

FINAL REPORT**DURATION: 1 YEAR****Project Title:** Apple maggot host attractants**PI:** Wee Yee
Organization: USDA-ARS
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State/Province/Zip: NY/14456**Cooperators:** Various homeowners, orchard owners, county parks personnel**Total Project Request: Year 1:** \$30,000**Other funding Sources****Agency Name:** Washington State Commission on Pesticide Registration (WSCPR)**Amount requested or awarded:** \$26,130**WTFRC Collaborative expenses:** None**Budget 1**

Organization: USDA-ARS	Contract Administrator: Bobbie Bobango		
Telephone: 509-454-6575	Email: Bobbie.Bobango@ARS.USDA.GOV		
Item	2008		
Salaries	0		
Benefits	0		
Wages	\$11,000 ¹		
Benefits	\$1,100		
Equipment	0		
Supplies	\$1,500 ²		
Travel	\$1,400 ³		
Miscellaneous	0		
Total	\$15,000		

¹ One GS-5 technician; ²Traps and components for lures; ³Fuel for 2 personal car for travel to field sites to collect fruit/pupae and to conduct trapping experiments.**Budget 2:**

Organization: Cornell University	Contract Administrator: Donna Loeb		
Telephone: 315-787-2325	Email: dr2@cornell.edu		
Item	2008		
Salaries	\$9,840		
Benefits	\$5,160		
Supplies	0		
Travel	0		
Miscellaneous	0		
Total	\$15,000		

RECAP OF ORIGINAL OBJECTIVES

1. To expand the survey of fly responses to traps baited with apple and other fruit volatiles at strategic host localities in central and western Washington.
2. To complete the identification of volatiles from black hawthorn, ornamental hawthorn, snowberry, and apple fruit.
3. To conduct extensive behavioral tests of the responses of apple maggot from Washington to volatile blends identified from black hawthorn, ornamental hawthorn, snowberry, and apple.

SIGNIFICANT FINDINGS

- Washington apple maggot (AM) flies reared from apple were more attracted in a flight tunnel to a newly identified apple blend (WA apple) than to the previously identified eastern apple blend.
- AM flies reared from black hawthorn fruit responded in a flight tunnel in much greater numbers to a newly identified WA black hawthorn blend (haw blend) than to eastern hawthorn, WA apple, and eastern apple blends.
- AM flies reared from ornamental hawthorn fruit did not respond in a flight tunnel in high numbers to any of the blends.
- Field tests showed that AM flies are attracted to the eastern apple blend and the WA apple blend in approximately equal numbers in apple, ornamental hawthorn, and black hawthorn trees, and generally were more attracted to them later than earlier in the season. On isolated apple trees, apple blends performed better than on apple trees in sites with hawthorn trees.
- On central WA black hawthorn trees, fruit volatiles attracted few flies, indicating that the fruit volatiles tested may be habitat, host, or population specific.
- However, in central Washington, fruit volatile-baited sticky red spheres were much more selective than ammonia-baited red spheres, as fruit volatiles attracted AM flies almost exclusively, whereas ammonia attracted both AM flies and high numbers of snowberry maggot flies, making fly identifications difficult.

RESULTS AND DISCUSSION

Objective 1. Expand Survey of Fly Responses to Traps Baited with Apple and Other Fruit Volatiles in Central and Western Washington. Trapping using new fruit volatiles identified and tested in objectives 2 and 3 (below) was conducted on three host trees: (1) apple; (2) ornamental hawthorn (*Crataegus monogyna*); (3) black hawthorn (*Crataegus suksdorfii* and *Crataegus douglasii*). Trees were trapped in three regions in Washington - Puyallup, Vancouver/Skamania, and Wenas. On apple in Puyallup, flies were attracted equally (statistically) to the eastern apple blend, newly identified WA apple blend, and AC treatments (**Table 1**).

On apple (**Table 1**) in Skamania, flies were attracted equally to the eastern apple and WA apple blends, although less than to AC. The differences between the control and eastern and WA apple blends at Puyallup were greater than those at Skamania. At Puyallup, there were 9.8 times more flies in the apple blend treatments than in the control, whereas at Skamania, there were only 1.9 and 1.3 times more. A likely explanation is that at Puyallup, an isolated apple orchard was trapped, whereas at Skamania, mixed stands of apple and hawthorn trees were trapped. Apple blend odors may have stood out more when the background odors were from apple only as opposed to being from apple and hawthorns combined, which may have an antagonistic effect on one another and make detection of

apple blend odors from lures more difficult for flies to detect. On apple at WSU, flies were not more attracted to the eastern apple blend than to the control. The population at this site was low, which may have contributed to the inability to detect a significant difference. The test here was conducted early in the season, before the WA apple blend was available.

Table 1. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in apple trees in Washington, 2008.

On Apple Trees					
Treat	Site 1 (Puyallup)	Treat	Site 2 (Skamania)	Treat	Site 3 (WSU)
Control	13.0B	Control	157.8C	Control	43.0B
AC	168.8A	AC	640.8A	AC	121.4A
East. Apple	122.0A	East. Apple	297.2B	East. Apple	63.2B
WA Apple	128.0A	WA Apple	213.0BC		
$F = 21.6; df = 3, 12; P < 0.0001$		$F = 23.7; df = 3, 12; P < 0.0001$		$F = 8.8; df = 2, 8; P = 0.0094$	

^bFrom 21 August to 9 October.

On ornamental hawthorn trees (**Table 2**) at Puyallup, flies were equally attracted to the eastern apple and WA apple blends, at 5.1 and 3.7 times more than the control. On ornamental hawthorn at Skamania, the eastern and WA apple blends were no more attractive than the control. This was also true on ornamental hawthorn at WSU. These results suggest that the host tree species affects responses by flies to apple blends. Flies that develop in ornamental hawthorn at some sites apparently are not attracted to the current apple blends that attract flies that develop in apples.

Table 2. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in ornamental hawthorn trees in Washington, 2008.

On Ornamental Hawthorn Trees					
Treat	Site 1 (Puyallup)	Treat	Site 2 (Skamania)	Treat	Site 3 (WSU)
Control	20.0C	Control	18.6B	Control	3.0A
AC	142.8A	AC	42.6A	AC	15.8A
East. Apple	101.2B	East. Apple	18.4B	East. Apple	10.2A
WA Apple	73.4B	WA Apple	15.2B		
$F = 32.2; df = 3, 12; P < 0.0001$		$F = 3.5; df = 3, 12; P = 0.0488$		$F = 3.4; df = 2, 8; P = 0.0831$	

On western black hawthorn trees (**Table 3**) at Saint Cloud, flies were 2.1 times more attracted to the black haw blend than to the control. The black haw blend lacked a component that needed to be synthesized in the laboratory, so the complete blend needs to be tested. By the time the black haw blend was identified in the lab (objective 2), the field season was underway and there was insufficient time to synthesize this component. On black hawthorn, flies were more attracted to the eastern apple than WA apple blend, but the WA apple blend was still 2.6 times more attractive than the control. On black hawthorn at WSU, the black haw blend did not attract more flies than the control.

Table 3. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in western black hawthorn trees in Washington, 2008.

On Western WA Black Hawthorn Trees			
Treat	Site 1 (Skamania)	Treat	Site 2 (WSU)
Control	40.2D	Control	8.6B
AC	518.8A	AC	18.0A
Black Haw	86.4C	East. Apple	4.4B
East. Apple	185.8B		
WA Apple	106.0C		
$F = 41.7; df = 4, 6; P < 0.0001$		$F = 12.2; df = 2, 8; P = 0.0037$	

On central WA black hawthorn trees (**Table 4**), flies were not attracted to the eastern apple and black hawthorn blends. Results suggest that responses by flies at different sites and from different hosts differed, as alluded to before. Because all apple maggot fly populations tested to date respond to fruit volatiles, it is likely that these flies will be attracted to the complete black haw blend that has yet to be tested. Importantly, however, 0% and 26.3% of flies caught on spheres baited with the eastern and WA apple blends, respectively, were snowberry maggot flies, whereas 76.8% of flies caught on ammonia-baited spheres were snowberry maggot flies, indicating much greater specificity of the apple blends for apple maggot flies. Because apple maggot and snowberry maggot flies are indistinguishable without laboratory examination, use of fruit volatiles that target apple maggot flies has a clear advantage over the use of ammonia.

Table 4. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in central black hawthorn trees in Washington, 2008.

On Central WA Black Hawthorn Trees			
Treat	Site 1 (Wenas) ^a	Treat	Site 1 (Wenas) ^b
Control	0.60B	Control	0.13C
AC	3.27A	AC	3.13A
East. Apple	0.07B	East. Apple	1.20AB
Black Haw	0.20B	WA Apple	0.93BC
<i>F</i> = 11.9; <i>df</i> = 3, 56; <i>P</i> < 0.0001		<i>F</i> = 6.7; <i>df</i> = 3, 56; <i>P</i> = 0.0006	

^aFrom 17 July to 21 August.

On apples, there were changes in attractiveness of the volatiles over the season (**Fig. 1**). At Puyallup (**Fig. 1A**), the eastern apple blend was slightly more attractive than the WA blend early in the season, but later they were equally attractive and on some dates the WA apple blend was more attractive. On apples in Skamania County (**Fig. 1B**), the WA apple blend was slightly more attractive than the eastern apple blend in early August, but the trend was reversed later in the month and into early September. Overall, both apple blends appeared less attractive at Skamania than at Puyallup, perhaps because there were mixed stands of apples and hawthorns in Skamania, whereas in Puyallup the apple orchard was isolated.

On ornamental hawthorn in Puyallup (**Fig. 2A**), the eastern apple blend was more attractive than the WA apple blend during mid season, but the WA apple blend was equally attractive later in the season. On black hawthorn at Skamania (**Fig. 2B**), the eastern apple blend was generally more attractive than the WA apple blend throughout the season, and the black haw blend was attractive late in the season.

Overall trapping results indicate that apple volatile blends identified as attractive in the flight tunnel (objectives 2 and 3 below) are also attractive in the field. Their effectiveness, however, seems to depend on the host and site where they are used. There may not be a general fruit volatile blend that is equally effective across all host trees, so several specific blends, specifically one from apple and one or two from hawthorns, may need to be identified for use in field detection surveys in the field. The inconsistencies in attractiveness of apple blends suggest that flies which develop in apple, ornamental hawthorn, and black hawthorn in Washington are genetically and behaviorally different from one another.

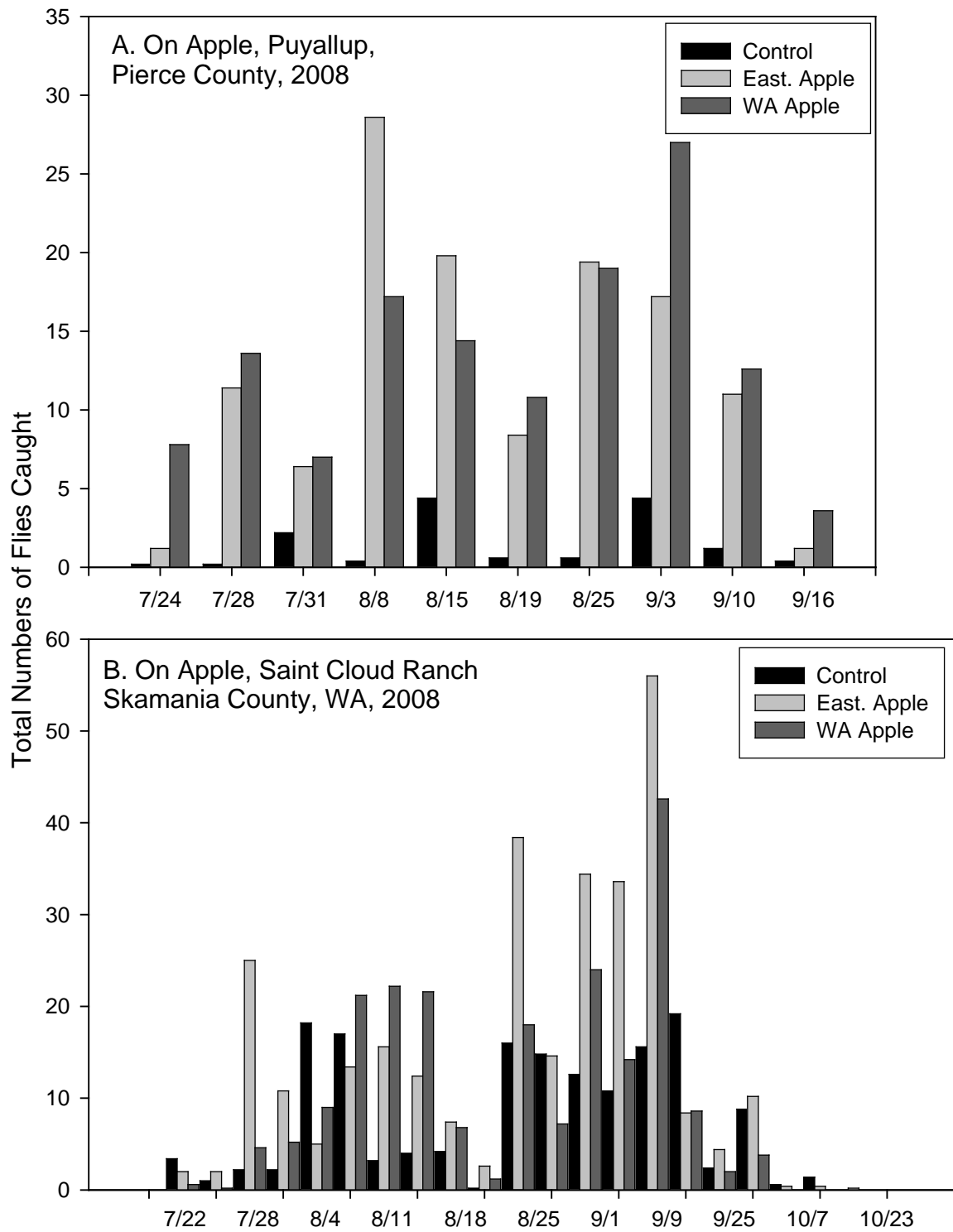


Fig. 1. Seasonal captures of apple maggot flies on sticky red spheres baited with different attractants on apple trees in 2008

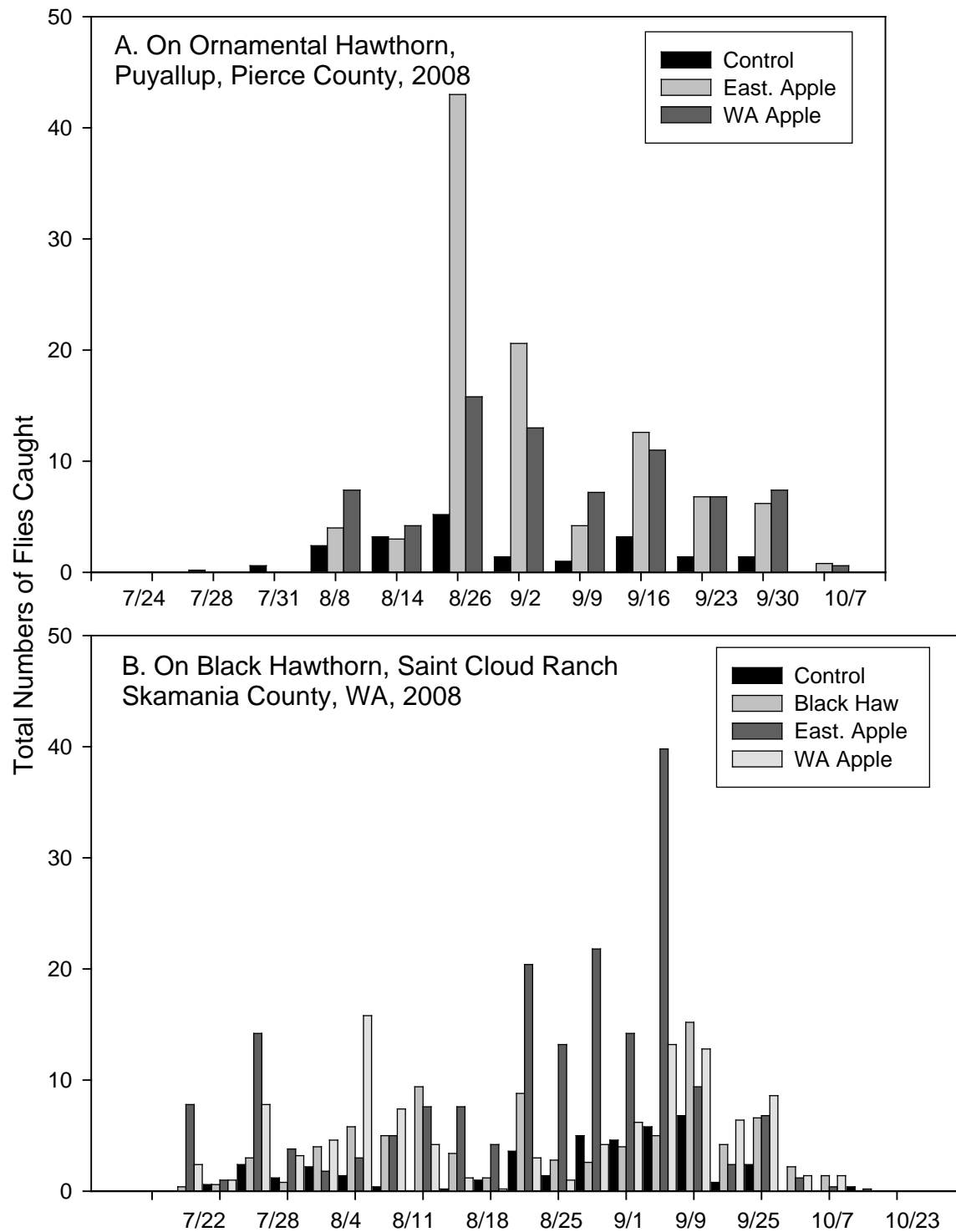


Fig. 2. Seasonal captures of apple maggot flies on sticky red spheres baited with different attractants on ornamental and black hawthorn trees in 2008

Objective 2. Complete Identification of Volatiles From Black hawthorn, Ornamental Hawthorn, Snowberry, and Apple Fruit. Characterization of fruit volatiles were performed using a combination of gas chromatography (GC) / electroantennagram (EAG) analysis of solid phase microextraction (SPME) samples from fruit. Synthetic blends of active compounds were iteratively tested in the flight tunnel to induce similar responses in flies as whole fruit extracts. Through repeated testing, a promising 9-component blend from apples and a 10-component blend from central WA black hawthorn fruit were isolated, which were then tested in the flight tunnel (objective 3).

Objective 3. Conduct Extensive Behavioral Tests of the Responses of Apple Maggot From Washington. In 2008 flight tunnel tests, apple maggot flies responded to the new blends of volatiles, which was the basis for the fruit volatile tests in the field (objective 1). Fly responses varied, but several clear patterns emerged (Figs. 3, 4, 5, 6): whereas NY apple maggots reared from apple were attracted to the eastern apple and WA apple blend equally (Fig. 3), Washington apple maggot flies reared from apple from St. Cloud (Skamania) (Fig. 4) and Puyallup (Fig. 5) were much more attracted to the WA apple blend than eastern apple blend. Apple maggot flies reared from black hawthorn fruit (Fig. 6) responded in the flight tunnel in much greater numbers to the newly identified WA black hawthorn blend than to eastern hawthorn, WA apple, and eastern apple blends. However, apple maggot flies reared from ornamental hawthorn from Puyallup (Fig. 7) did not respond in the flight tunnel in high numbers to any of the blends. Even though flies from apples tended to respond to apple blends and flies from hawthorns to the haw blend, some flies responded to several blends (Fig. 8). For example, four flies from apple from Washington responded to the eastern apple blend, WA apple blend, eastern hawthorn blend, and western hawthorn blend (BH). This suggests that within a fly population, some flies inherently are general responders, whereas other flies are more specific. Thus, any new attractant would be expected to attract some flies from apple and some from hawthorn. However, it remains true that flies from a particular host are most attracted to odors from that host. Snowberry fruit volatiles were isolated, but flies did not respond to them.

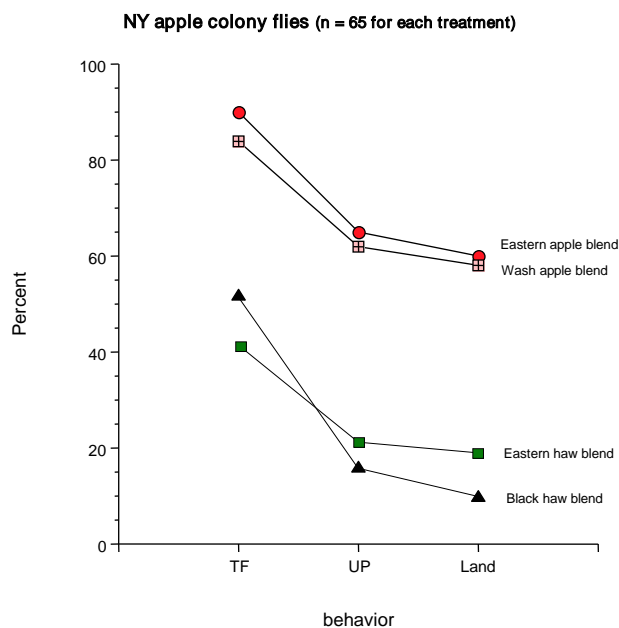


Fig. 3. Responses in a flight tunnel of NY apple maggot flies reared from apple to attractants.

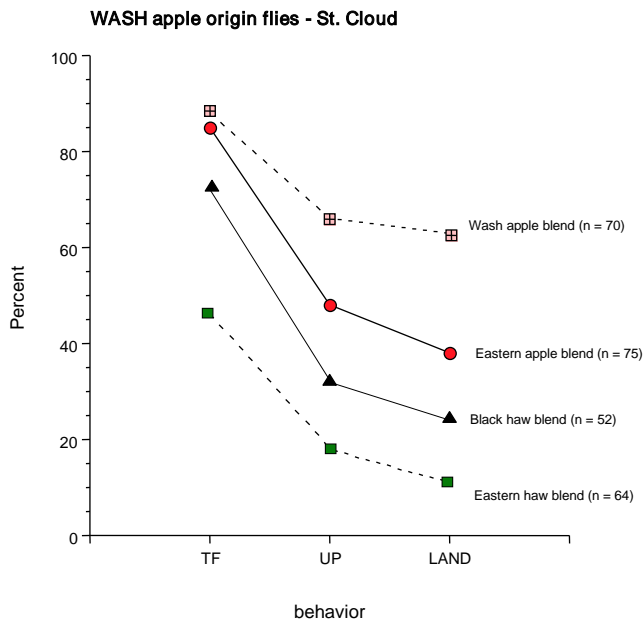


Fig. 4. Responses in a flight tunnel of WA (Skamania County) apple maggot flies from apple to attractants.

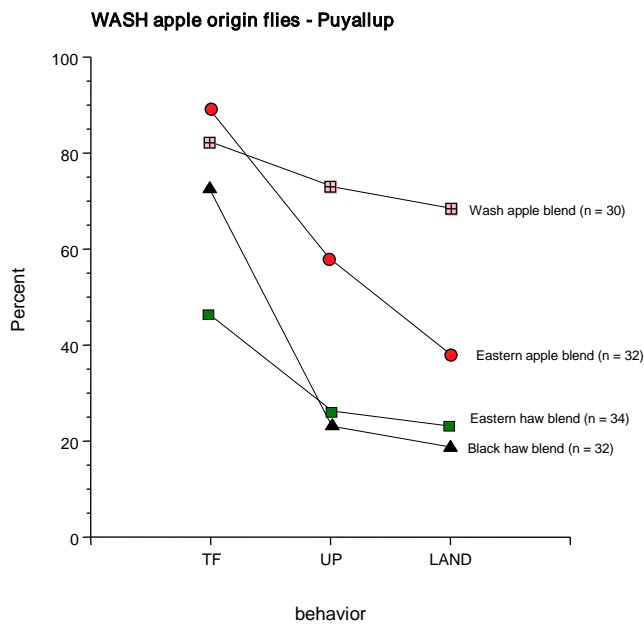


Fig. 5. Responses in a flight tunnel of WA (Puyallup) apple maggot flies reared from apple to attractants.

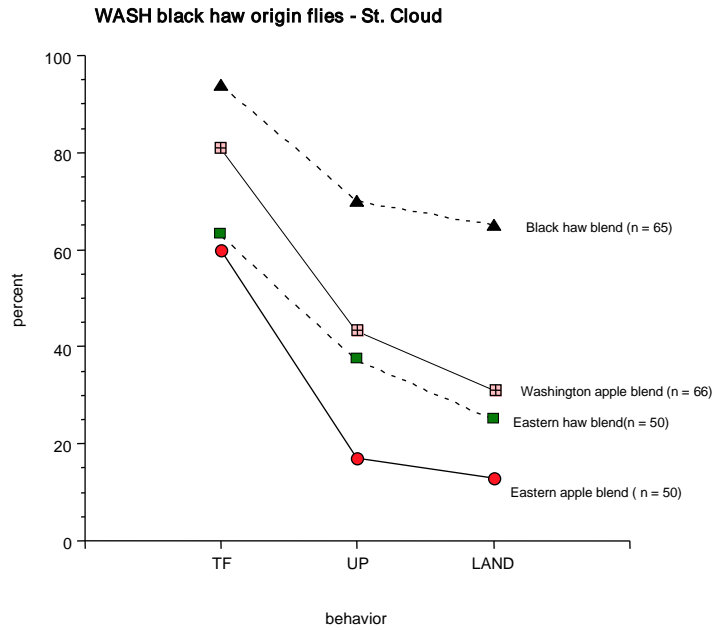


Fig. 6. Responses in a flight tunnel of WA (Skamania County) apple maggot flies reared from western WA black hawthorn to attractants.

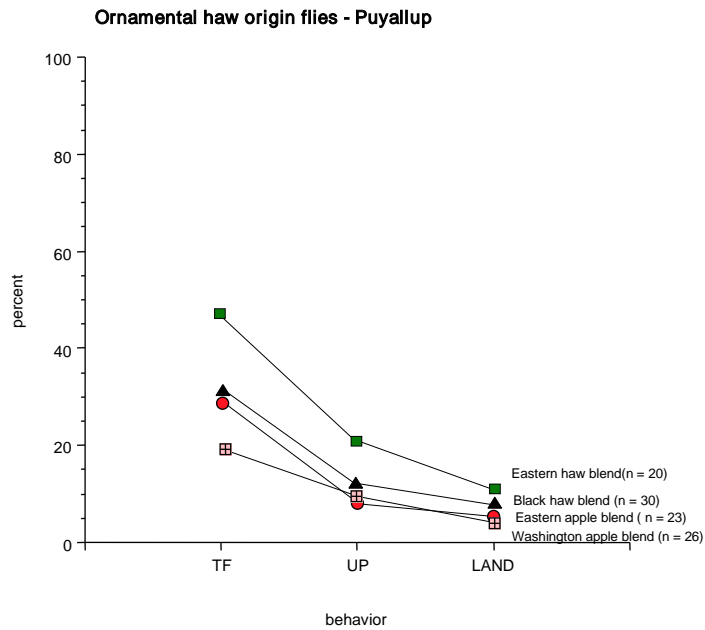


Fig. 7. Responses in a flight tunnel of WA (Puyallup) apple maggot flies reared from ornamental hawthorn to attractants.

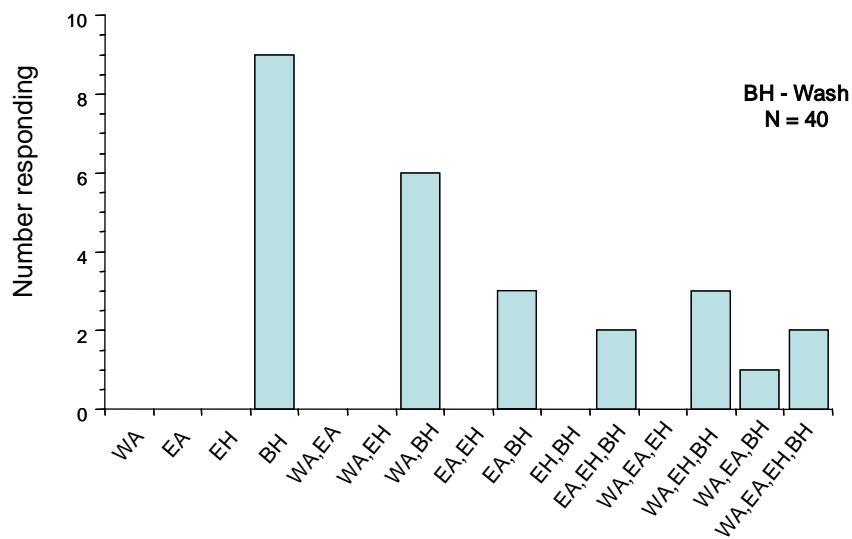
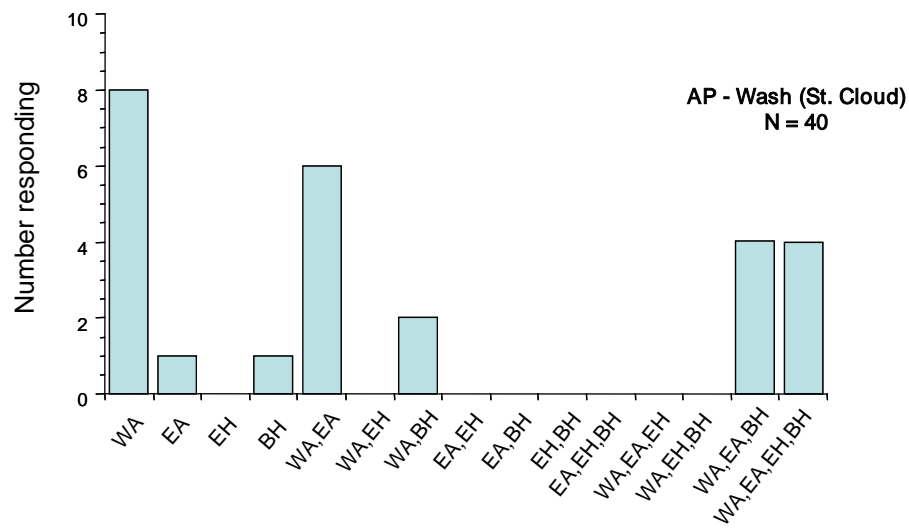
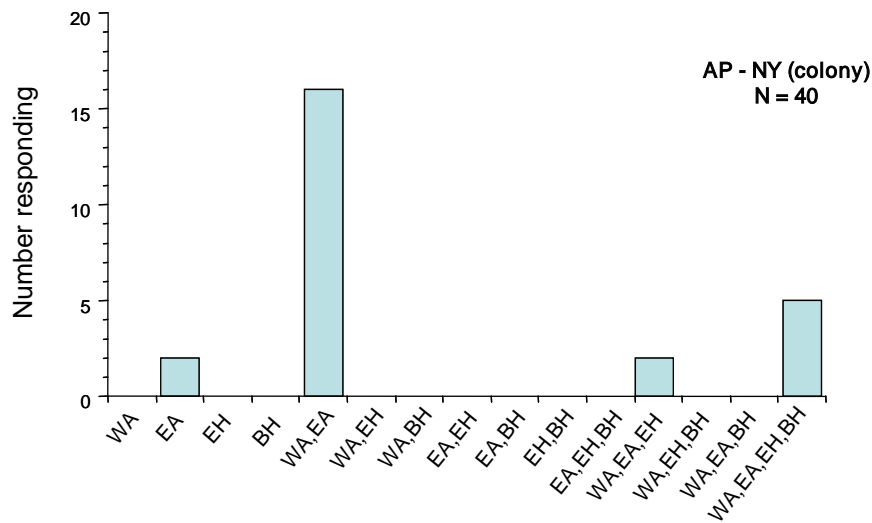


Fig. 8. Responses of NY and WA apple maggot flies to different fruit volatile blends.

Differences between field and laboratory results may be caused in part by the release rate of volatiles. In a flight tunnel, the flies need to fly only 1 m from the release point to the odor source. The amount of odor reaching the flies may be different than in the field, where many flies may be greater than 1 m away from the vials that contained the new blends. Thus, release rates may need to be increased in the field to elicit greater responses. Also, there are no competing host odors in a flight tunnel that could interfere with attractiveness, whereas these are present in the field and some odors may even be antagonistic with the newly identified blends. If so, blends that are not antagonized by other volatiles should be more attractive than current blends. Another possibility that may explain differences in the laboratory and field is that flies differed in their physiological state, i.e., their age, nutritional background, nutrient levels, prior experience with host odors, and mating status differed.

Significance to the Industry and Potential Economic Benefits

A highly effective attractant can be used to determine where apple maggot flies are located, and can document changes in their distribution, which is important because flies appear to be spreading into new regions in Washington. An insect population cannot be controlled if its distribution is unknown. A sensitive trap that can detect flies may help prevent their spread within the apple-growing regions. Keeping flies out of apple orchards can be accomplished if they are detected first and the positive sites are treated. Inaction because apple maggot flies are not detected due to insensitive traps may result in infested orchards and economic losses. There is no tolerance for larvae in fruit and shipments from any infested orchards likely would be banned.

A highly attractive and specific attractant can result in reduced labor costs needed to identify snowberry maggots caught on traps baited with ammonia lures that are intended to capture apple maggot flies. Costs can instead be re-directed to more in-field efforts of fly trapping in high-risk areas.

Present and future export markets will want to know if an area is apple maggot-free before accepting apples from the area. A highly attractive volatile that can detect low populations of flies can be used to provide evidence that an area is free of flies. This may help the industry gain access to markets that require areas be pest-free (fly-free) or that are considered to be low in pest prevalence.

EXECUTIVE SUMMARY

The presence of wide-spread breeding populations of the apple maggot fly in central Washington represents a serious threat to the apple industry, as there is a zero tolerance for larvae in exported apples. The fly continues to be found at new sites each year, suggesting the fly population is spreading. A major advance in managing the fly would be the development of a highly effective attractant that can be used to determine where the fly is located, and that can document changes in its distribution in central Washington. However, field survey tests using the fruit volatile attractants that are very effective in the eastern U. S. suggest they are not as effective for detecting the fly in Washington. An additional problem in developing an effective monitoring system is that we do not completely understand the identity of the various fly populations and their host/odor preferences in Washington. In 2007, with funding from the WTFRC, studies were initiated to establish whether fruit and flies from Washington displayed different volatile profiles/preferences. In a continuation of 2007 work, a project was conducted in 2008 to (1) to expand the survey of fly responses to traps baited with apple and other fruit volatiles at strategic host localities in central and western Washington; (2) to complete the identification of volatiles from black hawthorn, ornamental hawthorn, snowberry, and apple fruit, and (3) to conduct extensive behavioral tests of the responses of apple maggot from Washington to volatile blends identified from black hawthorn, ornamental hawthorn, snowberry, and apple. Washington apple maggot flies reared from apple were more attracted in a flight tunnel to a newly identified apple blend than to the previously identified eastern apple blend. AM flies reared from black hawthorn fruit responded in the flight tunnel in much greater numbers to a newly identified WA black hawthorn blend than to eastern hawthorn, WA apple, and eastern apple blends. AM flies reared from ornamental hawthorn fruit did not respond in the flight tunnel in high numbers to any of the blends. Snowberry fruit volatiles were isolated, but flies did not respond to them. Field tests showed that AM flies are attracted to the eastern apple blend and the newly identified WA apple blend in approximately equal numbers in apple, ornamental hawthorn, and black hawthorn trees, and generally were more attracted to them later than earlier in the season. On isolated apple trees, fruit volatiles performed better than on apple trees in sites with hawthorn trees. On central WA black hawthorn trees, fruit volatiles did not appear to be highly attract flies, indicating that the fruit volatiles tested may be habitat specific. Importantly, however, 0% and 26.3% of flies caught on spheres baited with the eastern and WA apple blends, respectively, were snowberry maggot flies, whereas 76.8% of flies caught on ammonia-baited spheres were snowberry maggot flies, indicating much greater specificity of the apple blends for apple maggot flies. Because apple maggot and snowberry maggot flies are indistinguishable without laboratory examination, use of fruit volatiles that target apple maggot flies has an advantage over the use of ammonia. There are other potential benefits of identifying attractive fruit volatiles. A sensitive trap that can detect flies may help prevent their spread within the apple-growing regions. Inaction because apple maggot flies are not detected due to insensitive traps may result in infested orchards and economic losses. There is no tolerance for larvae in fruit and shipments from any infested orchards likely would be banned. Finally, present and future export markets may want to know if an area is apple maggot-free before accepting apples from the area. A highly attractive volatile that can detect low populations of flies can be used to provide evidence that an area is free of flies. Results from the present flight tunnel tests show fruit volatiles are very promising but that further testing is needed to identify even more attractive blends for use in the field.