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ATTRACTION OF APPLE MAGGOT FLIES (DIPTERA: TEPHRITIDAE) TO SYNTHETIC FRUIT VOLATILE COMPOUNDS AND FOOD ATTRACTANTS IN MICHIGAN APPLE ORCHARDS

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ABSTRACT

The apple maggot, *Rhagoletis pomonella* (Walsh), is a serious pest of apples in the United States, requiring reliable monitoring and control programs. Various synthetic apple volatile lures with and without protein hydrolysate, ammonium acetate, or ammonium carbonate were evaluated from 1998-2000 for their attractiveness to *R. pomonella* adults with red sticky-sphere (9 cm diam.) monitoring traps. A blend consisting of butyl butanoate (10%), propyl hexanoate (4%), butyl hexanoate (37%), hexyl butanoate (44%), and pentyl hexanoate (5%) was the most effective lure tested for attracting both sexes of *R. pomonella* adults during all three field seasons. The addition of protein hydrolysate or ammonium compounds to spheres baited with a commercial attractant (BioLure) consisting of plastic dispensers containing butyl hexanoate, did not significantly increase apple maggot fly captures. Spheres baited with the blend or with butyl hexanoate in polyethylene vials and spheres baited with BioLure dispensers were highly selective in capturing *R. pomonella* flies relative to non-target insects. However, spheres baited with ammonium compounds with or without synthetic apple lures were non-selective with respect to apple maggot captures. Protein hydrolysate alone was ineffective for monitoring *R. pomonella* flies. We provide further evidence that baiting red-sticky sphere traps with the volatile blend without ammonium bait additives creates a highly effective and selective device for capturing apple maggot flies. The blend could be an important addition to current monitoring and control programs for apple maggot flies in Michigan orchards and other important apple growing regions.

Michigan ranks among the top states in apple production value in the U.S. Apples are grown commercially on more than 23,400 ha and more apples are produced by volume than all other Michigan fruits combined (Michigan Agricultural Statistics1999-2000). The apple maggot, *Rhagoletis pomonella* (Walsh), is an important fruit pest infesting commercially grown apples in Michigan and throughout the eastern U. S. There is zero tolerance for maggot infestation in apples bound for commercial sales. High numbers of apple maggot flies move from wild hosts into commercial orchards in Michigan, responding to visual and olfactory cues of host fruit, where mating and oviposition occurs (Liburd and Stelinski 1999). Sensitive and reliable monitoring techniques for the apple maggot fly are of extreme importance for making accurate and responsible control decisions.

Apple maggot flies are attracted to apple odors in the field (Prokopy et al.1973, Reissig 1974). Location of appropriate mating and oviposition sites by apple maggot flies is mediated in part by odors, which serve as primary cues for host site location and acceptance in fruit-infesting insects (Frey and Bush 1990). Among apple volatiles isolated from two apple varieties (Red Delicious and Red Astrachan), a blend consisting of a series of short chain carbon esters, including butyl hexanoate, is attractive to apple maggot flies (Fein et al. 1982). A comparison of visual stimuli showed that unbaited, 8 cm diam. red spheres captured significantly more apple maggot flies than unbaited, 10 cm diam. red spheres (Duan and Prokopy 1992).

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Furthermore, sticky spheres baited with vials releasing butyl hexanoate captured significantly more apple maggot flies than unbaited spheres, although there was no difference in catch with one, two, or four vials/sphere. A combination treatment containing one vial of ammonium carbonate and one vial of butyl hexanoate also significantly increased the capture of apple maggot flies compared to capture on unbaited spheres.

In addition to synthetic fruit volatiles, other odor baits are used in tephritid monitoring programs. Attraction of the Queensland fruit fly, *Dacus tryoni* Froggatt, to several hydrolysed proteinaceous baits is mainly due to the ammonia they release (Bateman and Morton 1981). Ammonia is a by-product of the bacterial decay of several adult tephritid food sources (Prokopy and Roitberg 1984) and is commonly used as an attractant in tephritid monitoring programs. Ammonia-producing baits may be attractive because flies are seeking a protein source important for egg maturation (Prokopy and Roitberg 1989, Prokopy 1993, and Prokopy et al. 1994).

The production of synthetic semiochemicals has led to the development of effective attractant-baited traps for monitoring and trapping of insects in agricultural production systems (Gut et al. in press). The approach is used to base management decisions on adult catches rather than taking a prophylactic calendar-based approach. Control sprays are typically applied only if catches exceed a predetermined level. Red sticky spheres baited with apple volatiles are often used to monitor apple maggot flies and alert pest managers for the need to apply control measures (Agnello et al. 1990, Stanley et al. 1987). An action threshold of 8 flies per trap was developed, which allowed for a 70% reduction in sprays with acceptable levels of control (Agnello et al. 1990).

More recently, Reynolds and Prokopy (1997) evaluated synthetic odor lures (butyl hexanoate, ammonium carbonate, or butyl hexanoate plus ammonium carbonate on red sticky spheres) for their attractiveness to apple maggot flies. Butyl hexanoate plus ammonium carbonate was more attractive than either odor alone. Finally, a recent study has shown that a lure consisting of a blend of synthetic apple volatiles, including butyl butanoate, propyl hexanoate, butyl hexanoate, hexyl butanoate, and pentyl hexanoate combined in specific proportions, was significantly more attractive to adult apple maggot flies than lures containing butyl hexanoate alone (Zhang et al. 1999).

Although a single published study showed evidence of increased attraction using the same blend we investigated, (Zhang et al. 1999), more research was needed to document information regarding the performance of this newly identified blend under different environmental conditions where apple maggot pressure is derived from within orchard plots and from surrounding abandoned fields. Furthermore, the selectivity of this blend to apple maggot flies as compared with other contemporary (butyl hexanoate, Biolure) lures and conventional (ammonium compounds) baits has not been documented. This information is relevant when developing monitoring programs for key pests since Drummond et al. (1984) and Liburd et al. (2000) have previously outlined the problems associated with trapping non-target insects on monitoring traps for *Rhagoletis* species.

The first objective of this research was to further investigate the attractiveness and selectivity of the newly identified blend. The second was to compare the attractiveness of the blend with contemporary butyl hexanoate lures and conventional ammonium baits. Our overall goal was to establish a sensitive, reliable, and selective monitoring device for apple maggot flies that could be implemented by apple growers in Michigan as well as other apple producing regions.

MATERIALS AND METHODS.

Research was conducted in apple orchards in Van Buren Co., Michigan during the 1998, 1999, and 2000 field seasons. Each treatment consisted of a 9 cm diam., red sphere coated with approximately 13 g of Tangle Trap (Great Lakes IPM, Vestaburg, MI). Spheres were hung approximately 25 m apart and 30 m between 1 ha blocks of Red Delicious and Golden Delicious. Treatments were arranged in a randomized complete block design (blocked by apple variety) with four replications. Trap positions were re-randomized weekly and old traps were replaced with new ones every 3 weeks. Apple maggot flies caught in traps were identified by sex, counted and removed biweekly. In addition, all non-target insect species captured on traps were counted and removed biweekly in 1998 and 2000. The non-target insects were separated taxonomically, and were used as a whole to estimate the proportion of apple maggot flies captured compared with non-targets.

1998. To evaluate the importance of protein hydrolysate and ammonium baits, we thoroughly mixed 2.0 g of ammonium acetate or ammonium carbonate and 0.5 g of protein hydrolysate into ca. 13 g of Tangle Trap before application to spheres. The experiment evaluated nine treatments that included red spheres coated with various synthetic protein, ammonia, and apple volatile sources (Table 1). BioLure dispensers (Consep. Inc., Bend, OR) containing a 1.8 g load rate of butyl hexanoate were also used in some treatments.

In a second study, we examined the attractiveness of different types of apple volatile blends with and without ammonium acetate (Table 2). In some

Table 1. Attraction of apple maggot flies to synthetic fruit volatiles and baits, Michigan, (1998).

Treatment	**Mean ± SEM flies per trap 25 June—9 August**
Volatile mix blend polyethylene vial	175.0 ± 35.5 a
Volatile mix blend polyethylene vial + ammonium acetate	60.5 ± 25.4 b
Biolure® dispenser	48.0 ± 2.9 b
Biolure® dispenser + ammonium acetate	55.8 ± 16.8 b
Butyl hexanoate polyethylene vial	75.3 ± 10.7 b
Butyl hexanoate polyethylene vial + ammonium acetate	71.3 ± 22.1 b

Means followed by the same letter are not significantly different (P = 0.05, LSD test).

Table 2. Attraction of apple maggot flies to synthetic fruit volatiles with and without ammonium-odor and protenaceous baits, Michigan, (1998).

Treatment	**Mean ± SEM flies per trap 3 July—19 August**
Ammonium acetate + protein hydrolysate + Biolure® dispenser	175.3 ± 39.6 a
Ammonium acetate + protein hydrolysate	143.8 ± 29.3 a
Ammonium acetate	215.5 ± 54.2 a
Ammonium carbonate + protein hydrolysate + Biolure® dispenser	174.3 ± 43.8 a
Ammonium carbonate + protein hydrolysate	156.3 ± 23.3 a
Ammonium carbonate	171.8 ± 35.9 a
Protein hydrolysate	34.3 ± 9.6 b
Biolure® dispenser	200.5 ± 53.5 a
Unbaited (control)	133.0 ± 26.1 a

Means followed by the same letter are not significantly different (P = 0.05, LSD test).

treatments, we used 5 ml polyethylene vials (Great Lakes IPM, Vestaburg, MI) containing 1.8 ml of butyl hexanoate or 1.8 ml of an apple volatile blend (1 g/ ml solution concentrations). The blend contained butyl butanoate (10%), propyl hexanoate (4%), butyl hexanoate (37%), hexyl butanoate (44%), and pentyl hexanoate (5%) as described in Zhang et al. (1999).

1999 and 2000. For our second and third field seasons, we chose four of the treatments evaluated in 1998 and compared them with unbaited (control) spheres (Table 3). The location of our study, experimental design, and sampling regime were the same as described for 1998.

Statistical analysis. This study was conducted using a randomized complete block design and all analyses were conducted using analysis of variance (ANOVA) (PROC GLM, SAS Institute 1989). Blocks in the experimental design were based upon known variability including tree cultivar differences. Data were square root transformed ($\sqrt{x} + 0.5$) to stabilize the variances. This was followed by mean separation using the least significant difference (LSD) test (SAS Institute 1989). Differences in captures between male and female flies were compared using *t*-test. Selectivity to apple maggot fly captures was calculated by determining the proportion of apple maggot flies captured relative to all non-target insect captures per trap. Percentage of apple maggot fly captures was also transformed (arcsine [square root (y)]) before analysis.

RESULTS

1998. Significantly ($F = 4.8$; df = 5,15; $P < 0.01$) more *R. pomonella* flies were captured on spheres baited with the blend compared with all other treatments tested (Table 1). There were no significant differences in total captures between spheres baited with a mixture of butyl hexanoate and ammonium acetate compared to catches with spheres baited with either compound alone (Table 1). Spheres having ammonium acetate bait with and without synthetic fruit volatiles captured significantly ($P < 0.05$) more females than males early in the season. This difference was not observed with spheres baited with synthetic fruit volatiles alone (Figure 1).

Spheres baited with protein hydrolysate captured significantly ($F = 4.1$; df = 8,24; $P = 0.03$) fewer *R. pomonella* flies in comparison to spheres baited with other lures (Table 2). With the exception of protein hydrolysate, there were no significant differences among catches with the other baits and lures evaluated in experiment 1. Spheres baited with BioLure (n = 15) alone were highly selective and captured an average of 70 ± 8.6 % apple maggot flies relative to non-target insects. Alternatively, spheres baited with ammonium carbonate and ammonium acetate were non-selective capturing on average 38 ± 3.6 % apple maggot flies, as well as many non-target insects.

1999. As observed in 1998, significantly ($F = 3.2$; df = 7,12; $P = 0.04$) more *R. pomonella* flies were captured on spheres baited with the blend compared

Table 3. Attraction of apple maggot flies to synthetic fruit volatiles and baits, Michigan, (1999).

Treatment	Mean ± SEM flies per trap
Volatile mix blend polyethylene dispenser	326.5 ± 67.2 a
Biolure® dispenser (Butyl hexanoate)	111.5 ± 13.8 b
Ammonium Acetate	164.3 ± 51.7 b
Butyl hexanoate polyethylene vial	150.3 ± 23.7 b
Unbaited (control)	105.5 ± 9.0 b

Means followed by the same letter are not significantly different ($P = 0.05$, LSD test)

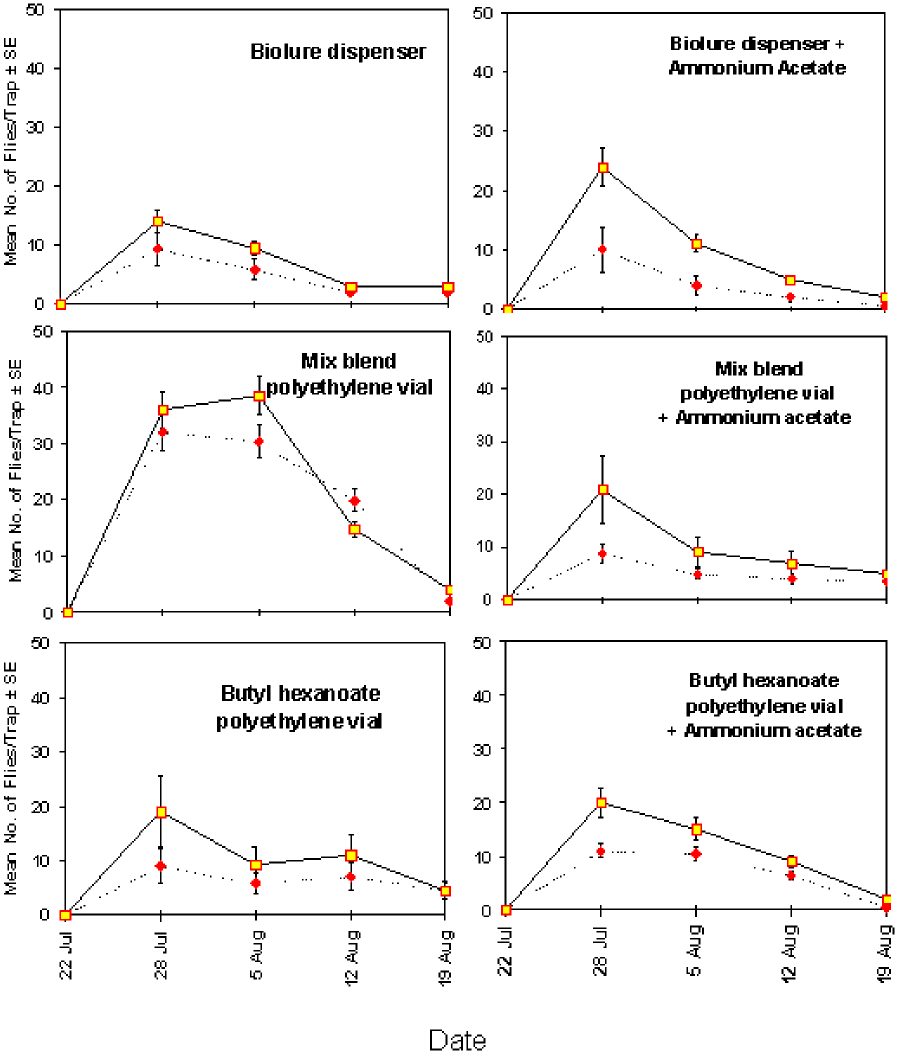

Figure 1. Response of *Rhagoletis pomonella* to red spheres baited with synthetic fruit volatiles and food-odor attractants in 1998 [···· Male; ———Female]

with all other treatments (Table 3). In fact, spheres baited with the apple volatile blend captured approximately twice as many apple maggot flies as spheres baited with the standard ammonium acetate bait and approximately three times as many apple maggot flies as unbaited (control) spheres. There were no differences in catches observed among the other treatments evaluated (Table 3).

2000. Spheres baited with the blend captured significantly ($F = 13.5$; df = 7,12; $P < 0.01$) more apple maggot flies compared with spheres baited with any of the other lures evaluated in 2000 (Table 4). Furthermore, we recorded significantly ($F = 13.5$; df = 7,12; $P < 0.01$) more *R. pomonella* flies captured on spheres baited with butyl hexanoate placed in polyethylene vials compared with spheres baited with the Biolure dispenser and unbaited (control) spheres. We observed a high degree of selectivity to apple maggot capture on spheres baited with the blend (polyethylene vial), butyl hexanoate (polyethylene vial), and the Biolure dispenser, which captured 74.7 ± 5.3 %, 84.2 ± 7.5 %, and 79.5 ± 7.1 %, respectively, of apple maggot flies in relation to non-target insect captures. We observed a moderate degree of selectivity for the unbaited (control) spheres, which captured 60.3 ± 12.9 % apple maggot flies relative to non-target insects. The lowest degree of selectivity was observed on spheres baited with ammonium acetate, which captured 19.8 ± 2.0 % apple maggot flies. Throughout the season, spheres baited with ammonium acetate captured significantly ($F = 17.5$; df = 3,4; $P = 0.05$) more *R. pomonella* females than males, while such a difference was not observed with the other treatments evaluated (Figure 2).

DISCUSSION

Over the course of three field seasons, the apple volatile blend was the most effective lure evaluated for attracting apple maggot flies. The apple maggot fly is known to use chemical stimuli in long-range orientation to their host plants. Chemical cues are also used to discriminate between host and non-host fruit (Prokopy and Roitberg 1984). When apple maggot flies detect an appropriate blend of odors, such as a synthetic fruit volatile lure, they move upwind in a series of flights that eventually culminate in the arrival at the odor source (Aluja and Prokopy 1992). The blend, as a fruit odor source, was significantly more attractive to apple maggot flies than butyl hexanoate alone. It is possible that the apple volatile blend may have produced a distinct, attractive odor due to a synergistic relationship among the individual compounds that elicit a greater response than individual compounds. Alternatively, differing release rates of compounds from vials loaded with the blend versus butyl hexanoate alone may have impacted trap attractiveness. More research is needed to further investigate this hypothesis.

The results from our first experiment in 1998 indicate that spheres baited with protein hydrolysate alone are not an effective means of monitoring *R. pomonella* fly populations. Spheres baited in this fashion were less effective than spheres baited with various other treatments tested, as well as unbaited spheres.

Table 4. Attraction of apple maggot flies to synthetic fruit volatiles and baits, Michigan, (2000).

Treatment	Mean ± SEM flies per trap
Volatile mix blend polyethylene dispenser	983.8 ± 203.5 a
Biolure® dispenser (Butyl hexanoate)	421.0 ± 32.1 cd
Ammonium Acetate	580.0 ± 60.8 bc
Butyl hexanoate polyethylene vial	610.5 ± 143.4 b
Unbaited (control)	336.3 ± 49.1 d

Means followed by the same letter are not significantly different ($P = 0.05$, LSD test).

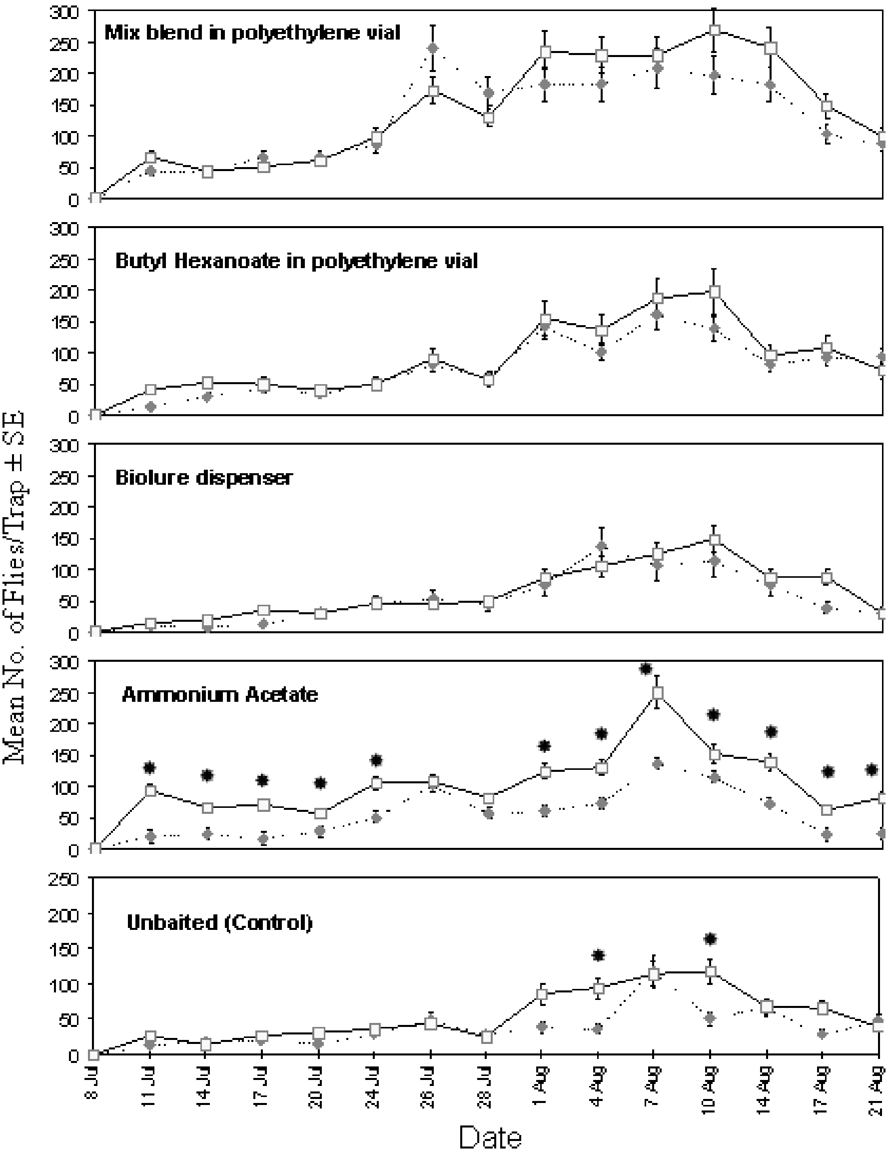

Figure 2. Response of *Rhagoletis pomonella* to red spheres baited with synthetic fruit volatiles and food-odor attractants in 2000 [···· Male; ——— Female]. Significant ($p<0.05$) differences between pairs of means are indicated by *.

Gow (1954) evaluated various protein hydrolysates and found that although they were initially very attractive to the oriental fruit fly, *D. dorsalis* Hendel, their attractiveness decreased when the protein hydrolysates experienced bacterial decay. Other studies have shown that hydrolysates are also attractive to apple maggot flies (Dean and Chapman 1973, Reissig 1974). We noticed that when protein hydrolysate was mixed into the Tangle-Trap and applied to a red sphere, the sphere became discolored, assuming a whitish appearance. Spheres baited in this fashion were possibly visually unattractive to *R. pomonella* flies. In addition, when spheres baited with protein hydrolysate were left in the field for two weeks, they began to lose their initial whitish color as the bait progressively dissipated. These aged spheres (2 wk. exposure) baited with protein hydrolysate, gradually caught increasing numbers of apple maggot flies as they began to regain their original dark red color.

Drummond et al. (1984) found that spheres baited with ammonium compounds were not selective for apple maggot flies. Instead, the traps used in their study attracted a wide variety of Dipteran (beneficial) insects, which decreased trap effectiveness due to the reduced trapping area caused by non-target insect captures. We recorded similar results in our study; spheres baited with ammonium compounds became saturated with insects after 7-10 d during all three field seasons. We observed that the average life span of a sphere baited with ammonium acetate/carbonate was at most three weeks, depending on fly pressure. After three weeks the decomposing insects caused a detectable odor that may have competed with the odor given off by the ammonia baits. Bateman and Morton (1981) studied the role of ammonium compounds and concluded that both ammonia and amino acids were essential to attract tephritid *(D. tryoni)* species, the former for olfactory stimulation and the latter for feeding response. Additional studies revealed that although ammonia is the major attractant, an accompanying increase in the pH level of the bait compound further increases its attractiveness. They concluded that at this increased pH level, additional unidentified volatiles may be produced, resulting in increased attractiveness to *D. tryoni* flies. These variables may have affected the results of our study; however, we did not measure these factors.

The addition of protein hydrolysate or ammonium compounds to spheres baited with BioLure did not significantly increase the attractiveness of these spheres to apple maggot flies. Similar results demonstrating limited effectiveness of red sphere traps baited with ammonium compounds have been documented previously (Reynolds and Prokopy 1997). In fact, the only difference that was recorded by combining an ammonium bait with an apple volatile lure in comparison to using the apple volatile alone, was an increased capture of female *R. pomonella* relative to males in early season. However, unbaited spheres and spheres baited only with BioLure, butyl hexanoate, or the blend, were far more selective in capturing *R. pomonella* flies than lures containing ammonium compounds. Such spheres containing no ammonia or protein bait, impregnated in the Tangle-Trap, maintained the original deep red color of the plastic. Spheres that were treated with baits impregnated into the Tangle-Trap tended to become discolored (lighter in color, whitish appearance).

The attractiveness of unbaited spheres observed in both 1998 and 2000 may be indicative of the visual stimulus, which mimics apples in both size and color (Prokopy 1968). The addition of apple volatiles to these spheres increased their attractiveness to apple maggot flies, while at the same time preserving their selectivity. The principal component of BioLure is butyl hexanoate, a fruit odor that attracts sexually mature apple maggot flies for mating and oviposition (Reynolds and Prokopy 1997). The possible reason why we did not observe an increased total trap capture by combining ammonium baits with BioLure, may be the decreased selectivity associated with this type of trap baiting system in comparison to using the synthetic apple volatile alone. Also, apple maggot flies immigrating into commercial orchards are sexually mature and therefore exhibit a greater response to host-fruit volatiles than to food-odor attractants (Rull and Prokopy 2000). Therefore, we feel that using synthetic apple volatiles without ammonium baits may produce more accurate and sensitive monitoring of both resident and immigrant populations of *R. pomonella* flies in Michigan commercial apple orchards.

We found that by using synthetic apple volatiles (apple volatile blend, butyl hexanoate, BioLure), the effectiveness of red, sticky sphere traps used in apple maggot monitoring programs could be extended beyond the three week life span characterizing monitoring traps baited with ammonium attractants. The combination of attractiveness and selectivity associated with using the blend may provide a high degree of effectiveness for monitoring both sexes of apple maggot flies in commercial apple orchards. Furthermore, the use of the apple volatile blend may produce the best possible results in apple maggot fly mass trapping programs.

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