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ATTRACTION, FEEDING, AND CONTROL OF *RHAGOLETIS POMONELLA* (DIPTERA: TEPHRITIDAE) WITH GF-120 AND ADDED AMMONIA IN WASHINGTON STATE

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ABSTRACT

Experiments were conducted in 2005 and 2006 in western Washington state to determine effects of adding ammonium carbonate (AC) and ammonium acetate (AA) to GF-120 NF Naturalyte® Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN) on attraction, feeding, and control of the apple maggot fly, *Rhagoletis pomonella* (Walsh). In the field, sticky yellow panel traps baited with GF-120 + 10% AC attracted more flies than those baited with GF-120 + 10% AA or GF-120 alone. In the laboratory, female flies responded more frequently to sucrose and 20% GF-120 than to water and 40% GF-120 + 10% AA on apples, perhaps because of the confined conditions during testing. In the field, fly attraction and feeding were greater for GF-120 + 10% or 2.5% AC or AA than GF-120 alone on apple leaves. In 2 spray tests with 100 mL of GF-120 alone and GF-120 + 2.5% AC or AA applied on single apple trees, larval infestations in fruit were reduced up to 99% compared with controls, but there were no differences among treatments, and none was different from spinosad alone. Results indicate the attractiveness of GF-120 to *R. pomonella* can be increased with added ammonia, but that this does not necessarily result in greater control, perhaps because the added ammonia volatilizes too quickly. Results suggest that at the spray volumes used, GF-120 alone or even spinosad alone can greatly reduce local *R. pomonella* populations in Washington.

Key Words: apple maggot fly, bait sprays, spinosad

RESUMEN

Se realizaron experimentos durante el 2005 y 2006 en el oeste del estado de Washington para determinar los efectos de añadir carbonato de amonio (CA) y acetate de amonio (AA) al cebo para moscas de la fruta, GF-120 NF Naturalyte® Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN) sobre la atracción, alimentación y el control del gusano de la manzana, *Rhagoletis pomonella* (Walsh). En el campo, trampas de paneles pegajosas de color amarillo con cebo GF-120 + 10% CA atrayeron mas moscas que las que estaban con solo GF-120 + 10% AA o GF-120. En el laboratorio, las hembras de moscas respondieron mas frecuentemente a la sucrosa y 20% de GF-120 que con agua y el 40% GF-120 + 10% AA sobre las manzanas, quizas por la condiciones confinadas del experimento. En el laboratorio, la atracción y la alimentación de las moscas fue mayor para el GF-120 + 10% o 2.5% CA o AA que solo para el GF-120 sobre las hojas de manzana. En 2 de las pruebas de asperción con 100 mL de solo GF-120 y GF-120 + 2.5% CA o AA aplicado sobre un árbol individual de manzana, las infestaciones de las larvas en el fruto fue reducida hasta el 99% comparado con el control, pero no hubo diferencias entre los tratamientos, y ninguno de ellos fue diferente con respecto al tratamiento con solo el spinosad. Los resultados indican que se puede aumentar la capacidad de GF-120 para atraer *R. pomonella* al añadir el amonio, pero este no necesariamente resulta en un mayor control, quizas porque el amonio añadido se volatiliza demasiado rapido. Los resultados sugieren que en el volumen de las asperciones usadas, solo con GF-120 o aun solo el spinosad puede reducir grandamente las poblaciones de *R. pomonella* en Washington.

Apple maggot fly, *Rhagoletis pomonella* (Walsh), is a major quarantine pest of apples, *Malus domestica* (Borkh.) Borkh., in Washington state and other parts of the Pacific Northwest of the U.S. Washington state is the leading producer of apples in the U.S., with a production value in 2005 of US$1.23 billion, at an estimated per hectare value of $3,078 over ~65,500 harvested ha (USDA 2006). To maintain apple production profits, insect pest management is vital. Until recently, *R pomonella* in Washington was believed to have established only in the western part of the state, along the lower Columbia River Gorge in the southern part, and in Spokane in the eastern part. However, larval infestations of hawthorns and non-commercial apples were detected in central Washington from 2003 to 2005, resulting in a partial quarantine of Kittitas County in 2004 and Yakima County in 2005 (Washington State Department of Agriculture 2005), near major apple-producing areas. There is zero tolerance for larvae in fresh market apples (Washington State Department of Agriculture 2001), so any trap captures of *R. pomonella* on isolated hawthorn or feral apple trees in central Washington trigger the spraying of trees to eliminate populations or prevent their spread (Klaus et al. 2007). The organophosphate insecticide phosmet has been effectively used for this purpose. To date, apple maggot has not been detected in commercial orchards in central Washington. Despite the effectiveness of phosmet, alternatives are needed because of impending restrictions on the use of this and other highly toxic organophosphates (Food Quality and Protection Act 1996).

Various new insecticides mixed with hydrolyzed protein baits may provide an effective alternative to conventional organophosphate sprays. Since around 2000, GF-120 NF Naturalyte® Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN) (GF-120) has been the most frequently tested bait against subtropical, tropical, and temperate fruit flies (e.g., Vargas et al. 2002; Prokopy et al. 2003; Barry & Polavarapu 2004). GF-120 is a modified, more concentrated and marketed version of Solbait originally developed for *Anastrepha* and *Ceratitis* fly control (Burns et al. 2001; Moreno & Mangan 2003). The frequent testing of this bait is the result of its effectiveness against representative tephritids, ease of use, low spray coverage needed, and organic labeling. GF-120 contains 0.02% spinosad (wt/vol), an insecticide derived from fermentation products of the bacterium *Saccharopolyspora spinosa* Mertz and Yao that has a high safety profile (Dow AgroSciences 2006), as well as 1% ammonium acetate as an attractant (Thomas & Mangan 2005).

Results with GF-120 against *Rhagoletis* species for control in the field have overall been positive. However, there are no published studies showing it can eliminate infestations after only one season of use. In New York, GF-120 was ineffective against *R. pomonella* (Reissig 2003), but in Michigan it was effective in one of two years (Pelz et al. 2005). In California, it was effective against the walnut husk fly, *R. completa* Cresson (Van Steenwyk et al. 2003), and in Washington and Utah it was effective against the western cherry fruit fly, *R. indifferens* Curran (Yee & Chapman 2005; Yee & Alston 2006).

Research has indicated that GF-120 is not or not highly attractive to *Rhagoletis* flies (Barry & Polavarapu 2004; Pelz et al. 2005; Yee & Chapman 2005; Yee 2006) and recent research has emphasized the need to make GF-120 more attractive (Pelz-Stelinski et al. 2006). A logical choice of materials to make the bait more attractive is ammonia, which is associated with protein-rich foods and has long been known to attract *R. pomonella* (Hodson 1948) and *R. indifferens* (Frick et al. 1954). Indeed, even though GF-120 has 1% ammonium acetate, adding more ammonium acetate to GF-120 enhanced its attractiveness to the eastern cherry fruit fly, *R. cingulata* (Loew) (Pelz-Stelinski et al. 2006). However, whether increasing the attractiveness of GF-120 with additional ammonia results in improved control of larval infestations in fruit is unclear.

In this study, the objectives were to determine the attraction, feeding, and control of *R. pomonella* with GF-120 and added ammonia in Washington. Three hypotheses were tested: (1) adding ammonium carbonate or ammonium acetate to GF-120 in lures increases trap captures compared with GF-120 alone in lures; (2) adding ammonia compounds to GF-120 also increases fly attraction and feeding responses when baits are sprayed on leaves; and (3) GF-120 containing additional ammonia compounds decrease larval infestations more than GF-120 alone.

Materials and Methods

Study Sites and Experimental Design

Four field and laboratory experiments consisting of various tests were conducted in western Washington in 2005 and 2006. A summary of the experimental sites, test years, replicate sizes, experimental settings, and experimental designs is shown in Table 1. Study sites were known to be infested with *R. pomonella*. Treatments and details for each of the 4 experiments follow.

Experiment 1: Effects of GF-120 with Ammonia Compounds on Attraction to Traps

For test 1A, various lures were tested with 14 × 23 cm sticky yellow panel traps (Trécé, Adair, OK). Ammonium carbonate (AC) (Keystone Universal Corp., Melvindale, MI) and ammonium acetate (AA) (EMD Brand, Barmstadt, Germany) were used as sources of additional ammonia. Comparisons were: (1) a control, (2) 10 g AC, (3) 17% GF-120, (4) 40% GF-120, (5) 40% GF-120 + 10% AC, and (6) 40% GF-120 + 10% AA. Percent GF-120 was based on vol/vol, but % AC and AA was based on wt/wt. Blank GF-120, without spinosad, was used. The 10 g AC was placed in a clear plastic vial (Thorton Plastic Co., No. 55-7, Salt Lake City, UT) with two 1-mm holes on the lid. Ten mL of each of the GF-120 treatments was placed in a 15-mL polypropylene narrow-mouth Nalgene® bottle (Nalge Nunc International, Rochester, NY) with the cap removed (13 mm diameter opening). A 0.25-g cotton ball was placed inside each bottle to prevent spillage. Each lure was hung 1 to 2.5 cm above the center of each trap, which was suspended from a branch ~2 m above the ground. Lures were not replaced during the season. Within each row, 1 trap was placed in every other tree (cv 'MacIntosh'). One block consisted of 2 seven- or eight-tree rows. Trees were 3.0 to 4.6 m wide and spaced 10 m apart. Traps were rotated each week among trees within a block. Flies were removed weekly and counted. The test was conducted 19 Jul to 22 Aug.

TABLE 1. SUMMARY OF *RHAGOLETIS POMONELLA* ATTRACTION, FEEDING, AND CONTROL EXPERIMENTS.

Experiment	Test	Site	Year	No. replicates	Apple tree setting	Experimental design
1. Attraction, traps	1A	V	2005	4	Orchard	RBD, blocks: tree rows
	1B	SCR	2005	4	Scattered trees	RBD, blocks: single trees
2. Feeding, containers	—	Lab	2005	10 to 27 single flies	—	CRD
3. Attraction, feeding	3A	SCR	2005	3 to 6, on ea. of 8 d	Scattered trees	RBD, blocks: single trees
	3B	SCR	2006	4, 5, on ea. of 11 d	Scattered trees	RBD, blocks: single trees
	3C	P	2005	4, all in 1 d[a]	Orchard	Each treatment on same four trees[a]
	3D	P	2006	10, 2 on ea. of 5 d	Orchard	RBD, blocks: single trees
4. Larval infestations	4A	P	2005	5	Orchard[b]	CRD, single trees
	4B	P	2006	5	Orchard[b]	CRD, single trees

V, Vancouver (Clark County) (45°37.45'N, 122°39.78'W); SCR, Saint Cloud Ranch (Skamania County) (45°35.88'N, 122°07.03'W); P, Puyallup (Pierce County) (47°11.76'N, 122°16.50'W).
RBD, randomized block design; CRD, completely randomized design.
[a]One or 2 treatments tested on single day (see text for details).
[b]Single tree replicates were sprayed.

Test 1B at Saint Cloud Ranch was conducted with similar methods, with the following differences. There was no 10-g AC lure. The test was set up in 4 scattered apple trees (cv 'Newtown', 'Winesap', 'Spitzenberg', and 'Early Transparent') with a single tree as a block. A replicate trap of the control and each treatment was hung in each tree. Positions of traps within trees were rotated weekly. Trees were 6.1 to 7.6 m wide and 9.1 to 15.2 m apart. The test was conducted 28 Jul to 22 Sep.

Experiment 2: Effects of GF-120 with Ammonia Compounds on Feeding Responses in the Laboratory

Infested apple and hawthorn fruit were collected in Vancouver and Puyallup in summer/fall 2004 and placed in tubs, where larvae emerged. The puparia subsequently formed were then collected and placed in moist soil and chilled at ~4°C for 6 months. Puparia were transferred to 20-27°C for adult emergence, after which flies were immediately placed in 473-mL paper containers with water and a 5% sucrose solution on a wick, but no other food. Flies were tested at 3 to 5 d after emergence. Five 50 µL drops of (1) water, (2) 13% sucrose (wt/wt), (3) 17% GF-120, (4) 20% GF-120, (5) 40% GF-120, (6) 40% GF-120 + 10% AC, or (7) 40% GF-120 + 10% AA were placed equidistant on top of 1 apple (cv 'Fuji') in a 1.9-liter paper container with an organdy screen that allowed viewing. Blank GF-120 was used. One male or female fly was then introduced into this test container. After 1 min, the fly was watched continuously for 60 min, and the numbers of feeding bouts and feeding durations were recorded. No water was provided in the test container. The test was conducted in a brightly lit room at 24 to 27°C.

Experiment 3: Effects of GF-120 with Ammonia Compounds on Attraction and Feeding in the Field

Test 3A at Saint Cloud Ranch in 2005 consisted of: (1) 13% sucrose, (2) 17% GF-120, 3) 40% GF-120, (4) 40% GF-120 + 10% AC, and (5) 40% GF-120 + 10% AA. Blank GF-120 was used. Ten mL of each treatment were sprayed on an apple branch with ~30 leaves with a 32-oz volume spray bottle (Consolidated Plastics Co., Twinsburg, OH). The five treatments were applied ~1 m apart within 1 tree and ~1.5 m above the ground. Trees were ~6.1 m wide. Numbers of flies ≤15 cm from spray drops and feeding on drops were recorded. Each treatment was observed for a continuous 2-min period, followed immediately by the next treatment for a total of 3 periods per treatment during 30 min. The sex of flies was recorded, but sexes were pooled for presentation and analyses because of the low numbers. Sprayed leaves and branches were removed after the completion of ~30 min. Observations were recorded from 0800-1300 h. On each day, observations were made on 3 to 6 replicate trees ≥23 m apart. The test was conducted on 8 dates from 26 Jul to 25 Aug. Positions of treatments within trees were randomized. For the entire test, there were 3.7 h of continuous observations for each of the 5 treatments (18.5 h total). Test 3B at Saint Cloud Ranch in 2006 was similar to test 3A, but the 17% GF-120 treatment was dropped, all GF-120 treatments contained 0.0096% spinosad (wt/vol) (Entrust® [80% spinosad], Dow AgroSciences, Indianapolis, IN), and a spinosad alone treatment (also 0.0096% wt/vol) was included. (In the rest of the tests in experiments 3 and 4 below, the spinosad concentration in all GF-120 and spinosad alone treatments was also 0.0096%.) The data collecting method was the same as in test 3A. The test was conducted on 11 dates from 10 Aug to 7 Sep. For the entire test, there were 5.4 h of continuous observations for each of the 5 treatments (27 h total).

Test 3C in Puyallup in 2005 consisted of: (1) a water control, (2) 13% sugar, (3) 17% GF-120, (4) 40% GF-120, (5) 40% GF-120 + 2.5% AC, (6) 40% GF-120 + 2.5% AA, and (7) spinosad alone. Ten mL of each were applied on a branch of an apple tree as before. Trees (3 unidentified varieties: striped, red/late, and yellow/early) in the orchard were ~5 to 7 m tall and wide. The design differed from that used in tests 3A and 3B because the initial thought was that odors among treatments compared simultaneously within trees could interfere with one another. Each treatment or control was tested on a single tree by itself, with 4 replicate trees each day. Observations were made on different days between 0900 and 1300 h: 8 Aug, 13% sucrose and 17% GF-120; 9 Aug, 40% GF-120 and spinosad alone; 10 Aug, 40% GF-120 + 2.5% AC; and 12 Aug, water control and GF-120 + 2.5% AA. Treated leaves were removed, and the same 4 trees were used for other treatments. Data recording also differed from that used in tests 3A and 3B. Flies seen every 2 min ≤15 cm from spray drops and feeding on drops within 30-min periods were recorded, for 16 instantaneous recordings over these periods. All 4 d were sunny, with high temperatures of 20.4 to 23.8°C, wind speeds of 75 to 140 m/min, and RH of 50 to 62%. Test 3D in Puyallup in 2006 was similar to test 3C, except that 17% GF-120 was dropped and all five treatments and the control were compared simultaneously within single trees, as in tests 3A and 3B. Data recording was the same as in test 3C. Observations from two replicate trees were made on each of five d from 24 Jul to 2 Aug, for 10 total replicates per treatment.

Experiment 4: Effects of GF-120 with Ammonia Compound Sprays on Larval Infestations

In all spray tests, single apple trees were sprayed. In Washington, flies of threat to commercial orchards are generally found in single feral trees or small patches of trees, and not in the orchards themselves, so spot instead of broadcast sprays were used. The label rates for spot sprays are 30 to 90 mL of undiluted GF-120 spray solution/tree (Dow AgroSciences 2006). Treatments in test 4A were delivered at 40 mL of undiluted GF-120 in 100 total mL of spray/tree with RL Flo-Master pressurized sprayers (Root-Lowell Manufacturing Co., Lowell, MI). Treatments in test 4A compared: (1) an unsprayed control, (2) 40% GF-120, (3) 40% GF-120 + 2.5% AC, (4) 40% GF-120 + 2.5% AA, and (5) spinosad alone. Trees (mostly cv. 'Jonagold') within rows in an orchard were used, but because of the irregular numbers of trees per row, a completely randomized instead of a randomized block design was used. Trees were 1.7 to 4.5 m tall and 1.5 to 3.8 m wide, most spaced 4 m from others. Sprays were initiated ≤7 d of first fly capture on AC-baited sticky yellow panel traps. After the first fly capture, AC lures were removed, leaving 1 unbaited panel on each tree throughout the test. Fly captures were either from within the trapped trees or from surrounding trees. Larval infestations could have originated from flies from either source. However, adult flies may have been caught before they able to oviposit, so there may be discordance between fly captures and larval infestations. Weekly applications were made 1 Jul to 6 Sep, for 11 total sprays. There is no maximum number of applications on the GF-120 label (Dow AgroSciences 2006), and in California, there can be up to 19 aerial or ground applications per acre per season made for control of exotic fruit flies (Cheney 2005). Forty nine to 108 apples were picked from each tree, except from two control trees, where only 8 and 9 apples were present. Test 4B compared the same treatments, also with 100 mL spray/tree and in a completely randomized design in an orchard (3 unidentified apple varieties: striped, red/late, and yellow/early) with 5.0 to 6.7 m tall and wide trees spaced 4 m apart. Weekly applications were made 11 Jul to 12 Sep, for 9 or 10 total sprays (some blocks were harvested before the last spray). One hundred apples were picked from each tree. In both tests, apples were placed in tubs and held for 2 months to allow larvae to emerge, after which counts of puparia were made.

Statistics

For experiment 1, data were analyzed by randomized block analysis of variance (ANOVA), followed by Fisher's LSD test for mean separation. Two-way ANOVA was also conducted to determine sex, bait, and sex × bait effects. For experiment 2, Fisher's exact test with R × C tables was used; differences among proportions also were analyzed with a Tukey-type multiple comparison test among proportions (Zar 1999). Experiment 3 tests were set up for ANOVA, but there were too many zero values in some replicates in tests 3A and 3B for this analysis. Thus, chi-square goodness of fit tests were used, with counts pooled from all replicates and dates and with equal ratios for all treatments as expected values. Numbers of flies near bait drops and numbers of flies that fed were pooled to give higher counts for analyses. In tests 3C and 3D, chi-square tests were performed as well, but there also were enough flies to conduct one-way ANOVA and randomized block ANOVA, respectively, followed by Fisher's LSD test. In experiment 3, data were not based on separate samples as in experiment 2, and some treatments had only 1 or 2 flies, so the Tukey-type multiple comparison test among proportions was not performed. For experiment 4, one-way ANOVA were conducted. Data were subjected to square-root (y) or square-root (y + 1) transformation (when counts were low and there were zeros) to stabilize the variance. The Statistical Analysis System (SAS Institute, Inc. 2004) was used for ANOVAs.

Results

Experiment 1: Effects of GF-120 with Ammonia Compounds on Attraction to Traps

In test 1A, the 10 g AC lure attracted more flies than the 40% GF-120 + 10% AC lure, which attracted more than the control and the 17% and 40% GF-120 lures, although not more than the 40% GF-120 + 10% AA lure (Table 2). The control and treatments attracted more females than males (two-way ANOVA, $F = 8.2$; $df = 1, 36$; $P = 0.0070$), although the sex response pattern across treatments was not different (bait: $F = 11.1$; $df = 5, 36$; $P < 0.0001$; sex × bait: $F = 2.2$; $df = 5, 36$; $P = 0.0736$). The percentages of females (n = 86) in the control, 10 g AC, 17% GF-120, 40% GF-120, 40% GF-120 + 10% AC, and 40% GF-120 + 10% AA treatments were 5.8, 54.7, 3.5, 5.8, 20.9, and 9.3%, respectively, and for males ($n = 37$), they were 0, 40.5, 18.9, 5.5, 27.0, and 8.1%, respectively.

In test 1B, the 40% GF-120 + 10% AC lure attracted more flies than the control and other GF-120 lures, including the 40% GF-120 + 10% AA lure (Table 2). There was no difference in the numbers of females and males caught (two-way ANOVA, $F = 1.8$; $df = 1, 30$; $P = 0.1878$), and the sex response pattern was similar across treatments (bait: $F = 19.9$; $df = 4, 30$; $P < 0.0001$; sex × bait: $F = 0.4$; $df = 4, 30$; $P = 0.8080$). The percentages of females ($n = 803$) in the control, 17% GF-120, 40% GF-120, 40% GF-120 + 10% AC, and 40% GF-120 + 10% AA treatments were 3.0, 4.9, 13.7, 65.7, and 12.7%, respectively, and for males (n = 614), they were 2.0, 4.9, 25.0, 57.5, and 10.6%, respectively.

Experiment 2: Effects of GF-120 with Ammonia Compounds on Feeding Responses in the Laboratory

Because of the low response to the baits, mean numbers of feeding bouts and feeding durations were not analyzed (e.g., there were no drinks on water). Fisher's exact test showed that numbers that fed were dependent on treatment in females ($P = 0.0054$), but not in males ($P = 0.2826$). Analyses of percentages (Table 3) indicated that female flies responded less to water and 40% GF-120 + 10% AA than to 13% sucrose and 20% GF-120. Unlike females, however, males did not respond differently to any treatment.

Experiment 3: Effects of GF-120 with Ammonia Compounds on Attraction and Feeding in the Field

In test 3A, fly sightings were infrequent given that there were 3.7 total h of continuous observations/treatment. Numbers of sightings of flies near or feeding on sucrose, 17% GF-120, and 40% GF-120 were similar (1 or 2), and lower than on 40% GF-120 + 10% AC or 10% AA (Table 4). Unlike in experiment 1, there were no evident differences in responses to GF-120 + 10% AC and 10% AA. In test 3B, fly responses were also low over 5.7 total h of observation/treatment and were similar to those in test 3A. Responses to sucrose, 40% GF-120, and spinosad alone were similar and lower than to GF-120 + 10% AC or 10% AA (Table 4).

In tests 3C and 3D, numbers of fly sightings were greater than in tests 3A and 3B. The numbers near or feeding on water, sucrose, and spinosad alone were similar, lower than on 17% or 40% GF-120, and much lower than on GF-120 + 2.5% AC or 2.5% AA (Table 5). No differences were seen between GF-120 + 2.5% AC and GF-120 + 2.5% AA treatments.

Experiment 4: Effects of GF-120 with Ammonia Compound Sprays on Larval Infestations

In test 4A, fewer flies were caught on traps in all treatments than in the control (Table 6). There were high levels of larval control with all the treatments, and GF-120 + 2.5% AC and 2.5% AA treatments did not perform better than GF-120 alone, and statistically no better than spinosad

Table 2. Mean total numbers of *Rhagoletis pomonella* ± SE caught over the season per sticky yellow panel trap with different lures on apple trees at 2 sites, WA, 2005.

Treatment	Test 1A: Vancouver 19 Jul to 22 Aug	Test 1B: Saint Cloud Ranch 28 Jul to 22 Sep
Control	1.2 ± 0.9 c	9.0 ± 2.0 b
10 g AC	15.5 ± 2.7 a	Not tested
17% GF-120	1.8 ± 1.1 c	17.3 ± 1.8 b
40% GF-120	1.8 ± 1.0 c	66.0 ± 47.9 b
40% GF-120 + 10% AC	7.0 ± 2.3 b	220.3 ± 39.1 a
40% GF-120 + 10% AA	2.8 ± 1.8 bc	41.8 ± 4.7 b
Randomized block	$F = 8.4$; $df = 5, 15$	$F = 13.0$; $df = 4, 12$
ANOVA	$P = 0.0006$	$P = 0.0003$

Blank GF-120 used.
Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

TABLE 3. PERCENT OF *RHAGOLETIS POMONELLA* THAT DRANK OR FED ON WATER OR GF-120 BAITS ON APPLES OVER 1-H OBSERVATIONS IN THE LABORATORY.

	Females		Males	
Treatment	*n*	% Drank or fed	*n*	% Drank or fed
Water	22	0.0 b	18	0.0 a
13% Sucrose	27	33.3 a	20	15.0 a
17% GF-120	25	20.0 ab	21	0.0 a
20% GF-120	22	27.3 a	18	11.1 a
40% GF-120	27	7.4 ab	24	16.7 a
40% GF-120 + 10% AC	21	9.5 ab	20	5.0 a
40% GF-120 + 10% AA	12	0.0 b	10	10.0 a

% followed by the same letter within columns are not significantly different (Tukey-type multiple comparison test among proportions, $P > 0.05$).
Blank GF-120 used.

alone (Table 6). In test 4B, fewer flies were caught in all treatments than in the control. Within treatments, fewest were caught in the spinosad alone and most in the GF-120 + 2.5% AC treatment (Table 6). Despite different effects on adult captures, all treatments again resulted in similarly high levels of larval control. Also, again GF-120 + 2.5% AC and 2.5% AA treatments did not perform better than GF-120 alone or spinosad alone (Table 6).

DISCUSSION

In experiment 1, addition of 10% AC to 40% GF-120 enhanced the attraction of *R. pomonella* to sticky yellow panel traps, supporting the first hypothesis and showing that GF-120 can be modified to increase fly responses, although it did not attract as many flies as the 10 g AC lure. Addition of ammonium bicarbonate also substantially increased the attractiveness of the commercial bait Nu-Lure to *R. pomonella* inside cages compared with Nu-Lure alone (Hendrichs et al. 1990). GF-120 + 10% AC was more attractive than GF-120 + 10% AA, suggesting that when amounts of the compounds in GF-120 are the same, AC releases more ammonia than AA. Females were more responsive to the GF-120 lures on traps than males in one test, likely because their need for protein is greater (Webster et al. 1979), but the lures affected the sexes similarly in terms of relative responses.

In experiment 2, feeding responses by flies to sucrose and GF-120 with or without added ammonia on apples in containers in the laboratory were low, but results demonstrated that sucrose and 20% GF-120 attracted females or caused them to feed more than on water. Female flies appeared

TABLE 4. NUMBERS OF SIGHTINGS OF *RHAGOLETIS POMONELLA* FEEDING ON OR NEAR SUCROSE, GF-120 BAITS, AND SPINOSAD ON APPLE LEAVES AT SAINT CLOUD RANCH, WA, 2005 AND 2006.

Treatment	No. feeding[a]	No. ≤15 cm from bait[b]	Total fly sightings
Test 3A: 2005			
13% Sucrose	1	0	1
17% GF-120	1	1	2
40% GF-120	1	1	2
40% GF-120 + 10% AC	8	6	14
40% GF-120 + 10% AA	4	12	16
Total fly sightings: Chi-Square = 30.9; *df* = 4; *P* < 0.0001.			
Test 3B: 2006			
13% Sucrose	4	0	4
40% GF-120	2	0	2
40% GF-120 + 10% AC	9	1	10
40% GF-120 + 10% AA	7	2	9
Spinosad alone	0	1	1
Total fly sightings: Chi-Square = 12.8; *df* = 4; *P* = 0.0121.			

2005, blank GF-120 used; 2006, GF-120 baits and spinosad alone both had 0.0096% spinosad (wt/vol).
[a]Expected cells <5, data not analyzed.
[b]Not feeding

TABLE 5. NUMBERS OF SIGHTINGS OF *RHAGOLETIS POMONELLA* DRINKING OR FEEDING ON OR NEAR WATER, SUCROSE, GF-120 BAITS, AND SPINOSAD ON APPLE LEAVES IN PUYALLUP, WA, 2005 AND 2006.

Treatment	No. drinking or feeding[a]	Total fly sightings	Mean fly sightings ± SE (per 30 min)
Test 3C: 2005			
Water	0	1	0.02 ± 0.02 d
13% Sucrose	0	1	0.02 ± 0.02 d
17% GF-120	0	13	3.20 ± 1.4 c
40% GF-120	3	41	10.20 ± 1.6 b
40% GF-120 + 2.5% AC	6	88	22.00 ± 3.4 a
40% GF-120 + 2.5% AA	6	89	22.02 ± 2.3 a
Spinosad alone	0	2	0.50 ± 0.3 d
Total fly sightings: Chi-Square = 286.7; $df = 6$; $P < 0.0001$.			
Mean fly sightings ± SE: One-way ANOVA: $F = 36.3$; $df = 6, 21$; $P < 0.0001$.			
Test 3D: 2006			
Water	0	1	0.10 ± 0.1 d
13% Sucrose	2	5	0.50 ± 0.2 cd
40% GF-120	1	14	1.40 ± 0.5 bc
40% GF-120 + 2.5% AC	6	36	3.60 ± 0.9 a
40% GF-120 + 2.5% AA	3	26	2.60 ± 1.0 ab
Spinosad alone	0	0	0.00 ± 0.0 d
Total fly sightings: Chi-Square = 51.8; $df = 4$; $P < 0.0001$; spinosad alone not included.			
Mean fly sightings ± SE: Randomized Block ANOVA: $F = 9.0$; $df = 4, 45$; $P < 0.0001$.			
2005 and 2006, GF-120 baits and spinosad alone had 0.0096% spinosad (wt/vol).			

[a]Expected cells <5, data not analyzed with Chi-Square.
Total fly sightings ± SE: means followed by the same letter are not significantly different ($P > 0.05$, Fisher's LSD test).

more responsive to sucrose and baits than males, based on overall response percentages, consistent with the attraction results in experiment 1. The lack of higher responses to GF-120 with added ammonia than to GF-120 alone was surprising given the results of experiment 1. In fact, addition of ammonia compounds numerically lowered responses of flies to GF-120. Perhaps release rates inside containers were initially very high and actually repelled rather than attracted flies. Had observations lasted longer, rates may have attracted for a period of time before dissipating to levels that no longer attracted. The environment inside containers in the laboratory probably lacked important cues flies need to respond to ammonia.

In 4 tests in experiment 3, GF-120 alone seemed not attractive or only slightly attractive when it was sprayed on apple leaves, but adding AC and AA to GF-120 clearly made it attractive. A 2.5% concentration of either AC or AA was sufficient to increase responses, but direct comparisons are needed to determine if it is as effective as the 10% concentration. Results support the second hypothesis and previous work showing attraction of *R. cingulata* to GF-120 enhanced with AA (Pelz-Stelinski et al. 2006). In tests 3A and 3B at Saint Cloud Ranch, GF-120 alone did not appear to be attractive compared with sucrose controls. The windiness at this site may partially explain the inability to detect any attractiveness of GF-120. In contrast, in the different environment in tests 3C and 3D in Puyallup, GF-120 alone did appear attractive and this may have been caused by olfactory cues, visual cues, or both. The higher fly activity in Puyallup than at Saint Cloud Ranch could have influenced results and increased the relative effectiveness of GF-120 alone in Puyallup. Despite the greater attraction to GF-120 + AC or AA, the low numbers of flies that responded suggest ammonia release from drops was too low to elicit strong or immediate responses from a large percentage of a fly population. High ammonia release from lures in experiment 1 is likely difficult to duplicate from small spray drops on leaves. The lack of differences between AC and AA could also be caused by the low amount of ammonia released from spray drops. In tests 3C and 3D, the numbers of feeding bouts on all GF-120 treatments were lower than numbers of non-feeding visits. This is consistent with the idea that GF-120 causes arrestment of flies, as with the blueberry maggot, *R. mendax* Curran (Pelz et al. 2005). More evidence is needed to confirm this with *R. pomonella*.

In experiment 4, adding AC or AA to GF-120 did not reduce fly numbers and larval infestations compared with GF-120 alone, thus not supporting the third hypothesis, but all GF-120 treatments resulted in very high levels of larval control in the 2 tests. In test 4B, more flies were caught on traps in trees sprayed with GF-120 + AC than in trees with GF-120 alone, suggesting ammonia from the

TABLE 6. MEAN NUMBERS OF *RHAGOLETIS POMONELLA* FLIES PER TRAP AND LARVAE PER APPLE FRUIT ± SE IN GF-120 BAIT SPRAY TESTS IN PUYALLUP, WA, 2005 AND 2006.

Treatment	No. flies/trap	% Decrease	No. larvae/fruit	% Decrease
Test 4A: 2005				
Control	35.2 ± 1.9 a	—	1.22 ± 0.59 a	—
40% GF-120	11.6 ± 3.1 b	67.0	0.07 ± 0.02 b	94.3
40% GF-120 + 2.5% AC	28.2 ± 2.2 b	19.9	0.08 ± 0.04 b	93.4
40% GF-120 + 2.5% AA	17.8 ± 2.6 b	49.4	0.16 ± 0.03 b	86.9
Spinosad alone	14.0 ± 2.8 b	60.2	0.05 ± 0.004 b	95.9
One-way ANOVA	$F = 12.5$		$F = 4.4$	
$df = 4, 20$	$P < 0.0001$		$P = 0.0098$	
Test 4B: 2006				
Control	119.4 ± 10.1 a	—	0.99 ± 0.23 a	—
40% GF-120	15.0 ± 1.4 c	87.4	0.08 ± 0.02 b	91.9
40% GF-120 + 2.5% AC	24.4 ± 1.6 b	79.6	0.05 ± 0.02 b	94.9
40% GF-120 + 2.5% AA	9.6 ± 1.5 c	92.0	0.13 ± 0.05 b	86.9
Spinosad alone	1.8 ± 0.8 d	98.5	0.01 ± 0.01 b	99.0
One-way ANOVA	$F = 158.7$		$F = 19.4$	
$df = 4, 20$	$P < 0.0001$		$P < 0.0001$	

GF-120 and spinosad alone had 0.0096% spinosad (wt/vol). 100 mL spray/tree.
Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

AC in GF-120 attracted more flies from surrounding trees than ammonia from GF-120 alone. This apparent influx of adults to the test trees did not increase larval infestations, however, suggesting flies were trapped or killed before they oviposited. With respect to larval infestations, one possible explanation for the lack of differences between GF-120 and GF-120 + AC or AA treatments is that ammonia release rates from enhanced drops decreased quickly after sprays, so after a few days or even less time the enhanced GF-120 was the same as GF-120 alone in attractiveness. Ingredients that prolong ammonia release may be beneficial for control. Perhaps at 100 mL of spray per tree, flies were able to find drops through normal foraging even after the drops lost their ammonia.

It was clear that GF-120 is very effective for fly control, even if it did not eliminate infestations. Coverage of all single trees in an area or of entire orchards with GF-120 may lead to even greater suppression than that obtained by spraying randomly selected single trees as in the present study or may even eliminate local fly populations over time. The success of the 2 tests with GF-120 against *R. pomonella* in this study contrasts with the bait's failure in New York (Reissig 2003). The amount of bait spray used in New York was only 32 mL per tree (compared with 100 mL in the present study), so perhaps this, climatic, and habitat differences explain the inconsistency.

The high levels of control obtained with GF-120 in experiment 4 were evident, but the use of 100 mL of spinosad alone per tree resulted in similarly high control levels, suggesting bait is not needed with spinosad for a spray to be effective. Spinosad seems unattractive compared with 40% GF-120 alone (experiment 3), so its effectiveness at this volume probably was unrelated to attraction. Perhaps no or little olfactory or visual stimulation is needed for flies to find the drops over time, so flies contacted drops while foraging indiscriminately on leaves. Spinosad probably remained on leaf surfaces long enough for flies to find them (before being absorbed or broken down). Some spray also may have landed directly on the flies. Spinosad drops were smaller than bait drops and likely covered larger surface areas. In Michigan, spinosad alone (SpinTor®) was also as effective as GF-120 in reducing larval infestation by *R. pomonella* in one of two years (Pelz et al. 2005). Future studies should determine if baits mixed with spinosad are more critical when spray volumes are <100 mL per tree than when >100 mL per tree.

In conclusion, overall results indicate the attractiveness of GF-120 to *R. pomonella* can be increased with added ammonia, but that this does not necessarily result in greater control, perhaps because the added ammonia volatilizes too quickly to make the enhanced GF-120 different over time than GF-120 alone. Addition of ammonia in GF-120 to increase attractiveness and control may be more critical at spray volumes lower than those used in this study. Results here suggest that at the spray volumes used, GF-120 alone or even spinosad alone can greatly reduce local *R. pomonella* populations in Washington, and should be useful for local horticultural pest and disease board treatments in residential areas where organic materials may be more acceptable than organophosphates. Future studies need to determine how long ammonia-enhanced baits remain attractive and if timed release of ammonia can improve GF-120 performance in eliminating larval infestations.

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