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Comparative Responses of *Rhagoletis zephyria* and *Rhagoletis pomonella* (Diptera: Tephritidae) to Commercial and Experimental Sticky Traps and Odors in Washington State

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Abstract

Rhagoletis zephyria Snow and *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) are morphologically similar flies that attack white-colored snowberry fruit (*Symphoricarpos* spp.) and yellow/red or dark-colored apple/hawthorn fruit (*Malus/Crataegus* spp.), respectively. The two flies are caught together on traps in *R. pomonella* surveys in the western United States, increasing labor needed to process catches. Comparing responses of the two species with different traps could help identify best practices for reducing *R. zephyria* captures in these surveys and could contribute to understanding population divergence in *Rhagoletis* flies. In Washington State, United States, we found that *R. zephyria* responded most to yellow rectangles and more to white than red spheres (RSs) baited with ammonium carbonate (AC), whereas *R. pomonella* responded most to RSs with AC. Yellow plastic rectangles with AC were more effective in capturing *R. zephyria* than cardboard rectangles, as has been found for *R. pomonella*. *R. zephyria* did not respond to apple fruit volatiles associated with RSs that were attractive to *R. pomonella*. In contrast, *R. zephyria* responded more to yellow rectangles with snowberry than apple volatiles. Both species responded to AC. Our results suggest that RSs are better than yellow rectangles for surveying *R. pomonella* when snowberries are abundant. However, if discrimination from *R. zephyria* is paramount, RSs with apple volatiles should be used. Differences in the species' responses to traps appear related to odor/color cues of the flies' host fruit, while commonalties appear related to visual/olfactory stimuli associated with protein feeding, for which AC is a general attractant.

Key words: snowberry, apple, fruit volatile, red sphere, white sphere

Rhagoletis zephyria Snow and the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), are morphologically similar fly species that attack white-colored snowberry fruit (*Symphoricarpos* spp.) and yellow/red or dark-colored apple/hawthorn fruit (*Malus/Crataegus* spp.), respectively (Bush 1966). *R. pomonella* is a major quarantine pest of apples (*Malus domestica* Borkhausen) in the western United States (Washington State Department of Agriculture 2016). Annual trapping surveys to detect the fly and prevent its spread in Washington State (WA) have been conducted since 1980 (Yee et al. 2012). Although *R. zephyria* is not a pest, it is caught on the same survey traps as *R. pomonella* when traps are deployed in apple and hawthorn trees near snowberry bushes (Yee and Klaus 2015), which are common in WA. This clutters traps and increases time and labor needed for processing fly captures, as positively identifying the two species requires careful examination of the genitalia (Bush 1966, Wescott 1982). At least six other *Rhagoletis* species are also caught on similar survey traps in western North America (Madsen 1970, Yee and Goughnour 2017), but their wing patterns are distinct and present no problem for identification.

Much is known about which traps and odors attract *R. pomonella*, but nothing is known about the relative attractiveness of different traps to *R. zephyria*. The only trapping data for *R. zephyria* are from yellow sticky rectangles (Madsen 1970, Tracewski and Brunner 1987, Yee and Klaus 2015), and only one study (Yee and Klaus

2015) reported concurrent captures of the two species. Determining which traps are attractive to *R. pomonella* and not to *R. zephyria* is important because proper trap selection could reduce captures of *R. zephyria*, greatly saving effort in surveys where thousands of traps are routinely deployed and monitored. It is also important because differential captures of flies on traps of varying colors and shapes baited with different lures could further support hypotheses about the importance of host fruit-related visual and olfactory cues in reproductively isolating *Rhagoletis* species (Cha et al. 2012, 2017).

Two types of commercially available sticky traps are commonly used for monitoring *R. pomonella*: the red sphere (RS), used mostly in the eastern United States, and the yellow rectangle, which is generally used in the western United States. Both are baited with external ammonium carbonate (AC) or fruit volatile odor lures to increase their attractiveness (Zhang et al. 1999, Yee and Landolt 2004, Yee et al. 2014). Neither trap type was developed for *R. zephyria*.

There are reasons to hypothesize that *R. zephyria* and *R. pomonella* may respond differently to different traps and odors in the field. In the laboratory, RSs baited with synthetic volatile blends developed from apple and hawthorn fruit are attractive to *R. pomonella*, while these same blends associated with clear spheres are not attractive to *R. zephyria*; in contrast, volatile blends developed from snowberry fruit are attractive to *R. zephyria* but not *R. pomonella* (Cha et al. 2017). However, yellow rectangles baited with AC elicit similarly high responses from both flies (Yee and Klaus 2015); AC probably acts as a protein source feeding cue for *Rhagoletis*. Because RSs mimic apples and, thus, attract *R. pomonella*, it is possible that white spheres (WSs) could mimic snowberries and differentially attract *R. zephyria*.

The main objective of the current study was to compare responses of *R. zephyria* and *R. pomonella* among different trap types and odor baits to test for differences between the species in the field. Specifically, we were interested in determining whether *R. zephyria* responds similarly or differently than *R. pomonella* to 1) yellow rectangle versus RS versus WS traps and 2) apple and hawthorn fruit volatiles; 3) whether *R. zephyria* responds to snowberry fruit volatiles; and 4) whether the two species respond differently to AC. We discuss the applied implications of our findings for implementing more effective methods for monitoring *R. pomonella* in detection surveys, as well as the basic implications of our results for understanding the ecology of population divergence and speciation in *Rhagoletis* flies.

Materials and Methods

Trap types and chemical compositions of different fruit volatile lures used in tests are given in references in Table 1. In total, 16 different trapping tests were conducted in southwestern and central WA from 2007 to 2016 using different combinations of these traps and lures. Six of the 16 tests were conducted in southwestern WA (noncommercial apple-growing area), because it has high numbers of *R. pomonella* and *R. zephyria*, facilitating comparisons between the two species. Of the 16 tests, 10 were conducted in central WA, even though *R. pomonella* is rare there (Yee et al. 2012), because this portion of the state contains the majority of commercial apple orchards and so is the focus of the annual apple maggot detection survey conducted by Washington State Department of Agriculture to

Table 1. Sticky traps and odors used in tests of *Rhagoletis zephyria* and *Rhagoletis pomonella* responses in Washington State

Sticky traps

Type	Abbreviation	General description	References for traps in
Red sphere	RS	9-cm diameter dark red plastic ball	Yee et al. (2014)
White sphere	WS	9-cm diameter white plastic ball	Made for Great Lakes IPM
Pherocon rectangle	PA1	Yellow cardboard, 23 × 14 cm, folded, b* = 78[a]	Yee and Goughnour (2017)
Multigard rectangle	PA2	Yellow cardboard, 23.5 × 14 cm, folded, b* = 81[a]	Yee and Goughnour (2017)
Alpha Scents rectangle	PA3	Yellow cardboard, 20 × 14 cm, one sheet, b* = 68[a]	Yee and Goughnour (2017)
Plastic yellow sticky strip	PL1	Yellow plastic rectangle, 23 × 14 cm, one sheet, b = 63[a,b]	Yee and Goughnour (2017)
Plastic Olson yellow strip	PL2	Yellow plastic rectangle, 23 × 14 cm, one sheet, b* = 57[a,b]	Yee and Goughnour (2017)
Blue rectangle	BR	Blue plastic rectangle, 25 × 10 cm, one sheet	Great Lakes IPM (2016)

Lures

Type of chemical (odor)	General description of lure	Chemical composition in:
Eastern apple (EA)	0.585, 0.9, or 2 ml volatiles in polyethylene vial[c]	Zhang et al. (1999)
Eastern downy (EH)	Volatiles in 0.8 mg wax in 15 mm high by 20 mm wide white cap	Nojima et al. (2003)
Modified apple (MA)	2 ml volatiles in polyethylene vial	–[d]
Western apple volatiles (WA)	0.585 or 0.96 ml volatiles in polyethylene vial[c]	Cha et al. (2012)
Black hawthorn (BH)	0.585 or 0.96 ml volatiles in polyethylene vial[c]	Cha et al (2012)
Ornamental haw (OH)	0.585 ml volatiles in polyethylene vial	Cha et al. (2012)
Snowberry (SB)	Volatiles in 0.92 mg wax in 15 mm high by 20 mm wide white cap	Cha et al. (2017)
Ammonium carbonate (AC)	10 g AC in clear plastic vial with two 1-mm holes in lid	Yee (2016)
Ammonium carbonate, Low	10 g AC in clear plastic vial with no holes in lid	Yee (2016)
Ammonium carbonate, 7 g AC	7 g AC in plastic pouch (RHAPOM, Alpha Scents)	Yee (2016)
Ammonium bicarbonate	27 g ammonium bicarbonate in plastic package (AgBio)	Yee (2016)

[a]b* value in Lab color space values (for human vision) represent the yellow/blue coordinate.

[b]Supplemented with Tanglefoot adhesive.

[c]Variations in volatile volumes in vials were due to limited availability of some chemicals because of cost.

[d]Nine component blend: 10% butyl butanoate; 10% hexyl acetate; 5% propyl hexanoate; 7% hexyl propionate; 7% 2-methylbutyl 2-methylbutyrate; 23% butyl hexanoate; 23% hexyl butanoate; 5% pentyl hexanoate; 10% hexyl hexanoate.

monitor the fly. Details of the 16 different trapping tests are given in Table 2, including site locations, dates of experiments, tree types on which traps were hung, types of traps deployed, trap lure treatments (i.e., control no odor, host fruit volatiles, AC), delivery systems for lures, and number of experimental replicates.

All traps used in the study (Table 1) had sticky adhesives added by the manufacturer or experimenter. Tests were set up as a randomized block design in 1) stands of apple or hawthorn (*Crataegus* spp.) trees and nearby nonhost trees, and 2) contiguous patches of snowberry bushes, with a given block of plants containing all trap treatments. Apple, hawthorn, and nonhost test trees were 1–5 m from the nearest *R. zephyria*-infested snowberry bush. Traps were hung at a height of 1.5–2 m (trees) or 0.8–2 m (snowberry bushes) above the ground and 3–6 m apart within blocks. One trap was hung per apple or hawthorn tree if the tree was <8 m in diameter and two traps in trees >8 m in diameter. Each test trial was run for 16–64 d. Traps were checked every 3–7 d, all *R. zephyria* and *R. pomonella* captured in a given monitoring period removed, and the positions of the traps rotated on trees/bushes. Traps were replaced when they became covered with nontarget insects, usually after 1 or 2 wk. Flies were preserved in 70% ethanol and later identified as *R. zephyria* or *R. pomonella* based on morphological traits (Wescott 1982, Yee et al. 2009). Morphological identification was required because captures of *R. zephyria* and. *R. pomonella* are not necessarily limited to traps on their natal host plants. Flies can disperse through a given field site and be attracted to and captured on traps hung on alternative non-natal hosts or nonhost trees. While placing traps on snowberry bushes and apple/hawthorn trees a minimum of 1 m apart at sites can reduce interhost movement modestly, this did not eliminate cross capture. Thus, we morphologically identified flies captured in the study to determine whether they were *R. zephyria* or *R. pomonella* and used these data in our analyses of response differences between the two species. Few other *Rhagoletis* species were caught.

The study had four major objectives (Table 2). The first objective addressed in tests 1–7 was to determine if *R. zephyria* responded the same or differently than *R. pomonella* to three yellow cardboard (PA) and two yellow plastic (PL) rectangle or blue-colored rectangle (BR)-shaped traps versus RS traps versus white sphere (WS) traps (Table 1). The three yellow PA and two yellow PL rectangles were tested because they are all commercially available and designed specifically for trapping *Rhagoletis* flies, as well as other insects (in the case of PL rectangles). Our rationale was that color and shape differences among the traps may differentially mimic the host fruits of *R. pomonella* (RSs) versus *R. zephyria* (WSs) or reflect some aspect of the biology of the two species that differs, resulting in greater discrimination in capture. Our second objective addressed in tests 8–13 was to determine if synthetic apple and hawthorn fruit volatile blends developed to be attractive to *R. pomonella* (see references in Table 1) increase the capture rate or discrimination in species captures compared with no odor controls. Similarly, our third objective addressed in tests 14–16 was to investigate whether a snowberry fruit blend developed for *R. zephyria* (Cha et al. 2017) was more attractive to *R. zephyria* than apple fruit volatiles in snowberry bushes. Finally, our fourth objective addressed in tests

Table 2. Trapping tests of *Rhagoletis zephyria* and *R. pomonella* conducted to address the four main objectives of the study; test 3 in Objective 1 and test 16 in Objective 3 also address Objective 2 but are listed only once under the heading for Objectives 1 and 3, respectively

Test	Study site	Dates	Plants	Traps	Odors	Replicates
			Objective 1. Responses of flies to red or white spheres and yellow (plastic and cardboard) or blue rectangles			
1	Skamania	6 Aug.–28 Sep. 2016	Apple, ornamental hawthorn, cherry, alder	RS, WS, PA1, BR	AC	12
2	Skamania	15 Aug.–28 Sep. 2016	Apple, alder	RS, WS, PA1	AC	8
3	Nile Valley	12 Aug.–6 Sep. 2016	Black hawthorn	RS, WA, PA1	EA, AC	4
4	Wenas	22 July–17 Sep. 2010	Black hawthorn	PA1, PA2, PA3	AC	8
5	Wenas	7 July–13 Aug. 2014	Snowberry	PA1, PA2, PA2, PL1, PL2	AC	3
6	Vancouver	27 June–20 Aug. 2014	Snowberry	PA1, PA2, PA2, PL1, PL2	AC	5
7	Nile Valley	9 July–3 Sep. 2014	Black hawthorn	PA1, PA2, PA2, PL1, PL2	AC	5
			Objective 2: Effects of *Rhagoletis pomonella* fruit volatile blends on fly captures			
8	Wenas	23 July–25 Sep. 2007	Black hawthorn	RS	Control, EA, EH, AC	15
9	Wenas	17 July–21 Aug. 2008	Black hawthorn	RS	Control, EA, AC	15
10	Wenas	21 Aug.–9 Oct. 2008	Black hawthorn	RS	Control, EA, MA, AC	15
11	Wenas	4 Aug.–29 Sep. 2009	Black hawthorn	RS	Control, EA, AC	10
12	Wenas	7 Aug.–13 Sep. 2013	Black hawthorn	RS	Control, WA, BH, OH, AC	4
13	Nile Valley	15 July–4 Sep. 2015	Black hawthorn	RS	Control, WA, BH, AC, AC low	4
			Objective 3: Effects of snowberry fruit volatile blend on *Rhagoletis zephyria*			
14	Woodland	5–25 Aug. 2010	Snowberry	PA1	Control, SB	5
15	Woodland	9–25 Aug. 2010	Snowberry	PA1	Control, SB	4
16	Vancouver	5 Aug.–1 Sep. 2010	Snowberry	PA3	Control, SB, EA, 7 g AC, 27 g AC	5
			Objective 4: Effects of ammonium carbonate lures on fly captures			
8–13	Tests 8–13 in which RS traps baited with AC versus odorless control RS traps were used to examine Objective 4					

All sites were in Washington State; Skamania, Vancouver, and Woodland are located in southwestern Washington, while Nile Valley and Wenas are in central Washington. Trap and odor abbreviations are listed in Table 1.

8–13 examined the amount of captures on RS traps baited with AC, a general feeding attractant for *Rhagoletis* flies. Our aim here was to assess whether AC when used in combination with RS traps could increase the amount of captures of flies compared with other lure treatments, while retaining any visual discrimination of *R. pomonella* versus *R. zephyria* for RSs.

Statistics

For directly comparing *R. zephyria* and *R. pomonella* in Objective 1 (where there were sufficient fly counts), data were analyzed using chi-square tests of independence, testing the null hypothesis of no difference in distributions between fly species responses to traps. In most other tests directly comparing *R. zephyria* and *R. pomonella* (Objectives 1 and 2), tests of two independent proportions (Zar 1999) were conducted comparing percentages of the two species within a trap treatment (out of totals added across all traps within species). For comparing within-species responses to control versus treatment traps or between particular treatment traps of interest to hypotheses, chi-square tests with an expected 50:50 ratio were conducted (Objectives 1–4). For Objective 1, in tests of PL versus PA yellow rectangles against *R. zephyria*, data were normal and had equal variances and the intent was to determine which traps caught the most *R. zephyria*, so randomized blocks analysis of variance was conducted within species, followed by LSD tests.

Results

Objective 1: Responses of Flies to Red or White Spheres, and Yellow (Plastic and Cardboard) or Blue Rectangles

The results for tests 1–7 addressing Objective 1 generally showed that *R. pomonella* preferred RS, followed by yellow rectangle, and then WS traps; while, in contrast, *R. zephyria* was captured in higher proportions on yellow rectangle, followed by WS and then RS traps.

In test 1 at Skamania in 2016 (Fig. 1A), significant differences were observed in the percentages of *R. zephyria* and *R. pomonella* flies captured on different trap types. In particular, 7.6% of *R. zephyria* versus 33.6% of *R. pomonella* were caught on RS traps (tests of two proportions: χ^2 = 193.62; P < 0.0001); while 57.3% of *R. zephyria* versus 29.0% of *R. pomonella* were caught on PA1 (χ^2 = 222.36; P < 0.0001) (total of 694 *R. zephyria* and 4,814 *R. pomonella* flies captured in test 1). Thus, there were relatively fewer *R. zephyria* captured on RS than on PA1 traps compared with *R. pomonella*. Also, the 398 to 53 ratio of *R. zephyria* captured on PA1 versus RS traps was significant (χ^2 = 263.91; P < 0.0001). However, there was no difference in the percentages of *R. zephyria* versus *R. pomonella* captured on WS or BR traps (P > 0.0500 in both cases).

Results from test 2 at Skamania in 2016 (Fig. 1B) were similar to those for test 1. No *R. zephyria* versus 54.4% of *R. pomonella* were caught on RS traps (χ^2 = 100.29; P < 0.0001), while 80.0% of *R. zephyria* versus 21.0% of *R. pomonella* were caught on PA1 traps (χ^2 = 158.90; P < 0.0001) (total of n = 90 *R. zephyria* and 1,396 *R. pomonella* flies captured in test 2). Again, there were relatively fewer *R. zephyria* on RS than PA1 traps compared with *R. pomonella*, and the counts of *R. zephyria* on PA1 versus RS traps were 72 versus 0 (χ^2 = 72.00; P < 0.0001). However, there was no difference in captures between the two species on WS traps (P > 0.0500). The response to BR traps was poor for both species (Fig. 1).

Combining Skamania tests 1 and 2 in 2016, 249 *R. zephyria* flies were captured on spheres baited with AC, of which 78.7% (n = 196) were trapped on WS versus RS (χ^2 = 82.13; P < 0.0001). In these two tests, 4,091 *R. pomonella* flies were captured on spheres baited with AC, of which 58.1% (n = 2378) were trapped on RS versus WS (χ^2 = 108.10; P < 0.0001). Unlike *R. zephyria*, *R. pomonella* also preferred the RS (58.3% of 4,076 total captures) over the PA1 trap (χ^2 = 117.09; P < 0.0001).

In contrast to tests 1 and 2 in which traps were hung on a variety of different host and nonhost plants, test 3 was conducted solely on black hawthorn trees at Nile Valley in 2016, where *R. pomonella* is relatively low in abundance. As a result, a total of only 60 *R. zephyria* and 73 *R. pomonella* were caught on the four different trap types (RS + eastern apple volatiles, RS + AC, WS + AC, and PA1 + AC) deployed in black hawthorn trees in test 3. Although there were too few total flies caught to conduct a test of independence (20% cells with expected n = 5), the percentage of *R. zephyria* on RS + AC (11.7%) was nevertheless almost significantly lower than that of *R. pomonella* (24.7%; χ^2 = 3.64; P = 0.0564). There were no differences between species on the other three trap types (P > 0.0500).

Test 4 conducted in black hawthorn trees at Wenas in 2010 was designed to assess the efficacy of different commercially available PA yellow rectangles for catching flies. The results showed that PA1 and PA3 traps caught 2.5 and 2.1 times more *R. zephyria*, repectively, than PA2 (494 vs 198; expected ratio of 50:50: χ^2 = 126.61; P < 0.0001; 395 vs 192; χ^2 = 70.20; P < 0.0001, respectively). The PA1 and PA3 traps caught similar numbers of *R. zephyria* (500 vs

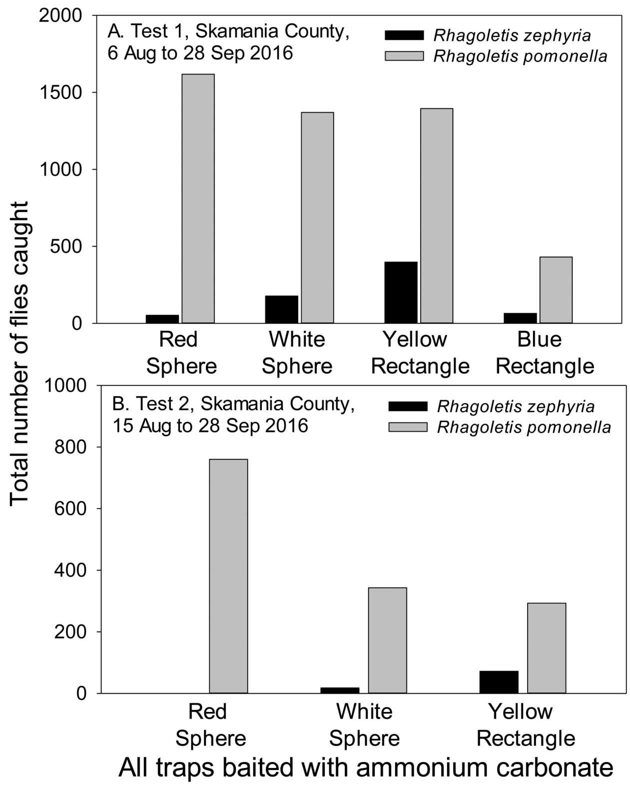

Fig. 1. Bar charts of results for tests 1 and 2 addressing Objective 1. Shown are total numbers of *R. zephyria* and *R. pomonella* flies caught on (A) four and (B) three different types of sticky traps baited with ammonium carbonate in Skamania County in western Washington State in 2016. Yellow rectangle = Pherocon AM = PA1 in this study. Test of independence: (A) χ^2 = 286.68; df = 3; P < 0.05; (B) χ^2 = 169.59; df = 2; P < 0.05. Test 1 was conducted in apple, ornamental hawthorn, cherry, and alder trees, while test 2 was performed in apple and alder trees.

503; $\chi^2 = 0.01$; $P = 0.9245$). Only 25 *R. pomonella* were captured in test 4 across all traps, too few to warrant analysis.

Tests 5–7 performed on snowberry bushes at Wenas, Vancouver, and Nile Valley in 2014 expanded the analysis of yellow rectangle traps to include those made out of PL as well as PA. In total, 2–47 times more *R. zephyria* were caught on PL1 and PL2 than on PA1, PA2, and PA3 traps at Wenas, Vancouver, and Nile Valley. At Wenas, the PL1 and PL2 traps caught 108.7 ± 19.4 (mean ± SE) and 88.3 ± 28.9 flies, respectively, compared with 2.3–36.3 for the three PA traps ($F = 43.04$; df = 2, 8; $P < 0.0001$); at Vancouver, 70.5 ± 14.8 and 41.8 ± 13.6 versus 2.5–22.8 flies ($F = 24.24$; df = 4, 12; $P < 0.0001$). Finally, in Nile Valley, 62.5 ± 20.2 and 45.8 ± 14.2 versus 7.6–25.8 ($F = 27.00$; df = 4, 16; $P < 0.0001$) flies were caught.

Objective 2: Effects of *R. pomonella* Fruit Volatile Blends on Fly Captures

Tests 8–13 addressing Objective 2 generally showed that apple and hawthorn fruit blends were more effective in attracting *R. pomonella* than *R. zephyria*. Percentages of *R. pomonella* caught on eastern apple, eastern downy hawthorn, modified apple, western apple, black hawthorn, and ornamental hawthorn volatile-baited RS traps in tests 8–13 conducted from 2007 to 2015 at Wenas and Nile Valley were all higher than the percentages of *R. zephyria* captured on these traps (Table 3). Combining the Wenas data for tests 8–11 from 2007 to 2009, significantly more *R. pomonella* were caught on RS traps baited with the eastern apple volatile ($N = 59$) than odorless control RS traps ($N = 20$; $\chi^2 = 19.25$; $P < 0.0001$ from predicted 50:50 ratio). In contrast, significantly fewer *R. zephyria* ($N = 1$) were caught on

Table 3. Objective 2: Effects of fruit volatile blends on fly captures using sticky red spheres in black hawthorn trees near snowberry bushes in Wenas and Nile Valley in central Washington, 2007–2015

Volatile/odor	Test 8: Wenas, 2007 (n = 15), 23 July–25 Sep.			Test 9: Wenas, 2008 (n = 15), 17 July–21 Aug.		
	R. zephyria	*R. pomonella*	χ^2; *P*-value	*R. zephyria*	*R. pomonella*	χ^2; *P*-value
Control	1.1 (2)	8.0 (6)	8.86; 0.0029	0 (0)	15.3 (9)	36.21; <0.0001
Eastern apple	0 (0)	38.7 (29)	82.49; <0.0001	0 (0)	1.7 (1)	3.91; 0.0479
Eastern downy hawthorn	0 (0)	12.0 (9)	23.60; <0.0001	–	–	–
AC	98.9 (188)	41.3 (31)	124.43; <0.0001	100 (230)	83.0 (49)	40.38; <0.0001
	N = 190	N = 75		N = 230	N = 59	

Volatile/odor	Test 10: Wenas, 2008 (n = 15), 21 Aug.–9 Oct.			Test 11: Wenas, 2009 (n = 10), 4 Aug.–29 Sep.		
	R. zephyria	*R. pomonella*	χ^2; *P*-value	*R. zephyria*	*R. pomonella*	χ^2; *P*-value
Control	2.2 (2)	2.4 (2)	0.1; 0.9267	1.9 (9)	3.8 (3)	2.09; 0.1484
Eastern apple	0 (0)	25.0 (21)	26.12;<0.0001	0.2 (1)	10.3 (8)	52.66; <0.0001
Modified apple	5.4 (5)	16.7 (14)	5.75; 0.0165	-----	-----	-----
AC	92.4 (85)	56.0 (47)	31.09; <0.0001	96.4 (449)	64.1 (50)	34.92; <0.0001
	N = 92	N = 84		N = 459	N = 61	

Volatile/odor	Test 12: Wenas, 2013 (n = 10), 7 Aug.–13 Sep.		
	R. zephyria	*R. pomonella*	χ^2; *P*-value
Control	0.6 (1)	19.3 (11)	28.23; <0.0001
Western apple	0	15.8 (9)	26.68; <0.0001
Black hawthorn	0	8.8 (5)	14.54; <0.0001
Ornamental hawthorn	0	8.8 (5)	14.54; <0.0001
AC	99.4(161)	47.4 (27)	93.88; <0.0001
	N = 162	N = 57	

Volatile/odor	Test 13: Nile Valley, 2015 (n = 4), 15 July–4 Sep.		
	R. zephyria	*R. pomonella*	χ^2; *P*-value
Control	0 (0)	4.0 (7)	2.08; 0.1496
Western apple	0 (0)	10.9 (19)	5.97; 0.0146
Black hawthorn	0 (0)	9.2 (16)	4.95; 0.0261
AC, low rate	18.0 (9)	9.8 (17)	2.56; 0.1093
AC	82.0 (41)	66.1 (115)	4.65; 0.0311
	N = 50	N = 174	

AC, ammonium carbonate; *R. zephyria*, *Rhagoletis zephyria*; *R. pomonella*, *Rhagoletis pomonella*.

All fruit volatiles were in polyethylene vials. Percentages between the two species within each date and volatile/odor treatment were compared.

n = number of treatment replicates in a test; N = total number of *R. zephyria* or *R. pomonella* flies caught in the test; values in parentheses are numbers of flies caught in a given treatment.

the eastern apple volatile-baited RS traps than the control RS traps (N = 13) (χ^2 = 10.29; P = 0.0013). Similar trends were observed for increased capture percentages of *R. pomonella* on RS traps baited with western apple (65.1%, N = 43 total captures) and black hawthorn volatiles (53.8%, N = 39) but not the ornamental hawthorn blend (31.3%, N = 16), compared with odorless control RS traps (Table 3). None of these comparisons were statistically significant, however, and additional experiments increasing the sample sizes for *R. pomonella* and *R. zephyria* are needed to make generalizations concerning the effects of the western apple, black hawthorn, and ornamental hawthorn volatiles on attracting or antagonizing these flies, respectively, compared to RS traps on black hawthorn trees in central WA.

Objective 3: Effects of Snowberry Fruit Volatile Blend on *R. zephyria* Captures

In tests 14 and 15, using PA1 traps on snowberry bushes at Woodland in 2010, the ratios of *R. zephyria* captured on traps baited with snowberry volatiles (N = 116 and 126, respectively) did not significantly differ from odorless control traps (N = 122 and N = 125; P > 0.0500 in both cases). However, in test 16 performed using PA3 traps in snowberry bushes at Vancouver, significantly more *R. zephyria* were caught on the snowberry volatile than control (χ^2 = 9.83; P = 0.0017) and eastern apple volatile-baited traps (χ^2 = 14.73; P < 0.0001), while there was no difference between the eastern apple volatile and control traps (χ^2 = 0.53; P = 0.4689) (Fig. 2).

Objective 4: Effects of Ammonium Carbonate Lures on Fly Captures

In tests 8–13, conducted to address Objective 4, RS traps baited with AC increased the amount of captures for both *R. zephyria* and *R. pomonella* compared with control RS traps (Table 3). There was discrimination by both fly species for AC-baited over control RS traps for all six tests, including in 2007–2009, and in 2013 at Wenas (tests 8–12), and 2015 at Nile Valley (test 13). Combining the results across all six tests (8–13) for *R. zephyria*, 1,154 versus 15 flies were captured on AC-baited compared with control RS traps (χ^2 = 1109.77; P < 0.0001). Similarly, for *R. pomonella*, 319 versus 38 flies were captured on AC-baited compared with control RS traps (χ^2 = 221.18; P < 0.0001) (Table 3). Thus, *R. zephyria* and *R. pomonella* flies were both highly attracted to AC, especially *R. zephyria*.

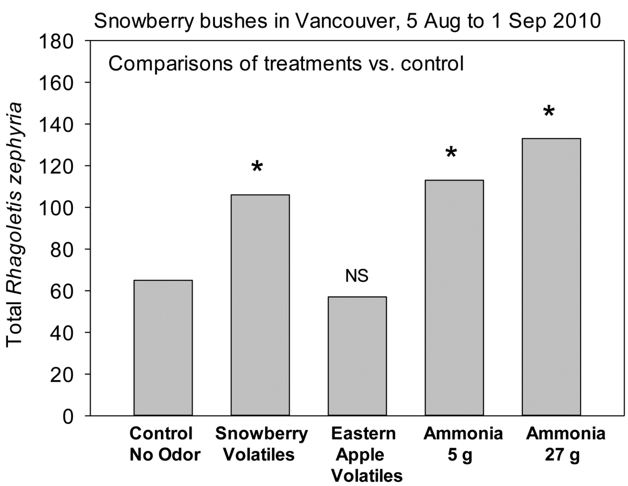

Fig. 2. Bar chart of results for test 16 addressing Objective 3. Shown are total numbers of *Rhagoletis zephyria* caught on yellow rectangle traps (Alpha Scents = PA3 in this study) baited with different odor lures in Vancouver in western Washington State in 2010. Odors compared with unbaited control. *Significant at alpha = 0.05; NS, P > 0.05. Snowberry volatiles versus control: χ^2 = 9.83; P = 0.0017; eastern apple volatiles versus control: χ^2 = 0.53; P = 0.4689; ammonia 5 g versus control: χ^2 = 12.94; P = 0.0003; ammonia 27 g versus control: χ^2 = 23.35; P < 0.0001.

Discussion

With respect to Objective 1, testing for response differences in flies related to different trap types, our results show that sticky RSs baited with AC are better to use than sticky yellow rectangles with AC in discriminating between *R. pomonella* and *R. zephyria*. Moreover, RSs caught more *R. pomonella* than PA1 yellow rectangles in direct comparison tests in 2016, so RSs are not just better at discriminating between species but also more effective at capturing *R. pomonella* in general. Unfortunately, AC can attract *R. zephyria* to RSs, so employing RSs with AC will not eliminate unwanted interspecific captures. However, the ratio of capture of *R. zephyria* to *R. pomonella* on RSs is still consistently lower than that on yellow rectangles. Indeed, in the 2016 tests, where RSs and yellow rectangles with AC were directly compared, RSs had 70%, 87%, or 100% fewer *R. zephyria* captures than on PA1 yellow rectangles. However, some field workers find that flies are easier to spot on yellow rectangles than on RSs and that rectangles are easier to handle. Thus, despite the greater effectiveness of RSs, trade-offs in their use exist such that when snowberry plants are absent, yellow rectangles could be preferred for surveys.

Objective 2 examined whether apple and hawthorn fruit odors may increase capture rates and minimize *R. zephyria* cross captures in surveys for *R. pomonella*. The results indicate that generally speaking, the use of apple volatiles increases capture rates of *R. pomonella* and increases the proportion of *R. pomonella* relative to *R. zephyria* captured compared with control, odorless traps. Hawthorn blend traps yielded too few captures to generalize. However, as seen in Objective 4, apple and hawthorn volatile blends are not as attractive to *R. pomonella* as AC, a result observed in a previous study of Yee et al. (2014) as well. Thus, a trade-off also exists with respect to the use of fruit volatiles versus AC to bait traps. Apple and hawthorn volatiles can increase discrimination in the trapping of *R. pomonella* relative to *R. zephyria* but will result in fewer overall numbers of *R. pomonella* caught compared with AC. Thus, if the primary concern is detecting rare *R. pomonella* in an area, then baiting RSs with AC may be preferable. But in areas where *R. pomonella* and *R. zephyria* are plentiful, then using apple fruit volatiles may be advisable. Another consideration, however, is that fruit volatiles attract high numbers of chloropid flies that clutter spheres (Yee et al. 2005); in some cases, rendering the spheres useless for monitoring *R. pomonella* after a week. Thus, the choice of fruit odor versus AC lures to attract *R. pomonella* must also balance the goal of the survey with practical labor considerations.

Although not an immediate goal of the study, our findings also implied that the most effective traps for catching *R. zephyria* are PL yellow rectangles baited with AC. PL yellow rectangle traps performed better than PA traps for capturing *R. zephyria*, similar to results that Yee and Goughnour (2017) found for *R. pomonella*. For both *R. zephyria* and *R. pomonella*, the same particular shade of yellow (color space b* = 57 or 63) or possibly translucence (thin for sunlight transmission) seems most attractive. Alternatively, the higher captures of both species on the PL rectangles may be due to the Tanglefoot adhesive applied on their surfaces, which may be tackier than manufacturer-applied adhesives on PA rectangles

(Yee and Goughnour 2017). Regardless, we recommend PL1 or PL2 with Tanglefoot adhesive as the best traps to use in phenology studies of *R. zephyria*, where large amounts of data are desirable.

The findings from Objectives 1–4 generally support the hypothesis that *R. zephyria* and *R. pomonella* preferentially respond to traps emulating olfactory and visual cues that these flies use to identify and distinguish between their respective host plants. With respect to olfactory cues, odorless control RSs captured 13 times more *R. zephyria* than RSs with eastern apple volatiles, suggesting that the fly avoided or was even antagonized by non-natal volatiles. Also, PA3 traps baited with the snowberry blend caught 1.9 times more *R. zephyria* than traps with the eastern apple volatiles in test 16 (Fig. 2). This latter finding is consistent with flight tunnel assays where 66.7% of western WA *R. zephyria* responded to the nine-component snowberry blend versus only 4.3% to the eastern apple blend (Cha et al. 2017). Our current results therefore suggest that either the snowberry blend is attractive to and/or apple volatiles or repellent to *R. zephyria* in the field. The reason(s) for the lack of a difference between control and snowberry volatile-baited PA1 traps in tests 14 and 15 is unclear but may reflect location effects (e.g., higher winds at the Woodland site next to the Lewis River compared with the more sheltered Vancouver location along the Burnt Bridge Creek Greenway), higher trap visibility due to small snowberry bushes with low fruit loads at Woodland versus Vancouver, or the difference in trap type (Woodland = PA1, Vancouver = PA3). These possibilities warrant further study.

In regard to olfaction in *R. pomonella*, no test in the current study directly compared the response of *R. pomonella* to snowberry versus apple or hawthorn blends. However, *R. pomonella* was captured in greater numbers on traps baited with eastern apple volatiles compared with no odor controls, a finding also reported in several previous field trapping studies (Forbes et al. 2005, Forbes and Feder 2006, Sim et al. 2012). Moreover, flight tunnel tests have shown that *R. pomonella* does not respond to the snowberry blend but strongly does to natal apple or hawthorn volatiles (Linn et al. 2003, 2004, 2005, 2012; Powell et al. 2012; Cha et al. 2017). Thus, fruit odor discrimination appears to commonly play an important role in generating prezygotic reproductive isolation, and sometimes postzygotic isolation (Linn et al. 2004, Dambroski et al. 2005), between *Rhagoletis* flies specialized on different plants, contributing to their behavior of mating only on or near the fruit of their respective hosts (Feder et al. 1989, 1994).

With respect to visual cues, the prediction that *R. zephyria* should be preferentially captured on white versus RSs due to WSs mimicking the white color of snowberry fruit was supported. It is possible that the visual discrimination *R. zephyria* displays for white is even greater than that measured in our study. AC was used to bait red and WSs in Skamania tests 1 and 2 in 2016. AC is a powerful attractant for *Rhagoletis,* and so the use of the compound may have drawn more *R. zephyria* to the RSs than would normally be the case. Moreover, the size of the spheres used as traps (9 cm diameter) was much larger than the natural size of a snowberry fruit (1 cm), which could have reduced the preference of *R. zephyria* for white in the study. However, snowberries do occur in clusters of ~5–10 mature fruit, so it is possible that the spheres reasonably mimicked a cluster of fruit. Further work is needed to address these possibilities. In addition, despite the significant attraction of *R. zephyria* to white versus RSs, PA1 traps caught more *R. zephyria* than WSs, suggesting that the color yellow and/or shape of the rectangles may be more powerful visual stimuli to snowberry flies than red or white large-sized spheres. More accurate fruit mimics must be tested to clarify this issue.

The effects of visual cues for *R. pomonella* also conformed to predictions. Although the differences for *R. pomonella* were not as pronounced among trap types as for *R. zephyria*, RSs were more attractive to *R. pomonella* than the other traps tested, as expected. Moreover, the preferences by *R. pomonella* we detected may underestimate the flies' visual attraction to RSs, as similar considerations discussed above for *R. zephyria*, including the use of AC to bait traps, may also apply to *R. pomonella*.

Overall, our results suggest that differences in *R. zephyria* and *R. pomonella* responses to traps in the field are related to odor/color cues of host fruit, while commonalties are related in part to visual/olfactory stimuli associated with protein feeding (i.e., the use of AC as a lure). This is consistent with the hypothesis that specific host fruit cues reproductively isolate fly species, whereas adult food cues do not. Work using fruit mimics more closely resembling snowberries, apples, and hawthorns coupled with appropriate fruit odor blend baits is needed to further test this hypothesis.

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