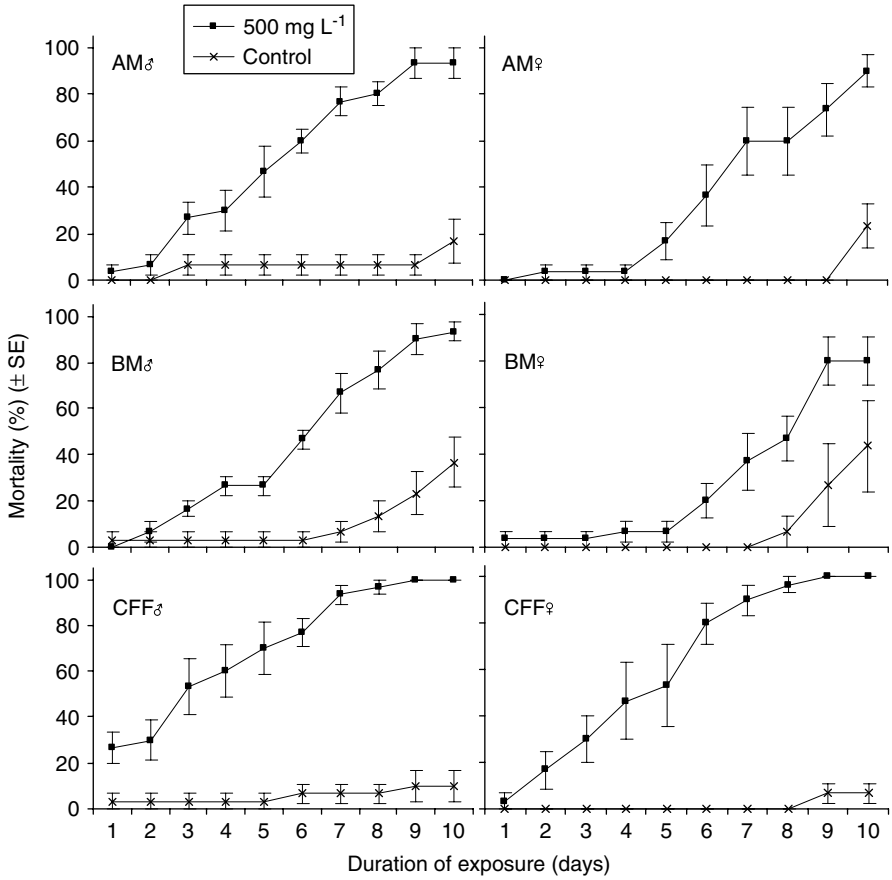

Figure 2. Mortality of *Rhagoletis* fruit flies in ingestion toxicity assays for apple maggot male (AM♂) and female (AM♀), blueberry maggot male (BM♂) and female (BM♀) and cherry fruit fly male (CFF♂) and female (CFF♀) exposed for 10 days to 500 mg L^{-1} of chlorantraniliprole.

Table 1. Number of eggs, number of eggs per day, number of eggs per fly per day and percentage of egg hatch of apple maggot female or male flies exposed to 500 mg L^{-1} of chlorantraniliprole (Female + Male+) or not (Female − Male−) in treated food, and observed for 14 days (trial 1) or 12 days (trial 2)[a]

	Eggs (± SE)	Eggs day^{-1} (± SE)	Eggs day^{-1} fly^{-1} (± SE)	Egg hatch (%) (± SE)
Trial 1				
Female + Male−	67 (±21)	11.1 (±3.5)	0.8 (±0.2)	76 (±6)
Female − Male+	74 (±24)	12.3 (±4.0)	0.9 (±0.3)	75 (±2)
Female − Male−	89 (±13)	14.8 (±2.2)	1.1 (±0.2)	85 (±3)
Trial 2				
Female + Male−	96 (±17)	16.0 (±2.8)	1.1 (±0.2)	49 (±10)
Female − Male+	162 (±31)	27.0 (±5.1)	1.9 (±0.4)	60 (±4)
Female − Male−	119 (±27)	19.8 (±4.4)	1.4 (±0.3)	68 (±4)

[a] In each trial, means in the same column followed by the same letter are not significantly different (LSD, $P > 0.05$).

4 DISCUSSION AND CONCLUSIONS

This study showed that chlorantraniliprole is active against apple maggot, blueberry maggot and cherry fruit fly. Significant tarsal contact and ingestion toxicity, evidence of delayed egglaying and significant field activity of chlorantraniliprole towards the three fruit fly species were found. Overall, contact mortality occurred slowly and only reached moderate levels, even when apple maggot flies were exposed to deposits of chlorantraniliprole for 10 days. Male apple maggot or blueberry maggot were more sensitive to chlorantraniliprole than female maggots, but differences between male and female cherry fruit flies were small. With apple maggot, ingestion toxicity was generally higher than contact toxicity, especially after long exposure to food containing the insecticide. With the other species, mortality after 4 days was similar in the tarsal contact and ingestion assays. Among the three species, cherry fruit fly was the most sensitive to ingestion toxicity. There were no significant sex-related differences in mortality after ingestion of food containing the insecticide. These results indicate that chlorantraniliprole is slow acting and has moderate tarsal contact and ingestion toxicity against *Rhagoletis* fruit flies.

With respect to sublethal effects, the present evaluation did not show a significant effect of ingesting chlorantraniliprole on the number of eggs laid or the percentage hatching. However, female flies exposed to chlorantraniliprole showed a tendency to initiate egglaying later than unexposed female flies. Chlorantraniliprole acts on the ryanodine receptors of muscle cells, and intoxication of insects by this insecticide results in rapid muscle paralysis.[14] It is possible that females experienced ovipositor dysfunction, thus delaying egg deposition. Chlorantraniliprole has been shown to have a significant disruptive effect on mating behavior of *Cydia pomonella* L. (Lepidoptera: Tortricidae), but, as this study also showed with fruit flies, only a small effect on adult survival or fertility.[18] Other studies have shown

Table 2. Number of eggs (mean ± SE) laid 1–5 days after apple maggot female or male flies were exposed to 500 mg L^{-1} of chlorantraniliprole (Female + Male+) or not (Female − Male−) in treated food[a]

	Days after exposure				
	1	2	3	4	5
Trial 1					
Female + Male−	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	1.0 (±0.4) a
Female − Male+	0.2 (±0.2)	0.7 (±0.7)	2.5 (±1.5)	1.3 (±0.6)	4.2 (±1.1) b
Female − Male−	0.0 (±0.0)	0.0 (±0.0)	0.7 (±0.3)	1.3 (±0.7)	6.7 (±1.6) b
Trial 2					
Female + Male−	0.0 (±0.0) a	0.0 (±0.0) a	0.7 (±0.7) a	7.2 (±1.3)	6.8 (±2.1)
Female − Male+	0.0 (±0.0) a	5.5 (±2.5) b	7.0 (±2.7) b	8.5 (±2.6)	14.8 (±4.8)
Female − Male−	0.5 (±0.2) b	1.8 (±0.9) ab	3.5 (±1.2) ab	4.8 (±2.3)	18.2 (±6.8)

[a] In each trial, means in the same column followed by the same letter are not significantly different (LSD, $P > 0.05$).

Table 3. Number of apple maggot pupae (mean ± SE) recovered from apples treated with chlorantraniliprole at 73.6 g AI ha^{-1}, with azinphos-methyl at 1.1 kg AI ha^{-1}, with imidacloprid at 101.7 g AI ha^{-1} or with an unsprayed control, and the number of cherry fruit fly pupae recovered from cherries treated with chlorantraniliprole at 122.6 g AI ha^{-1}, with imidacloprid at 174 g L^{-1} or with an unsprayed control[a]

Treatment	Apple maggot pupae per 35 L of fruit (± SE)	Cherry fruit fly pupae per kg of fruit (± SE)
Untreated control	209.8 (±124.0) a	80.2 (±35.3) a
Chlorantraniliprole	29.3 (±10.8) b	5.1 (±1.8) b
Azinphos-methyl	5.5 (±3.7) b	–
Imidacloprid	10.8 (±5.0) b	1.8 (±0.7) b

[a] Means in the same column followed by the same letter are not significantly different (LSD, $P > 0.05$).

that combining chlorantraniliprole with the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar caused additive or synergistic increases in mortality when used against white grubs (Coleoptera: Scarabeidae), likely resulting from the chlorantraniliprole disrupting larval defensive or evasive behaviors.[19] In addition, chlorantraniliprole caused concentration-dependent repellency when used against Asian subterranean termites *Coptotermes gestroi* (Wasman) (Isoptera: Rhinotermitidae).[20] In the present study there was no evidence of behavioral effects of chlorantraniliprole in terms of fly movement or food repellency (L Teixeira, personal observation).

Field trials with apple maggot and cherry fruit fly showed significant reductions in fruit infestation from treatment with chlorantraniliprole, similar to the level of protection achieved using standard insecticides. The chlorantraniliprole concentration in the mixtures used in the field trials with apple maggot and cherry fruit fly was 78.7 mg L^{-1} and 218.4 mg L^{-1} respectively. These concentrations are substantially lower than the 500 mg L^{-1} concentration used in most of the present bioassays. However, in the field, mortality can occur from exposure to chlorantraniliprole by several routes simultaneously. It is also possible that chlorantraniliprole can penetrate the fruit epidermis and kill larvae in the fruit, because it has been shown that chlorantraniliprole has translaminar activity.[21] Other insecticides can penetrate apple skin and cause significant larval mortality of plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), in apple pulp.[22] It remains to be determined whether chlorantraniliprole has the same type of activity against larval *Rhagoletis* fruit flies in apple or tart cherry. Overall, data presented here indicate that chlorantraniliprole will provide control of *Rhagoletis* fruit flies, acting primarily through direct toxicity of adult flies. Given the relatively slow action of this insecticide compared with standard organophosphate or pyrethroid insecticides, applications should be timed soon after fly emergence to allow time for control before females become reproductively mature.

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