Sucrose Mixed with Spinosad Enhances Kill and Reduces Oviposition of *Rhagoletis indifferens* (Diptera: Tephritidae) Under Low Food Availability[1]

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Abstract Whether sugar mixed with insecticides enhances kill of western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), may depend on insecticide rate and food availability. Here, we tested the hypothesis that sucrose mixed with the insecticide spinosad (in the Entrust® SC formulation) enhances kill of adults and reduces oviposition when food is scarce. Three- to 5-d-old flies were exposed to a low or high rate of dried spinosad or spinosad mixed with sucrose in the presence of (a) supplemental food in the form of yeast extract + sucrose (YE + S) and sweet cherries (*Prunus avium* [L.] L.) or (b) only sweet cherries. Cherries were a food source and an oviposition substrate. At the low spinosad rate, sucrose enhanced fly kill over spinosad alone under both food conditions the first 4 d or during all 7 d of experiments. At the high spinosad rate, sucrose enhanced kill only when supplemental food was absent. Sucrose-enhanced spinosad did not reduce oviposition versus spinosad alone at either spinosad rate when supplemental food was present but it did at both spinosad rates when only cherries were present. Results suggest that sucrose mixed with the formulation of spinosad tested here in low-volume sprays could be useful for managing *R. indifferens* in low-food environments, but it offers no benefit in preventing oviposition when applied in food-rich environments.

Key Words western cherry fruit fly, spinosyn insecticide, yeast extract + sucrose, sweet cherries, oviposition

Insecticides mixed with proteinaceous bait alone, with or without sugar, have been shown to be effective in killing or controlling tephritid fruit flies in numerous studies (e.g., Barnes and Ortega 1959; Burns et al. 2001; Mangan et al. 2006; McQuate et al. 2005; Steiner 1952; Yee 2006). Fewer papers have reported on the effects of insecticides mixed with sugar alone for managing or killing flies. In some reports, sugar added to insecticides did not enhance mortality of tephritid flies, for example, against oriental fruit fly *Bactrocera dorsalis* (Hendel) (Steiner 1952, Steiner and Hinman 1952), but the majority of reports suggests it does (e.g., Barry and Polavarapu 2004; Duan and Prokopy 1993; Myburgh and Stubbings 1950; Yee 2009; Yee and Alston 2012). Whether complex baits or simple sugar baits are used, a main goal of mixing them with insecticides is to reduce insecticide output into the

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environment. Baits with insecticides could attract flies to entice greater feeding on, or increase contact with, insecticides, reducing the need for high volume cover sprays (Chambers et al. 1974; Prokopy et al. 1992, 2003).

Western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), is a major quarantine pest of sweet cherry (*Prunus avium* [L.] L.) and tart cherry (*Prunus cerasus* L.) in the northwestern United States. The fly needs to be managed using insecticides because of the zero tolerance for larval infestations in fruit (Anonymous 1968). GF-120 NF Naturalyte Fruit Fly Bait (GF-120) and Entrust SC (Dow AgroSciences, Indianapolis, IN, USA) are relatively safe (Kollman 2002) and organic spinosad-based products that are widely used in cherry orchards to safeguard fruit from *R. indifferens* infestations. They can also be used to control *R. indifferens* in cherry trees outside orchards to reduce the threat of flies entering orchards (Alston and Murray 2015). In the case of GF-120, sugar is a key ingredient of a complex bait (Mangan et al. 2006) that stimulates feeding and ingestion of spinosad by *R. indifferens*. Synthetic pyrethroids kill *R. indifferens* faster than does spinosad (Yee 2009, 2011), but they may be harsher on nontarget insects such as honeybees (Sánchez-Bayo 2012).

Sucrose mixed with spinosad has been tested against *R. indifferens* (Yee 2009). Feeding on sucrose–insecticide baits may cause *R. indifferens* to ingest more toxin, killing flies more quickly than external contact alone (Yee and Alston 2006). However, mortality responses to insecticide–sucrose mixes in the laboratory can vary (Yee 2006, 2008, 2011) for unknown reasons. Sources of variability in fly responses to sucrose–insecticide mixes may include the rate of insecticides used and the hunger state of the flies, which is affected by food availability. Effects of hunger state alone on behavioral responses to baits or food-associated odors in tephritids have been examined, mostly by depriving flies of a protein source (Barry et al. 2003; Hendrichs et al. 1990; Prokopy et al. 1992; Prokopy et al. 2003; Vargas et al. 2002; Vargas and Prokopy 2006; Yee 2006, 2008) but also by depriving them of food altogether for short periods (Yee 2011; Yee and Chapman 2009). Despite this, how insecticide rate and hunger state together affect kill, as well as egg laying responses in *R. indifferens*, has never been determined.

In this study, the objective was to test the hypothesis that sucrose mixed with spinosad enhances kill of adults and reduces oviposition when food is scarce. This was accomplished by determining the effects of spinosad (in the Entrust SC formulation), and of sucrose mixed with spinosad, on kill and oviposition of *R. indifferens* under low and high food availability conditions at two spinosad rates.

Materials and Methods

Insect source and pretest conditions. *Rhagoletis indifferens* originated as larvae infesting sweet cherries collected in June and July 2012 from Kennewick in central Washington, United States. Pupae were stored at 3–4°C for 6–7 mo and then transferred to 21–23°C, 20–35% relative humidity (RH) and 16:8 L:D for adult emergence (same conditions for the experiments). Before testing, 30 males and 30 females were held together inside individual 1.9-l (10.2 cm diameter × 16.2 cm high) paper containers (cages) covered with tulle fabric. Food was provided on paper strips as dry 20% yeast extract (Hy-Yest© 412, powder, Sigma-Aldrich, St. Louis,

Table 1. Experimental conditions for Experiments (Expt) 1–4 evaluating mortality and oviposition of 3–5-d-old *Rhagoletis indifferens* after exposure to spinosad alone, sucrose alone, and spinosad + sucrose mix with and without yeast + sucrose (YE + S) food present.[a]

	Treatments				
Expt	1. Spinosad alone, mg/633 cm^2	2. Sucrose alone, mg/633 cm^2	3. Spinosad + sucrose mix, mg/633 cm^2		YE + S food present
			Spinosad	Sucrose	
1	0.0015 (low)	0.125	0.0015 (low)	0.125	Yes
2	0.0015 (low)	0.125	0.0015 (low)	0.125	No
3	0.030 (high)	0.125	0.030 (high)	0.125	Yes
4	0.030 (high)	0.125	0.030 (high)	0.125	No

[a] 633 cm^2 is the surface area inside test cages. YE + S, dried 20% yeast extract + 80% sucrose on a 30-cm^2 paper strip. Nine replicates for each of the three treatments in each experiment. Ripe sweet cherries present in all treatments.

MO, USA) combined with 80% sucrose (w:w) (YE + S). Water was provided in a glass vial through a cotton wick.

Experimental design. Four experiments were conducted using two spinosad rates in the presence or absence of YE + S food (Table 1). Entrust SC, which is 80% (w:w) spinosad (comprising 85% spinosyn A and 15% spinosyn D), was used as the source of spinosad (in this study, Entrust SC is referred to by the name of the active ingredient, "spinosad"). For each experiment, treatments were dried (a) spinosad alone, (b) 0.25% (w:w) sucrose-alone control, and (c) spinosad + 0.25% sucrose. Five 10-μl drops of each treatment solution were applied onto a 78.5-cm^2 (10 cm diameter × 0.8 cm high) plastic dish to simulate low spray coverage. Drops were dried at 21–22°C and ~30% RH for 24 h, at which time they became spots, before testing against flies. The two spinosad rates were 0.0015 mg and 0.030 mg per 633 cm^2, the area inside a 1.9-l cage, excluding the top. The 0.25% sucrose rate was chosen because it was practical for field use (cost effective, low sucrose residues when applied onto leaves) and tests had indicated it did not differ from 0.5% sucrose when used with spinosad for killing flies (data not shown). The 0.25% sucrose in solution translated to a total of 0.125 mg sucrose/633 cm^2.

For treatments with YE + S present, a 30 cm^2 (3.5 × 8.5 cm) paper strip with YE + S was clipped onto the side of a 1.9-l cage (same type as for pretest flies); no strip was placed for YE + S absent treatments (Table 1). Ten females and 10 males aged 3–5 d post eclosion were transferred into each experimental cage. At 23.9°C, *R. indifferens* begin laying eggs at 6 d post eclosion (Frick et al. 1954). Although only females damage fruit, males were included to simulate a natural condition where the sexes had access to each other for continued mating. A plastic dish with spinosad, sucrose alone, or spinosad + sucrose spots was slid into each cage. Five ripe ('Bing') sweet cherries (*P. avium*, obtained commercially from Chile) that had

Table 2. Kruskal-Wallis test results of numbers of dead female and male *R. indifferens* at days 1–7 after exposure to spinosad alone, sucrose alone, and spinosad + sucrose treatments with and without YE + S food present in Experiments 1–4. (df = 2 for all experiments and days.)

Kruskal-Wallis	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Experiment 1							
χ^2	9.57	15.29	18.03	17.42	16.79	17.90	19.28
P	0.0083	0.0005	0.0001	0.0002	0.0002	0.0001	<0.0001
Experiment 2							
χ^2	21.83	19.69	15.77	19.60	16.92	16.39	15.82
P	<0.0001	<0.0001	0.0004	<0.0001	0.0002	0.0003	0.0004
Experiment 3							
χ^2	17.49	16.64	17.40	22.02	21.11	21.18	19.34
P	0.0002	0.0002	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
Experiment 4							
χ^2	20.18	23.52	20.44	20.43	21.08	21.08	21.95
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

been washed with water and dried were placed next to the dish. Cherries were the only food source for flies in treatments without YE + S. Flies feed on juice from punctures made by females in cherries using their ovipositors or on juice that seeps out as cherries ripen (Yee 2003). At day 3, the five cherries were replaced with a new set of five for the remaining four test days (7 d total test period). Numbers of dead flies were recorded each day. After removal from the cage, cherries were preserved in 70% ethanol. Eggs, located ~1 mm beneath the cherry skin, were counted beneath the skin using a stereomicroscope (at 50×). Eggs from the 10 cherries per replicate were combined for analyses. For each of the four experiments, three trials were run, each on a separate day and each set up using three replicates.

Data analyses. Most data were not normal or had unequal variances (due to many zeroes in data collected), even after transformations, so analyses of variance were not performed. Instead, for each experiment, numbers of dead flies within each day of the 7-d experimental period and numbers of eggs laid were analyzed using the Kruskal-Wallis test. No differences were detected among the three trials (three replicates per trial) ($P > 0.05$, days as factors within treatments, Kruskal-Wallis test; e.g., in Experiment 1, numbers of dead flies at 7 d in the spinosad + sucrose treatment: $\chi^2 = 0.27$; df = 2; $P = 0.8722$), so analyses utilized nine replicates. Means of the ranks among treatments (rather than the data themselves)

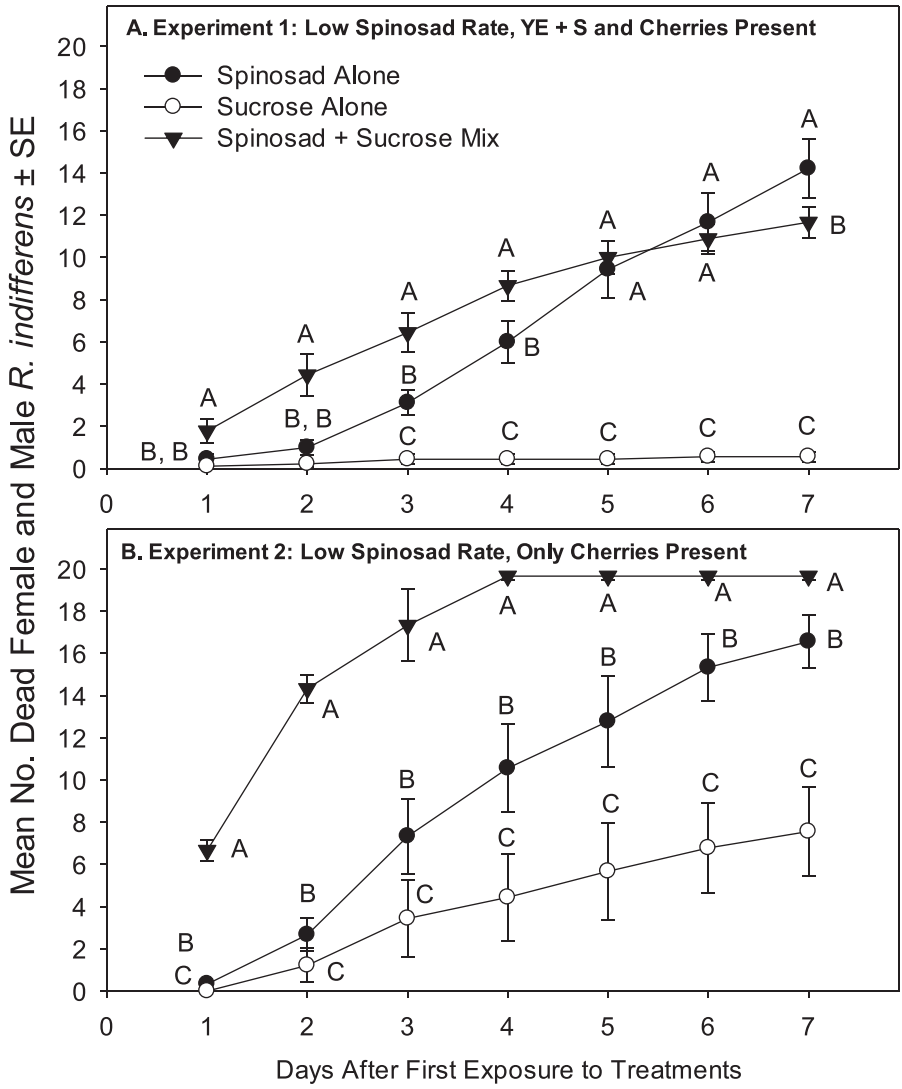

Fig. 1. Mean cumulative numbers of dead female and male *Rhagoletis indifferens* ± SE (maximum of 20 per replicate) exposed to a low spinosad rate (0.0015 mg/633 cm^2 cage): (A) Experiment 1 with yeast extract + sucrose (YE + S) and cherries present; (B) Experiment 2 with only cherries present. Although means are shown, letters closest to means with the same letters within each day indicate that mean rank scores are not significantly different (Tukey HSD test of ranks, $P > 0.05$).

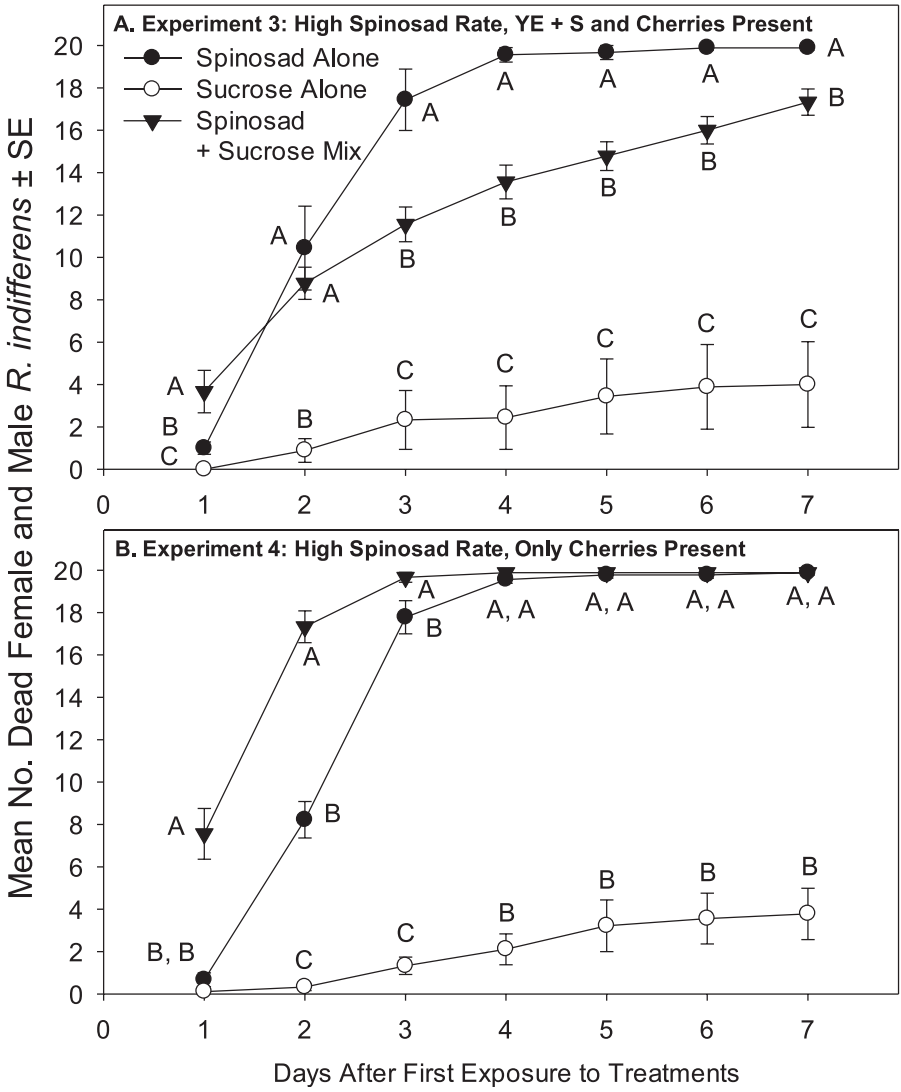

Fig. 2. Mean cumulative numbers of dead female and male *Rhagoletis indifferens* ± SE (maximum of 20 per replicate) exposed to a high spinosad rate (0.030 mg/633 cm^2 cage): (A) Experiment 3 with yeast extract + sucrose (YE + S) and cherries present; (B) Experiment 4 with only cherries present. Although means are shown, letters closest to means with the same letters within each day indicate that mean rank scores are not significantly different (Tukey HSD test of ranks, $P > 0.05$).

were separated using HSD Tukey tests following the rationale of Conover (1980). Data were analyzed using SAS Version 4.3 (SAS Institute Inc. 2010).

Results

Sucrose-enhanced kill. Female and male data are presented together, as kill patterns did not differ by sex ($P > 0.5$, Kruskal-Wallis test; e.g., in Experiment 1, numbers of dead female and male flies at 7 d in the spinosad alone treatment: $\chi^2 = 0.10$; df = 1; $P = 0.7516$). In all experiments, differences in adult mortality among treatments were detected on all days of the 7-d trial period (Table 2). Fly kill in the sucrose-alone control over 7 d was low in all experiments (Figs. 1, 2). In Experiment 1, using the low spinosad rate in the presence of YE + S and cherries (Fig. 1A), sucrose-enhanced kill of flies over spinosad alone was detected on days 1–4 but not days 5–7. However, in this experiment, mean kill in the spinosad + sucrose treatment never reached 100% (with a maximum kill of 20 flies). In Experiment 2, using the low spinosad rate in the presence of only cherries (Fig. 1B), sucrose-enhanced kill of flies over spinosad alone was detected on days 1–7 and mean kill in the spinosad + sucrose treatment reached 100% by day 4. In Experiment 3, using the high spinosad rate in the presence of YE + S and cherries (Fig. 2A), sucrose-enhanced kill of flies over spinosad alone was detected only on day 1. On days 3–7, more flies were killed in the spinosad alone than with the spinosad + sucrose treatment, the opposite of that seen in Experiment 2. Mean kill in the spinosad alone treatment reached 100% by day 4 whereas it peaked at 85% on day 7 in the spinosad + sucrose treatment. In Experiment 4, using the high spinosad rate in the presence of only cherries (Fig. 2B), sucrose-enhanced kill of flies over spinosad alone was detected on days 1–3; however, mean kill in both spinosad alone and spinosad + sucrose treatments reached or nearly reached 100% by day 4.

Sucrose-enhanced reduction in oviposition. Sucrose mixed with spinosad did not reduce ovposition compared with spinosad alone in Experiments 1 and 3 where YE + S and cherries were present, but it did in Experiments 2 and 4 where only cherries were present (Fig. 3). Specifically, in Experiment 1 (Fig. 3A), there were no differences in oviposition levels among the three treatments ($\chi^2 = 0.22$; df = 2; $P = 0.8980$). In Experiment 2 (Fig. 3B), flies in the spinosad + sucrose treatment laid fewer eggs than did flies in the spinosad alone and sucrose alone treatments ($\chi^2 = 14.29$; df = 2; $P = 0.0008$), and spinosad alone did not differ from the sucrose alone control. In Experiment 3 (Fig. 3C), flies in the spinosad alone treatment laid no eggs in cherries (corresponding to the greater kill in this treatment) whereas flies in the spinosad + sucrose treatment laid more eggs; flies in the sucrose alone treatment laid the most eggs ($\chi^2 = 14.52$; df = 2; $P = 0.0007$). In Experiment 4 (Fig. 3D), no eggs were laid by flies in the spinosad + sucrose treatment, which was statistically fewer than eggs laid by flies in the spinosad alone treatment; flies in the sucrose alone treatment laid the most eggs ($\chi^2 = 21.48$; df = 2; $P < 0.0001$).

Discussion

In this laboratory study, low food availability and low or high spinosad rates were identified as conditions that allowed for sucrose-enhanced kill of *R. indifferens*

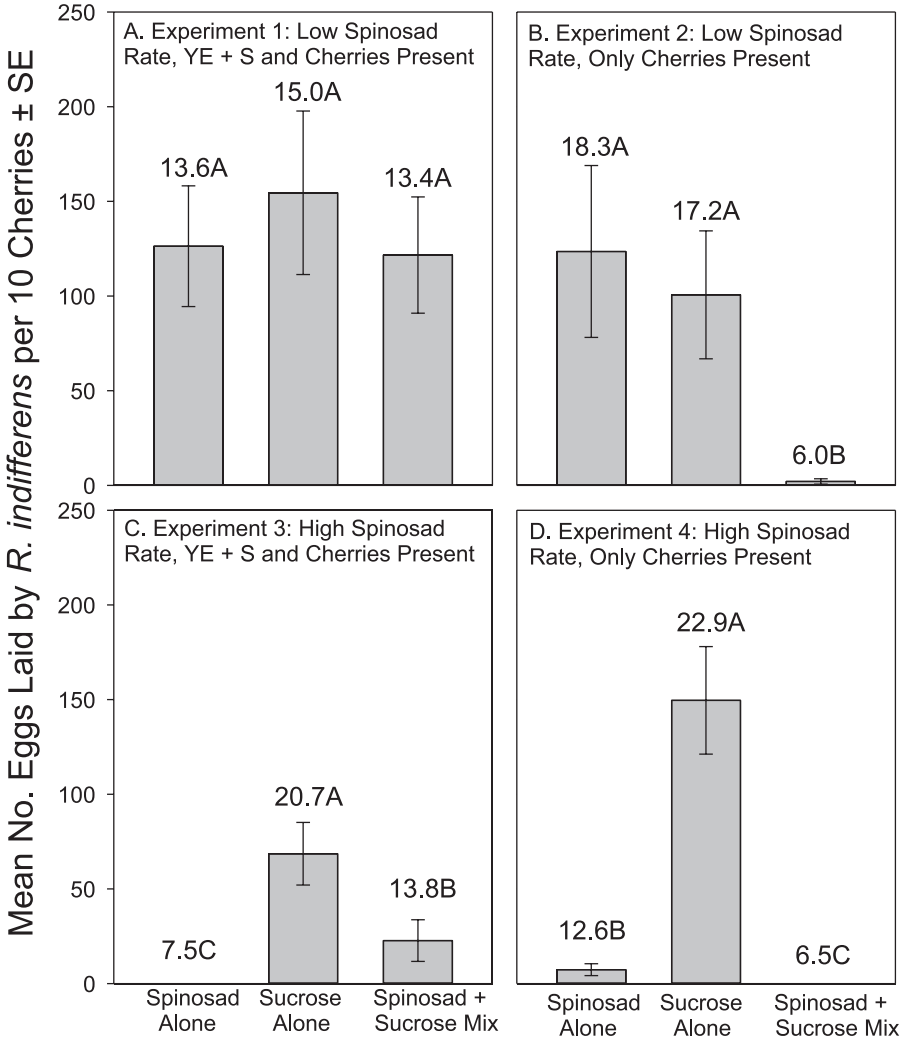

Fig. 3. Mean numbers of eggs laid by *Rhagoletis indifferens* ± SE per 10 cherry fruits in (A) Experiment 1, (B) Experiment 2, (C) Experiment 3, and (D) Experiment 4. Mean rank scores above bars with same letters are not significantly different (Tukey HSD test of ranks, $P > 0.05$). Low spinosad rate = 0.0015 mg/633 cm^2 cage; high spinosad rate = 0.030 mg/633 cm^2 cage.

under simulated conditions of low spinosad spray volume coverage. In Experiments 2 and 4 where supplemental food (YE + S) was absent, exposure to the low or high spinosad rate resulted in 100% fly kill in the spinosad + sucrose treatment. Correspondingly, there was sucrose-enhanced reduction in oviposition levels compared with spinosad alone in these experiments, with the high spinosad rate +

sucrose being the only treatment to eliminate oviposition altogether in Experiment 4. When YE + S was present in Experiments 1 and 3, sucrose-enhanced kill was observed for 1–4 d, but it was not sustained over time. For the low spinosad rate in Experiment 1, only 55% and 65% total mortality was achieved in the spinosad + sucrose and spinosad alone treatments, respectively.

Fly hunger state and foraging behaviors (Hendrichs et al. 1990, Prokopy et al. 1992) are likely responsible for the sucrose-enhanced kill and reduction in oviposition in Experiments 2 and 4. Juice from ripe sweet cherries provides *R. indifferens* with nutrients to survive 20–23 d and produce some eggs but provides less nutrition than YE + S (Yee 2003). Foraging behaviors, characterized by flies rapidly protruding their mouthparts onto surfaces while walking (Yee 2006, 2008), must be more intense in flies with access to cherries only, leading to more-lethal encounters with spinosad + sucrose. Such behaviors are probably common in all tephritids deprived of rich food sources (Prokopy et al. 1992, Barry et al. 2003).

Spinosad has residual contact and ingestion activity (Adán et al. 1996, Yee and Alston 2006), which means flies either walking or feeding on spinosad can be killed. Tarsal contact with spinosad + sucrose by flies deprived of YE + S may have resulted in more-immediate feeding responses than by flies with access to YE + S (e.g., dropping of labella in the eastern cherry fruit fly, *Rhagoletis cingulata* (Loew) [Frings and Frings 1952]). Flies ingesting spinosad are paralyzed within 4 h and 100% kill occurs within 24 h of feeding (Yee 2006, 2008, 2009). Therefore, flies deprived of YE + S died quickly and were unlikely to lay many if any eggs. Tarsal contact with spinosad alone at low or high rates by flies deprived of YE + S probably elicited no or weaker feeding responses. Consequently, no or less toxin was ingested, kill was reduced or delayed, and more eggs were laid.

Unlike Experiments 1, 2, and 4 where sucrose enhanced adult kill on at least 3 d of the 7-d trial period, in Experiment 3 at the high spinosad rate where YE + S and cherries were present, more flies were killed in the spinosad alone than spinosad + sucrose treatment from days 3–7, and spinosad alone also eliminated oviposition. A possible explanation is that spinosad in the spinosad alone treatment was more exposed to flies than in the spinosad + sucrose treatment using the Entrust SC formulation. Sucrose may have covered some of the spinosad in the mix because there was four times more of it by weight than the spinosad in Experiment 3.

Spinosad may have occurred unevenly in the dried spinosad + sucrose spots, with less of it in the edges than in the center. The center of spots was cloudy due to small particles that occupied 68% of the area, surrounded by a clear ring; spinosad alone spots were cloudy and sucrose alone spots were entirely clear. The cloudy center was likely mostly spinosad, as Entrust SC is 80% spinosad (mixed with 5.4% kaolin and silica gel) (Dow AgroSciences 2013), and the clear outer ring likely sucrose. There was probably less spinosad in the sucrose ring than in the center because it is less water soluble than sucrose (Charles 1960, Dow AgroSciences 2001). Unless it is mostly consumed, the spinosad + sucrose may not be as lethal to flies as spinosad alone, given equal numbers of contacts, especially if only parts of the clear ring are ingested.

Similar to Experiment 3, fly kill in the high rate spinosad alone treatment in Experiment 4 reached 100% by day 5. However, kill before day 5 was lower in Experiment 4, and some oviposition occurred in the spinosad alone treatment while none did in Experiment 3. The spinosad alone result in Experiment 4 could be due to some flies deprived of YE + S touching the spinosad alone spots more briefly

than did flies with access to YE + S. A greater hunger state could lead flies to walk more rapidly over the spots, avoiding prolonged contact and rapid kill.

Results suggest that sucrose mixed with the formulation of spinosad tested here in low volume sprays could be useful for managing *R. indifferens* in low-food environments but offers no benefit in preventing oviposition when applied in food-rich environments. In commercial orchards and unmanaged cherry trees, food probably is not as abundant as in this laboratory study, where YE + S was constantly available, or as limited as when only ripe cherries were available in Experiments 2 and 4. Thus, determining food availability in managed cherry orchards and unmanaged urban and feral cherry trees, for example, by analyzing cherry leaf surfaces for sugars (Yee and Chapman 2008), would be a useful step for assessing the value of using sucrose with spinosad for managing *R. indifferens* in a variety of cherry tree settings.

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