AUG 6 2009

Reduction in Emergence of *Rhagoletis indifferens* (Diptera: Tephritidae) from Sweet Cherries with Different Egg and Larval Distributions Using Newer Insecticides[1]

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J. Entomol. Sci. 44(3): 171-186 (July 2009)

Abstract Western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), is the major insect pest of sweet cherry, *Prunus avium* (L.) L., in the Pacific Northwest of the U.S. To reduce fly populations in unharvested fruit following the completion of commercial harvest, it is important to control immature stages in cherry fruit. In this study, the goals were to (1) identify the susceptibility of the eggs and larvae to neonicotinoid and other insecticides and (2) determine the effects of these insecticides on larval emergence from sweet cherries with different egg and larval distributions (the relative percentages of different stages). Only 3.3% of eggs exposed for 15 sec to thiacloprid hatched, whereas 25.0 - 41.0% of eggs exposed to water only, spinosad, and imidacloprid hatched. Larval mortalities in cherries 48 - 52 h after being treated with imidacloprid, thiacloprid and acetamiprid were 50.0 - 66.7%, significantly higher than the 24.0% mortality in untreated cherries. Imidacloprid, thiamethoxam, thiacloprid, acetamiprid, and clothianidin were equally effective in reducing larval emergence when sprayed on cherries in which eggs were 94.2% of the immature stages, but imidacloprid and acetamiprid appeared to be the most effective when sprayed on cherries in which eggs were only 19.7% and 53.8% of the immature stages. Results indicate neonicotinoids are toxic to eggs and larvae of *R. indifferens* and that all are more effective in cherries against eggs than larvae. For fly control, the time interval between the last spray directed against adult flies and the first postharvest spray of neonicotinoids should be no more than 1 wk, to reduce chances eggs hatch and larvae develop.

Key Words western cherry fruit fly, post harvest, spinosad, neonicotinoid insecticides

Western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), is the major insect pest of sweet cherry, *Prunus avium* (L.) L., in the Pacific Northwest of the U. S. Because of its quarantine pest status in cherries exported to domestic and overseas markets, the fly until recently had been controlled by using organophosphate insecticides (Zwick et al. 1970, 1975). However, because of the Food Quality and Protection Act of 1996, use of organophosphate insecticides considered harmful to the environment and human health has become more restricted. At the same time, the continued zero tolerance for larvae in commercial fruit (State of Washington Department of Agriculture, Permanent Order No. 1099, effective 30 September 1968) makes the use of insecticides necessary. Newer insecticides have been tested against adult *R. indifferens* and other *Rhagoletis* species in response to the need for using

[1]Received 08 September 2008; accepted for publication 28 December 2008.
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less toxic materials for fly control (vanRanden and Roitberg 1998, Liburd et al. 2003, Reissig 2003, Barry and Polavarapu 2005, Barry et al. 2005, Yee and Alston 2006).

Insecticides used against adult *R. indifferens* are usually applied weekly to kill the flies before they develop and lay eggs, which occurs in sweet cherry throughout the season after cherries begin ripening. Eggs are laid in slits made through the cherry skin. Usually one egg is laid per fruit, but up to 14 eggs and larvae have been found in a single fruit (Yee 2005). At 25°C, most eggs hatch in 6 d. The larval stage, consisting of 3 instars, lasts an average of 11 d. The first (1 - 2 mm long), second (2.1 - 4.5 mm), and third instars (4.6 - 8 mm) last 2 - 5, 2 - 6, and 4 - 10 d, respectively (Frick et al. 1954). Eggs and larvae are frequently found in the same cherry, but ratios of eggs to larvae decrease as the season progresses (Yee 2005). Larvae tunnel toward the center of the fruit, feed around the seed, and loosen it from the pulp. The third instar emerges from the fruit (on the tree or ground) by making an exit hole and crawling through it. Larvae pupate within 8 h after emergence from the fruit (Frick et al. 1954).

Fruit in unmanaged residential cherry trees or in abandoned cherry orchards can be heavily infested with eggs and larvae of *R. indifferens* (Yee 2005), and unpicked fruit left in managed orchards can become infested after sprays against adults end (Frick and Simkover 1953, Frick et al. 1954). The importance of controlling immature stages in unharvested fruit to reduce fly populations the following season was recognized long ago. The organophosphates parathion, dimethoate, demeton, EPN-300, and azinphos-methyl, the organochlorine methoxychlor, and the inorganic arsenical lead arsenate have lethal effects on eggs or larvae of *R. indifferens* or eastern cherry fruit fly, *R. cingulata* (Loew), and have been recommended for controlling these stages (Sherman 1951, Cox 1952, Frick 1952, Zwick et al. 1975, Washington State University 2005). These materials, however, are not desirable because of their high toxicity and harmful effects on the environment. Newer and safer insecticides need to be evaluated, as currently, for *R. indifferens*, dimethoate and the neonicotinoid imidacloprid are the only 2 insecticides recommended for postharvest control (Washington State University 2008). Limited published results suggest that neonicotinoid insecticides can reduce larval emergence from late-season sweet cherries (Yee and Alston 2006). Also, recent work has shown that imidacloprid can reduce larval emergence from fruit (Smith 2007). It was unclear, however, whether its effects on eggs and larvae differed, because numbers of eggs and larvae in fruit before treatment were not documented. It is important to know this because it will determine when at postharvest insecticides can be effective.

In this study, two specific goals were to (1) identify the susceptibility of the eggs and larvae of *R. indifferens* to neonicotinoid and other insecticides and (2) determine the effects of these insecticides on larval emergence from sweet cherries with different egg and larval distributions, defined as the relative percentages of different stages. The hypothesis that insecticides are more effective in cherries against eggs than larvae was tested.

Materials and Methods

Experiment 1: Effects of insecticides on egg hatch. Three insecticides were tested for their ability to reduce or prevent egg hatch. Eggs were directly exposed to (1) water (control); (2) spinosad (Entrust®, Dow AgroSciences, Indianapolis, IN) (36 ppm, in mg/liter); (3) imidacloprid (Provado®, Bayer CropScience, Research Triangle Park, NC) (30 ppm); and (4) thiacloprid (a neonicotinoid) (Calypso® 4 Flowable, Bayer

CropScience, Research Triangle Park, NC) (75 ppm). All rates in this and the other 3 experiments are those on labels. To obtain eggs, 17 - 20 female and 10 - 20 male flies that were ≥ 14 d old and reared from field-collected larvae were placed inside a 1.9-L container with 12 cherries for 1 - 2 d. Eggs were extracted from cherries by breaking the skin with forceps. In test 1, eggs were transferred with a brush directly onto filter paper (4.3 cm diam, Whatman no. 2, Maidstone, England) saturated with water (control) or 400 µL of insecticide solutions for 15 sec, and then onto filter paper with water in a Petri dish (5.0 cm diam) until there were 50 eggs per dish, with 6 dishes per treatment. Each dish was then sealed with Parafilm® (Pechiney Plastic Packaging, Chicago, IL). In test 2, methods were the same as test 1, except eggs were placed and kept on papers saturated with insecticides continuously. Eggs in dishes were checked for hatch under a microscope 3 - 7 d after cherries were removed from the containers with flies [as most hatch in 6 d (Frick et al. 1954)], and then daily for 13 - 15 d. Filter papers were moistened with water as needed. Each replicate was composed of eggs from different flies.

Experiment 2: Effects of insecticides on larval mortality. In this experiment, treatments were: (1) water (control); (2) imidacloprid (Provado®), 11.3 g ai/378.5 l (30 ppm); (3) thiacloprid (Calypso® 480 SC, Bayer CropScience, Research Triangle Park, NC), 168.1 g ai/378.5 l (180 ppm); and (4) acetamiprid (a neonicotinoid) (Assail® 70 WP, Cerexagri-Nisso LLC, King of Prussia, PA), 67.5 g ai/378.5 l (178 ppm). Infested cherries (unknown varieties) were picked from 3 trees in Roslyn, WA (altitude 722 m), on 29 July 2008. Fruit were orange and red and were brought to a shaded outdoor facility next to a building at the Yakima Agricultural Research Laboratory (YARL) in Wapato, WA, where they were placed on hardware cloth on plastic wash tubs. On 4 August, 80 cherries were randomly placed on each of 4 tubs, and sprayed with 10 ml of water or 1 of the 3 insecticides until runoff using a squirt bottle and held at the facility in the shade. At 24 - 28 h after treatment, half of the cherries were dissected under a microscope using forceps and scored for living and dead first, second, and third instars. At 48 - 52 h after treatment, the remainder of the cherries was dissected. Larvae were considered dead if they did not move when probed with the tip of jeweler's forceps.

Experiment 3: Larval emergence from detached cherries treated with insecticides. Two tests (3A and 3B) were conducted in 2006 and 2008 to determine if insecticides sprayed on detached sweet cherries can reduce larval emergence. In test 3A in 2006, treatments were: (1) water (control); (2) spinosad (Entrust®), 13.6 g ai/378.5 l (36 ppm); (3) spinosad bait [40% (v/v) GF-120® Naturalyte® Fruit Fly Bait, Dow AgroSciences, Indianapolis, IN], 36.3 g ai/378.5 l (96 ppm); (4) imidacloprid (Provado®), 11.3 g ai/378.5 l (30 ppm); (5) thiamethoxam (a neonicotinoid) (Actara®, Syngenta Crop Protection, Greensboro, NC), 39.0 g ai/378.5 l (103 ppm); (6) indoxacarb (an oxadiazine) (Avaunt®, E. I. du Pont de Nemours and Company, Wilmington, DE), 51.0 g ai/378.5 l (135 ppm); and (7) azinphos-methyl (Guthion® 50W, Gowan Company, Yuma, AZ), 340 g ai/378.5 l (899 ppm). Azinphos-methyl was listed along with dimethoate as a postharvest product (Washington State University 2005). Infested cherries (unknown varieties) with stems attached were removed from 7 replicate trees in Kennewick, WA, on 31 May, 7 June, and 14 June. On these respective dates, fruit were mostly yellow, rosy/orange, and red. On each date, 30 cherries from each tree were spread on hardware cloth on tubs and sprayed with 3 ml of water or insecticides using a squirt bottle. A second spray was applied 1 wk later. At pretreatment, 3 representative cherries of the 30 were removed, dissected under a microscope,

and the numbers of eggs and larvae (all 3 instars) in them recorded, leaving 27 cherries per replicate for larval emergence. Cherries were held at the outdoor facility at YARL in the shade for 30 d. Total numbers of larvae that emerged were recorded. In test 3B and experiment 4 that follow, pretreatment cherries were dissected and cherries were held for larval emergence in the same manner as in test 3A. In test 3A, pupae were chilled at 3°C for 6 months and then transferred to 27°C to determine percent emergence of adult flies as a measure of possible insecticide effects on survival of pupae.

In test 3B in 2008, treatments were: (1) water (control); (2) spinosad; (3) imidacloprid; (4) thiamethoxam; (5) thiacloprid; (6) acetamiprid; and (7) clothianidin (a neonicotinoid) (Clutch® 50 WDG, Valent USA Corp., Leland, MS), 42.5 g ai/378.5 l (112 ppm). Rates of thiacloprid and acetamiprid were the same as in experiment 2, and those of spinosad, imidacloprid, and thiamethoxam the same as in test 3A. Infested orange-to-red cherries (unknown varieties) with attached stems were removed from 3 trees in Yakima, WA, on 2 July (1 tree) and 8 July (2 trees) and sprayed with the insecticides. There were 51, 61, and 80 pretreatment cherries from each tree. One hundred (2 July) or 70 (8 July) cherries from each tree were spread on hardware cloth and sprayed with 10 or 7 ml of water or insecticides, respectively, on the day of collections. Numbers of larvae that emerged 1 d after treatment were recorded, in addition to total numbers that emerged 30 d after treatment.

Experiment 4: Larval emergence from cherries treated while on trees. Three tests (4A, 4B, and 4C) were conducted on sweet cherries on trees, one in 2007 and two in 2008. It is unknown if cherries in trees uptake insecticides differently than detached cherries (experiment 3). In test 4A in 2007, treatments were: (1) water (control); (2) spinosad; (3) spinosad bait; (4) imidacloprid; (5) thiamethoxam; (6) thiacloprid; and (7) azinphos-methyl. Thiacloprid was Calpyso® 4 Flowable (28.4 g ai/378.5 l, 75 ppm). Rates of the other insecticides were the same as in test 3A. Trees in an unmanaged 'Bing' cherry orchard in Selah, WA, were sprayed on 6 July. Cherries were dark purple or black and ripe. The test was set up as a randomized blocks design with 7 replicate rows (13 trees/row), each with the control and 6 treatments (49 total trees). Every other tree within a row was used. Trees were 4 - 6 m diam and 5 - 6.7 m tall. There were 20 pretreatment cherries removed from each test tree (980 total). A volume of 3.785 L of spray was applied onto cherries until runoff on the south side of each tree, using a power hand gun at 689 kpa attached to a Nifty Pul-Tank (Rear's Mfg. Co., Eugene, OR). Spinosad bait was used at the same volume as other treatments to facilitate comparisons. Five or 6 d after applications, 200 cherries were collected from each tree. Larval emergence was then recorded. Pupae were stored at 3°C for 9 months, and then transferred to 27°C to determine percent adult emergence.

In test 4B in 2008, treatments were: (1) water (control); (2) spinosad; (3) imidacloprid; (4) thiamethoxam; (5) thiacloprid (Calypso® 480 SC); (6) acetamiprid; and (7) clothianidin. Rates were the same as in previous tests. The test was conducted at an experimental cherry orchard at the USDA-ARS Research Farm near Moxee, WA, that was infested with a low population of flies. The orchard had 29 'Van' and 209 'Bing' cherry trees. The test was set up as a randomized blocks design with 5 replicate blocks (2 of 'Vans' and 3 of 'Bing'), each with the control and 6 treatments (35 trees). To generate cherries with mostly eggs, fruit on trees were infested using laboratory-reared flies from 2007 cherry collections. Flies were maintained in mixed sex cages on yeast extract (EZ Mix™, Sigma-Aldrich, St. Louis, MO) and sugar diet for ~14 - 30 d before use to ensure high egg loads. An organdy sleeve cage (72 cm long × 50 cm

wide) was tied onto a branch with 10 - 30 orange to red cherries on each test tree. On 26 June, 8 female flies were released inside each cage (with a water wick and yeast extract and sugar food) and left in it for 7 d. After cages were removed, 2 - 4 pretreatment cherries from 20 of the 35 trees (47 total) were picked, and then 5 - 10 ml of water or insecticides were sprayed on the cherries until runoff. Cherries were removed 4 d after sprays, and then larval emergence was recorded.

In test 4C in 2008, treatments were the same as in test 4B. The test was conducted at the same orchard at the USDA-ARS Research Farm. The test was set up as a randomized blocks design with 3 replicate blocks, 2 of 'Bing' and 1 of 'Van' trees (19 trees). In 2 trees, there were 2 treatments. On 14 July, 5 - 10 pretreatment dark red or purple cherries were removed from each test tree (183 total), and then the cherries on 2 - 4 branches on each tree [29.1 ± 2.3 (mean ± SE) per replicate] were sprayed with ~5 - 10 ml of water or insecticides until runoff. One day after the sprays, cherries were removed, and then larval emergence recorded.

Statistics. Data from experiment 1 were analyzed using one-way analysis of variance (ANOVA). Data from experiment 2 were analyzed using a Tukey-type multiple comparison test among proportions (Zar 1999). Data from experiments 3 and 4 were analyzed with ANOVA using a randomized blocks design, testing for insecticide treatment effect only. Larval counts were square-root (y + 0.5) transformed before analyses. Means were separated using Fisher's LSD test (SAS Institute Inc. 2004). Percent adult emergence from pupae in test 3A was analyzed using a Tukey-type multiple comparison test, and in test 3A it was analyzed using ANOVA.

Results

Experiment 1: Effects of insecticides on egg hatch. Eggs exposed for 15 sec to thiacloprid had lower hatch than eggs exposed to water, spinosad, and imidacloprid (Table 1). Eggs in all treatments hatched between 3 and 7 d. There were no visible sclerotized mouthparts and body form in the eggs immediately after extraction from cherries, but unhatched eggs exposed to spinosad, imidacloprid, and thiacloprid contained fully developed (seen through the chorion) but apparently dead larvae. Eggs exposed continuously to insecticides did not hatch (Table 1). As with 15-sec exposures, eggs exposed continuously to insecticides contained developed but apparently dead larvae, indicating insecticides did not prevent some development inside eggs.

Experiment 2: Effects of insecticides on larval mortality. Data from all instars were combined to increase numbers for statistical analysis. At 24 - 28 h after treatment (Table 2), mortalities of larvae inside thiacloprid- and acetamiprid-treated cherries were higher than inside control cherries, and mortality inside acetamiprid-treated cherries was higher than inside imidacloprid-treated cherries. At 48 - 52 h (Table 2), mortality rates in all insecticide treatments were higher than in the control. At 48 h, 11 pupae were found in the tub with 80 control cherries, and 7 in each of the 3 tubs with insecticide treatments, indicating the treatments did not prevent larval emergence.

Experiment 3: Larval emergence from detached cherries treated with insecticides. In test 3A, pretreatment cherries in the early, middle, and late collections (Fig. 1A, 1B, and 1C) had different egg and larval distributions. In particular, cherries in the early collection had only eggs. In the early collection (Fig. 2A), there were no differences ($P > 0.05$) in larval emergence among the control and treatments, but numerically, there were 82.5 - 100% less in the spinosad bait, imidacloprid, thiamethoxam,

Table 1. Percent egg hatch ± SE of *Rhagoletis indifferens* exposed to insecticides for two durations after 14 d

Treatment	15-sec Exposure	Continuous Exposure
Water Control	41.0 ± 5.8a (123/300)	42.4 ± 9.0a (106/250)
Spinosad	37.0 ± 6.9a (111/300)	0.0 ± 0.0b (0/250)
Imidacloprid	25.0 ± 4.7a (75/300)	0.0 ± 0.0b (0/250)
Thiacloprid	3.3 ± 1.6b (10/300)	0.0 ± 0.0b (0/250)

15-sec exposure: six replicates, 50 eggs each; Continuous exposure: five replicates, 50 eggs each. Total numbers hatched/total eggs inside parentheses.
ANOVA: 15-sec exposure: $F = 15.3$; d.f. = 3, 20; $P < 0.0001$; continuous exposure: $F = 55.7$; d.f. = 3, 16; $P < 0.0001$. Means followed by the same letter within columns are not significantly different (Fisher's LSD test, $P > 0.05$).

Table 2. Percent mortality of *Rhagoletis indifferens* larvae in cherry fruit treated with different insecticides

Treatment	first Instar	second Instar	third Instar	All Instars
		24-28 h After Treatment		
Water Control	11.1 (18)	0.0 (6)	9.5 (21)	8.9 (45)c
Imidacloprid	35.3 (17)	16.7 (12)	41.7 (12)	31.7 (41)bc
Thiacloprid	43.5 (23)	44.4 (9)	50.0 (10)	45.2 (42)ab
Acetamiprid	50.0 (14)	60.0 (5)	73.3 (15)	61.8 (34)a
		48-52 h After Treatment		
Water Control	35.0 (20)	17.8 (17)	15.4 (13)	24.0 (50)b
Imidacloprid	52.6 (19)	36.4 (11)	56.2 (16)	50.0 (46)a
Thiacloprid	61.5 (13)	40.0 (10)	64.7 (17)	57.5 (40)a
Acetamiprid	71.4 (7)	44.4 (9)	81.8 (11)	66.7 (27)a

Numbers of larvae inside parentheses; 40 cherries for each treatment and time.
Percents followed by the same letters within last columns are not significantly different (Tukey-type multiple comparison among proportions, critical $q_{0.05, \infty, 4} = 3.633$).

and azinphos-methyl treatments than in the control. In the middle collection (Fig. 2B), larval emergence in spinosad bait, imidacloprid, and azinphos-methyl treatments was 71.1 - 100% less than in the control, and in the late collection (Fig. 2C), it was 63.4 - 100% less in spinosad bait, imidacloprid, and azinphos-methyl treatments than in the control, with azinphos-methyl being most effective.

Total pupae that developed into adult flies across the 3 dates were pooled to increase sample sizes. The percent from spinosad bait [14.9% ($n = 87$)] was lower than from the control [31.1% ($n = 312$)] ($q = 4.433$; critical $q_{0.05, \infty, 6}, = 4.030$), but there were

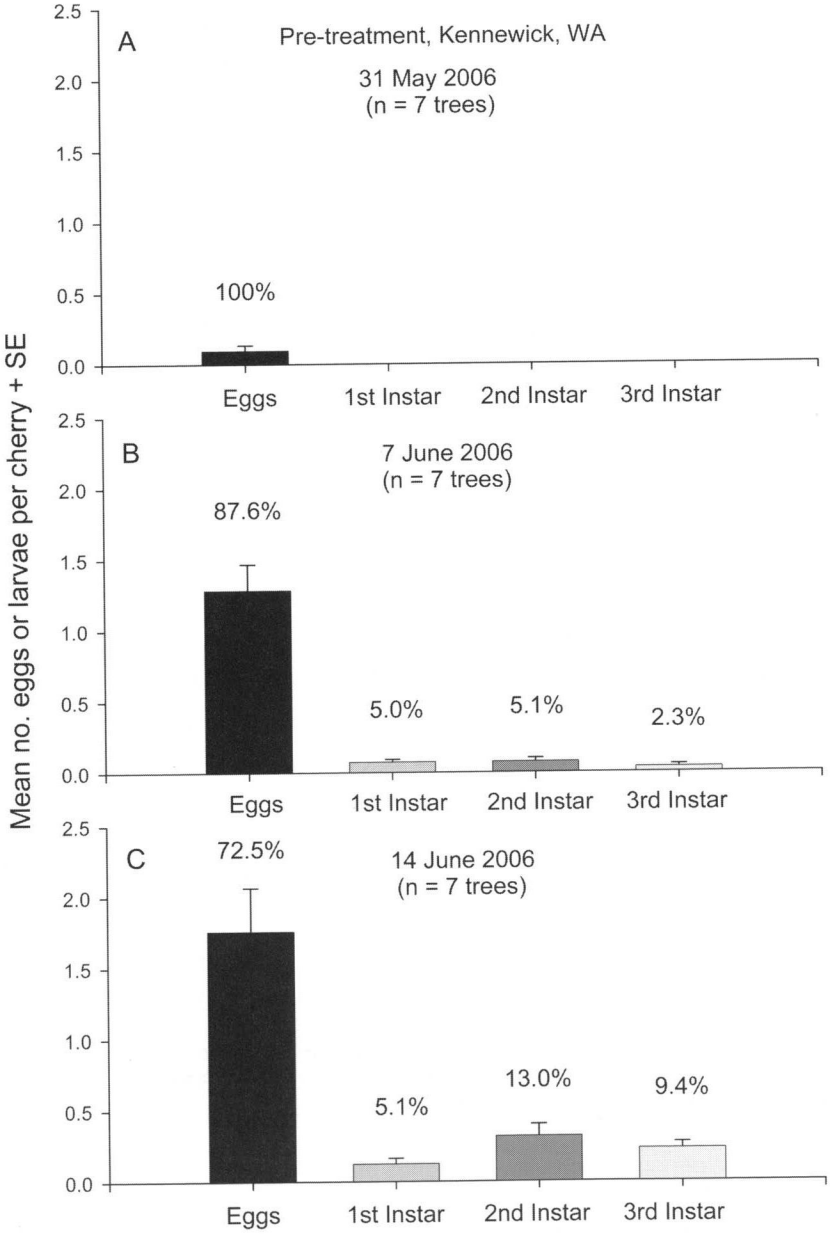

Fig. 1. Experiment 3, test 3A: pretreatment *R. indifferens* egg and larval distributions in cherries from Kennewick, WA in (A) early, (B) mid, and (C) late season, 2006.

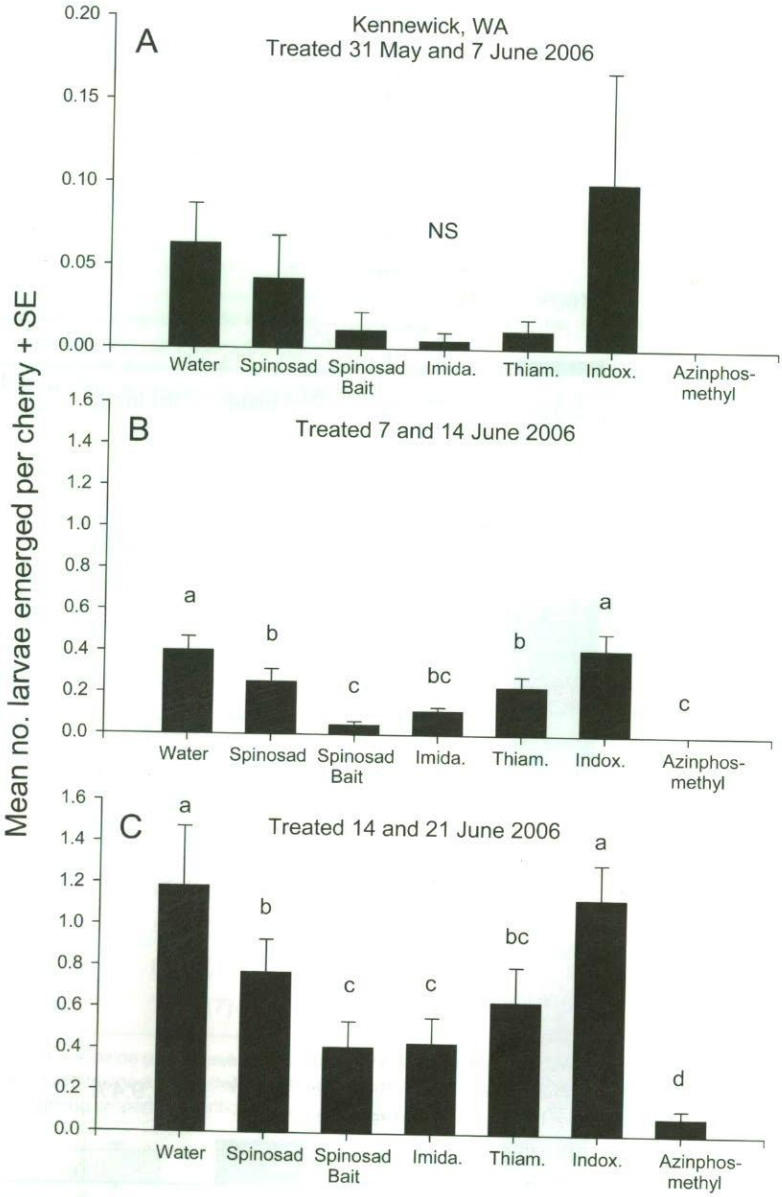

Fig. 2. Experiment 3, test 3A: numbers of *R. indifferens* larvae that emerged from detached cherries that were treated with insecticides after removal from trees in Kennewick, WA: (A) early, (B) middle, and (C) late season collections, 2006. (ANOVA, early: $F = 2.0$; d.f. = 6, 36; $P = 0.0931$; middle: $F = 11.7$; d.f. = 6, 36; $P < 0.0001$; late: $F = 16.1$; d.f. = 6, 35; $P < 0.0001$.) Means with same letters are not significantly different (LSD test, $P > 0.05$). Note Y-axis in (A) is different from (B) and (C) to show trend.

no other differences. Azinphos-methyl was not included because of the low sample size.

In pretreatment cherries in test 3B, there were more eggs than larvae, but larvae comprised 34.2% of the stages, with most being third instars (Fig. 3A). Larval emergence in imidacloprid and acetamiprid treatments was 70.2 and 77.2% lower, respectively, than in the control (Fig. 3B). Statistically, imidacloprid, thiamethoxam, thiacloprid, and clothianidin did not differ, but acetamiprid had lower numbers than thiamethoxam. Despite differences in larval emergence among treatments after 30 d, no differences were detected 1 d after treatment ($P > 0.05$).

Experiment 4: Emergence of larvae from cherries treated while on trees. In pretreatment cherries in test 4A, eggs comprised only 19.7% of stages, with many second and third instars (Fig. 4A). Larval emergence in insecticide treatments was 30.6 - 94.2% lower than in the control (Fig. 4B), with azinphos-methyl and imidacloprid being most effective and showing no difference. Spinosad, spinosad bait, and thiacloprid did not differ from imidacloprid, but were less effective than azinphos-methyl. Thiamethoxam was less effective than imidacloprid.

Percents of pupae that developed into adults in spinosad (mean ± SE, 12.1 ± 4.9%), spinosad bait (11.7 ± 2.9%), and thiacloprid (19.3 ± 4.6%) treatments were lower than in the control (34.7 ± 5.8%) ($P < 0.05$). Imidacloprid and thiamethoxam did not differ from the control and other treatments. Azinphos-methyl had few pupae and was not included.

In test 4B, pretreatment cherries had 94.2% eggs and few larvae (Fig. 5A). The egg number per cherry was much higher than in previous tests. Despite this high egg number, all neonicotinoids were equally effective, as larval emergence in neonicotinoid treatments was 94.9 - 99.3% lower than in the control and all were more effective than spinosad (Fig. 5B).

In test 4C, pretreatment cherries had about equal numbers of eggs and larvae, with similar numbers of second and third instars (Fig. 6A). All insecticides reduced larval emergence (Fig. 6B), but not as high as when the egg was the predominant stage (test 4B). Larval emergence in imidacloprid, thiacloprid, and acetamiprid treatments was 89.0 - 92.1% lower than in the control (Fig. 6B).

Discussion

Results of experiment 1 indicate that thiacloprid had a greater effect on the eggs of *R. indifferens* than spinosad and imidacloprid, suggesting thiacloprid was more toxic to eggs or that movement of thiacloprid through the chorion was faster than of spinosad and imidacloprid. In eggs of the plum curculio, *Conotrachelus nenuphar* (Herbst), thiacloprid may be able to move through the chorion better than thiamethoxam because of its positive octanol-water partitioning coefficient (Hoffman et al. 2008). Results using other insects are consistent with those reported here. Spinosad did not have ovicidal activity against *C. capitata*, even when eggs were exposed to it for 48 h at high doses (Adán et al. 1996), and imidacloprid had low ovicidal activity against the whitefly, *Bemisia tabaci* (Gennadius) (Horowitz et al. 1998). However, thiacloprid had high ovicidal activity against codling moth, *Cydia pomonella* (L.) (Elbert et al. 2001), and plum curculio (Hoffman et al. 2008).

Results of experiment 2 indicate that imidacloprid, thiacloprid and acetamiprid are toxic to *R. indifferens* larvae, but that thiacloprid and acetamiprid may act more quickly than imidacloprid. None of the 3 insecticides caused 100% mortality of any larval

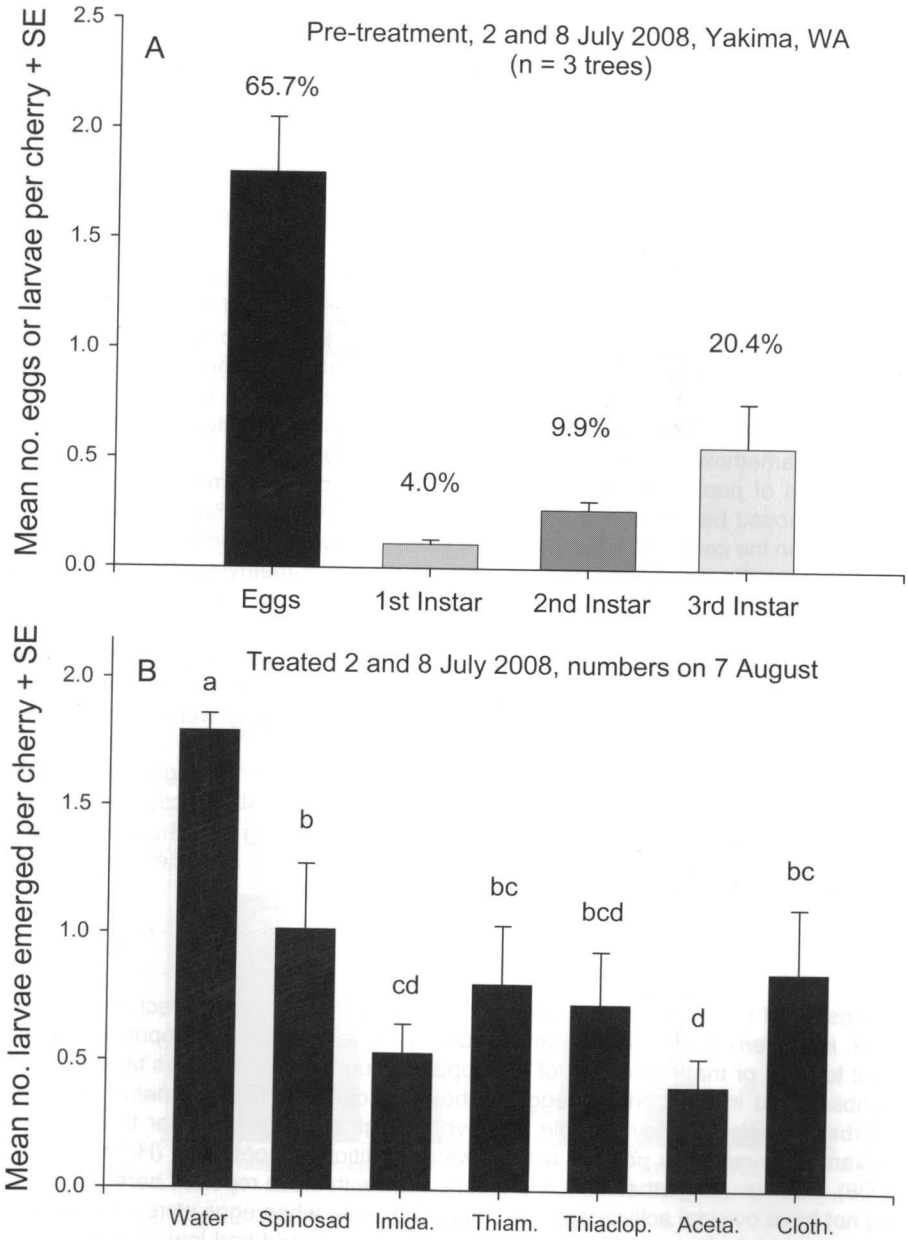

Fig. 3. Experiment 3, test 3B: (A) pretreatment *R. indifferens* egg and larval distributions in cherries and (B) numbers of larvae that emerged from detached cherries that were treated with insecticides after removal from trees in Yakima, WA, 2008. (ANOVA, $F = 9.4$; d.f. = 6, 12; $P = 0.0006$.) Means with same letters are not significantly different (LSD test, $P > 0.05$).

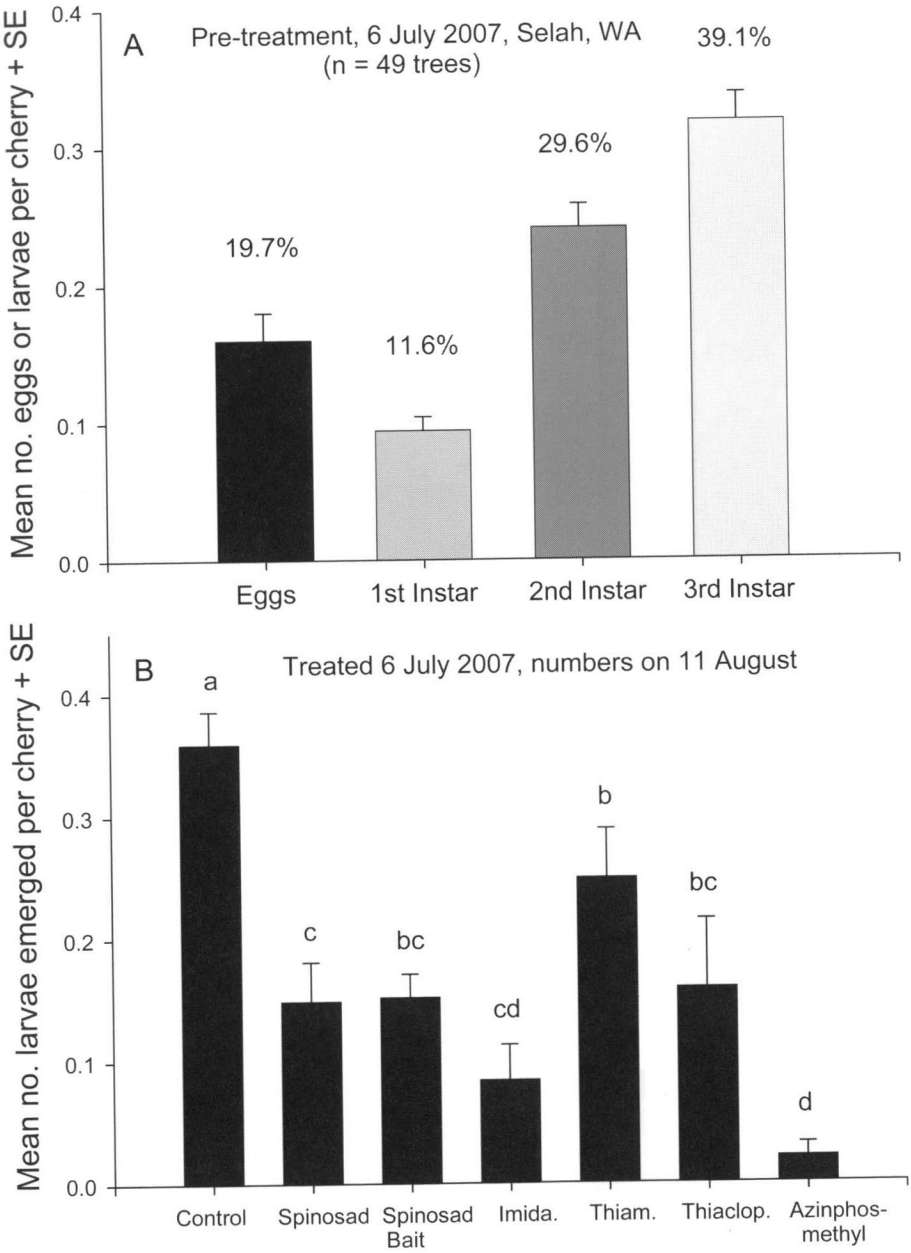

Fig. 4. Experiment 4, test 4A: (A) pretreatment *R. indifferens* egg and larval distributions in cherries and (B) numbers of larvae that emerged from cherries that were treated with insecticides while on trees in Selah, WA, 2007. (ANOVA, $F = 5.8$; d.f. = 12, 36; $P < 0.0001$.) Means with same letters are not significantly different (LSD test, $P > 0.05$).

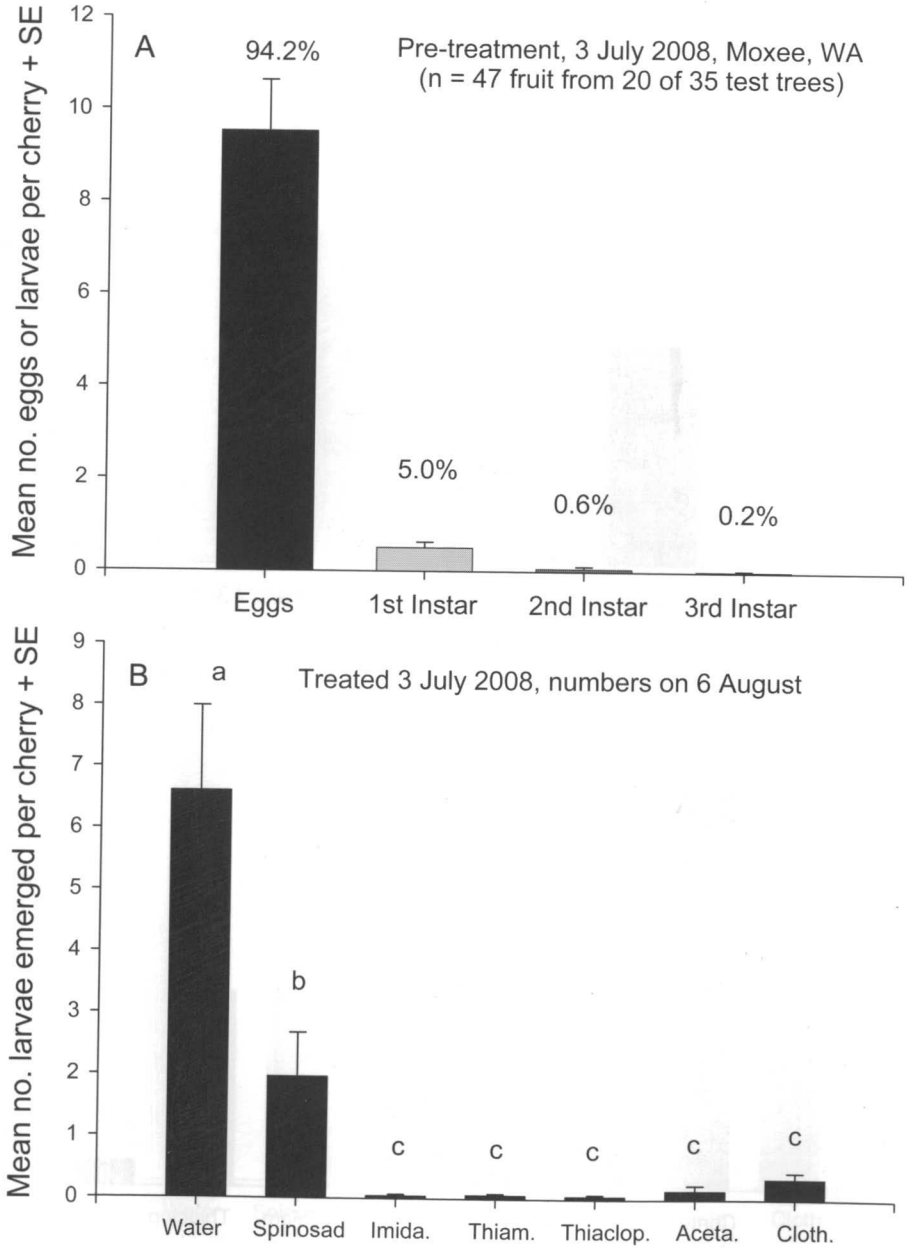

Fig. 5. Experiment 4, test 4B: (A) pretreatment *R. indifferens* egg and larval distributions in cherries and (B) numbers of larvae that emerged from cherries that were treated with insecticides while on trees near Moxee, WA, 2008. (ANOVA, $F = 44.3$; d.f. = 6, 21; $P < 0.0001$.) Means with same letters are not significantly different (LSD test, $P > 0.05$).

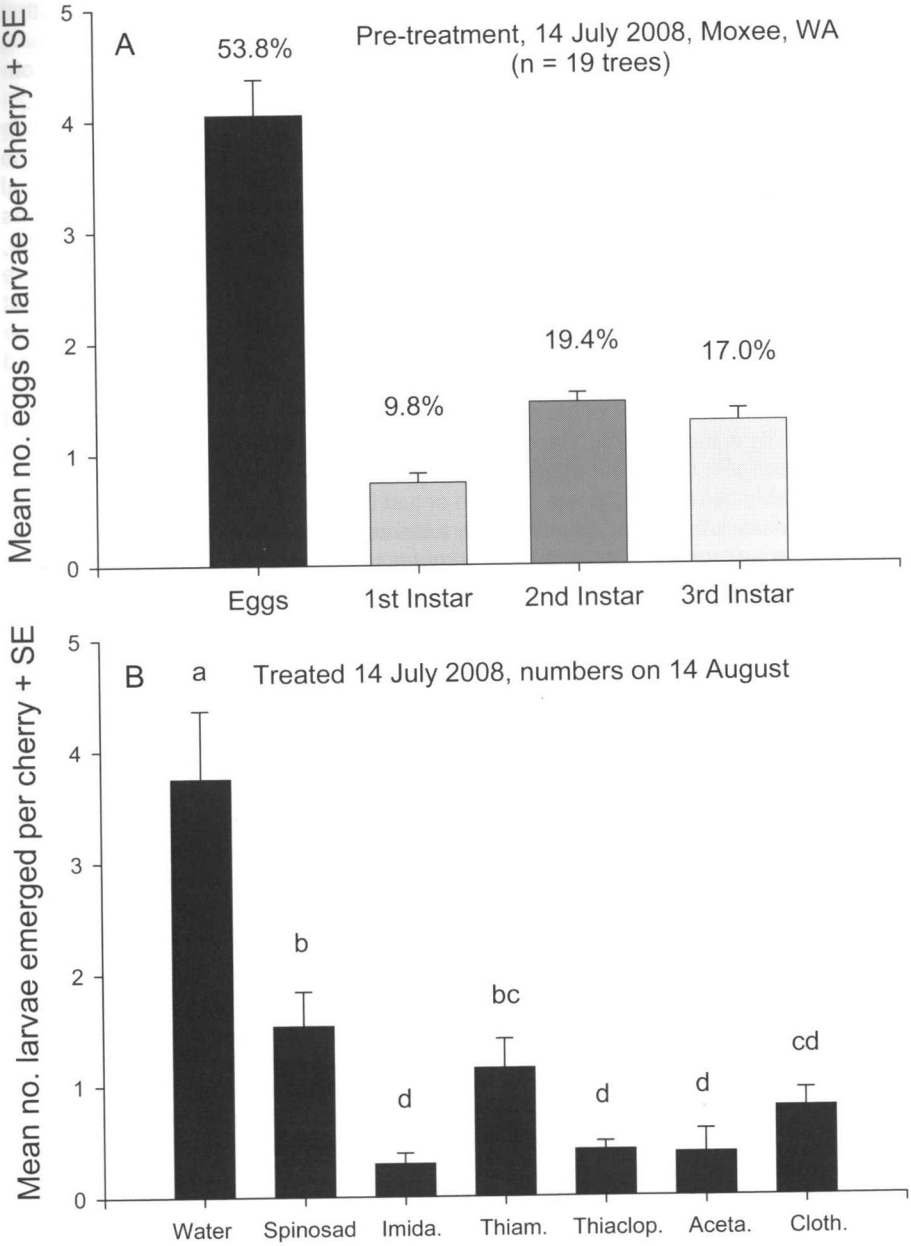

Fig. 6. Experiment 4, test 4C: (A) pretreatment *R. indifferens* egg and larval distributions in cherries and (B) numbers of larvae that emerged from cherries that were treated with insecticides while on trees near Moxee, WA, 2008. (ANOVA, $F = 23.4$; d.f. = 6, 12; $P < 0.0001$.) Means with same letters are not significantly different (LSD test, $P > 0.05$).

stage, suggesting larvae are not highly susceptible to the amounts of insecticides that they were exposed to or that 48 h was insufficient exposure time. In studies using parathion and demeton against *R. indifferens*, 1-d mortality was lower than that obtained after 5 d (Frick and Simkover 1953).

In experiments 3 and 4, there was evidence that insecticides are more effective in cherries against eggs than larvae. In test 3A, differences among treatments in the early collection were not significant probably because of low larval numbers, but larval emergence (as percentages of controls) from early cherries that had only eggs was lower than from middle and late cherries that had larvae. In the imidacloprid treatment, larval emergence was 92.1, 71.1, and 63.4% lower than the control in the 3 respective collections. In test 3B, the lack of insecticide effects 1 d after treatment indicates that it may not be possible for insecticides to kill all third instars that are ready to emerge from fruit. In tests 4A and 4C, where only 19.7% and 53.8% of stages were eggs and the remainder larvae, imidacloprid reduced larval emergence by 76.6.% and 92.1%, but in test 4B, where 94.2% of the stages were eggs, all neonicotinoids reduced larval emergence by almost 100%. Based on experiment 1, thiacloprid was expected to have the strongest effect, but eggs apparently are highly susceptible to all neonicotinoids. Possibly because eggs are found in or just beneath the skin, they are exposed to higher insecticide concentrations than are larvae. Also, oviposition punctures in the cherry skin may provide insecticides direct routes to the eggs. In apples, >70% of total thiamethoxam, thiacloprid, indoxacarb, and azinphos-methyl were from the skin, and ~10% were in the outer 2 mm of flesh (Wise et al. 2007).

In experiments 3 and 4, effects of the various insecticides sprayed on cherries with moderate to high numbers of larvae differed. In general, spinosad bait and neonicotinoids appeared equally effective. In test 3A, spinosad bait was more effective than spinosad alone, because of the higher spinosad concentration in bait or perhaps because bait components acted as adjuvants that increased spinosad absorption. Use of spinosad bait as a cover spray for larval control might be limited because of the potential damage it could cause to leaves (DeLury et al. 2008) (depending on cultivar, even though growers have not reported phytotoxicity through its use) and costs associated with using it this way, but results do suggest use of some adjuvants may help absorption of insecticides into cherries. Within the neonicotinoids, larval emergence numbers alone suggest imidacloprid and acetamiprid are the best candidate materials to use against larvae in cherries, in agreement with work conducted in north central Washington (Smith 2007), although azinphos-methyl was the most effective. In addition to higher toxicity, insecticides should be more effective against larvae if they can move deep into cherries where larvae are found. In apples, thiamethoxam did not move as deep as thiacloprid and azinphos-methyl, which were found from the apple skin to the core (Wise et al. 2007), suggesting one reason why larval emergence was lower from thiacloprid- than thiamethoxam-treated cherries and why azinphos-methyl was so effective in the present study.

In tests 3A and 4A, spinosad, spinosad bait, and thiacloprid seemed to reduce the emergence of adult flies from pupae. This suggests that even though eggs or larvae exposed to insecticides may develop into pupae, there is a carry-over toxic effect within puparia. Further work is needed to confirm this finding.

Results of the present study have implications for the use of neonicotinoid insecticides to reduce larval emergence of *R. indifferens* from cherries. They indicate neonicotinoids are toxic to eggs and larvae, and that all are more effective in cherries against eggs than larvae. For fly control, the time interval between the last spray directed

against adult flies and the first postharvest spray of neonicotinoids should be no more than 1 wk, to reduce chances that eggs hatch and larvae develop. Neonicotinoids also may kill eggs laid by flies not killed by insecticides at preharvest. The possibility of mixing adjuvants with imidacloprid and acetamiprid to increase their rate of uptake into postharvest cherries and to further increase larval kill needs to be explored.

Acknowledgments

The author thanks Pete Chapman, Janine Jewett, and Julie Schlaman for field and laboratory assistance and Joseph Munyaneza (USDA-ARS, Wapato, WA) and Mike Willett (Northwest Horticultural Council, Yakima, WA) for helpful comments on earlier drafts of the manuscript. This research was partially supported by the Washington Tree Fruit Research and Oregon Sweet Cherry Commissions and a Technical Assistance for Specialty Crops Grant from the Foreign Agricultural Service (USDA). This article presents the results of research only. Mention of a proprietary product does not constitute an endorsement for its use by USDA.

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