Spinosad Versus Spinetoram Effects on Kill and Oviposition of Rhagoletis indifferens (Diptera: Tephritidae) at Differing Fly Ages and Temperatures

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Abstract

Western cherry fruit fly, Rhagoletis indifferens Curran (Diptera: Tephritidae), is a major quarantine pest of cherries (Prunus spp.) in western North America that is often managed using the organic insecticide spinosad, but there is a question of whether its semisynthetic relative spinetoram is more toxic and better to use for controlling the fly. Here, spinosad and spinetoram effects on R. indifferens kill and oviposition were determined by exposing 3–4, 7–10, or 14–18 d old flies to dry spinosad and spinetoram (0.21 or 0.33 mg active ingredient [a.i.] per dish) and untreated cherries or to insecticide-treated cherries at 15.6, 22.5, and 29.4°C. Kill was not affected by fly age. Spinetoram killed more female flies by day 1 than at all temperatures. In both treatments, kill was lower at 15.6°C than 22.5 and 29.4°C, although a difference between 22.5 and 29.4°C was detected more often in spinosad treatments. Both insecticides killed 3–4 d old flies quickly enough to prevent oviposition, but neither prevented oviposition by 7–10 and 14–18 d old flies. Significantly, oviposition by flies exposed to spinosad and spinetoram did not differ at any temperature. Results indicate spinetoram is more toxic to R. indifferens than spinosad. However, this higher toxicity is not needed to prevent oviposition by younger flies. Furthermore, spinetoram residues are not sufficiently toxic to kill older flies quickly enough to reduce oviposition more than spinosad. Taken together, these conclusions imply that spinosad and spinetoram are equal for controlling R. indifferens infestations.

Key words: western cherry fruit fly, Entrust SC insecticide, Delegate WG insecticide

Western cherry fruit fly, Rhagoletis indifferens Curran (Diptera: Tephritidae), is a major quarantine pest of cherries (Prunus spp.) in western North America, including the Pacific Northwest of the United States (Washington, Oregon, Idaho, Montana, and Utah) and British Columbia in Canada. In the Pacific Northwest in 2017, the value of sweet cherry (Prunus avium [L.] L.) was US$1 billion (Northwest Horticultural Council 2018). The fly must be controlled using insecticides because of the zero tolerance for its larvae in cherries destined for all domestic and foreign markets (Smith 2005). The organic insecticide spinosad is often used to manage the fly in organic and conventional systems. Spinosad is derived from fermentation products of the bacterium Saccharopolyspora spinosa Mertz & Yao and consists of spinosyns A and D in a 5.67:1 ratio (Dripps et al. 2008a). It is desirable to use due to its low mammalian toxicity, safe environmental profile (Dow AgroSciences 2001), high toxicity to R. indifferens (Yee 2011, 2015; Yee and Alston 2016), and its effectiveness in the field (Flye Sainte Marie 2003). However, spinosad may not always eliminate infestations in trees when there are outside sources of flies (Yee 2006).

In 2007, the semisynthetic spinosyn spinetoram, consisting of spinosyns J and L in an approximate 3:1 ratio, was registered under Environmental Protection Agency’s Reduced Risk Pesticide Initiative (Dripps et al. 2008a,b). Spinetoram is reportedly more potent, faster-acting, and longer-lasting than spinosad and controls a wider range of insect pests in fruits and vegetables (Dripps et al. 2008a,b; Bacci et al. 2016). However, spinetoram and spinosad are considered toxicologically equivalent (EPA 2009) with apparently the same mechanism of action (Shimokawatoko et al. 2012). This raises the question of whether spinetoram is more toxic to R. indifferens and better to use for controlling it. There are no studies comparing the two against R. indifferens.

Work comparing spinosad with spinetoram against a wide range of insect taxa has recently been published (e.g., Abdel-Latif and Abdu-Allah 2011, Besard et al. 2011, Hamm et al. 2015, Bhatta et al. 2016, Siebert et al. 2016, Andreazza et al. 2017). However, little work in this area has been done for tephritid fruit flies. Against apple maggot fly, Rhagoletis pomonella (Walsh), spinetoram aged for 7 d in the field caused less than or as much mortality as spinosad in GF-120 bait aged for 7 d (Yee et al. 2007), but it is unknown if the...
protein and sugar components in GF-120 increase or decrease the toxicity of spinosad. Spinosad and spinetoram in soil drenches were similarly effective in preventing emergence of three tropical fruit fly species (Stark et al. 2013). In a recent study, spinosad was found to be 2–36 times as toxic as spinetoram when combined with different hydrolyzed proteins against South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Schutze et al. 2018). However, as alluded to, the role of protein bait in affecting the efficacies of spinosyns is unknown.

For *R. indifferens* control, timings of spinosad and spinetoram applications during the cherry season from May to July are critical. Timing of applications affects control of flies of different ages and possible efficacies of applications due to variations in seasonal temperatures. While it is unknown if kill caused by insecticides is affected by fly age, fly kill caused by spinosad is lower at cooler than higher temperatures in the laboratory (Yee 2015, 2017). The mechanism for lower kill appears to be reduced movement by flies at cooler temperatures, such that these flies contact localized spinosad residues less rapidly than more active flies at warmer temperatures (Yee 2015). Fly contact with scattered spray residues through movement could determine control levels in the field, as insecticide spray coverage of cherry trees is not likely to be complete and is dependent on application method and row locations (Rothwell et al. 2017). Whether temperature influences the effects of spinetoram on kill

![Graphs showing mean cumulative numbers of dead female Rhagoletis indifferens ± SE in controls and low and high rate spinosad and spinetoram treatments at 15.6°C for (A) 3–4 and (B) 14–18 d old flies, at 22.5°C for (C) 3–4 and (D) 14–18 d old flies, and at 29.4°C for (E) 3–4 and (F) 14–18 d old flies at 1, 3, and 5 d after exposure. Maximum is 10 females.](image)

*Fig. 1.* Experiment 1: mean cumulative numbers of dead female *Rhagoletis indifferens* ± SE in controls and low and high rate spinosad and spinetoram treatments at 15.6°C for (A) 3–4 and (B) 14–18 d old flies, at 22.5°C for (C) 3–4 and (D) 14–18 d old flies, and at 29.4°C for (E) 3–4 and (F) 14–18 d old flies at 1, 3, and 5 d after exposure. Maximum is 10 females.
and oviposition of *R. indifferens* the same way it does of spinosad is unknown. Temperature effects on the more toxic malathion (vs spinosad) were absent (Yee 2017). If spinetoram is more toxic than spinosad, then its effect on kill may be less impacted by temperature than that of spinosad.

For *R. indifferens*, oviposition is the measure of damage done by the fly, assuming the eggs hatch and produce larvae that feed on cherry flesh. Timing of and levels of oviposition by *R. indifferens* are affected by fly age and temperature (Frick et al. 1954; Yee 2015, 2017). At 26.7, 32.2, and 15.6–18.3°C in the laboratory, the minimum preoviposition periods of *R. indifferens* are 5–8 (post-eclosion), 5–7, and 7–21 d, respectively (Frick et al. 1954). Thus, to prevent oviposition, toxicities of spinosad and spinetoram need only be high enough to kill flies before they reach 5 d old. Lower temperatures reduce oviposition levels by *R. indifferens* exposed to spinosad, and they may also do this to flies exposed to spinetoram, potentially affecting fly control, although there are no data for this.

The objective of this study was to determine spinosad versus spinetoram effects on kill and oviposition of *R. indifferens* at differing fly ages and temperatures. Four hypotheses were tested: 1) spinetoram is more toxic than spinosad to *R. indifferens* of all fly ages; 2) for spinosad and spinetoram, lower temperatures result in lower kill and oviposition; 3) the performance of spinosad is more affected by temperature than that of spinetoram; 4) spinosad is toxic enough despite the greater toxicity of spinetoram to kill and prevent oviposition by younger flies. Possible significance of results for control of *R. indifferens* is discussed.

**Materials and Methods**

**Files and Pretest Conditions**

Files used in experiments originated as larvae infesting cherries in backyard trees in Kennewick in central Washington in June and July 2015 and 2017. Puparia were held at 3–4°C for ~6 mo and then transferred to 21–22°C for adult emergence before testing. Flies upon emergence were maintained inside 1.9-liter (10.2 cm diameter × 16.2 cm high) paper containers with tulle fabric covering at a density of ~30 males and 30 females at ~23°C, 16:8 L:D h, and 20–35% RH. Dry 80% sucrose and 20% yeast extract (by weight) (Hy-Yest 412, powder, Sigma-Aldrich, St. Louis, MO) food on paper strips and water in cotton wicks were provided to flies up to and during testing.

**Insecticide Sources and Rates and Source of Cherries**

The source of spinosad was Entrust SC (suspension concentrate) insecticide (22.5% spinosad; 77.5% other ingredients; 0.24 kg active ingredient [a.i.] per liter); the source of spinetoram was Delegate WG insecticide (water dispersible granule) (25% spinetoram; 75% other ingredients) (both Dow AgroSciences, Indianapolis, IN). Low and high rates tested were equivalent to label rates for Entrust of 292 and 584 ml/ha and for Delegate of 315 and 490 g/ha, both at 935 liters water per hectare. Sweet cherries (~2.5 cm diameter) used for tests originated from Chile and were purchased from a local market and were held in modified atmosphere packaging at 3°C to retain their freshness. Cherries were not organic and were washed in water to remove potential insecticide residues and then dried for 1–2 h at 21°C before use. Any insecticides that penetrated the fruit cuticle would not have affected results, as insecticides inside fruit would affect larval development rather than oviposition, which was measured here. In addition, control flies laid many eggs in these cherries. Finally, flies can survive >50 d when exposed to such cherries (Yee 2003), indicating no detrimental effect of using them. All cherries were firm at beginning of tests.

**Experiment 1: Spinosad and Spinetoram Effects at Three Temperatures, 3–4 and 14–18 d Old Flies**

This experiment was conducted in 2016 using 10 male (for mating) and 10 female flies held inside a 1.9-liter container (same type as during pretesting with food and water as described above), five cherries, and a dish with dry spinosad or spinetoram. Each container was placed in a BOD low temperature incubator (VWR, Radnor, PA) set to a constant 15.6, 22.5, or 29.4°C (~0.5°C, range). Controls and low and high rates of spinosad and of spinetoram were tested (four insecticide treatments) at each of the three temperatures using 3–4 d old and 14–18 d old flies. Five replicates of controls and treatments were set up.

Low or high rate spinosad or spinetoram solutions were applied as five 500-µl drops onto a 9.8-cm diameter Petri dish and then allowed to dry on the dish for 1 d at 21–22°C. The amount of both dry spinosad and dry spinetoram per dish for the low rate was 0.21 mg a.i.; for the high rate, 0.33 mg a.i. The 2,500 µl total volume was chosen because past work using 1.9-liter containers (Yee 2015) suggested that lower volumes (maximum of 1,200 µl) were insufficient to cause most flies to contact spinosad quickly enough to be killed. In the current study, volumes >2,500 µl were not tested.

**Table 1. Experiment 1: analysis of treatment effects on 3–4 and 14–18 d old female *Rhagoletis indifferens* exposed to dry residues of spinosad and spinetoram in dishes: three-way ANOVA for numbers of dead flies (spinosad and spinetoram treatments only) and two-way ANOVA for oviposition (control, spinosad, and spinetoram treatments; fly age not included because 3–4 d old flies laid no eggs)**

| Source                                      | F-value | df     | P-value |
|----------------------------------------------|---------|--------|---------|
| No. dead females by day 1 (three-way ANOVA) |         |        |         |
| Treatment                                   | 9.17    | 3, 96  | <0.0001 |
| Fly age                                     | 1.13    | 1, 96  | 0.2894  |
| Temperature                                 | 322.59  | 2, 96  | <0.0001 |
| Treatment × fly age                         | 0.43    | 3, 96  | 0.7349  |
| Treatment × temperature                     | 0.86    | 6, 96  | 0.5259  |
| Fly age × temperature                       | 1.00    | 2, 96  | 0.3709  |
| Treatment × fly age × temperature           | 0.86    | 6, 96  | 0.5261  |
| Oviposition—14–18 d old flies only (two-way ANOVA) |         |        |         |
| Treatment                                   | 141.69  | 4, 60  | <0.0001 |
| Temperature                                 | 52.09   | 2, 60  | <0.0001 |
| Treatment × temperature                     | 15.18   | 8, 60  | <0.0001 |
| Treatment × temperature effect sliced by temperature | 15.6°C | 7.30   | 4, 60   | <0.0001 |
| 22.5°C                                      | 65.75   | 4, 60  | <0.0001 |
| 29.4°C                                      | 99.00   | 4, 60  | <0.0001 |
| Treatment × temperature effect sliced by treatment | Control | 99.46  | 2, 60  | <0.0001 |
| Low spinosad                                | 2.91    | 2, 60  | 0.0624  |
| High spinosad                               | 3.83    | 2, 60  | 0.0272  |
| Low spinetoram                              | 5.71    | 2, 60  | 0.0054  |
| High spinetoram                             | 0.90    | 2, 60  | 0.4108  |

aExcluded control because there were zero deaths by day 1 in five of six control treatments (three temperatures × two fly ages).

bSliced because of significant treatment × temperature interaction (terminology of Schabenberger et al. 2000).
because the aim was to tease apart any toxicity differences between spinosad and spinetoram, which may not have been detectable had higher volumes been used.

The control dish or dish with insecticide was placed on the bottom of a container and then cherries placed around the dish. Flies were introduced into the container, and the container placed in one of the three incubators. Each test ran for 10 d. A new set of five cherries was placed into the container at day 3 and at day 7. Numbers of dead flies were recorded on days 1, 3, 5, 7, 9, and 10. Flies were considered dead if they were unable to walk. All cherries upon removal from containers were preserved in 70% ethanol. After at least 3 d, cherries were examined under a microscope at 50× for eggs. Storage in ethanol removed the dark cherry skin pigments, allowing the white eggs found below the surface (≤1 mm) to be easily counted.

**Experiment 2: Spinosad and Spinetoram Effects at Three Temperatures, 7–10 and 14–18 d Old Flies**

An experiment similar to Experiment 1 was conducted in 2018. The major difference was that 7–10 d old flies were tested in place of 3–4 d old flies. Flies 14–18 d old were tested as before. In addition, numbers of dead flies were checked on fewer days, at days 1, 3, 7, and 10, because results of Experiment 1 indicated that most flies in treatments (dependent on temperature) were dead by day 3. Five replicates of controls and treatments were set up.

**Fig. 2.** Experiment 1: mean numbers of eggs laid by *Rhagoletis indifferentens* ± SE starting at 3–4 d old for (A) controls and (B) low and high rate spinosad and spinetoram treatments at 15.6, 22.5, and 29.4°C and at 14–18 d old for (C) controls and (D) low and high rate spinosad and spinetoram treatments at 15.6, 22.5, and 29.4°C. Note 12 times greater y-axis scale for control than treatments.

**Statistical Analyses**

Numbers of dead female flies and numbers of eggs laid were square-root transformed to normalize the data and homogenize the variances (males do no damage, so male data were not analyzed). Data of dead females by day 1 after exposure in Experiments 1 and 2 were analyzed using a three-way analysis of variance (ANOVA), with treatment (control and the four insecticide treatments), temperature, and fly age as factors. In Experiment 1, there were no dead females in the five control
groups, so controls had no variance and were not included in the analysis. Also, in Experiments 1 and 2, data of dead females by day 3 were analyzed using one-way ANOVA for just 15.6°C because all flies in some treatments at 22.5 and 29.4°C were dead by then (and after 3 d), resulting in no variance. Data of dead females in Experiment 3 for days 1–4 were analyzed using two-way ANOVA. Oviposition data from Experiment 1 were analyzed using a two-way ANOVA, dropping fly age as a factor because flies in the 3–4 d old treatment laid no eggs. Oviposition data from Experiments 2 and 3 were analyzed using a three-way and two-way ANOVA, respectively. When there were significant interactions, simple main effects were analyzed using slices following methods described in Schabenberger et al. (2000).

Pairwise comparisons were then conducted within significant slices using one-way ANOVA and LSD tests. Analyses were performed using the PROC GLM procedure in SAS 9.4 (SAS Institute Inc. 2015).

Results

Experiment 1: Spinosad and Spinetoram Effects at Three Temperatures, 3–4 and 14–18 d Old Flies

Kill by insecticides at different fly ages

Numbers of dead females are shown for days 1, 3, and 5 and not for days 7–10 (Fig. 1), as almost all flies across treatments were dead.

Fig. 3. Experiment 2: mean cumulative numbers of dead female Rhagoletis indifferens ± SE in controls and low and high rate spinosad and spinetoram treatments at 15.6°C for (A) 7–10 and (B) 14–18 d old flies, at 22.5°C for (C) 7–10 and (D) 14–18 d old flies, and at 29.4°C for (E) 7–10 and (F) 14–18 d old flies at 1, 3, and 7 d after exposure. Maximum is 10 females.
by day 7. There was no fly age effect on kill (Table 1) and for both 3–4 and 14–18 d old flies the two spinetoram treatments killed more females by day 1 than the spinosad treatments at 15.6 and 22.5°C (Table 1, Fig. 1). In addition, the high spinosad treatment killed more females by day 1 than the low spinosad treatment (critical \( t = 1.985; \) LSD = 0.2301; \( P < 0.05 \)). By day 3, numbers of dead 3–4 d old females at 15.6°C (Fig. 1A) were greater in the high spinetoram than both spinosad treatments (\( F = 17.37; \) df = 4, 20; \( P < 0.0001 \)). By day 3, numbers of dead 14–18 d old females at 15.6°C (Fig. 1B) were greater in both spinetoram treatments than the low spinosad treatment (\( F = 3.42; \) df = 3, 16; \( P = 0.0428 \); controls left out, no variance). However, by days 3 and 5 at 22.5 and 29.4°C, there were >9 dead females (maximum of 10) across all insecticide treatments (Fig. 1C–F), which can be considered similar and not significant.

**Experiment 2: Spinosad and Spinetoram Effects at Three Temperatures, 7–10 and 14–18 d Old Flies**

**Kill by insecticides at different fly ages**

Numbers of dead females are shown for days 1, 3, and 7 (Fig. 3) and not for day 10 because most flies were dead by then. There was no age effect on fly kill (Table 2). In both 7–10 and 14–18 d old flies by day 1, low and high spinetoram treatments killed more females than low and high spinosad treatments at all temperatures (no age × treatment interaction) (critical \( t = 1.980; \) LSD = 0.2211; \( P < 0.05 \)) (Table 2, Fig. 3).

By day 3 at 15.6°C, both spinetoram treatments had killed more 7–10 d old females than both spinosad treatments (\( F = 4.43; \) df = 3, 16; \( P = 0.0190 \); controls left out, no variance) (Fig. 3A), as was the case at 15.6°C for 14–18 d old females (\( F = 26.04; \) df = 4, 20; \( P < 0.0001 \)) (Fig. 3B). However, by day 3 at 22.5 and 29.4°C, there were no numerical differences in kill among treatments (Fig. 3C–F).

**Oviposition**

Within the low spinetoram treatment, oviposition was lower at 15.6°C than at 29.4°C (\( F = 5.78; \) df = 2, 12; \( P = 0.0088 \)). Within the high spinosad treatment, oviposition was between the control and all insecticide treatments (Fig. 3B), which can be considered similar and not significant.

Within 15.6 and 22.5°C, there were no differences in oviposition among spinosad and spinetoram treatments (\( P > 0.05 \)). However, at 29.4°C, oviposition was lower in the low spinetoram than high spinosad treatment (\( F = 82.38; \) df = 4, 20; \( P < 0.0001 \)). There was no oviposition effect in the control than insecticide treatments (only 14–18 d old flies: 15.6°C: \( F = 9.00; \) P = 0.0003; 22.5°C: \( F = 66.61; \) P < 0.0001; 29.4°C: \( F = 82.38; \) P < 0.0001; df for all = 4, 20). Within 15.6 and 22.5°C, there were no differences in oviposition among spinosad and spinetoram treatments (\( P > 0.05 \)). However, at 29.4°C, oviposition was lower in the low spinetoram than high spinosad treatment (\( F = 82.38; \) df = 4, 20; \( P < 0.0001 \)) (Fig. 2D), the only case in this study where spinetoram significantly reduced oviposition more than spinosad.

Within control 14–18 d old females was lower at 15.6°C than at 22.5 and 29.4°C (no difference between 22.5 and 29.4°C) (\( F = 58.71; \) df = 2, 12; \( P < 0.0001 \)) (last subheading in Table 1, Fig. 2C). Within the high spinetoram treatment, oviposition was lower at 15.6°C than at 29.4°C (\( F = 7.20; \) df = 2, 12; \( P = 0.0088 \)). Within the low spinetoram treatment, oviposition was lower at 15.6°C than at 22.5°C (\( F = 5.78; \) df = 2, 12; \( P = 0.0175 \)) (Table 1, Fig. 2D).

**Experiment 3: Spinosad and Spinetoram Effects at Three Temperatures, 7–8 and 7–10 d Old Flies**

**Kill by insecticides**

There were no differences in kill between the control and spinosad and spinetoram treatments (\( P > 0.05 \))
However, at 22.5°C by day 1, spinetoram killed more females than spinosad (both more than the control) \( F = 28.67; \text{df} = 2, 12; P < 0.0001 \) (Fig. 5B), although at 29.4°C by day 1, there was no difference between spinosad and spinetoram (although both more than the control; \( F = 80.33; \text{df} = 2, 12; P < 0.0001 \)) (Table 3, Fig. 5C). By days 2–4, there were no differences between spinosad and spinetoram at any temperatures \( P > 0.05 \).

### Kill among temperatures

By day 1, there was no difference in deaths within control flies among the three temperatures (Table 3, Fig. 5), but kill by spinosad at 15.6°C was lower than at 22.5°C, which was lower than at 29.4°C \( F = 29.45; \text{df} = 2, 12; P < 0.0001 \). In contrast, for spinetoram by day 1, kill at 15.6°C was lower than at 22.5 and 29.4°C \( F = 14.96; \text{df} = 2, 12; P = 0.0005 \), but kill at 22.5 and 29.4°C did not differ (Fig. 5). This same pattern was true by day 2, but by days 3 and 4, differences in kill by spinosad and spinetoram across temperatures did not differ significantly \( P > 0.05 \).

### Oviposition

There were differences in oviposition between the control and two insecticide treatments, but there was also a significant interaction (Table 3, Fig. 6). Specifically, there was no difference at 15.6°C but there were significant differences at 22.5 and 29.4°C, but only between the control and the two insecticide treatments \( F = 19.09; \text{df} = 2, 12; P = 0.0002; 29.4°C: F = 24.29; \text{df} = 2, 12; P < 0.0001 \) (penultimate subheading in Table 3, Fig. 6). Within the control (last subheading in Table 3, Fig. 6A), oviposition was greater at 22.5 and 29.4°C than at 15.6°C \( F = 15.61; \text{df} = 2, 12; P = 0.005 \).

However, no statistically significant temperature effects within each insecticide treatment were detected \( P > 0.05 \), even though oviposition was numerically greater at higher temperatures (Fig. 6B).

### Discussion

Results show that spinetoram is more toxic to *R. indifferentes* than spinosad, as measured by fly kill using the protocol here, and that all age groups of flies are similarly affected. Spinetoram is also more...
toxic than spinosad against many insects (Dripps et al. 2008a,b; Abdel-Latif and Abdu-Allah 2011; Hamm et al. 2015; Siebert et al. 2016; Andreazza et al. 2017), but possibly not against all (Besard et al. 2011, Athanassiou and Kavallieratos 2014, Bhatta et al. 2016), including fruit flies (Schutze et al. 2018). For nontarget insects, whether spinetoram is more toxic than spinosad appears to depend on the insect taxa (Lefkaditis et al. 2017). In sum, results here were not entirely predictable based on the literature.

The reason for the greater toxicity of spinetoram against \emph{R. indifferens} is not clear based on its reported mechanism of action. Spinetoram affects nicotinic acetylcholine receptors and \( \gamma \)-aminobutyric acid receptors on postsynaptic membranes in insect nervous systems, causing abnormal neural transmission and death (Shimokawatoko et al. 2012). This is the same mechanism proposed earlier for spinosad (Salgado 1998, Orr et al. 2006, 2009). Spinetoram may disrupt the function of nicotinic acetylcholine and \( \gamma \)-aminobutyric acid receptors in \emph{R. indifferens} faster than spinosad does.

Cooler temperatures resulted in lower kill of \emph{R. indifferens} exposed to both spinosad and spinetoram. Based on results at 18.3°C versus 23.9°C (Yee 2015), this was probably due to flies being less active at 15.6°C than at 22.5 and 29.4°C. Less active flies contacted the insecticides in the dishes or on the cherries later or at lower doses than more active flies. An alternative explanation that toxicities of insecticides were reduced at cooler temperatures is less likely. For example, the toxicity of spinosad fed to houseflies (\emph{Musca domestica} L.), (Diptera: Muscidae) decreased 3.16-fold as temperatures increased from 20 to 34°C (Khan and Akram 2014). When pyrethroids were applied onto houseflies, kill was greater at 18°C than 32°C (Scott and Georgiou 1984).

Table 3. Experiment 3: analysis of treatment effects on 7–8 old female \emph{Rhagoletis indifferens} exposed to cherries with dry residues of spinosad and spinetoram: two-way ANOVA for numbers of dead female flies and oviposition (control, spinosad, and spinetoram treatments for both)

| Source | F-value | df | P-value |
|--------|---------|----|---------|
| No. dead females by day 1 (two-way ANOVA) | | | |
| Treatment | 60.76 | 2, 36 | <0.0001 |
| Temperature | 29.69 | 2, 36 | <0.0001 |
| Treatment × temperature | 10.01 | 4, 36 | <0.0001 |
| Treatment × temperature effect sliced by temperature<sup>a</sup> | | | |
| 15.6°C | 2.16 | 2, 36 | 0.1302 |
| 22.5°C | 23.40 | 2, 36 | <0.0001 |
| 29.4°C | 55.22 | 2, 36 | <0.0001 |
| Treatment × temperature effect sliced by treatment<sup>a</sup> | | | |
| Control | 0.29 | 2, 36 | 0.7502 |
| Low spinosad | 26.84 | 2, 36 | <0.0001 |
| Low spinetoram | 22.58 | 2, 36 | <0.0001 |

Oviposition (two-way ANOVA)

| Source | F-value | df | P-value |
|--------|---------|----|---------|
| Treatment | 40.23 | 2, 36 | <0.0001 |
| Temperature | 19.52 | 2, 36 | <0.0001 |
| Treatment × temperature | 8.56 | 4, 36 | <0.0001 |
| Treatment × temperature effect sliced by temperature<sup>a</sup> | | | |
| 15.6°C | 0.49 | 2, 36 | 0.6142 |
| 22.5°C | 22.12 | 2, 36 | <0.0001 |
| 29.4°C | 34.74 | 2, 36 | <0.0001 |
| Treatment × temperature effect sliced by treatment<sup>a</sup> | | | |
| Control | 34.44 | 2, 36 | <0.0001 |
| Low spinosad | 0.52 | 2, 36 | 0.5996 |
| Low spinetoram | 1.69 | 2, 36 | 0.1992 |

Only results of dead females by day 1 of the 4-d experiment are shown. Sliced because of significant treatment × temperature interaction (terminology of Schabenberger et al. 2000).

Fig. 5. Experiment 3: mean cumulative numbers of dead female \emph{Rhagoletis indifferens} ± SE in controls and low rate spinosad and spinetoram treatments starting at 7–8 d old at (A) 15.6°C, (B) 22.5°C, and (C) 29.4°C at 1, 2, 3, and 4 d after exposure. Maximum is 10 females.

Differences in kill of \emph{R. indifferens} caused by spinosad versus spinetoram by day 1 or 3 were most detectable at 15.6°C but less or not detectable at 22.5 and 29.4°C. As alluded to in the previous paragraph, at lower temperatures, flies probably contacted smaller spinosad and spinetoram doses, allowing for a detectable separation due to differential toxicity. At higher temperatures, greater fly activity levels may have resulted in contact with higher doses of spinosad, making its effects on kill similar to that of spinetoram.

Temperature effects on kill seemed less important for spinetoram than spinosad. In Experiment 1, kill within spinosad and spinetoram was successively greater at each of the three temperatures, but in Experiments 2 and 3 this was true only for spinosad. Possibly the amount of spinetoram picked up by flies at 22.5°C, even if lower than at 29.4°C, reached a threshold that maximized kill. It would be valuable to determine if in general more toxic insecticides such as spinetoram, as well as malathion (Yee 2017), are less affected by temperature than less toxic insecticides.

In Experiment 1, unlike for kill, there was a clear age effect on oviposition of \emph{R. indifferens}, in that 3–4 d old flies exposed to both spinosad and spinetoram laid no eggs while 14–18 d old flies exposed to both laid many eggs in similar numbers. Despite its lower toxicity, spinosad was poisonous enough to kill 3–4 d old flies before
egg development and oviposition occurred. Even at 15.6°C, the 3–4 d old flies (Experiment 1) were apparently active enough to contact spinosad and suffer 100% mortality before they could oviposit, perhaps aided by delayed oviposition at that temperature. Thus, to prevent oviposition, it did not matter if spinetoram killed all flies (exposed at 3–4 d old) 1–3 d earlier than spinosad.

Significantly, oviposition within 7–10 and 14–18 d old flies exposed to spinosad and spinetoram did not differ at any temperature. Cooler temperatures suppressed oviposition by both, even though spinetoram residues on cherries could not knock flies down quickly enough to eliminate oviposition at the lowest temperature tested (although close in Experiment 3, Fig. 6B). There was only one case where spinetoram caused lower oviposition, at 29.4°C in Experiment 2. The lack of oviposition differences caused by the two insecticides was unexpected, as greater (faster) kill by spinetoram should result in lower oviposition. Conversely, more flies survived in the spinosad treatment at day 1, so oviposition should have been greater in this than in the spinetoram treatment. One possible explanation is that most flies that contacted spinosad and survived after day 1 were sickened and rendered incapable of ovipositing or oviposited only at low levels during the short time they were alive. In a previous study, 20% of R. indifferens that fed on a spinosad-protein/sugar bait (GF-120) oviposited, but they laid only 0.8 eggs per fly before dying within 1 d (Yee 2010).

Lack of oviposition differences in spinosad and spinetoram treatments could also be due to the very low oviposition levels in treatments relative to controls, and the high variability inherent in oviposition levels among individual R. indifferens. The differences in fly kill, though significant, were not large enough to overcome this variability. For instance, at day 1 in Experiment 2 within the high rate spinosad and spinetoram treatments (Fig. 3D), the difference in numbers of live flies was about two. However, flies in these respective treatments laid 6–43 and 7–122 eggs per replicate, contributing to the lack of significant differences. These ranges probably were dependent in part on whether females that had a tendency to oviposit at high levels, highly variable itself, were killed early or not.

Results have implications for control of R. indifferens and for further study. They support the specimen label instructions for spinosad (Entrust SC) and spinetoram (Delegate WG) against R. indifferens to ‘maintain protective sprays at 7-day intervals while adults are present and fruit is susceptible to attack’ (Dow AgroSciences 2017a,b). Sprayed at these intervals, spinosad and spinetoram residues should be effective for preventing infestations of fruit when used against flies <4 d old. Because flies emerge from soil from late May to early July in central Washington (Frick et al. 1954), weekly sprays need to be applied during this period up until 7 d (for both insecticides) before harvest. There may be no reason to use spinetoram over spinosad when the 7-d spray interval is followed, except if residual activity of spinosad is much <7 d. Residual activity durations of spinosad and spinetoram may depend on heat, sun exposure, rainfall, and residue amounts on surface versus bottom of leaves, and will require field study. Preventing oviposition may be more difficult or impossible when flies are approaching reproductive maturity even though it may be easier when it is cooler. Eliminating oviposition by older (7–10 and 14–18 d) flies using spinosad and spinetoram may be possible only if one or the other is sprayed directly on the flies (Yee and Alston 2012). Faster-acting knockdown insecticides such as pyrethrins (Scott and Georghiou 1984) or some neonicotinoids (Yee 2010) may be better options to use than spinosyns or should be included in spray programs when there is a threat of many reproductively mature flies moving into cherry orchards from outside infested trees.

In summary, results provide the first comparisons of spinosad versus spinetoram effects on kill and oviposition of R. indifferens. Results indicate spinetoram is more toxic to R. indifferens than spinosad. However, this higher toxicity is not needed to prevent oviposition by younger flies. Furthermore, spinetoram residues are not sufficiently toxic to kill older flies quickly enough to reduce oviposition more than spinosad. Taken together, these conclusions imply that spinosad and spinetoram are equal for controlling R. indifferens infestations, a hypothesis that needs to be tested in the field.

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