Effects of Several Newer Insecticides and Kaolin on Oviposition and Adult Mortality in Western Cherry Fruit Fly (Diptera: Tephritidae)[1]

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Abstract Effects of newer insecticides and kaolin-based particle film (Surround® WP Crop Protectant) on oviposition and mortality in the western cherry fruit fly, *Rhagoletis indifferens* Curran, were determined. In a no-choice experiment, azinphos-methyl sprayed on cherries reduced oviposition by 98.5% compared with the control, whereas spinosad bait, imidacloprid, and thiamethoxam reduced it by 74.4% to 91.8% and indoxacarb reduced it by 61.7%. Despite reducing oviposition to similar levels, imidacloprid did not cause as much mortality as spinosad bait, spinosad, or thiamethoxam. Imidacloprid reduced oviposition more than indoxacarb, even though the two caused similar mortality. In a choice experiment, there were no differences in oviposition in untreated and insecticide-treated cherries, except fewer eggs were laid in spinosad-bait treated than untreated cherries. In a no-choice experiment using kaolin, flies laid up to 36 times more eggs in control than treated cherries. In a choice experiment, flies laid 10 times more eggs in untreated than kaolin-treated cherries. Results show oviposition by *R. indifferens* can be reduced to similar levels by materials with significantly different toxicities, and that none except spinosad bait is an oviposition deterrent. The high levels of oviposition reduction indicate these insecticides (except indoxacarb) can protect fruit from mature flies, but more work is needed to modify or improve them so that they can reduce oviposition as much as azinphos-methyl. In residential trees where none of these insecticides is desirable, kaolin may be useful in reducing oviposition and preventing buildup of *R. indifferens* populations.

Key Words *Rhagoletis indifferens,* spinosad, neonicotinoids, barrier films, infestations

The western cherry fruit fly, *Rhagoletis indifferens* Curran, is the major pest of sweet cherries, *Prunus avium* (L.) L., in the Pacific Northwest of the U. S. and in British Columbia. Because of its quarantine pest status in cherries exported to domestic and overseas markets, the fly until recently had been controlled almost solely by using conventional organophosphate insecticides (Zwick et al. 1970, 1975). However, provisions in the Food Quality and Protection Act (FQPA) of 1996 have resulted in the phase-out of these insecticides in the near future, even when continued use of insecticides is necessary because of the zero tolerance for larvae in commercial fruit (State of Washington Department of Agriculture, Permanent Order No. 1099, effective 30 September 1968). Newer insecticides have been tested against *R. indifferens* in response to the need for using nonorganophosphates for fly control (vanRanden and

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Roitberg 1998, Yee and Alston 2006). Currently, spinosad bait (GF-120 Naturalyte® Fruit Fly Bait, Dow AgroSciences, Indianapolis, IN) is commonly used for fly control in Washington state. Insecticides are typically applied at 7-d intervals because of their residual activities and because flies need to be killed before they develop eggs at about 7 d postemergence. However, the usefulness of an insecticide is increased if it can also kill reproductively mature flies quickly enough to prevent oviposition, especially because infestations in commercial cherry orchards are likely often caused by these flies moving into them from surrounding unmanaged trees. Newer insecticides studied (spinosad, imidacloprid, and thiacloprid) have not prevented oviposition into fruit by mature *R. indifferens* (Yee and Alston 2006), but it is not known if even azinphos-methyl, a highly toxic organophosphate standard, can prevent oviposition.

Research has been conducted on the effects of insecticides on oviposition and/or on adult mortality of *Rhagoletis* flies (e.g., Reissig et al. 1983, Hu and Prokopy 1998, Reissig 2003, Liburd et al. 2003, Barry and Polavarapu 2005, Barry et al. 2005, Yee and Alston 2006), but the relationship between oviposition and mortality caused by insecticides in *R. indifferens* remains unclear. Oviposition should be reduced more by using more toxic than less toxic insecticides. Oviposition by the apple maggot, *Rhagoletis pomonella* (Walsh), was greatly reduced by the organophosphate azinphos-methyl (Guthion), which is highly toxic to adults (Reissig et al. 1983). However, oviposition may not necessarily be greater when using less toxic insecticides if these insecticides have sublethal effects such as paralyzing flies or deterring oviposition.

There also has been work on the use of a noninsecticide alternative for protecting fruit from oviposition by fruit flies, although not against *R. indifferens*. A kaolin-based particle film, which includes proprietary spreader stickers, marketed in 1999 as Surround® WP Crop Protectant (Englehard Corp, NJ) is certified for use in organic fruit production and has shown promise in deterring oviposition in tephritids. Kaolin reduced oviposition in apples by apple maggot, *R. pomonella* (Wright et al. 2000), in blueberries by blueberry maggot, *Rhagoletis mendax* Curran (Liburd et al. 2003), in nectarines, apples, and persimmons by Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Mazor and Erez 2004), and in olives by olive fly, *Bactrocera oleae* Gmelin (Saour and Makee 2004). Kaolin is a white aluminosilicate mineral that acts as a physical particle film barrier and may act as a deterrent to settling responses in some insects because it adheres to the tarsi, resulting in irritation and causing the insects to avoid treated surfaces (Glenn et al. 1999).

In this study, the main objective was to determine the effects of various newer insecticides and kaolin on oviposition by *R. indifferens* in sweet cherry. The relationship between oviposition and mortality caused by various insecticides and the ability of flies to choose between treated and untreated fruit were examined. Results are compared with previous findings reported in the literature for other *Rhagoletis* and fruit fly species.

Materials and Methods

Fly source and experimental conditions. Flies used in all laboratory experiments were collected as larvae from field-infested sweet and sour cherry fruit in central Washington in June 2001 or 2005. Cherries were laid on hardware cloth placed on top of tubs with moist soil (equal volumes of vermiculite, peat moss, and sand). Larvae dropped into the soil and pupated. Pupae were stored inside sealed plastic containers with soil at 3°C for 6-8 months before being transferred to 27°C for adult emergence. Prior to experiments, adults were maintained on dry 20% yeast extract-80% sucrose (wt/wt) food on ~10 × 5 cm paper strips. This was the same food used in all experiments. Experiments were conducted at 25-27°C, 30-40% RH, and under a 16 h light: 8 h dark cycle.

Experiment 1: Effects of insecticides on oviposition. The main objectives in this no-choice experiment were to determine the effects of various insecticides on oviposition into cherries by simulating a situation where insecticides are applied on fruit on trees and then attacked by gravid flies from unmanaged trees outside an orchard and to compare the effects of these insecticides on oviposition and mortality. Label insecticide rates were used. Spray volumes for all treatments below except for (2) were 935 l/ha. Comparisons were: (1) a water control; (2) spinosad bait (GF-120 Naturalyte® Fruit Fly Bait, 0.24 g spinosad/liter; 1 l bait/1.5 l recommended rate for single trees; g/ha dependent on numbers of trees/ha); (3) spinosad (800 g/kg, Entrust®, Dow AgroSciences, Indianapolis, IN), 17.0 g/ha (13.6 g a.i./ha); (4) imidacloprid (119.8 g/l, Provado®, Bayer CropScience, Research Triangle Park, NC), 59.1 ml/ha (11.3 g a.i./ha); (5) thiamethoxam (250 g/kg, Actara®, Syngenta Crop Protection, Greensboro, NC), 155.9 g/ha (39.0 g a.i./ha); (6) indoxacarb (300 g/kg, Avaunt®, E. I. du Pont de Nemours and Company, Wilmington, DE), 170.1 g/ha (51.0 g a.i./ha); and (7) azinphos-methyl (500 g/kg, Guthion®, Gowan Company, Yuma, AZ), 680.4 g/ha (340.2 g a.i./ha), used as a conventional insecticide comparison, but not included as a material to be used in the field. The toxins in spinosad are spinosyns A (85%) and D (15%), fermentation products derived from the actinomycete *Saccharopolyspora spinosa* Mertz and Yao. Imidacloprid and thiamethoxam are neonicotinoids, which are chloronicotinyl nitroguanidines, and indoxacarb is an oxadiazine, a relatively new class of a pyrazoline-type insecticide.

Insecticides were applied on cherries the day before testing. Cherries (cv 'Bing') had been stored at 3°C for 2-3 months before use. Thirty cherries were spread as one layer on a piece of aluminum foil on a plastic tray in a fume hood. A total of 4.8 ml of water or insecticide solution was evenly applied onto the 30 cherries using a spray bottle set to deliver 6 squirts of 0.8 ml each. Cherries were left on the tray for 24 h at 20-21°C.

Flies were aged inside 1.9-L white paper containers (12.5 cm high × 16.2 cm diam) (Sweetheart Cup, Owings Mills, MD) containing food and water. Each container held up to 50 flies (about equal number of males and females). Flies were tested at 14-16 d old, when they were sexually mature and females had developed eggs. On test day, the 30 cherries were gently poured onto the bottom of a 1.9-L paper container. The container had two water wicks and a food strip. Even though flies had access to mates for >14 d, 6 males and 6 female flies were placed together into each container to allow for continual mating, which may affect oviposition. The additional mating opportunities primarily may have benefited control flies, as insecticides may have killed flies too quickly for mating to take place. Female fly mortality was checked at 1, 2, and 3 d after exposure to cherries. At 3 d, all 30 cherries were removed and stored in 70% ethanol. Cherries were inspected for eggs located ~1 mm below the surface. There were 5 replicates (1 replicate = 1 container with 30 cherries) of the control and all treatments.

Experiment 2: Effects of insecticides on oviposition choice. The major objective of this choice experiment was to determine oviposition in treated versus untreated cherries. Insecticide effects on oviposition and mortality also were compared. Methods were essentially the same as experiment 1, except that 15 treated and 15 untreated cherries were placed on the bottom of the same container. A strip of stiff white paper ~3 cm wide and 16 cm long separated the 2 types of cherries. All materials were used at the label rates in experiment 1, except for spinosad, which was used at 136 g a.i./ha. There were 5 replicates of the control and treatments.

Experiment 3: Effects of kaolin on oviposition. In a no-choice experiment, the degree of oviposition deterrence caused by kaolin (950 g/kg, Surround®) was determined. Because kaolin is not a toxic insecticide and was not expected to cause high adult mortality, methods differed from those used for experiments 1 and 2. Six female and 6 male newly-emerged flies were placed into 3.8-L paper containers with water wicks and exposed to (1) 16 untreated cherries and no food (other than cherries, control), (2) 16 kaolin-treated cherries and no food, or (3) 16 kaolin-treated cherries and a food strip. The food was included to examine the possibility that kaolin also deters feeding. Prior studies show that *R. indifferens* will feed on intact ripe cherries, obtaining sugar from the surfaces (Yee 2003a). If flies do not survive when exposed to treated cherries with no food but do survive when exposed to treated cherries with food, then kaolin probably prevents the flies from feeding. For all treatments, 'Bing' sweet cherries that had been stored in 100% nitrogen for four months were used. Two sets of 8 fruit, each in a separate Petri dish (1.4 cm high × 8.9 cm diam), were placed on the bottom of the containers. Each set of treated fruit was sprayed with 7 ml of kaolin solution (57.0 g kaolin/l), and dried using a fan for 45 min. Every 3 d, old fruit were removed and 16 new control or treated fruit were introduced into containers until day 21, when the test ended and fly mortality was recorded. Fruit were stored in 70% ethanol and later dissected to determine numbers of eggs. There were 3 replicates of each treatment.

Experiment 4: Effects of kaolin on oviposition choice. In a choice test, 6 male and 6 female flies were exposed to 8 untreated and 8 kaolin (Surround®) treated cherries, with each of the 8 in separate dishes inside the same container. There was no food strip. There were 3 replicates. Test conditions and other methods were the same as those in experiment 3.

Experiment 5: Effects of kaolin on adult and larval infestations. A field spray experiment using kaolin (Surround®) was conducted in an experimental sweet cherry orchard in Moxee, WA, from 14 June to 12 July 2002. Untreated control trees, trees treated with kaolin once, and trees treated with kaolin twice were compared. There were 6 replicate trees for each. Trees were 2.5 m tall, 2 m in diam, and were arranged in a randomized block design, with each block being a row of trees, for 6 total rows. There was 1 buffer row between treatment rows and 1 buffer tree between each test tree within a row. A rate of 57.0 g kaolin/l of spray was applied on each treatment tree using a Nifty Pul-Tank (Rear's Mfg Co., Eugene, OR) and a handgun at 689 kpa until trees were fully covered (about 3.7 L per tree). Pretreatment adult numbers were determined by hanging one 14 × 23 cm sticky yellow panel trap (Scenturion, Clinton, WA) on the south side of a tree 1.5-2 m above ground from 14-17 June, when flies began emerging, and removed before the first application. The first and second applications were made on 19 and 26 June, respectively. A trap was hung on each tree 1 d after the spray and then removed 24 h later to assess the immediate effects of the spray on adult numbers. Trees were stripped of all their fruit on 12 July. Fruit were laid on screens over large tubs containing water outdoors. Numbers of larvae that dropped into the water each day over 39 d (12 July to 19 August) were counted to determine infestation rates.

Statistics. For experiments 1 and 2, one-way analysis of variance (ANOVA) was conducted. For experiment 1, correlation of ranks (1 = lowest, 6 = highest) between numbers of eggs and percentage mortality at day 1 within insecticides was performed. For experiment 2, two sample paired *t*-tests also were conducted for choice comparisons. For experiments 3 and 4, repeated-measures ANOVA (Littell et al. 1996) was conducted. For experiment 3, two-way ANOVA using treatment and date as factors also was conducted. For experiment 5, randomized block ANOVA was conducted, followed by orthogonal contrasts (a priori) (contrast 1: control versus mean of 1 and 2 kaolin sprays; contrast 2: 1 versus 2 kaolin sprays) (Steel and Torrie 1980), using the experimental error mean square from a randomized block ANOVA to calculate F values. In all ANOVAs except in experiment 5, means were separated using Fisher's LSD test (a posteriori). Counts and percentages were square-root or square-root + 1 and square-root arcsine-transformed, respectively. Data were analyzed using the Statistical Analysis System (SAS Institute Inc. 2003).

Results

Experiment 1: Effects of insecticides on oviposition. In the no-choice experiment, all materials reduced oviposition compared with the control, although none prevented it (Table 1). When percentage of cherries infested was the measure, azinphos-methyl was the most effective insecticide, reducing it by 98.5% compared with the control. Spinosad bait, imidacloprid, thiamethoxam, and spinosad were equally effective, reducing it by 74.4% to 91.8%, whereas indoxacarb was statistically less effective than all except spinosad, at 61.7%. When total numbers of eggs was the measure, reductions were greater, at 99.7% by azinphos-methyl, 94.1% to 97.9% by spinosad bait, spinosad, imidacloprid, and thiamethoxam, and 80.3% by indoxacarb. When mean numbers of eggs per cherry was the measure, results were similar (Table 1).

Oviposition was related to percentage female mortality in only a few cases, so lower oviposition was not necessarily a result of greater or earlier kill. Fewest eggs were laid in azinphos-methyl treatment, which had the highest mortality at day 1. Within insecticides, highest numbers of eggs were laid in the indoxacarb treatment, which had the lowest mortality at day 1. However, oviposition in spinosad, spinosad bait, and thiamethoxam treatments were similarly low, even though mortality in the imidacloprid treatment was lower than in the others on day 1. Also, indoxacarb reduced oviposition less than imidacloprid, even though the 2 caused similar mortality on day 1. Thus, overall, there was no significant inverse correlation in ranks between oviposition and mortality using the 6 materials ($r = -0.600$, $P = 0.2080$). Mortality increased with days after exposure, with all materials except indoxacarb causing mortality similar to that of azinphos-methyl by day 3.

Experiment 2: Effects of insecticides on oviposition choice. In the choice experiment, contrary to expectations, there were no differences between oviposition in treated and untreated cherries, except with spinosad bait, where more eggs were laid in untreated than treated cherries (Table 2). Similar to experiment 1, imidacloprid reduced oviposition as much as spinosad bait, spinosad, and thiamethoxam, even though it caused less mortality on days 1-3 or 2 and 3. Similar to experiment 1, indoxacarb did not reduce oviposition as much as imidacloprid, even though it caused as much mortality as imidacloprid on days 1-3 (Table 2).

Experiment 3: Effects of kaolin on oviposition. In the no-choice experiment using kaolin, higher percentages of control than treated fruit were infested (Table 3).

Table 1. No-choice experiment: effects of insecticides on mean oviposition measures and mortality ± SE in *Rhagoletis indifferens*

	% Cherries			Cumulative % female mortality[b]		
Treatment	Infested[a]	Total no. eggs[a]	No. eggs/cherry	Day 1	Day 2	Day 3
Control	88.7 ± 3.1a	141.2 ± 19.4a	4.70 ± 0.65a	0.0 ± 0.0e	3.3 ± 3.3d	3.3 ± 3.3c
Spinosad Bait	9.3 ± 2.7c	4.6 ± 1.3cd	0.15 ± 0.04c	76.7 ± 8.5c	96.7 ± 3.3a	100.0 ± 0.0a
Spinosad	22.7 ± 6.4bc	8.4 ± 2.7c	0.28 ± 0.09c	96.7 ± 3.3ab	100.0 ± 0.0a	100.0 ± 0.0a
Imidacloprid	7.3 ± 1.9c	3.0 ± 0.8cd	0.10 ± 0.03c	42.5 ± 7.9d	81.7 ± 5.5b	94.2 ± 3.6a
Thiamethoxam	14.7 ± 4.4c	7.0 ± 2.3cd	0.23 ± 0.08c	80.0 ± 12.3bc	86.7 ± 9.7ab	93.3 ± 6.7a
Indoxacarb	34.0 ± 7.8b	27.8 ± 9.9b	0.93 ± 0.33b	23.3 ± 6.7d	56.7 ± 10.0c	73.3 ± 4.1b
Azinphos-methyl	1.3 ± 1.3d	0.4 ± 0.4d	0.01 ± 0.01c	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
One-way ANOVA						
F (df = 6, 28)	27.4	39.9	43.7	30.1	32.6	61.5
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

5 replicates of 6 females and 6 males; [a] Per 30 cherries; [b] Days after exposure to treatments.
Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

Table 2. Choice experiment: effects of insecticides on mean oviposition measures on treated and untreated cherries and mean mortality of *Rhagoletis indifferens*

	% Cherries infested[a]			Total no. eggs[a]			Cumulative % female mortality[b]		
Treatment	Treated	Untreated	*t*-test, P^c	Treated	Untreated	*t*-test, P^c	Day 1	Day 2	Day 3
Control	96.0a[d]	98.7a[d]	—	105.6a[d]	153.8a[d]	—	0.0e	0.0c	0.0c
Spinosad Bait	12.0d	24.0cd	0.0780	2.4cd	10.0c	0.0154	66.7b	100.0a	100.0a
Spinosad[e]	18.7cd	17.3d	0.4817	3.0c	3.2cd	0.9382	76.7ab	100.0a	100.0a
Imidacloprid	30.7c	20.0cd	0.2946	5.0c	4.6cd	0.8044	32.9cd	67.6b	86.6b
Thiamethoxam	25.4c	33.4c	0.1780	5.0c	9.6c	0.1381	66.7bc	93.3a	100.0a
Indoxacarb	60.0b	62.7b	0.7206	25.0b	41.2b	0.1021	33.4d	60.0b	83.3b
Azinphos-methyl	4.0e	6.7e	0.9012	0.6d	1.4d	0.4805	96.7a	100.0a	100.0a
One-way ANOVA									
F (df = 6, 28)	63.5	51.5	—	81.6	59.8	—	14.2	78.9	133.2
P	<0.0001	<0.0001	—	<0.0001	<0.0001	—	<0.0001	<0.0001	<0.0001

5 replicates of 6 females and 6 males; [a] Per 15 cherries; [b] Days after exposure to insecticides; [c] Treated versus untreated; [d] Both not treated, but each in separate half of container. [e] Using 136 g a.i./ha (only rate different than in Table 1).
Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

Table 3. No-choice experiment: effects of kaolin on mean percentages of cherry fruit infested and mean numbers of eggs laid per fruit ± SE by *Rhagoletis indifferens* during 3-d periods

	% Cherries infested		
Fly age (days)	Control[a]	Kaolin[a]	Kaolin + food
4-7	12.5 ± 6.3	0.0 ± 0.0	0.0 ± 0.0
7-10	81.2 ± 18.8	14.6 ± 14.6	35.4 ± 23.2
10-13	100.0 ± 0.0	0.0 ± 0.0	20.8 ± 20.8
13-16	100.0 ± 0.0	8.3 ± 8.3	27.1 ± 21.1
16-19	97.9 ± 2.1	33.3 ± 18.2	8.3 ± 4.2
19-21	97.9 ± 2.1	4.2 ± 4.2	0.0 ± 0.0
Overall Means/3 d	81.6 ± 14.1a	10.1 ± 5.2b	15.3 ± 6.0b
Repeated-measures	Treatment	$F = 35.5$, df = 2, 6	$P = 0.0005$
ANOVA	Days	$F = 4.1$, df = 1, 44	$P = 0.0481$
	No. Eggs per Cherry		
Fly age (days)	Control[a]	Kaolin[a]	Kaolin + food
4-7	0.13 ± 0.06	0.0 ± 0.0	0.0 ± 0.0
7-10	4.39 ± 1.67	0.37 ± 0.37	0.87 ± 0.63
10-13	8.92 ± 1.23	0.0 ± 0.0	1.00 ± 1.00
13-16	8.79 ± 0.68	0.10 ± 0.10	0.98 ± 0.86
16-19	11.15 ± 1.22	0.52 ± 0.46	0.10 ± 0.06
19-21	5.40 ± 1.45	0.06 ± 0.06	0.0 ± 0.0
Overall Means/3 d	6.46 ± 1.62a	0.18 ± 0.09b	0.49 ± 0.21b
Repeated-measures	Treatment	$F = 45.4$, df = 2, 6	$P = 0.0002$
ANOVA	Days	$F = 5.3$, df = 1, 44	$P = 0.0265$

3 replicates of 6 male and 6 females and 16 cherries per replicate.
[a] No sucrose-yeast food in container.
Overall means followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

Nearly 100% of control fruit were infested at ≥10 d, whereas only 0-33.4% of treated fruit were infested at ≥10 d. Flies overall laid 36 and 13 times more eggs in untreated than in kaolin-treated cherries in the absence and presence of food strips, respectively. Flies exposed to control cherries laid the most eggs at 16-19 d of age, but flies exposed to treated cherries laid eggs irregularly (Table 3). At the conclusion of the 21-d test, 8.3 ± 8.3% (mean ± SE) of flies in the control had died, compared with 61.2 ± 5.9% and 57.8 ± 13.5% of flies in the treatment with food strips and in the treatment without food strips, respectively ($F = 9.3$; df = 2, 6; $P = 0.0146$).

Table 4. Choice experiment: effects of kaolin on mean percentages of cherry fruit infested and mean numbers of eggs laid per fruit ± SE by *Rhagoletis indifferens* during 3-d periods

	% Cherries infested		No. eggs per cherry	
Fly age (days)	Untreated	Kaolin treated	Untreated	Kaolin treated
4-7	4.2 ± 4.2	0.0 ± 0.0	0.04 ± 0.04	0.0 ± 0.0
7-10	58.3 ± 30.1	0.0 ± 0.0	3.25 ± 2.13	0.0 ± 0.0
10-13	91.7 ± 8.3	8.3 ± 4.2	5.96 ± 2.43	0.08 ± 0.04
13-16	87.5 ± 12.5	16.7 ± 4.2	11.50 ± 5.02	0.25 ± 0.08
16-19	95.8 ± 4.2	53.0 ± 14.2	7.72 ± 1.08	2.13 ± 1.09
19-21	95.8 ± 4.2	25.0 ± 12.5	5.73 ± 1.73	0.98 ± 0.56
Overall Means/3 d	72.2 ± 14.8a	17.2 ± 8.2b	5.70 ± 1.59a	0.57 ± 0.35b
Repeated-	Treatment	Days	Treatment	Days
Measures *F*	15.1	22.9	23.5	30.3
ANOVA df	1, 4	1, 29	1, 4	1, 29
P	0.0178	<0.0001	0.0083	<0.0001

3 replicates of 6 male and 6 females and 8 untreated and 8 treated fruit per replicate. Overall means followed by the same letter within parameters are not significantly different (Fisher's LSD test, $P > 0.05$).

Experiment 4: Effects of kaolin on oviposition choice. In the choice experiment using kaolin, flies overall laid 10 times more eggs in untreated than treated cherries (Table 4). Most eggs were laid in untreated and treated cherries at 16-19 d. During this period, up to 95.8% of untreated cherries but only 53.0% of treated cherries at most were infested (Table 4). Mean fly mortality ± SE at 21 d was 21.2 ± 11.6%.

Experiment 5: Effects of kaolin on adult and larval infestations. In the field experiment using kaolin, numbers of flies on control trees posttreatment were significantly greater than on kaolin-treated trees (orthogonal contrasts, $P < 0.05$) (Table 5). However, there were no differences in numbers trapped in 1 and 2 application treatments ($P > 0.05$). Numbers of fruit per tree did not differ among treatments ($P > 0.05$), but control trees produced 29 and 152 times more larvae than trees treated once and twice, respectively ($P < 0.05$). Trees treated twice yielded 5.2 times fewer larvae than trees treated once ($P < 0.05$). Trees treated once or twice yielded similar numbers of larvae/fruit ($P > 0.05$), and 96.1% or 99.0% fewer than in the control ($P < 0.05$) (Table 5).

Discussion

Experiment 1 results indicated that oviposition by *R. indifferens* can be reduced to similarly low levels using various insecticides with different toxicities. Although oviposition was reduced most using the most toxic insecticide, azinphos-methyl, oviposition was reduced to similar levels using spinosad bait, spinosad, imidacloprid, and thiamethoxam, even though imidacloprid was the least toxic of the 4 as measured by mortality at day 1. Compared with imidacloprid, spinosad and thiamethoxam were highly toxic to *R. indifferens* based on mortality rates on day 1. Results suggest that

Table 5. Effects of kaolin on mean numbers of adult *Rhagoletis indifferens* trapped on sticky yellow panels and on mean numbers of larvae ± SE in control and treated cherry trees in Moxee, WA, 2002

	No. Flies/Trap/Day		
Treatment	Pre-treatment	1 d after first spray	1 d after second spray
	14 to 17 June	20 to 21 June	27 to 28 June
Control	1.3 ± 0.5	3.7 ± 2.1	3.3 ± 1.4
1 Spray	1.7 ± 0.7	0.7 ± 0.5	0.2 ± 0.2
2 Sprays	1.1 ± 0.3	0.8 ± 0.5	0.2 ± 0.2
Randomized Block ANOVA			
F (df = 2, 10)	0.5	1.6	6.6
P	0.6012	0.2531	0.0148
	Larvae		
Treatment	No. Fruit/Tree[a]	No. Total Larvae/Tree	No. Larvae/Fruit
Control	998.8 ± 65.5	197.5 ± 14.7	0.205 ± 0.024
1 Spray	865.3 ± 81.3	6.7 ± 2.9	0.008 ± 0.003
2 Sprays	941.7 ± 97.8	1.3 ± 0.9	0.002 ± 0.001
Randomized Block ANOVA			
F (df = 2, 10)	0.8	241.9	78.4
P	0.4554	<0.0001	<0.0001

6 trees per treatment; [a] Picked 12 July.
Differences between control and treatments and between treatments analyzed using orthogonal contrasts (results in text).

spinosad bait, spinosad, and thiamethoxam reduced oviposition in *R. indifferens* mostly by killing females quickly (≤1 d), compared with imidacloprid, which apparently killed flies more slowly. Consistent with the results with *R. indifferens*, in *R. pomonella*, imidacloprid caused a relatively slow kill, with mortality highest 4 d after exposure (Hu and Prokopy 1998), and it reduced oviposition by ~95% in this species, even though only ~50% of females were killed after 48 h of exposure (Reissig 2003). Imidacloprid probably reduced the flies' tendency or ability to oviposit before killing them. In *R. pomonella*, it was suggested that imidacloprid does not kill eggs that are ready to be laid, although it may inhibit egg development (Hu and Prokopy 1998).

Indoxacarb, which caused similar mortality as imidacloprid after 1 d of exposure in experiment 1 and on all 3 d in experiment 2, did not reduce oviposition by *R. indifferens* as much as imidacloprid. Indoxacarb also did not reduce oviposition by *R. pomonella* except at very high concentrations (Reissig 2003). Imidacloprid mimics the action of acetylcholine in the nerve synapse (Cox 2001), whereas indoxacarb blocks neuronal sodium channels (Lapied et al. 2001), indicating different mechanisms of kill.

In observations during 2-min exposures of 10 individual female *R. indifferens* to dried indoxacarb (50 μl when wet) inside 5.0 × 1.4 cm glass vials, none showed effects, despite the flies feeding on stains of the indoxacarb, and all were alive after 1 d. However, 6 out of 10 females similarly exposed to imidacloprid suffered uncontrolled, rapid wing beating 0.12-1.93 min after exposure, followed by paralysis or no movement, although 4 of these 6 flies recovered 1 d later (W.L.Y., unpubl.). Because imidacloprid had more immediate (≤2 min) effects than indoxacarb, the similar mortality caused by the 2 probably resulted from the high numbers of feeds on indoxacarb over time.

The effects of the various insecticides on oviposition by *R. indifferens* were generally similar to those reported for other *Rhagoletis*, but some differences were notable. In *R. mendax*, oviposition scars were significantly more numerous in blueberries treated with imidacloprid than with spinosad and thiamethoxam, although fly mortality was not recorded (Liburd et al. 2003). In *R. pomonella*, thiamethoxam (label rate) killed only ~50% of flies after 48 h exposure, and unlike in *R. indifferens* in experiment 2, indoxacarb (above label rate) was less toxic to *R. pomonella* than imidacloprid (below label rate) (Reissig 2003). These comparisons suggest results using one *Rhagoletis* species cannot always be extrapolated to another, although variations in insecticide amounts will affect the results, as above label concentrations reduce oviposition more than label concentrations (Reissig 2003). Because it is undesirable to use higher insecticide rates, mixing insecticides with ingredients such as the synergist piperonyl butoxide (Environmental Protection Agency 2006) or the adjuvants SM-9, Kinetic, or Tween 60 (Mangan and Moreno 2001) to increase their toxicity, spread, or deterrent effect may be a preferred way to make them more effective.

In experiment 2, flies did not make a choice between untreated and treated cherries, except when the choice was untreated versus spinosad-baited cherries. This indicates that for materials other then spinosad bait, the insecticides did not immediately alter the oviposition behaviors of the flies. Except with spinosad bait, there was no deterrence, as deterrence would have resulted in higher infestations in untreated cherries. Mortalities in choice versus no-choice experiments were similar, suggesting that 100% insecticide coverage of cherries is not needed to reduce oviposition to low levels. Spinosad bait deterred oviposition likely because of its stickiness, and flies likely fed on the bait rather than oviposited.

In experiment 3, kaolin deterred oviposition by *R. indifferens* even after 21 d, when flies likely had many fully-developed eggs. High oviposition drive did not always overcome the deterrent effect of kaolin. Given no alternative, most flies still did not lay into the treated cherries. Flies probably retained their eggs, even in the presence of sucrose-yeast food, which increased egg loads, although the walls and bottoms of containers were not inspected to determine whether flies dumped their eggs. Despite the deterrence, flies were not prevented from ovipositing, suggesting that the irritant properties of kaolin need to be increased, that a thicker layer of kaolin needed to be applied, or that the cherries need to be completely covered with kaolin to achieve complete control.

Although kaolin primarily acted as an oviposition deterrent, it also caused mortality. Flies feed on sugar daily (Yee 2003b) and in feeding on sugars in the cherry juice, they probably also ingested kaolin particles daily. Mortality also was seen in the walnut husk fly, *Rhagoletis completa* Cresson, that ingested particles of diatomaceous earth, and it was suggested that undissolved particles ingested by flies caused mortality by abrading tissues in the alimentary canal (Boyce 1932).

In experiment 4, unlike with insecticides, most flies chose to oviposit in untreated rather than kaolin-treated cherries. Flies did oviposit in treated cherries when >88% of untreated cherries were infested, possibly because the oviposition-deterring pheromone (Mumtaz and AliNiazee 1983) deterred further oviposition on most kaolin-free cherries. It is unlikely, though, that the pheromone is a stronger deterrent than kaolin, given that 10 times more eggs were laid in untreated fruit and up to 45 eggs were laid within a single untreated fruit. In a choice test using *C. capitata*, no eggs was laid in kaolin-treated nectarines (Mazor and Erez 2004). The mortality of *R. indifferens* in the choice experiment was higher than that of controls in the no-choice experiment probably because flies tested were less vigorous. It is unlikely flies in the choice experiment encountered more kaolin.

In experiment 5, kaolin reduced adult and larval infestation rates in the field. Laboratory data suggest this was caused by kaolin deterring flies from ovipositing. There is a small possibility that flies were removed from trees during the sprays and did not return, or that flies did not recognize the trees because of the white residues. One kaolin application provided season-long suppression of larval *R. indifferens* in the trees, but 2 applications were more effective. This suggests kaolin from 1 application were lost even in the absence of rain and that the kaolin layer on fruit needs to be thicker than that achieved from 1 spray to maximize deterrence. Similar to the positive results in the present study, kaolin reduced the percentage apple damage by *R. pomonella* by 95.0% (Wright et al. 2000), and reduced numbers of *R. mendax* larvae in blueberry (Liburd et al. 2003). Also, kaolin almost completely prevented larval infestations of *C. ceratitis* in nectarines, apples, and persimmons (Mazor and Erez 2004), and reduced infestations by *B. oleae* in olives by 85.3% (Saour and Makee 2004). Kaolin is an option for homeowners who do not wish to spray their trees with insecticides, and may help reduce oviposition and prevent increases in *R. indifferens* populations. Isolated trees in residential areas are the ones most likely to be infested with flies, and because fruit from them are often never used, the residues should not present a problem. Most flies that are deterred may be unable to find other isolated cherry trees, and thus may not oviposit and add flies to populations.

In summary, results show oviposition by *R. indifferens* can be reduced to similar levels by materials with significantly different toxicities, such that spinosad bait, spinosad, imidacloprid, and thiamethoxam are equally effective. None except spinosad bait is an oviposition deterrent. The high levels of oviposition reduction indicate these insecticides can protect fruit from mature flies, even though they are likely more effective in preventing oviposition when used against reproductively immature flies. More work is needed to modify or improve the insecticides so that they can reduce oviposition as much as azinphos-methyl, and to determine if laboratory results extend to the field. In residential trees where none of these insecticides is desirable, kaolin may be useful in reducing oviposition and preventing buildup of *R. indifferens* populations.

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