Ammonium Carbonate Is More Attractive Than Apple and Hawthorn Fruit Volatile Lures to *Rhagoletis pomonella* (Diptera: Tephritidae) in Washington State

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ABSTRACT The apple maggot fly, *Rhagoletis pomonella* (Walsh), is an introduced, quarantine pest of apple (*Malus domestica* Borkhausen) in the Pacific Northwest of the United States. In the eastern United States where the fly is native, fruit volatiles have been reported to be more attractive than ammonia compounds to *R. pomonella*. However, the opposite may be true in the western United States. Here, we determined whether newly identified western apple and western hawthorn fruit volatiles are more attractive than ammonium carbonate (AC) to *R. pomonella* in apple, black hawthorn, and ornamental hawthorn trees in western Washington State. In all three host trees, sticky red sphere or yellow panel traps baited with AC generally caught more flies than traps baited with lures containing the four newly developed fruit blends (modified eastern apple, western apple, western ornamental hawthorn, and western black hawthorn) or two older blends (eastern apple and eastern downy hawthorn). Fruit volatiles also displayed more variation among trapping studies conducted at different sites, in different host trees, and across years than AC. The results imply that traps baited with AC represent the best approach to monitoring *R. pomonella* in Washington State.

KEY WORDS apple maggot fly, ammonia, western apple fruit volatile, ornamental hawthorn fruit volatile, western Washington

The apple maggot fly, *Rhagoletis pomonella* (Walsh), is a quarantine pest of apple (*Malus domestica* Borkhausen) in the Pacific Northwest of the United States, where it was apparently introduced from the eastern United States <60 yr ago. The fly was first detected in the western United States in Oregon in 1951 (AliNiazee and Wescott 1986) and then again in Portland in 1979 (AliNiazee and Penrose 1981), after which it was discovered in Washington and then California, Idaho, and Utah (Brunner 1987). Washington produces ≈60% of the United States apples, with an annual value of ≈US$1.8 billion (Washington State Department of Agriculture 2013). To help protect apple exports in Washington, the Washington State Department of Agriculture (WSDA) monitors *R. pomonella* in an annual survey to appraise the presence and spread of the fly near commercial orchards (Yee et al. 2012). Ammonium carbonate (AC), first shown to attract *R. pomonella* almost 70 yr ago in Minnesota (Hodson 1948), has been the only odorant used by the WSDA to bait sticky traps for large-scale monitoring of the fly.

In the eastern United States where *R. pomonella* is native, fruit volatile lures are attractive or have been reported to be more attractive to flies than AC or other ammonia compounds (Reissig et al. 1985, Agnello et al. 1990, Reynolds and Prokopy 1997, Zhang et al. 1999, Rull and Prokopy 2000, Stelinski and Liburd 2002). As a result, fruit volatiles are favored over ammonia lures for detecting *R. pomonella* in the eastern United States. In contrast, in Washington and Oregon, AC-baited traps were found to be more attractive than traps baited with apple volatiles (Yee et al. 2005). However, the fruit blends used in the study of Yee et al. (2005) were developed specifically for apple- and downy hawthorn (*Crataegus mollis* Scheele)-infesting flies from the eastern United States. Recently, volatile blends have been developed for *R. pomonella* infesting apple and native black (*Crataegus douglasii* Lindley), and introduced ornamental (*Crataegus monogyna* Jacquin) hawthorns from the state of Washington. These newly identified blends for western *R. pomonella* have been shown to be more attractive to flies than the older eastern blends in flight tunnel behavioral assays (Cha et al. 2012, Linn et al. 2012). The new blends have

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Table 1. Trapping experiments for *R. pomonella* comparing AC and fruit volatiles in western Washington State from 2007 to 2013

Site	Tree	No. replicate trees[a]	Date started	Date ended	Test duration (d)	No. trap rotations
Experiment 1 (2007): Red spheres with no odor, AC; EA Vial Lure, EH wax lure						
Skamania	Apple	3	1 July	27 Sept.	88	24
	Black hawthorn	3	1 July	27 Sept.	88	24
	Ornamental hawthorn	3	22 July	27 Sept.	67	19
Vancouver	Apple	3	3 July	25 Sept.	84	12
	Black hawthorn	3	3 July	11 Sept.	70	9
	Ornamental hawthorn	3	24 July	25 Sept.	63	9
Puyallup	Apple (Orchard 1)	5	25 Aug.	1 Oct.	37	10
	Apple (Orchard 2)	5	1 July	27 Aug.	57	11
	Ornamental hawthorn	5	2 July	28 Aug.	57	11
Experiment 2 (2008): Red spheres with no odor, AC; EA vial lure						
Vancouver	Apple	5	7 July	24 Sept.	79	11
	Black hawthorn	5	7 July	2 Sept.	57	8
	Ornamental hawthorn	5	7 July	9 Sept.	64	9
Experiment 3 (2008): Red spheres with no odor, AC; EA, MA vial lures						
Skamania	Apple	5	16 July	23 Oct.	99	21
	Black hawthorn	5	16 July	23 Oct.	99	21
	Ornamental hawthorn	5	16 July	24 Sept.	70	8
Puyallup	Apple	5	20 July	25 Aug.	36	7
	Ornamental hawthorn	5	20 July	9 Sept.	51	9
Experiment 4 (2011): Yellow panels with no odor, AC; EA, WA, OH, BH wax lures						
Puyallup	Apple	5	20 July	15 Sept.	57	16
	Ornamental hawthorn	4	15 Aug.	18 Oct.	64	16
Experiment 5 (2012): Red spheres with AC; EA, WA, OH, BH vial lures						
Skamania	Black hawthorn	4	30 July	11 Sept.	43	6
	Black hawthorn	4	11 Sept.[b]	19 Oct.	38	4
Puyallup	Apple	5	18 July	14 Sept.	58	22
	Ornamental hawthorn	5	20 Aug.	19 Oct.	60	20
Experiment 6 (2013): Red spheres with no odor, AC; EA, WA, OH, BH rubber septa lures						
Puyallup	Apple	5	19 July	8 Aug.	20	6
	Ornamental hawthorn	5	16 Aug.	10 Sept.	25	7
Experiment 6 (2013): Red Spheres with no odor, AC; EA, WA, OH, BH vial lures						
Puyallup	Apple	5	8 Aug.	13 Sept.	36	10
	Ornamental hawthorn	5	10 Sept.	24 Oct.	44	13

EA, eastern apple; EH, eastern hawthorn; MA, modified eastern apple; WA, western apple; OH, western ornamental hawthorn; BH, western black hawthorn.

[a] Trap positions rotated among trees each time after flies were removed from traps.

[b] OH lure removed.

not been compared with AC in the field, however, to determine if they may have greater efficacy for use in fly monitoring.

At the current time in the western United States, the use of AC is appealing because it is less expensive than fruit volatiles and is highly attractive to *R. pomonella.* However, AC is disadvantageous because it also attracts many nontarget flies, including other *Rhagoletis* species that could be confused morphologically with *R. pomonella* (Yee et al. 2011), reducing the efficiency of apple maggot fly identification and monitoring. In contrast, western fruit volatiles could be appealing despite their higher cost if they are more selective for *R. pomonella* relative to other fly species.

Flies originating from apples and downy hawthorns in the eastern United States preferentially orient to the volatiles of their respective host fruit (Linn et al. 2003, 2004; Forbes and Feder 2006). The same has been found for *R. pomonella* in southwestern Washington (Linn et al. 2012, Sim et al. 2012). In flight tunnel tests, flies reared from apple, black hawthorn, and ornamental hawthorn collected in Washington showed higher levels of upwind-directed flight to their respective natal than nonnatal fruit volatile blends (Linn et al. 2012). These results are consistent with the presence of host races in Washington. Therefore, the possibility exists that using different host odors may generate increased specificity of fly capture near apple orchards for individuals originating from apple, which pose a greater threat to apple than flies from alternative hosts.

Here, we test whether apple and hawthorn volatiles developed for alternative host fruit of eastern and western populations of *R. pomonella* are more attractive for trapping the fly in western Washington State than AC. We also investigate the related question of whether fly responses to AC and fruit volatiles differ in apple and hawthorn trees in Washington.

Materials and Methods

AC and Fruit Volatiles. The AC lure was a clear 2.6 cm in width by 5.5 cm in height plastic vial (7 dram Crystal, Thornton Plastics, Salt Lake City, UT) containing 10 g of powdered AC (Keystone Universal Corp., Melvindale, MI). A white plastic cap with two 1-mm holes for odor release was snapped onto the top of the vial. The AC lure releases ≈5–7 mg of ammonia + CO_2 + water per hour for up to 30 d in the laboratory (Yee et al. 2005); from days 42–55, the rate is 2.2 mg/h. The ammonia released attracts more flies than controls for up to 3 mo in western Washington (W.L.Y., unpublished).

Two new apple and two new hawthorn volatile blends were tested. The two new apple volatile blends were the modified eastern apple (MA) and western apple (WA) blends. The MA consists of nine different compounds: 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, and 10% hexyl hexanoate. At the time this blend was tested, it was the most attractive of the newer apple blends in flight tunnel behavioral assays (C.E.L., unpublished). The components of the other three new blends are described in Cha et al. (2012). WA is the "WA3" in Cha et al. (2012), the most attractive of five WA volatiles subsequently tested after MA in the flight tunnel. The two new hawthorn volatiles were the western ornamental hawthorn (OH) and western black hawthorn (BH) blends. OH is the "OH1" and BH is the "BH2" in Cha et al. (2012), the most attractive of four ornamental hawthorn and seven black hawthorn blends, respectively, tested against western *R. pomonella* in flight tunnel behavioral assays. Two additional previously developed volatiles were also tested in the study: the eastern apple (EA) and eastern downy hawthorn (EH) blends. EA is the five-component apple blend of Zhang et al. (1999), which was the apple blend previously tested against western *R. pomonella* by Yee et al. (2005). EH is the six-component hawthorn blend of Nojima et al. (2003). Because of limitations in blend availability, not all odor delivery methods (see following) were used for all volatile fruit blends against AC.

Volatiles were released by one of three delivery methods from 1) vials, 2) wax, or 3) rubber septa lures. The vials were 2-cm in width by 5 cm in height (8.5 ml volume) polyethylene cylinders that contained either 1 or 2 ml of the blend and were sealed on their open ends with a screw-on cap. The vials were the same as the commercially available EA lures from Great Lakes IPM (Vestaburg, MI). Release rate from vials with 2 ml of blend was ≈488 μg/h (at 21°C, 30% relative humidity), similar to ≈500 μg/h from vials with fruit esters (Averill et al. 1988). In 2013 only, each vial contained only 1 ml of fruit volatiles because of the high cost (US$536 per gram) of 3 (*E*)-4, 8-dimethyl-1, 3, 7-nonatriene, a key component of the hawthorn blends. Release rates over 32 d from vials with 1 ml of WA, EA, or OH were 308–315, 274–286, or 204–220 μg/h, respectively. The wax lure (Suterra Corp., Bend, OR) was a 1.8 cm in width by 1.5 cm in height white cap half-filled with 700 mg of a 1:3 fruit volatile: wax mix. Release rate from wax lures was ≈30–35 μg/h (Forbes et al. 2005). Red rubber release septa (stoppers; Ace Glass Inc., Vineland, NJ) were 1.7 cm in width by 2.5 cm in length and 2.31 g. Each septum was soaked in acetone for 24 h and dried for 2 d before being loaded with 205 μl of a blend, followed by 200 μl of hexane to increase the absorption of the blend in

Table 2. Randomized complete block design ANOVA results for ammonium carbonate and fruit volatile lure effects on captures of *R. pomonella* in western Washington State, 2007–2013

Tree Species	Sex	*F*	df	*P* value
Experiment 1: 2007 (Skamania)				
Apple	F + M	39.26	3, 6	0.0002
Black hawthorn	F + M	27.50	3, 6	0.0007
Ornamental hawthorn	F + M	32.27	3, 6	0.0004
Experiment 1: 2007 (Vancouver)				
Apple	F + M	11.95	3, 6	0.0061
Black hawthorn	F + M	14.63	3, 6	0.0036
Ornamental hawthorn	F + M	7.04	3, 6	0.0216
Experiment 1: 2007 (Puyallup)				
Apple (Orchard 1)	F	53.13	3, 12	<0.0001
	M	68.50	3, 12	<0.0001
Apple (Orchard 2)	F	12.88	3, 12	0.0005
	M	12.28	3, 12	0.0006
Ornamental hawthorn	F	7.82	3, 12	0.0037
	M	2.65	3, 12	0.0966
Experiment 2: 2008 (Vancouver)				
Apple	F + M	8.84	2, 8	0.0094
Black hawthorn	F + M	12.22	2, 8	0.0037
Ornamental hawthorn	F + M	3.45	2, 8	0.0831
Experiment 3: 2008 (Skamania)				
Apple	F + M	23.75	3, 6	<0.0001
Black hawthorn	F + M	45.08	3, 6	<0.0001
Ornamental hawthorn	F + M	3.52	3, 6	0.0488
Experiment 3: 2008 (Puyallup)				
Apple	F	22.36	3, 12	<0.0001
	M	18.85	3, 12	<0.0001
Ornamental hawthorn	F	28.71	3, 12	<0.0001
	M	21.25	3, 12	<0.0001
Experiment 4: 2011 (Puyallup)				
Apple	F	69.84	5, 20	<0.0001
	M	35.43	5, 20	<0.0001
Ornamental hawthorn	F	15.57	5, 15	<0.0001
	M	47.48	5, 15	<0.0001
Experiment 5: 2012 (Skamania) early Season				
Black hawthorn	F + M	17.50	4, 12	<0.0001
Experiment 5: 2012 (Skamania) late season				
Black hawthorn	F + M	1.65	3, 9	0.2468
Experiment 5: 2012 (Puyallup)				
Apple	F	33.93	4, 16	<0.0001
	M	34.72	4, 16	<0.0001
Ornamental hawthorn	F	22.82	3, 9	0.0002
	M	34.09	3, 9	<0.0001
Experiment 6: 2013 (Puyallup: Rubber septa lure test)				
Apple	F	9.91	5, 20	<0.0001
	M	6.78	5, 20	0.0008
Ornamental hawthorn	F	10.97	5, 20	<0.0001
	M	6.78	5, 20	0.0008
Experiment 6: 2013 (Puyallup: vial lure test)				
Apple	F	10.86	5, 20	<0.0001
	M	5.77	5, 20	0.0019
Ornamental hawthorn	F	11.57	5, 20	<0.0001
	M	19.35	5, 20	<0.0001

F, female; M, male.

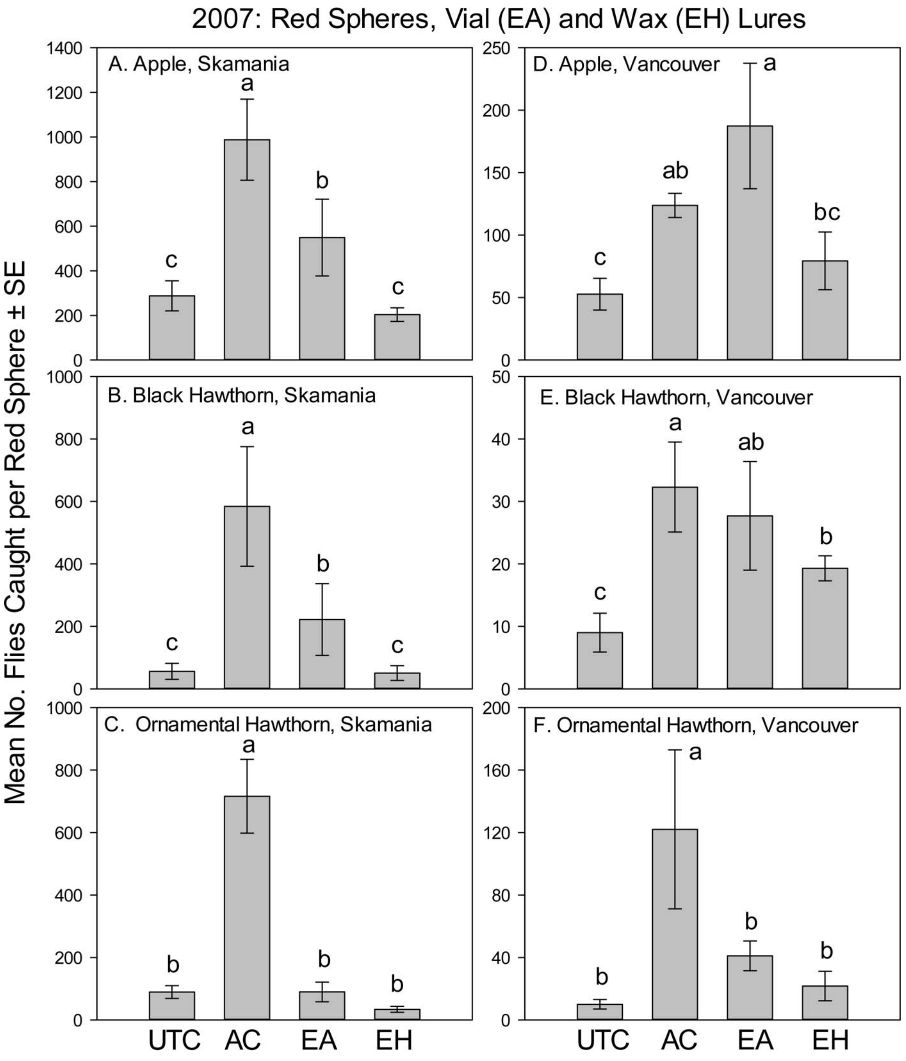

Fig. 1. Captures of *R. pomonella* (sexes combined) on spheres baited with odors in 2007 in (A) apple, (B) black hawthorn, and (C) ornamental hawthorn trees in Skamania, and in (D) apple, (E) black hawthorn, and (F) ornamental hawthorn in Vancouver, WA. UTC, untreated control; AC, ammonium carbonate; EA, eastern apple; EH, eastern hawthorn. Means with same letters are not significantly different ($P > 0.05$).

the septum. Release rates from septa were 270–630 μg/h during the first week and 23–47 μg/h during the second week (septa were only used for ≈3 wks in the field, see General Design of Experiments). The vial and wax lures retain potency for at least 2 mo (W.L.Y., unpublished), while the septa lures retained potency for at least 3 wks in the current study (see results).

Study Sites. Three sites in the coast forest ecosystem in western Washington were used in this study: Saint Cloud Park in Skamania County (45.58° N, 122.16° W; Skamania), Washington State University Research and Extension Center in Vancouver in Clark County (45.68° N, 122.65° W; Vancouver), and Puyallup within 3.2 km of the Washington State University Research and Extension Center in Pierce County (47.18° N. 122.29° W; Puyallup). All three sites were outside the major commercial apple-growing areas of central Washington. The Skamania County ("Skamania" hereafter) site is a former homestead along the Columbia River with apple, black hawthorn, and ornamental hawthorn trees spaced from ≈2 to 0.5 km from one another. The Vancouver site is a riparian zone on the Washington State University-Vancouver campus with scattered apple, black hawthorn, and ornamental hawthorns within 0.5 km of one another located along a series of nature trails. The Puyallup site is an urban area with small apple orchards and scattered ornamental hawthorn trees located from 0.8 to 3.2 km away from the orchards. Black hawthorn was not found in Puyallup.

General Design of Experiments. Six experiments comprising a total of 27 different trapping trials were performed in a randomized complete block design at the three study sites with three to five replicates con-

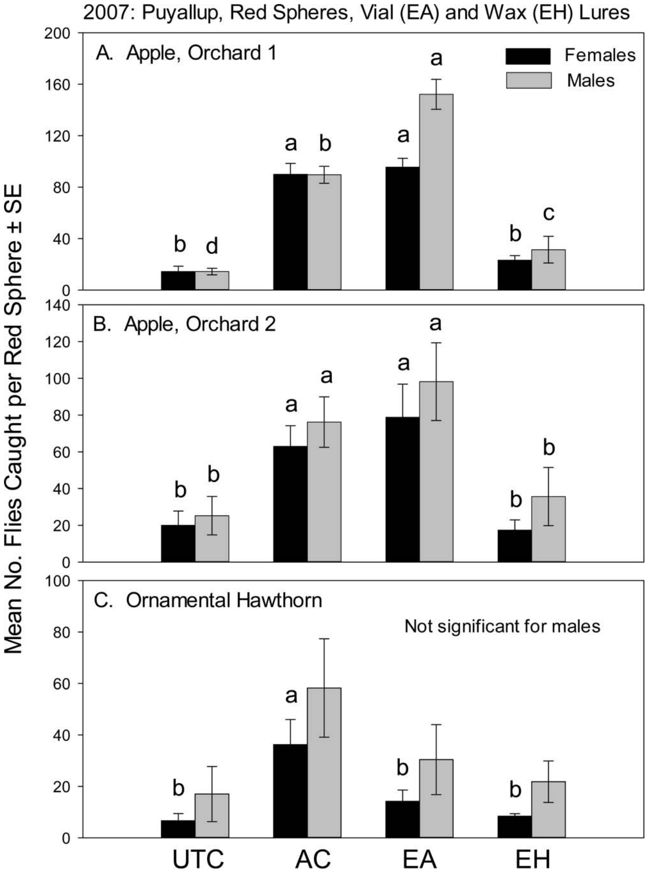

Fig. 2. Captures of *R. pomonella* on spheres baited with odors in 2007 in (A) apple in Orchard 1, (B) apple in Orchard 2, and (C) ornamental hawthorn trees in Puyallup, WA. UTC, untreated control; AC, ammonium carbonate; EA, eastern apple; EH, eastern hawthorn. Means within sexes with same letters are not significantly different ($P > 0.05$).

ducted for each odor treatment. Tests were set-up simultaneously or at overlapping times during the 2007–2013 field seasons in apple, black hawthorn, and ornamental hawthorn trees (Skamania and Vancouver) or in apple and ornamental hawthorn trees (Puyallup). Treatments included an untreated or no odor control (UTC; except in four cases), and traps baited with AC or one of six different fruit volatile lures (EA, MA, WA, EH, OH, or BH). The UTC was a vial with a ball of cotton placed inside. AC, control, and fruit volatile blend lures were hung 1 cm above traps. One trap was placed per test tree on the southern side of the tree ≈2 m above the ground, with traps on different trees separated by a minimum of 3 m. In experiment 4, sticky 14 by 23 cm yellow panels (Pherocon AM; Trecé, Adair, OK) were used to trap flies. In all other experiments, 8-cm-diameter sticky Tartar Red spheres were used as traps (Great Lakes IPM, Vestaburg, MI). Our tests using one trap per tree mirrored that of WSDA to monitor the presence and spread of the fly, although we primarily used red spheres instead of yellow panels because spheres have been shown to be more attractive (Yee and Landolt 2004). Every 3 to 7 d, flies were removed from traps and the traps rotated among trees (except once in Skamania in 2012, when trap positions were left unchanged for 17 d). Traps were replaced 3 wk postdeployment if needed due to buildup of nontarget insects on trap surfaces. Flies collected on each collection date were counted. In Puyallup, flies were also sexed. Tests lasted from 20 to 99 d.

A summary of the tests and treatments performed in each of the six experiments is given in Table 1. In general, experiments 1–3 were designed to retest the previous findings that AC was more attractive to *R. pomonella* in the western United States than the EA blend developed for eastern flies as well as the EH and MA blends. Experiments 4–6 were conducted to expand these results by testing whether WA, BH, and OH blends developed specifically for *R. pomonella* from Washington State were more or less attractive to flies than AC. Certain aspects of experiments 5 and 6 varied from the protocol used in the other experiments and are noted as follows. For experiment 5 in 2012 at the Skamania site in black hawthorn trees, the OH treatment was removed after 43 d for another study (not reported here); the remaining traps continued to be monitored through the second half of the season. In the second part of experiment 6 in Puyallup, rubber septa were replaced with vial lures. The change was made because the septa lost odor after 3 wk; in addition, we wanted to determine if flies responded differently to septa versus vial lures.

Statistics. Randomized complete block analysis of variance (ANOVA) was conducted on total counts of flies (sexes combined; Skamania and Vancouver sites) or for each sex separately (Puyallup site) over the 2007–2013 seasons in experiments 1–6. Counts were first square root-transformed to normalize their distribution and standardize their variance. Means for treatments were analyzed using Fisher least significant difference test ($P = 0.05$; SAS Institute Inc. 2008, Cary, NC). To determine if captures of flies on traps baited with fruit volatiles versus with AC differed during the season, ANOVA was also conducted on numbers of flies caught during early, mid-, and late season trapping periods within experiments. For this analysis, trapping duration within each test was divided as evenly as possible into three sampling periods, each comprising three to eight monitoring dates. However, for the second half of experiment 5 in 2012 in Skamania, there were only two sampling periods comprising two monitoring dates each.

Results

Overview of ANOVA Results. ANOVAs for 25 of the 27 tests performed for the six experiments indicated significant differences in the numbers of trapped flies on a host tree species among odor treatments (Table 2). The only exceptions were in ornamental hawthorn in experiment 2 in 2008 in Vancouver and in black hawthorn in experiment 5 in 2012 in Skamania. In Puyallup where flies were sexed, statistically significant differences were detected between females and males in certain experiments, so separate tests were performed for the sexes. The significant differences found in the ANOVA analyses among treatments allowed us to test for response differences between particular pairs or combinations of odor treatments in the studies. We highlight general findings and discuss important details of the trapping results for experiments 1–6 below.

Experiments 1–3. Results showed some variation among experiments 1–3 and between host trees within experiments, but traps baited with AC were nevertheless generally more or as effective at capturing flies as the EA blend (Figs. 1–4), as previously reported by Yee et al. (2005), and the EH blend. The only exception was for males in Puyallup apple Orchard 1 in 2007 in experiment 1, where significantly more flies were captured on EA than AC traps (Fig. 2A). Little difference was found between using EA versus the MA blend in traps (Fig. 4).

Experiments 4–6. Although the results displayed some variation among experiments and across sites, years, and host trees, traps baited with AC were nevertheless generally more or as effective at capturing flies as both the eastern and western volatile fruit blends (Figs. 5–7). Moreover, traps with apple and hawthorn fruit volatiles did not capture more flies than traps with AC during early, mid-, or late trapping periods ($P > 0.05$, results not shown). Thus, AC was the most consistent and effective attractant of western *R. pomonella* across time (years and seasons), space (sites), and host plants in the study.

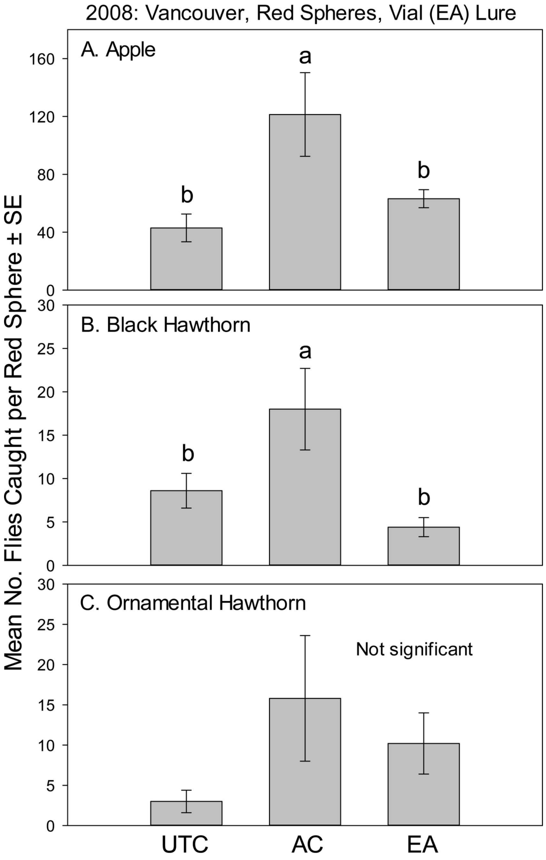

Fig. 3. Captures of *R. pomonella* (sexes combined) on spheres baited with odors in 2008 in (A) apple, (B) black hawthorn, and (C) ornamental hawthorn trees in Vancouver, WA. UTC, untreated control; AC, ammonium carbonate; EA, eastern apple. Means with same letters are not significantly different ($P > 0.05$).

Differences were observed among the different volatile blends in their attractiveness to flies. A portion of the variation was consistent with flies preferentially orienting to volatiles of their natal apple and hawthorn host fruit. In apple trees in experiments 4 and 5 at the Puyallup site in 2011 and 2012, more flies were captured using the EA and WA apple fruit blends than on traps baited with OH and BH blends (EH, BH, or OH; Figs. 5A, 6C). This pattern was observed regardless of whether yellow sticky panels in experiment 4 (Fig. 5A) or red sticky spheres in experiment 5 (Fig. 6C) were used to trap flies. The same trend was present in Puyallup for apple flies being more attractive to apple than hawthorn blends in the early part of the season in 2013 from 19 July to 8 August in experiment 6 (Fig. 7A), with the exception that males captured on BH did

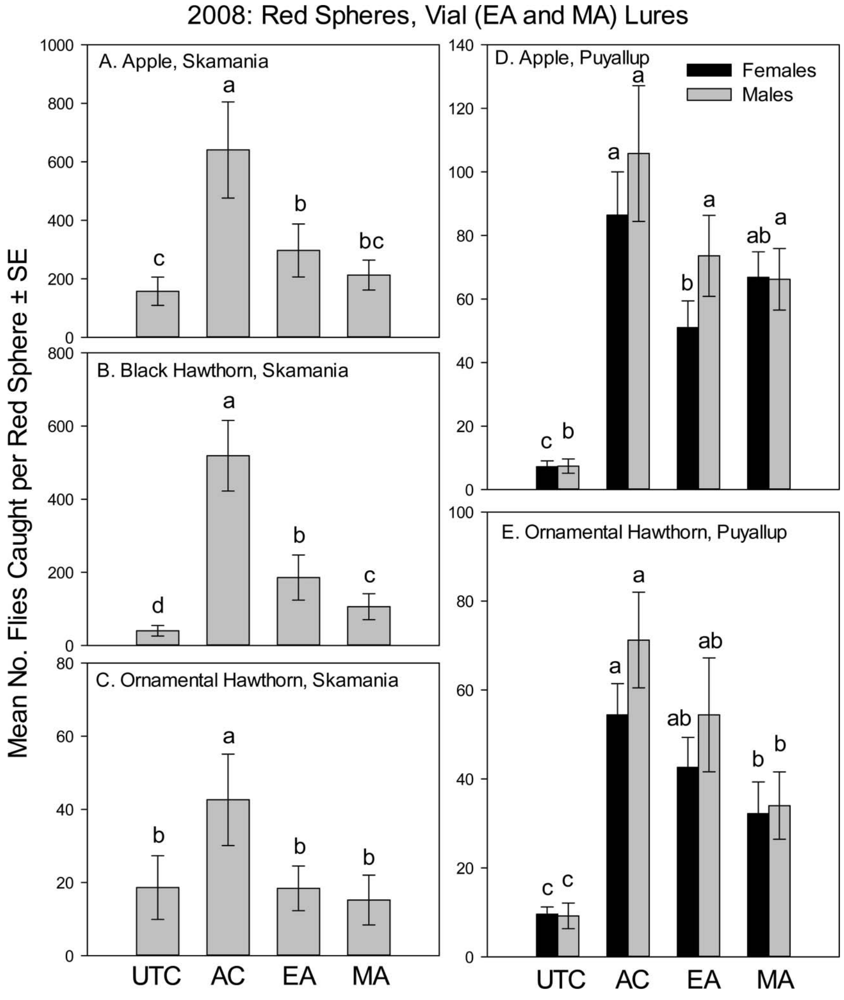

Fig. 4. Captures of *R. pomonella* on spheres baited with odors in 2008 in (A) apple, (B) black hawthorn, and (C) ornamental hawthorn trees in Skamania (sexes combined), and in (D) apple and (E) ornamental hawthorn in Puyallup, WA. UTC, untreated control; AC, ammonium carbonate; EA, eastern apple; MA, modified eastern apple. Means or means within sexes with same letters are not significantly different ($P > 0.05$).

not differ significantly in numbers from males trapped on EA traps. Discrimination of apple flies for the WA blend versus the hawthorn blends increased in the latter part of the season in Puyallup from 8 August to 13 September in 2013 (Fig. 7C). However, while the relative attractiveness of the WA blend to apple flies increased in the latter half of the 2013 season in Puyallup, this was not the case for EA. Instead, EA traps caught statistically fewer flies than WA traps from 8 August to 13 September and more similar numbers as BH and OH traps (Fig. 7C).

In contrast to flies trapped in apple, flies trapped in ornamental hawthorn trees in Puyallup in experiments 4–6 displayed a general trend to be captured significantly more often on traps baited with hawthorn than apple fruit volatiles (Figs. 5B, 6D, 7D). The only exception to this pattern was the early season result in experiment 6 (Fig. 7B). In experiment 4 in 2011, ornamental hawthorn flies also showed increased attractiveness for their natal OH versus the BH blend (Fig. 5B). However, this pattern was not significant in experiment 6 (Figs. 7B and D).

In contrast to the results for apple and hawthorn trees, flies captured in black hawthorn trees at the Skamania site in experiment 5 in 2012 did not display increased attractiveness for hawthorn (including their natal BH blend) compared with apple volatiles (Figs. 6A and B).

Discussion

Our results indicate that AC is more attractive than various formulations of fruit volatile-based blends to western *R. pomonella* in Washington State. Newly developed western fruit volatiles displayed a range of variation in their effectiveness from being unattractive compared with controls, more attractive than controls but less than AC, to as attractive as AC. However,

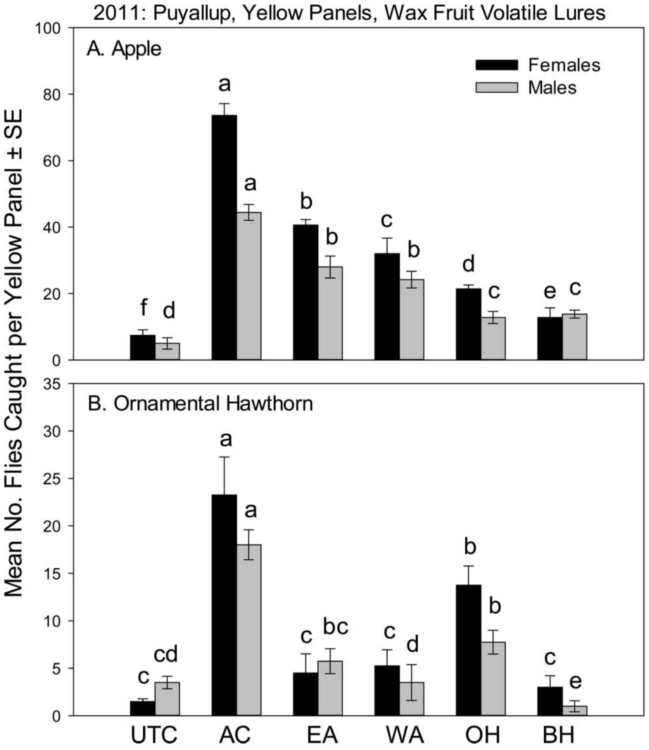

Fig. 5. Captures of *R. pomonella* on yellow panels with odors in 2011 in (A) apple and (B) ornamental hawthorn trees in Puyallup, WA. UTC, untreated control; AC, ammonium carbonate; EA, eastern apple; WA, western apple; OH, western ornamental hawthorn; BH, western black hawthorn. Means within sexes with same letters are not significantly different ($P >$ 0.05).

neither eastern nor western fruit blend (whether apple or hawthorn) traps captured more western flies than AC traps. Thus, AC was the most consistent attractant for *R. pomonella* in the study, implying that it should be continued to be used as the standard for annual monitoring of apple maggot flies in the western United States. Fruit volatiles can still be useful in surveys, however, for helping determine the possible origin and existence of *R. pomonella* host races in this region (Linn et al. 2012, Sim et al. 2012). This also has practical value because it can show the fly populations that are more or less likely to attack commercial apples.

Reports that AC performs poorly against *R. pomonella* in the eastern United States compared with lures based on fruit volatiles are perplexing given our current findings and older reports in the literature showing that eastern flies are attracted to ammonia or proteinaceous compounds (Hodson 1943, 1948; Neilson 1960; Reissig 1974, 1975). In Massachusetts, red spheres baited with AC lures performed no better than control, odorless spheres (Reynolds and Prokopy 1997, Rull and Prokopy 2000). On this basis, researchers concluded that "ammonium carbonate should no longer be considered strongly as a potent attractant" for use in orchards against *R. pomonella* (Rull and Prokopy 2000). It is possible that release rates of 650–700 μg/h from AC lures used in Reynolds and Prokopy (1997) were too low, as higher ammonia release rates have been clearly shown to lead to increased catch rates of *R. pomonella* in the western United States and of other tephritids (Bateman and Morton 1981; Mazor et al. 1987, 2002; Yee and Landolt 2004). How traps are baited with ammonia may also affect responses. In a Michigan study where AC performed no better than the control (Stelinski and Liburd 2002), 2 g AC or ammonium acetate and 0.5 g protein hydrolysate were mixed in Tangle Trap and applied to spheres. This mixture was similar to what was used in an earlier Massachusetts study, except that 1 g instead of 2 g of ammonium acetate was used per sphere (Prokopy and Hauschild 1979). Mixing ammonium acetate, protein hydrolysate, and Tangle Trap may be convenient for coating traps, but an ineffective way of dispensing ammonia odors compared with external vial lures (Jones 1988). Another contributing factor may be that humid conditions in the eastern United States cause the hygroscopic AC to quickly lose potency (Reynolds and Prokopy 1997). In the dry conditions of Utah, yellow panels with lures containing 10 g of AC outperformed red spheres with the "Fein" blend of apple volatiles for catching *R. pomonella* (Jones and Davis 1989). However, western Washington is humid like the eastern United States, yet the AC lures performed well in attracting western *R. pomonella.*

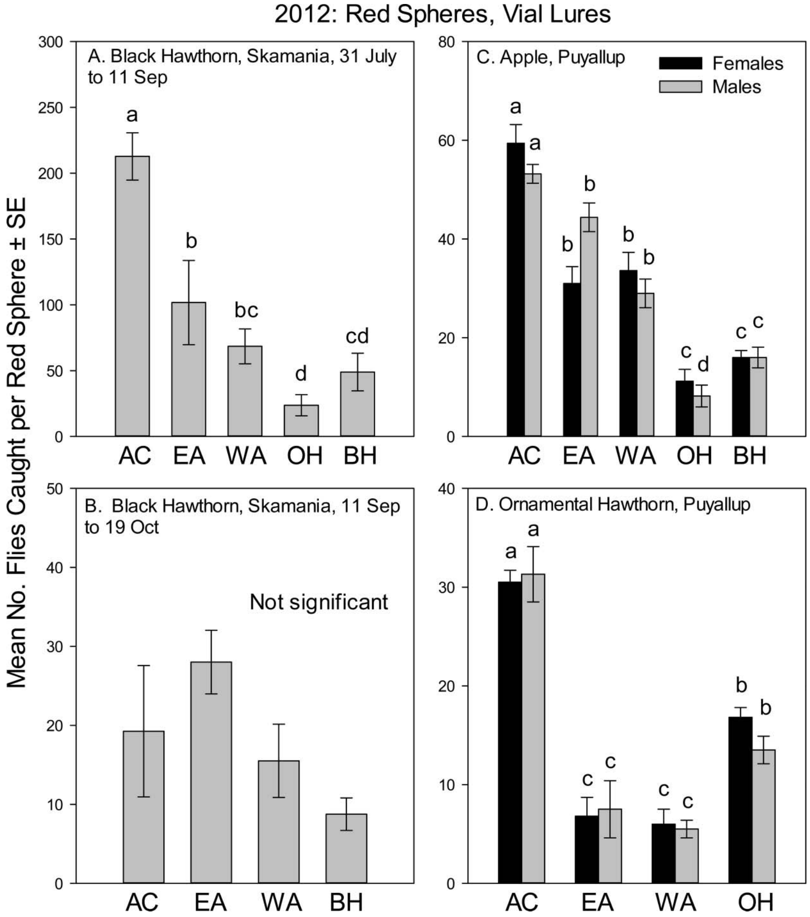

Fig. 6. Captures of *R. pomonella* on spheres baited with odors in 2012 in (A) apple and (B) black hawthorn trees in Skamania (sexes combined), and in (C) apple and (D) ornamental hawthorn in Puyallup, WA. AC, ammonium carbonate; EA, eastern apple; WA, western apple; OH, western ornamental hawthorn; BH, western black hawthorn. Means or means within sexes with same letters are not significantly different ($P > 0.05$).

Differences in the responses to AC could also represent genetically based variation in the sensitivity of eastern and western flies to ammonia. Possibly the high response of western flies to AC is associated with the introduction of *R. pomonella* to the Pacific Northwest from its native eastern North America. A small subset of introduced flies from the east may have by chance had a higher than average response to AC. A similar "bottleneck" explanation was made for differential responses by eastern and western *R. pomonella* to the newly identified WA volatile blend (Linn et al. 2012). Studies of the antennal and flight tunnel responses of eastern and western *R. pomonella* to ammonia and the use of standardized AC lures in the field are needed to resolve the issue of variation in attractiveness of ammonia across North America.

Another possible explanation for inconsistencies between results here and those from the eastern United States is the difference in environments where trap studies took place. In eastern North America in apple where fruit volatiles and spheres work well, flies that are trapped apparently originate outside orchards (Prokopy et al. 1990, Bostanian et al. 1999), perhaps drawn in by volatiles acting as a longer-range attractant than AC. In the current study, short-range attraction to AC may effectively trap flies because they overwintered as pupae below their hosts. Some of the variation among the three Washington experimental sites may have been related to fly location. Long-range attraction may not have been as important at the Skamania site, where flies were abundant under each host and hosts were close together, than in Puyallup. The Puyallup apple sites may have been more like eastern North American orchards, in that proportionately more flies originated outside of them. Indeed the apple volatiles did well at this site.

Fruit volatiles did not become more attractive than AC later in the season in western Washington, even though in the eastern United States responses to synthetic blends may be higher when fruit are unripe or overripe (Reissig et al. 1982, Carle et al. 1987). In New York, responses by *R. pomonella* to fruit volatiles increased later in the season, when apples dropped (Reissig et al. 1982), but ammonia compounds were not

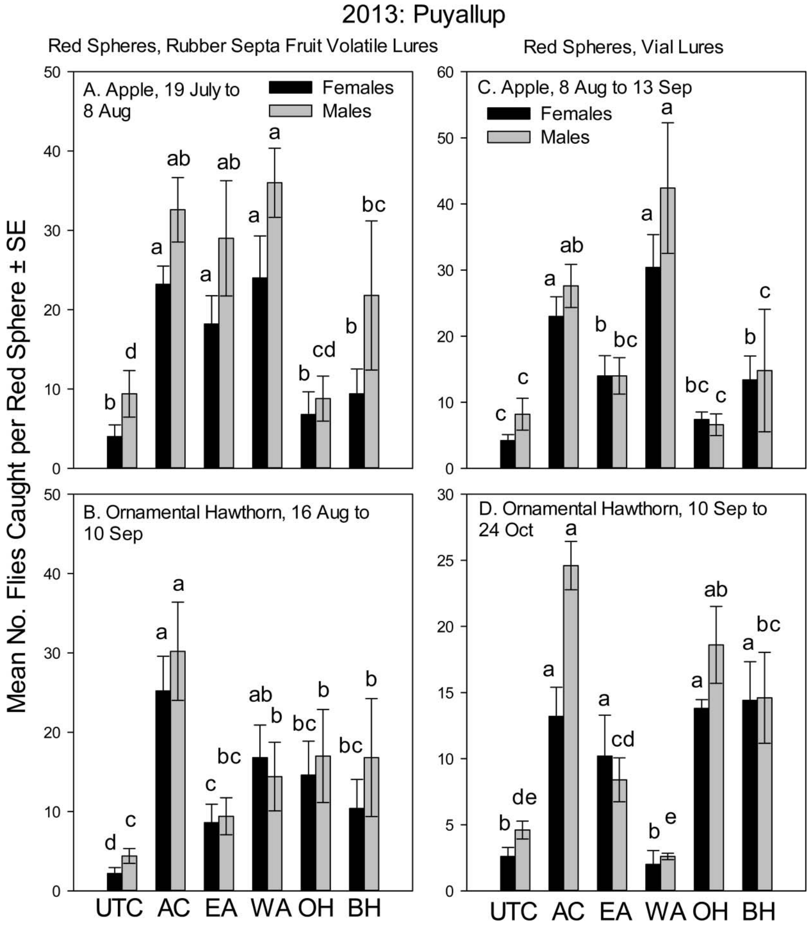

Fig. 7. Captures of *R. pomonella* on spheres baited with odors in 2013 in (A) apple and (B) ornamental hawthorn trees using rubber septa fruit volatile lures, and in (C) apple and (D) ornamental hawthorn trees using vial lures in Puyallup, WA. UTC, untreated control; AC, ammonium carbonate; EA, eastern apple; WA, western apple; OH, western ornamental hawthorn; BH, western black hawthorn. Means within sexes with same letters are not significantly different ($P > 0.05$).

tested. In Massachusetts, butyl hexanoate, a key component of the EA blend, was more attractive to *R. pomonella* than AC throughout the season (Reynolds and Prokopy 1997), which differs from the results presented here for western flies. Some data indicated differences in responses to fruit volatiles over the season. Specifically, fly responses in ornamental hawthorn trees to hawthorn volatiles seemed greater later than earlier in the season in experiment 6 in Puyallup (Fig. 7B and D). The difference could be explained by either seasonal variation in the odor discrimination behavior of flies or by the changing of the volatile delivery method from rubber septa to vials that altered release rates.

Fly responses to fruit volatiles among tree species differed, so even if fruit volatile lures were used for monitoring, no one blend can be effectively used on apple, black hawthorn, and ornamental hawthorn trees. Previous laboratory (Linn et al. 2005, 2012) and field trapping studies (Sim et al. 2012) have found that apple-, black hawthorn-, and ornamental hawthorn-origin flies in Washington prefer their natal fruit blends and tend to be antagonized by nonnatal volatiles. Here, we report similar trends for flies in apple and ornamental hawthorn trees, but not black hawthorn trees (Fig. 6A and B). Variable responses by *R. pomonella* to fruit volatile odors from lures within and across field studies are common (e.g., Reissig et al. 1982, 1985; AliNiazee et al. 1987; Jones and Davis 1989; Reynolds and Prokopy 1997; Stelinski and Liburd 2002) and could be affected by many factors, including the physiological state of flies (age and hunger) and the environment (fruit loads and ripeness, competing odors, temperature, precipitation or humidity, and wind currents). Also, the blends may not show the specificity in mixed-tree settings such as Skamania that they do in single tree settings because of fly movement among different trees. This is suggested by the high fly numbers on apples and hawthorns from August to September at Skamania (Tracewski et al. 1987).

In addition to the above factors affecting responses to fruit volatiles, the trapping methods we used in the current study were not set up to determine differences in host fruit odor preference by flies. This requires more elaborate configurations of control versus odor-baited traps within trees (Forbes and Feder 2006, Sim et al. 2012) than the single trap per tree design we used. However, the one-trap-per-tree design is the most practical for field monitoring of *R. pomonella* and was used in our study to maximize the portability of our findings to pest management and control of the fly.

Tests using fruit volatiles versus AC in the current study in western Washington have not been performed in central Washington, where major commercial apple orchards are found and monitoring is most important. The low abundance of *R. pomonella* in central Washington has made it difficult to conduct trapping studies in this region (W.L.Y., unpublished data). However, it is possible that the drier climate, presence of mostly black hawthorn-adapted flies (Yee 2008), and higher numbers of nontarget chloropid flies cluttering traps in central Washington (Yee et al. 2005) could produce different results from those we report here.

Most of the results we report were obtained using red spheres, which are commonly used in the eastern United States. Although spheres are more attractive to *R. pomonella* than yellow panels in Washington (Yee and Landolt 2004), WSDA continues to use the panels. Results for experiment 4, however, imply that our findings for AC are generally transferable and applicable regardless of which trap is used.

In conclusion, our results indicate that in western Washington, AC is more attractive than the newly identified fruit volatile blends as they are formulated in the current study and at the release rates tested. While certain fruit volatile blends in some tests were as attractive as AC, they performed inconsistently across experiments and host plants compared with AC, possibly because of their greater sensitivity to a combination of factors, including the existence of fly host races, variation in fly physiology, and differences in environmental conditions. Studies in central Washington are needed to determine the extent to which the current findings can be applied to *R. pomonella* present in the margins of commercial apple-growing regions. However, at the present time the continued use of AC as the standard attractant for monitoring *R. pomonella* in Washington appears warranted.

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