Behavioral Responses of *Rhagoletis cingulata* (Diptera: Tephritidae) to GF-120 Insecticidal Bait Enhanced with Ammonium Acetate

K. S. PELZ-STELINSKI, L. J. GUT, AND R. ISAACS

Department of Entomology, Michigan State University, East Lansing, MI 48824

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ABSTRACT GF-120 is a baited formulation of the insecticide spinosad containing 1% ammonium acetate, developed for control of economically important fruit flies. The response of feral cherry fruit flies, *Rhagoletis cingulata* Loew, to GF-120 augmented with 0, 5, or 10% ammonium acetate was evaluated under orchard conditions. Significantly more flies were observed within 30 cm of bait droplets with 10% ammonium acetate added compared with standard bait or to a water control. These fly visits to GF-120 enhanced with 10 or 5% ammonium acetate lasted an average of 263.2 ± 85.2 and 337.6 ± 72.6 s, respectively, compared with 50.3 ± 36.4 s for standard GF-120. Droplets containing additional ammonium acetate also were contacted by more flies, and more flies fed upon these droplets than on GF-120 or the water control. Furthermore, the duration of feeding on GF-120 bait enhanced with either level of additional ammonium acetate was significantly greater compared with standard GF-120 or water. Feeding events lasted between 61.5 ± 30.7 and 73.4 ± 21.0 s on enhanced GF-120 compared with 6.8 ± 5.7 s on standard GF-120. Collectively, these results indicate that the interaction of feral *R. cingulata* with GF-120 droplets and the toxicant spinosad can be increased by addition of ammonium acetate.

KEY WORDS cherry fruit fly, protein bait, reduced-risk insecticide, fruit flies, ammonium acetate

The cherry fruit fly, *Rhagoletis cingulata* (Loew) (Diptera: Tephritidae), is an important pest of cherry (*Prunus* spp.) in the United States. Larval feeding damages the internal tissues of fruit, making infested fruit unsuitable for sale and leading to a zero tolerance for *R. cingulata* in harvested fruit. The Food Quality Protection Act (Anonymous 1996) has mandated restrictions on broad-spectrum insecticides in the United States, including organophosphates and carbamates, which have been effective in controlling tephritid fruit flies in the past (Liburd et al. 2003). Many of the new reduced-risk insecticides registered for use against *Rhagoletis* flies, such as spinosyns and chloronicotinyls, require ingestion for optimal activity (Mangan and Moreno 1995, Vargas et al. 2002); therefore, the development of phagostimulant bait sprays for deployment of these new insecticides is an important aspect of optimizing their effective integration into pest management programs. Flies must contact the droplets and feed such that they obtain a lethal dose of the insecticide.

Female tephritids require a posteclosion protein meal for ovarian development and egg production (Hagen 1953, Christenson and Foote 1960). Consequently, proteinaceous bait sprays have shown activity against many tephritid species (Wakabayashi and Cunningham 1991; King and Hennessey 1996; Peck and McQuate 2000; Burns et al. 2001; Vargas et al. 2001, 2002; Barry et al. 2003; Moreno and Mangan 2003; Prokopy et al. 2003). Despite their activity, recent studies have shown that efficacy may be limited by the low capacity of baits to attract flies in the field (Prokopy et al. 2003, Pelz et al. 2005). In a recent field observational study (Pelz et al. 2005), we demonstrated that the bait used in a commercial formulation of spinosad, GF-120 Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN), acts as an arrestant to the blueberry maggot, *Rhagoletis mendax* Curran, rather than as an attractant. Flies spent significantly more time within a 5-cm range of bait droplets compared with water droplets, but they did not approach within 5 cm of bait droplets more frequently. Similar results were obtained in a study by Barry et al. (2003), which showed that GF-120 attracts Mediterranean fruit flies, *Ceratitis capitata* (Weidemann), only when they are within several centimeters of the droplets. Despite evidence that it does not attract over long distances , GF-120 has shown promise against *Rhagoletis* flies in field trials, providing 67, 98, and 84% reduction of infestation of apples, blueberries, and cherries by *Rhagoletis pomonella* (Walsh), *R. mendax*, and *R. cingulata*, respectively (Kostarides 2002, Wise and Gut 2002, Wise et al. 2002, Pelz et al. 2005). However, given the zero tolerance for these flies in fruit at harvest, a higher level of control is required. Increasing attractiveness of GF-120 is critical to its development for use against temperate fruit flies, because this increase would be expected to promote greater likelihood that flies ingest spinosad, thus improving the efficacy of this bait spray.

Ammonium acetate is an attractant for both male and female *Rhagoletis* flies (Prokopy and Coli 1978; Prokopy and Hauschild 1979; Liburd et al. 1998, 2001) and is present at only 1% (wt:vol) in the bait component of GF-120 (Moreno and Mangan 2003). Because of its high volatility, ammonium acetate dissipates rapidly. This volatilization is likely the cause of the loss of GF-120's attractiveness to the melon fly, *Bactrocera curcurbitae* Coquillett, observed within the first day after application (Prokopy et al. 2003). Increasing the concentration of ammonia in lures associated with Pherocon AM or red sphere traps has been shown to significantly increase attraction of the apple maggot, *Rhagoletis pomonella* (Walsh), to traps (Yee and Landolt 2004). It is therefore possible that increasing the concentration of ammonium acetate in GF-120 also would increase its attractiveness to *Rhagoletis* flies.

The purpose of this study was to determine whether the attractiveness of GF-120 to feral *R. cingulata* flies could be enhanced through the addition of ammonium acetate. Our objective in this study was to determine how experimental formulations of GF-120 containing additions of 5 or 10% (wt:wt) ammonium acetate affect *R. cingulata* interaction with bait droplets.

Materials and Methods

Study Location. Research was conducted during summer 2004 in an unmanaged orchard of sour cherries, *Prunus cerasus* L., located in Coloma, MI. The trees at this site were mature with canopies ≈3–4.6 m in height and 3–4 m in width. This orchard was chosen for its historically robust *R. cingulata* population.

Preparation of Treatments. To evaluate the performance of GF-120 Fruit Fly Bait enhanced with ammonium acetate against *R. cingulata,* solid ammonium acetate (Sigma-Aldrich, St. Louis, MO) was added to GF-120 Fruit Fly Bait (Dow AgroSciences) at 5 and 10% (wt:wt). Ammonium acetate was added after diluting the GF-120 with water (1 part GF-120 to 1.5 parts water by volume) according to the manufacturer's label recommendation, for a final volume of 2.5. Two control treatments also were used: GF-120 diluted as described above without additional ammonium acetate and water. In the diluted mixture, GF-120 contains 0.4% ammonium acetate (wt:vol) such that the final prepared treatments contained 0, 0.4, 5.4, or 10.4% ammonium acetate. All treatments were prepared daily before application.

Observations of *R. cingulata.* The behavioral response of feral cherry fruit flies to the treatments was evaluated by conducting daily observations of flies in the orchard. Observations were carried out at least four times per week between 0900 and 1400 hours during peak flight activity (22 June–13 July), on sunny days at ≈20–27°C. Treatments were replicated by day for a total of 12 d. Each treatment was applied daily to one of four individual trees (one treatment per tree), with trees separated by at least 15 m. Five droplets (2–3 mm in diameter) of a treatment were applied with a 5-ml syringe to the top surface of leaves on the individual trees. The treatment selected for application to a tree was randomized on each day of observation, and all treated foliage was removed from the site after observations. In total, 300 flies were observed during this study, with 12 replicates of each treatment. The number of flies observed during a single observation ranged from 0 to 22 flies.

Behavior of feral *R. cingulata* flies was assessed by two investigators standing ≈0.5 m from a treated tree and observing a 3-m-diameter surrounding the treatment droplets. Flies observed within this distance but >30 cm from the treatments were considered nonresponsive to calculate the percentage of flies responding to treatments. All other measurements were based on flies observed within 30 cm of treatment droplets. Investigators were assigned randomly to each treatment on each observation day. To observe the specific interactions of *R. cingulata* flies with droplets, one investigator observed individual flies for the duration of their presence in the observation area. This investigator recorded 1) duration(s) of fly visit(s) within 5 cm of treatment droplets and 2) number and duration(s) of feeding events on treatment droplets. Flies physically touching the treatment droplets (0 cm from droplets) were recorded as contacting the treatment regardless of whether flies exhibited feeding. Proboscis contact with the droplet surface was considered a feeding event (Yee 2003, Pelz et al. 2005). Observations of individual flies were conducted for 30 min or until the fly under observation left the observation area. Investigators recorded behaviors of individual flies by using the software program Observer Mobile (Noldus Information Technology, Wageningen, The Netherlands) loaded onto Axim X3 hand-held pocket PCs (Dell, Austin, TX). The Observer Mobile program provides internal timekeeping, resulting in precise assessments of behavior durations. Additionally, this software allows investigators to customize and predefine categories for observation (e.g., location, feeding, and distance from treatment), promoting objective recording of behaviors. Data were downloaded directly from the Observer Mobile into Microsoft Excel (Microsoft, Redmond, WA).

To quantify fly responses to droplets, a second investigator observed all flies visiting droplets for 30 min per treatment, and recorded 1) total number of fly visits within a 3-m diameter of the treatments, 2) nearest proximity of all flies to treatment droplets, and 3) occurrence (presence or absence) of feeding events by individual flies. These data were recorded by the first observer using Microsoft Notepad software, also loaded onto the hand-held pocket PCs described above. Thus, the first observer was able to record the individual fly observations and the total fly observations. After observations, flies were removed from the tree to prevent being scored more than once.

Statistical Analysis. Data on the number of flies visiting droplets, the number feeding on droplets, and duration of fly visits to droplets of each treatment were subjected to a one-way analysis of variance (ANOVA). Before analysis, all data were square root

Table 1. Number ± SE of *R. cingulata* flies observed within 30 cm of droplets during 30-min observations on cherry trees treated with water or GF-120 containing different concentrations of ammonium acetate

Bait treatment	Flies observed
GF-120 + 10% ammonium acetate	16.8 ± 2.4a
GF-120 + 5% ammonium acetate	12.6 ± 2.4ab
GF-120	6.8 ± 2.0bc
Water (control)	3.7 ± 1.8c

Means followed by the same letter are not significantly different (α = 0.05, ANOVA followed by Fisher's protected least significant difference test). Untransformed values are shown.

transformed $[(x + 0.5)^{1/2}]$ to normalize the distribution of values. Fisher protected least significant difference (LSD) test was used to separate means (significance level $\alpha = 0.05$) (SAS Institute 2000).

Results

Fly behavior was affected by the composition of droplets deployed on cherry foliage; interaction of flies with droplets was greater when droplets contained increased ammonium acetate. A significantly ($F = 7.29$; df = 3, 16; $P = 0.003$) greater number of *R. cingulata* alighted on cherry foliage within 30 cm of treatment droplets consisting of GF-120 with 5 or 10% ammonium acetate compared with control water droplets (Table 1). There was no significant difference between the number of flies alighting on foliage within 30 cm of GF-120 droplets and the number alighting within 30 cm of water droplets (Table 1). Significantly more *R. cingulata* contacted treatment droplets (0 cm from droplet) containing GF-120 with 5 or 10% ammonium acetate than water or GF-120 droplets ($F = 39.55$; df = 3, 16; $P < 0.0001$) (Fig. 1). The number of *R. cingulata* approaching within 5 cm of droplets containing GF-120 with 5 or 10% ammonium acetate was significantly greater than the number approaching water droplets ($F = 4.84$; df = 3, 16; $P = 0.014$) (Fig. 1). At distances >5 cm of droplets, there were no significant differences among treatments in the number of *R. cingulata* observed approaching various treatment droplets.

The duration *R. cingulata* flies spent within 5 cm of GF-120 + 5% or GF-120 + 10% ammonium acetate treatments was significantly ($F = 4.15$; df = 3, 65; $P = 0.009$) greater than the duration spent within the same distance of GF-120 or water (Table 2). Greater time was spent within 5 cm of the GF-120 + 5% ammonium acetate treatment than the GF-120 + 10% ammonium acetate treatment, although the values were not significantly different. Flies spent approximately six-fold and five-fold more time within 5 cm of GF-120 + 5% and GF-120 + 10%, respectively, compared with GF-120. Only 4 and 6% of the flies observed were seen to feed on water and GF-120, respectively. In contrast, 28 and 24% of the flies exhibited feeding behavior on GF-120 + 5% and GF-120 + 10%, respectively. How-

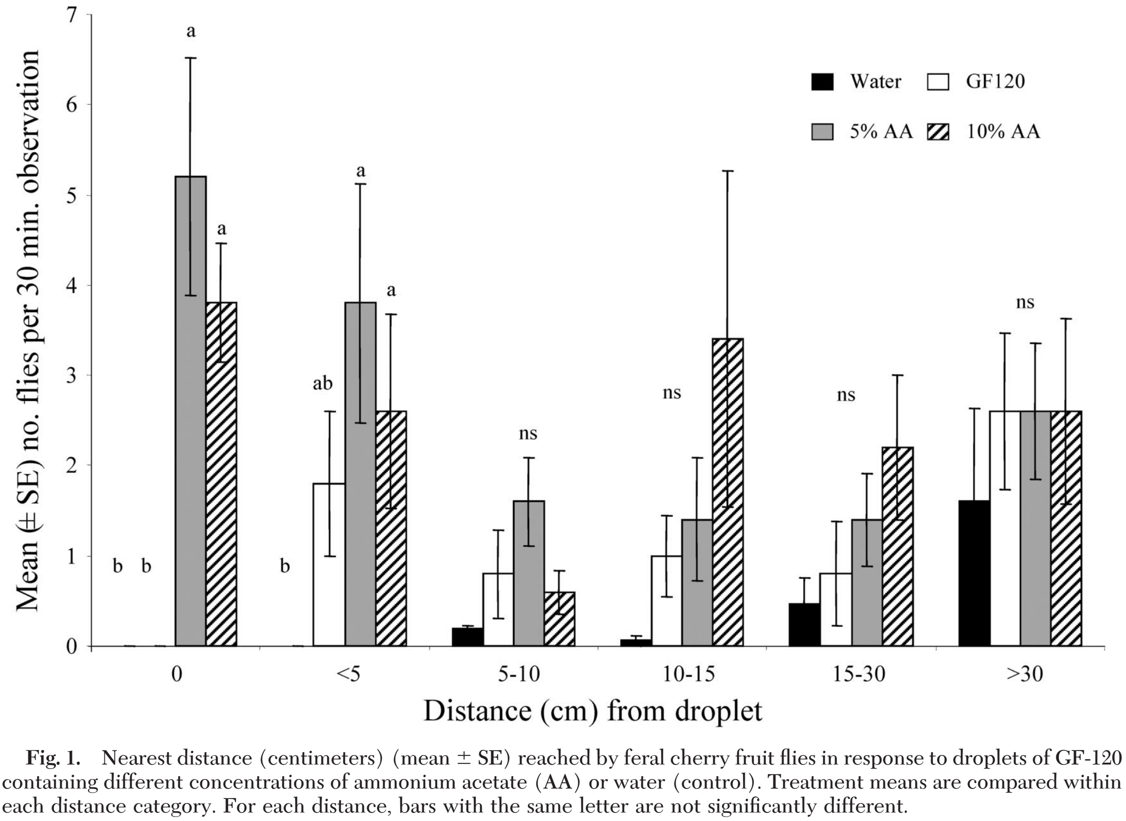

Fig. 1. Nearest distance (centimeters) (mean ± SE) reached by feral cherry fruit flies in response to droplets of GF-120 containing different concentrations of ammonium acetate (AA) or water (control). Treatment means are compared within each distance category. For each distance, bars with the same letter are not significantly different.

Table 2. Average duration of behavior exhibited by *R. cingulata* flies observed for 30 min on cherry trees treated with bait or water droplets

Bait treatment	n	Mean ± SE duration (s)	
		Within 5 cm of treatment droplet	Feeding on treatment droplet
GF-120 + 10% ammonium acetate	17	263.2 ± 85.2a	61.5 ± 30.7a
GF-120 + 5% ammonium acetate	29	337.6 ± 72.6a	73.4 ± 21.0a
GF-120	15	50.3 ± 36.4b	6.8 ± 5.7b
Water (control)	8	101.2 ± 81.8b	8.6 ± 6.1b

ever, because of the low number of flies observed feeding, there were no significant differences among the treatments. The duration of feeding behavior was significantly greater on GF-120 + 5% and GF-120 + 10% ammonium acetate treatments compared with GF-120 and water. This duration represents a 10-fold increase in the duration of feeding compared with GF-120 and water ($F = 3.03$; df = 3, 65; $P = 0.036$) (Table 2). In addition, the proportion of total time spent feeding increased with ammonium acetate concentration. The *R. cingulata* flies that fed during observations did so for 18% (GF-120 + 5%) and 23% (GF-120 + 10%) of the total observation period, whereas flies fed on GF-120 or water for only 1 and 2%, respectively, of the total observation period.

Discussion

Our results indicate that interaction of *R. cingulata* flies with droplets of GF-120 can be enhanced with additional ammonium acetate, improving fly attraction to droplets, arrestment at droplets, and interaction with droplets. The addition of 10% ammonium acetate significantly increased attraction to the bait spray droplets compared with standard GF-120 containing 1% ammonium acetate. Flies spent more time in proximity to all bait treatments compared with water, suggesting that flies became arrested at droplets, similar to earlier observations of *R. mendax* (Pelz et al. 2005). In addition, flies spent more time in proximity to droplets with 5 and 10% additional ammonium acetate than near standard GF-120, suggesting that higher levels of ammonium acetate in GF-120 can increase arrestment of foraging flies.

The current study demonstrates that an addition of 5–10% ammonium acetate rendered GF-120 substantially more attractive to *R. cingulata* in a field setting compared with the standard concentration (1%) of ammonium acetate. Similarly, Yee and Landolt (2004) found that captures of *R. pomonella* flies on traps increased as concentrations of ammonia in lures increased from 0 to 29.3%, resulting in higher release rates. Limited improvement in attractiveness to *R. pomonella* also was reported for ammonium bicarbonate added to the protein hydrolysate bait NuLure (Hendrichs et al. 1990). In contrast, an increase in the percentage of ammonium acetate concentration in water (0–4%), presented as 50-μl droplets on artificial *Ficus* leaves, deterred feeding by *R. mendax* in laboratory bioassays (Barry and Polavarapu 2004). Together, the results of these studies suggest that the optimal concentration of ammonium acetate needed to attract *Rhagoletis* flies may be species-specific. In addition, the studies with *R. pomonella* and *R. mendax* used ammonia compounds presented in solution (Barry and Polavarapu 2004, Yee and Landolt 2004). Future work is needed to determine whether the effect of ammonia released by these solutions translates to a similar effect when the ammonia source is incorporated into a protein–sugar bait and deployed under field conditions. Furthermore, the release rate of ammonia from bait is likely to be dependent on the size of the droplet. Additional field and laboratory observations of fly behavior in response to varying levels of ammonium acetate in bait droplets of different sizes are necessary to clarify the importance of these factors.

Baited insecticides offer an attractive alternative to conventional pesticides used for fruit fly control, in part because the environmental impact is reduced compared with broadcast foliar sprays. For GF-120, there is a much lower concentration of active ingredient than in broadcast spray formulations, such as the unbaited SpinTor formulation of spinosad (0.2% compared with 22.8% in SpinTor) and minimal effect on nontarget insects (Vargas et al. 2001, 2002; Mazor et al. 2003). Bait-and-kill formulations of spinosad have been effective against several tropical tephritids, such as Caribbean fruit fly, *Anastrepha suspensa* (Loew) (King and Hennessey 1996, Burns et al. 2001); Mexican fruit fly, *Anastrepha ludens* (Loew) (Moreno and Mangan 2003); melon fly (Wakabayashi and Cunningham 1991, Prokopy et al. 2003); and Mediterranean fruit fly (Peck and McQuate 2000; Vargas et al. 2001, 2002; Barry et al. 2003). Studies of temperate tephritids (Reissig 2003, Pelz et al. 2005, Yee and Chapman 2005) have not shown the same level of success with bait-and-kill formulations of spinosad; thus, in its current formulation, GF-120 carries a greater risk of fruit contamination by larvae if used in place of broad-spectrum insecticides (Pelz et al. 2005). The effectiveness of GF-120 will depend on increasing fly attraction to, and feeding on, the bait. A further potential complication is that the responses of temperate tephritid species to ammonium acetate in bait droplets may differ by species. This is suggested by the response seen in this study and in laboratory studies of *R. cingulata* compared with laboratory studies of *R. mendax* (K.S.P., unpublished data; Barry and Polavarapu 2004). Our results provide evidence that GF-120 can be enhanced to maximize its efficacy against *R. cingulata;* however, work is needed to determine whether increased interaction and ingestion of ammonium acetate-enhanced GF-120 will reduce fruit infestation by *R. cingulata* compared with unaltered GF-120, if the longevity of ammonium acetate in enhanced GF-120 is sufficient enough as to remain volatile in between applications, and whether enhanced efficacy will extend to other economically important *Rhagoletis* species.

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