Captures of *Rhagoletis mendax* and *R. cingulata* (Diptera: Tephritidae) on Sticky Traps Are Influenced by Adjacent Host Fruit and Fruit Juice Concentrates

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ABSTRACT   Field trapping studies were conducted to determine whether feral blueberry maggot flies, *Rhagoletis mendax* Curran, and Eastern cherry fruit flies, *R. cingulata* (Loew), respond to natal host-fruit volatiles. Subsequent experiments were conducted to evaluate the potential of using simple and inexpensive fruit juice concentrate lures to monitor these key pests of blueberries and cherries, respectively. The presence of ripe blueberries and ripe tart cherries in enclosures that permitted escape of fruit volatiles significantly increased captures of *R. mendax* and *R. cingulata*, respectively, on adjacent sticky traps compared with traps without adjacent fruit. *R. mendax* were not affected by addition of blueberry concentrate to Pherocon AM traps with or without ammonium acetate. Pherocon AM boards prebaited with ammonium acetate directly incorporated into the sticky Tangle-Foot coating were equally effective as unbaited Pherocon AM boards deployed with separate ammonium acetate dispensers for monitoring *R. mendax*. A cherry juice concentrate increased captures of *R. cingulata* on unbaited Pherocon AM but not Rebell traps. In addition, combining cherry juice concentrate and ammonium acetate on Pherocon AM traps increased captures of *R. cingulata* compared with captures on traps with either stimulus alone. Adding ammonium acetate lures to Rebell traps more than doubled captures of *R. cingulata* over that on unbaited Rebell traps. Our results show that feral *R. mendax* and *R. cingulata* flies are attracted to the volatiles emitted from their host fruit and that this may be exploited to improve monitoring of these important fruit pests.

KEY WORDS   monitoring, trapping, blueberry maggot fly, cherry fruit fly, host fidelity

THE BLUEBERRY MAGGOT FLY, *Rhagoletis mendax* Curran, and the Eastern cherry fruit fly, *Rhagoletis cingulata* (Loew), are key pests of blueberries and cherries, respectively, in the United States. Larvae of both species feed internally, destroying fruit tissues and creating contamination concerns for producers. Zero tolerance for fruit fly infestation necessitates accurate monitoring of fly presence in crops and prompt deployment of control measures. Broad-spectrum contact insecticides such as organophosphates and carbamates have successfully controlled these pests to date. However, restrictions of these insecticides in response to the Food Quality Protection Act (Anonymous 1996) have required the evaluation and adoption of newer "reduced-risk" insecticide chemistries for control of fruit flies (van Randen and Roitberg 1998a, b, Liburd et al. 2003). These newer compounds (e.g., spinosad) are effective in controlling *Rhagoletis* flies, but often require ingestion rather than contact to kill flies (Liburd et al. 2003, Pelz et al. 2005). Accurate

timing of insecticide applications is necessary to ensure that flies will ingest the toxicant before oviposition.

Thus, the cornerstone of an effective fruit fly management program is reliable and sensitive monitoring for fly presence. *Rhagoletis* flies are monitored with sticky traps that exploit behaviorally relevant visual and olfactory stimuli. These attractive cues may mimic host foliage, host odor, or natural food sources (Prokopy and Coli 1978, Prokopy and Hauschild 1979, Neilson et al. 1981, Duan and Prokopy 1992, Liburd et al. 1998a, 2001). Pherocon AM boards baited with release devices containing ammonium acetate are highly effective monitoring tools for both *R. cingulata* and *R. mendax* (Prokopy and Coli 1978, Liburd et al. 1998a, 2001), as well as other *Rhagoletis* species (Prokopy and Hauschild 1979). Current commercially available lures for these species consist of plastic dispensers (chargers) containing 2.0 g of ammonium acetate that are affixed to traps. Additionally, prebaited Pherocon AM traps are available, containing ammonium acetate and protein hydrolysate impregnated directly within the sticky Tangle-Foot coating. This latter approach is common in Michigan because of the reduced time required for trap deployment.

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Host-volatile lures have been developed for trapping the apple maggot fly, *R. pomonella* (Walsh). These lures provide a more accurate indication of fly presence than ammonium-based lures (Rull and Prokopy 2000, Stelinski and Liburd 2002) and show promise for development of attract-and-kill approaches to control this pest. A seven-component blend of apple volatiles was developed through field, wind tunnel, and olfactometer bioassays to attract *R. pomonella* (Fein et al. 1982, Reissig et al. 1982, Averill et al. 1988). Further experiments showed that a five-component blend (Zhang et al. 1999) combined with a red sphere is the optimal trap for attracting the maximum number of *R. pomonella* (Stelinski and Liburd 2002). Before the development of an attractive synthetic apple volatile lure, Prokopy et al. (1973) showed that *R. pomonella* were attracted to the odor of apples. In contrast, attractive and effective host-fruit volatiles for use in monitoring *R. mendax* and *R. cingulata* have not been identified. Several blueberry volatiles elicit electroantennogram responses from *R. mendax* (Lugemwa et al. 1989) and have shown promise for improving capture of *R. mendax* (Liburd 2004). To date, it has not been determined whether *R. cingulata* are attracted to volatiles specific to their host fruit: cherries.

The objectives of this study were to (1) determine whether feral *R. mendax* and *R. cingulata* are attracted to natal host-fruit volatiles in the field, (2) determine the response of *R. mendax* and *R. cingulata* to host-fruit juice concentrates in an attempt to develop a host-fruit lure to attract adults of these species, and (3) determine the optimal method of ammonium acetate deployment (plastic dispenser versus prebaited Pherocon AM boards) for monitoring *R. mendax.*

Materials and Methods

Field Sites. Experiments in highbush blueberry, *Vaccinium corymbosum* L., were conducted at two sites chosen because of their historically high levels of *R. mendax* infestation. The experimental sites were located at the University of Rhode Island, East Farm Experimental Station in Kingston, RI, described in Liburd et al. (1998b) and at an unsprayed blueberry plantation (cultivar Jersey) in southwest Michigan (Allegan Co.) described in Stelinski and Liburd (2001) and Pelz et al. (2005).

Experiments in tart cherries, *Prunus cerasus* L., were conducted in southwest Michigan (Van Buren Co. and Allegan Co.) and northwest Michigan (Leelanau Co.). These orchards consisted of unsprayed, mature trees (cultivar Montmorency) of ≈4.6-m canopy height.

Response of *R. mendax* to Odor of Blueberries. This experiment tested the hypothesis that *R. mendax* respond to the volatiles of their host fruit: blueberries. Blueberries or glass marbles of identical size (control) were placed inside 0.5-cm erosion-mesh bags (Bemis Co., St. Louis, MO), at 1 kg per bag. Bags containing ripe blueberries and marbles were placed in collapsible cages (BioQuip Products, Gardena, CA) and hung ≈2 m from *V. corymbosum* bushes that had all fruit

removed. Blueberries (cultivar Berkley and Collins) were obtained from the Kingston Experimental Station described above. Bags were tied (with twist-ties) to the aluminum frame of the collapsible cage. Cages were constructed with 32-mesh Lumite-screens (Bio-Quip Products), and each cage was 20.3 by 20.3 by 20.3 cm in size. Enclosures were designed to prevent flies from contacting fruit, yet allow the emission of olfactory stimuli. The top of each cage was lined with Velcro to allow easy access for handling specific treatments. Four unbaited green sticky-sphere traps used for monitoring *R. mendax* were hung 15 cm from the face of each side of the cage. Treatments were arranged in a randomized complete block design with five replicates. Traps were separated by at least 15 m, with 15 m between blocks. To prevent bias among treatments, fresh blueberries were replaced in each cage every 3 d, and cages within blocks were rotated three times per week.

Response of *R. cingulata* to Odor of Cherries. This experiment tested the hypothesis that *R. cingulata* respond to the volatiles of their natal host fruit: cherries. For each treatment, 1 kg of fruit (described below) was washed and placed in 0.5-cm cheesecloth bags. These bags were placed in cylindrical enclosures (30.5 cm length by 15.2 cm diameter) constructed of 1-mm mesh aluminum window screening. Cheesecloth fruit bags were suspended from a wire hanger in the enclosures with a twist tie such that fruit was ≈3 cm from the cage walls. Enclosures were hung 2.1 m above ground. All foliage and cherries within a 0.5 and 2 m radius, respectively, were removed to prevent competition with caged fruit. These fruit enclosures were designed to prevent flies from contacting fruit, yet allow the emission of olfactory stimuli. In addition, the contents within enclosures were not visible from the outside. Two Pherocon AM traps (Trécé, Adair, OK) were hung on opposite sides of the fruit enclosures 3 cm from their edge.

The following treatments were tested: (1) unripe tart cherries, (2) unripe sweet cherries, (3) ripe tart cherries, (4) ripe sweet cherries, and (5) no fruit (control). Ripe fruit was obtained from a local fruit market, whereas unripe fruit was obtained from research orchards at Michigan State University's Trevor Nichols Research Complex (Fennville, MI). Five replicates of each treatment were arranged in a randomized block design. Traps were separated by at least 20 m, with 30 m between blocks. *R. cingulata* flies captured on traps adjacent to the enclosures containing fresh fruit were counted and removed every 3 d. After each trap inspection, fresh fruit was replaced, and treatment positions were rotated within blocks.

Fruit Lure Evaluation: Chemicals and Release Devices. These experiments tested the hypothesis that concentrated solutions of blueberry and cherry juice are attractive to *R. mendax* and *R. cingulata*, respectively. Blueberry and cherry concentrates were obtained from Milne Fruit Products (Prosser, WA). For the cherry fruit lure, 5% tart cherry concentrate (Brix 68 g sugar/100 ml; lot JEL-02–092-MI), 5% sweet cherry concentrate (Brix 68 g sugar/100 ml; lot MFP-

92–074-M3), and 85% water were blended. Because of the high sugar content, ethanol (5% vol:vol) was also added to preserve freshness under field conditions. Milne Fruit Products removed all nonsoluble components, including sugars, from the blueberry concentrate; therefore, it was not necessary to add ethanol to this purified extract. Five milliliters of each fruit concentrate was dispensed into 2.5 by 5.0-cm plastic bags and heat-sealed. A 6.4-cm-long cotton wick was inserted into the dispenser before sealing to draw the concentrate out for release. Fruit concentrate dispensers were attached to the top of traps with 0.6-cm binder clips.

Captures of flies on traps baited with fruit concentrate lures were compared with captures on traps baited with ammonium acetate lures, because these are known to be highly attractive to both *R. mendax* (Liburd et al. 1998a) and *R. cingulata* (Liburd et al. 2001). In treatments where ammonium acetate was used, plastic yellow dispensers (chargers; Great Lakes Integrated Pest Management [IPM], Vestaburg, MI) were filled with 2 g of solid ammonium acetate (Sigma Aldrich, St. Louis, MO) immediately before field deployment. A single dispenser was attached to the upper corner of the Pherocon AM trap with a yellow twist tie.

An evaluation of lures for *R. mendax* was conducted by comparing five treatments in a randomized complete block design with six replicates. The five treatments were (1) Pherocon AM traps prebaited with ammonium acetate (2 g) and protein hydrolysate (0.5 g; Trécé) mixed within the sticky Tangle-Foot, (2) unbaited Pherocon AM traps with a blueberry concentrate lure, (3) unbaited Pherocon AM traps with an ammonium acetate lure, (4) unbaited Pherocon AM traps with both a blueberry concentrate and an ammonium acetate lure, and (5) unbaited Pherocon AM traps (control). Traps were hung at the optimum height within blueberry bushes ≈15 cm below the uppermost canopy and in a vertical orientation on the south side of the blueberry bushes with foliage and fruit cleared within a 0.5-m radius (Liburd et al. 2000). Traps were separated by at least 15 m with 10 m between blocks. *R. mendax* were counted and removed weekly, and treatments were rotated within each block to account for positional bias. Treatments and traps were replaced biweekly for an 8-wk period.

Two trapping experiments were conducted to evaluate lures for *R. cingulata*. In the first experiment, Pherocon AM traps were baited with (1) cherry concentrate lure, (2) ammonium acetate lure, (3) cherry concentrate and ammonium acetate lures, or (4) blank (control). In the second experiment, the four lure treatments were identical, but they were evaluated using Rebell traps (Great Lakes IPM) rather than Pherocon AM traps. Rebell traps were used because these are highly attractive to *R. cingulata* (Liburd et al. 2001). The experimental design was a randomized complete block with six replicates for the experiment conducted with Pherocon AM traps and five replicates for the experiment conducted with Rebell traps. Traps were hung vertically on the south side of trees with

Table 1. Season-long captures of *R. cingulata* on Pherocon AM boards hung adjacent to enclosures containing ripe or unripe sweet or sour cherries in Michigan cherry orchards

Treatment (cherry type)	Flies per trap
Ripe tart	216.0 ± 32.5a
Ripe sweet	129.3 ± 31.1b
Unripe tart	97.0 ± 46.9b
Unripe sweet	78.0 ± 8.7c
No fruit (control)	94.7 ± 21.7b

Mean ± SEM; means within each column followed by the same letter are not significantly different ($P > 0.05$, Fisher's LSD test). Untransformed values are shown.

foliage cleared within a 0.5-m radius of the traps. Traps were separated by at least 20 m, with 30 m between blocks. Trap maintenance protocols were identical to those described for blueberries above.

Statistical Analysis. For each experiment, data from subweekly trap captures were pooled across the season for analysis. Data were normalized by square root-transformation $(x + 0.5)^{1/2}$ and subjected to analysis of variance (ANOVA). Means separation was conducted using Fisher's least significant difference (LSD) test (SAS Institute 2000). In all cases, the significance level was $\alpha < 0.05$.

Results

Response of *R. mendax* to Odor of Blueberries. Significantly ($F = 9.8$; df = 1,4; $P = 0.04$) more flies were captured on spheres adjacent to collapsible cages containing blueberries (20.4 ± 4.0) compared with those placed adjacent to marbles (surrogate blueberries; 6.4 ± 1.6), representing a more than three-fold increase in fly captures. In addition, significantly ($F = 11.8$; df = 1,4; $P = 0.02$) more female *R. mendax* were captured on spheres placed near blueberries (12.0 ± 3.2) compared with spheres near marbles (3.0 ± 1.2) within collapsible cages.

Response of *R. cingulata* to Odor of Cherries. Significantly ($F = 5.3$; df = 4,8, $P < 0.05$) more *R. cingulata* were captured on Pherocon AM traps placed adjacent to cages containing ripe tart cherries compared with the other treatments (Table 1), representing a more than two-fold increase in fly captures. There were no significant differences between the following treatments: ripe sweet, unripe tart, and no fruit (control; Table 1). Treatments with unripe sweet cherries caught significantly fewer *R. cingulata* than the control (Table 1).

Fruit Lure Evaluation: *R. mendax* Experiment. Significantly ($F = 10.7$; df = 4,20; $P < 0.05$) fewer *R. mendax* were captured on Pherocon AM boards baited with blueberry concentrate lures compared with any of the treatments containing ammonium acetate (Table 2). In addition, fly captures on traps baited with the blueberry concentrate lures were not significantly ($P > 0.05$) different from those on unbaited Pherocon AM traps. Similar numbers of *R. mendax* were captured on Pherocon AM traps con-

Table 2. Effect of different lure formulations on season-long captures of adult *R. mendax* on Pherocon AM traps

Lure type	Flies per trap
Ammonium acetate (dispenser) + blueberry concentrate	66.3 ± 12.2a
Ammonium acetate + protein hydrolysate (impregnated in adhesive)	63.0 ± 11.8a
Ammonium acetate (dispenser)	58.2 ± 15.0a
Blueberry concentrate	18.8 ± 4.9b
None (control)	15.5 ± 2.9b

Mean ± SEM means within each column followed by the same letter are not significantly different ($P > 0.05$, Fisher's LSD test). Untransformed values are shown.

taining ammonium acetate lures alone and ammonium acetate lures combined with blueberry essence lures (Table 2).

Fruit Lure Evaluation: *R. cingulata* Experiment. Significantly ($F = 9.7$; df = 3,15; $P < 0.05$) more *R. cingulata* were captured on Pherocon AM traps baited with ammonium acetate and cherry concentrate lures combined than were captured on Pherocon AM traps containing the cherry concentrate lure alone (Table 3). However, captures of *R. cingulata* on Pherocon AM traps baited with either the cherry concentrate or ammonium acetate lures alone were not significantly different (Table 3). All treatments captured significantly ($P < 0.05$) more *R. cingulata* than unbaited Pherocon AM traps (Table 3). Although there was no significant ($P > 0.05$) difference between mean fly captures on Pherocon AM boards baited with the combination of cherry concentrate and ammonium acetate lures and the ammonium acetate lures alone, the former treatment captured ≈30% more flies than the latter (Table 3).

The response of *R. cingulata* to the same lure treatments using Rebell traps differed from that observed with Pherocon AM boards. Significantly ($F = 9.8$; df = 3,12; $P < 0.005$) fewer flies were captured on Rebell traps baited with cherry concentrate lures than were captured on traps baited with ammonium acetate lure treatments (Table 3). The addition of a cherry concentrate lure to the Rebell trap did not increase fly captures relative to unbaited Rebell traps (Table 3). In addition, there was no significant ($P < 0.05$) difference between captures of *R. cingulata* on Rebell traps containing ammonium acetate and ammonium acetate

Table 3. Effect of different lure formulations on season-long captures of adult *R. cingulata* on Pherocon AM and Rebell traps

Lure type	Flies per trap	
	Pherocon AM	Rebell
Ammonium acetate + cherry concentrate	51.7 ± 9.4a	43.6 ± 9.2a
Ammonium acetate (dispenser)	37.2 ± 9.9ab	32.8 ± 9.9a
Cherry concentrate	28.7 ± 5.1b	16.0 ± 3.4b
None (control)	15.8 ± 4.5c	17.4 ± 4.8b

Mean ± SEM means within each column followed by the same letter are not significantly different ($P > 0.05$, Fisher's LSD test). Untransformed values are shown.

plus cherry concentrate lures (Table 3). At least twice as many flies were captured on Rebell traps baited with either of the ammonium acetate treatments than on the other treatments evaluated (Table 3).

Discussion

Our results indicate that feral *R. mendax* and *R. cingulata* flies are attracted to the volatiles emitted by their respective host fruit. Specifically, ripe blueberries and ripe tart cherries increased captures of *R. mendax* and *R. cingulata*, respectively, on adjacent sticky traps. Host races of the related *R. pomonella* are attracted to exact blends of volatiles given off by their specific host fruit (Linn et al. 2003, Nojima et al. 2003a, b). Such volatiles mediate long-range attraction of *R. pomonella* to the location of host fruit (Prokopy et al. 1973). Within the genus *Rhagoletis*, mating, oviposition, and larval development are strictly associated with species-specific, unabscised host fruit. This association between the location of larval development and the location of mating and oviposition is referred to as "host fidelity" and is believed to impart a premating isolation mechanism for sympatric speciation (Feder et al. 1994, Feder 1998). Our data provide direct evidence for host-fruit association mediated by olfactory cues for two more species within the fruit-infesting *Rhagoletis* sibling complex.

Ripe cherries increased captures of *R. cingulata* on adjacent traps, whereas unripe fruit did not (Table 1). This suggests that cherries may become maximally attractive to *R. cingulata* at a specific phenological stage, perhaps increasing chances of successful oviposition and larval fitness. Alternatively, the ripe cherry treatments may have attracted more flies given that store-bought fruit released a greater amount of volatiles after a period of aging compared with freshly picked unripe fruit.

Increased captures of *R. mendax* on traps adjacent to ripe blueberries observed in this study corroborates previous work suggesting that this species responds to host fruit volatiles. As measured by electroantennograms (EAGs), *R. mendax* are more sensitive to fruit extracts of blueberries than that of apples, indicating that antennal sensitivity may be adapted to the species-specific host (Frey and Bush 1990, Frey et al. 1992). Furthermore, hybrids of *R. mendax* and *R. pomonella* exhibit significantly reduced EAG responses to host odor compounds compared with parental lines, suggesting host-odor perception is an important factor in species isolation (Frey and Bush 1996). In addition, the two species have evolved divergent egg-laying responses to chemical stimuli on the fruits of their respective hosts (Bierbaum and Bush 1990). Specifically, *R. mendax* lay more eggs compared with *R. pomonella* when stimulated by extracts from blueberries, whereas the opposite is true for *R. pomonella*.

Both *R. mendax* and *R. cingulata* are key pests of their respective host fruit (Liburd et al. 1998a, 2001; Teixeira and Polavarapu 2001). Visual and olfactory traps are used to monitor these pests and time insec-

ticide applications. Ammonium acetate is known to be highly attractive to *Rhagoletis* species, but this lure is nonselective resulting in substantial nontarget insect captures predominated by various Diptera (Stelinski and Liburd 2002). Lack of selectivity reduces monitoring effectiveness because traps become inundated with nontarget insects, removing effective trapping surface area. This increases labor input, given the need for biweekly trap cleaning and replacement (Stelinski and Liburd 2002) and the need to sort through flies to find the target pest species. Fruit volatile lures developed for *R. pomonella* (Zhang et al. 1999) are highly selective to this species and perform significantly better than ammonium acetate lures (Stelinski and Liburd 2002). Developing highly attractive and selective monitoring lures for both *R. mendax* and *R. cingulata* would represent an important advance for management of these species.

These results show the potential for developing synthetic host-fruit lures for these *Rhagoletis* flies, given that both species are attracted to their natal host fruit. Our initial attempt to develop a simple and inexpensive fruit-based lure using fruit concentrates did not prove highly effective. The lack of response by *R. mendax* to the blueberry concentrate lure suggests that ripe blueberries give off a specific blend of volatiles, which was not preserved in the blueberry juice concentrate we evaluated. For *R. cingulata,* there was evidence that the cherry juice concentrate was attractive when deployed with Pherocon AM boards, but this lure was not more attractive than ammonium acetate. In addition, combining the cherry concentrate lure with an ammonium acetate lure increased fly captures on Pherocon AM traps relative to similar traps baited with either lure type deployed individually. However, the cherry concentrate lure did not increase captures of *R. cingulata* when evaluated with Rebell traps. These traps provide a highly attractive visual stimulus to cherry fruit fly species and capture more cherry fruit flies than ammonium acetate–baited Pherocon AM boards (Liburd et al. 2001). Thus, the extraordinary visual attractiveness of these traps may have maximized fly response, precluding further stimulation by olfactory cues.

Ammonium acetate is currently the standard lure used for monitoring *R. mendax* and *R. cingulata* (Liburd et al. 1998a, 2001). Although this compound is nonselective, it is a highly potent food-based attractant (Liburd et al. 1998a, Stelinski and Liburd 2002). Our results showed that Pherocon AM boards prebaited with ammonium acetate directly impregnated into the sticky Tangle-Foot coating were just as effective in capturing *R. mendax* as were initially unbaited Pherocon AM boards with separately attached ammonium acetate dispensers (Table 2). Given that the former trap type is easier to handle and deploy in the field, we recommend using prebaited Pherocon AM boards when monitoring *R. mendax* with ammonium acetate. In addition, our results showed that adding an ammonium acetate lure to a Rebell trap more than doubled captures of *R. cingulata* compared with captures on unbaited traps (Table 3). Rebell traps deployed without bait are highly attractive and effective traps for both *R. cingulata* and the black cherry fruit fly, *R. fausta* (Osten Sacken) (Liburd et al. 2001). The addition of ammonium acetate lures to these traps may improve their monitoring sensitivity and practicality as attract-and-kill devices.

The response of *R. mendax* and *R. cingulata* to ripe blueberries and ripe tart cherries, respectively, suggests an odor-mediated interaction between these insects and their host fruit. Future experiments should focus on elucidating how sexual maturity of flies affects the behavioral response to host fruit. These findings open the possibility for identification of the specific volatile blends mediating this attraction. This has been recently completed for *R. pomonella* host races (Linn et al. 2003, Nojima et al. 2003a, b). Future work should focus on the collection, isolation, and identification of headspace volatiles associated with unharvested host fruits. Identifying the odors mediating host-fruit location by *R. mendax* and *R. cingulata* will improve monitoring tactics for these important fruit pests and provide insights into the mechanisms by which they have speciated.

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