

## SHORT COMMUNICATION

### A Simple and Effective Method for Capturing Viable Adult Blueberry Maggot Flies, *Rhagoletis mendax* (Diptera: Tephritidae)

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The blueberry maggot fly, *Rhagoletis mendax* Curran, (Diptera: Tephritidae) is a widely distributed pest of low- and highbush blueberries (*Vaccinium angustifolium* Aiton and *Vaccinium corymbosum* L., respectively) (Vacciniaceae) within the northeastern and midwestern United States, as well as Canada. Given its economic importance, the fly has been the target of considerable research with respect to the development of monitoring and control strategies within the context of integrated pest management (Prokopy and Coli, 1978; Liburd *et al.*, 1998a, b, 2000). Several attempts have been made to develop and refine blueberry maggot fly monitoring programs in order to forecast the presence of infestations in commercial blueberry plantings (Liburd *et al.*, 1998a, 2000). The current monitoring practices employed by commercial growers of low- and highbush blueberries involve the deployment of ammonium-baited Pherocon AM yellow sticky panels or green and red sticky spheres (Prokopy and Coli, 1978; Liburd *et al.*, 1998a). Such practices allow growers to detect fly emergence for more accurate timing of sprays. The blueberry maggot is also a member of the *R. pomonella* (Walsh) sibling species complex and has been used as part of a long-running study system by evolutionary biologists for investigating the mechanisms of speciation in sympatry (Feder and Bush, 1989).

The blueberry maggot fly completes larval development inside blueberry fruit and undergoes an obligate annual diapause, making laboratory-rearing difficult. To our knowledge, a continuously-reared laboratory colony of blueberry maggot does not exist. The blueberry maggot has been the subject of many laboratory studies investigating toxicity of potential insecticides (Ayyappath *et al.*, 2000; Stelinski *et al.*, 2001; Liburd *et al.*, 2003). Other laboratory investigations have focused on the antennal sensitivity and oviposition preference of blueberry maggot flies with reference to host-plant related chemical cues (Frey and Bush, 1996). In these laboratory studies, the investigators have relied on the use of adult flies reared from larvae collected the previous year from infested fruit. We describe here a simple and effective method for capturing viable male and female blueberry maggot fly adults in both abandoned and commercial highbush blueberry *Vaccinium* spp. plantations. Such field-captured, live blueberry maggot flies could be used in the laboratory for toxicological studies, electrophysiological investigations, behavioral assays or in genetic analyses of specific populations. Potentially, this technique could also be adopted for other tephritid species of broad interest.

#### Materials and Methods

##### General

Research was conducted in the summers of 2000 in a 0.5 ha abandoned plantation of highbush blueberries and in 2001 in the same abandoned site as well as a 1.4 ha commercially maintained plantation of highbush blueberries in Fennville, MI. The abandoned plantation had not been treated with insecticide or maintenance sprays and had not been pruned for more than 5 years. The commercial site was pruned annually and received regular applications of organophosphate and pyrethroid insecticides throughout the blueberry growing season.

##### 2000

In 2000, we conducted the initial test of a funnel-trap for capturing live adult blueberry maggot flies. The trap consisted of a wire mesh funnel connected to a perforated plastic vial (Fig. 1). The interior portion of the funnel was lined with bright yellow laminated paper. The yellow paper was colored only on one side such that only the funnel's interior is yellow. The outer opening of the funnel was ca. 20 cm in diameter and tapered down to a diameter of ca. 0.5 cm. The funnel-traps were evaluated with two bait-treatments and unbaited funnel-traps served as a control.

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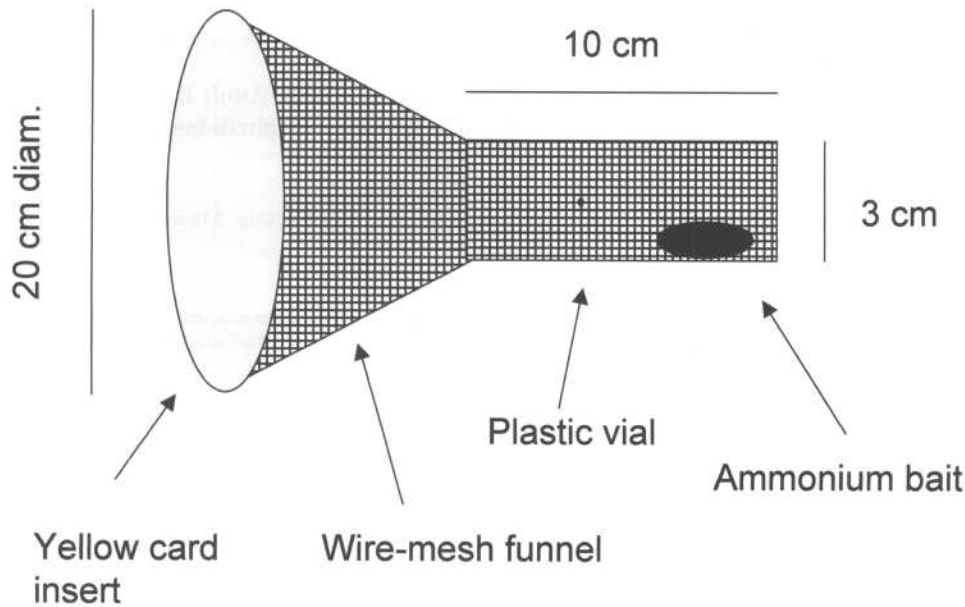


Fig. 1. Schematic diagram of the funnel-trap.

The traps containing the various treatments were arranged in a randomized complete block design with five replicates. Treatments were placed ca. 10 m apart within rows and 15 m between blocks. The traps were attached to branches within blueberry bushes using twist-ties in a horizontal orientation as shown in Fig. 1 and ca. 15-cm from the uppermost bush. This location within the blueberry bush canopy has been found to be the most effective position for trapping blueberry maggot (Liburd *et al.*, 2000). The two bait treatments evaluated were: 1) 2.0 g of solid ammonium acetate (Aldrich Chemical Company Milwaukee, WI) placed into 5 ml scintillation vials (National Diagnostics, Atlanta, GA), and 2) 2.0 g of ammonium acetate dissolved in 3 ml of water placed into 5 ml scintillation vials. These baits were chosen because blueberry maggot exhibit an attraction to ammonia (Liburd *et al.*, 1998a), apparently seeking a protein source that may be important for egg maturation (Prokopy and Roitberg, 1984; Prokopy *et al.*, 1994). The aqueous ammonium acetate treatment was tested because Liburd *et al.* (2001) found that aqueous ammonium baits are more attractive to cherry fruit fly species (*R. cingulata* and *R. fausta*) than solid formulations.

Traps were sampled weekly. Flies found captured within traps were counted by sex and removed. Ammonium acetate lures were replaced every three weeks or as needed.

#### 2001

The following season all funnel-traps evaluated were baited with 2.0 g of solid ammonium acetate in scintillation vials because traps baited with solid and aqueous formulations of this bait captured equal numbers of blueberry maggot flies in 2000 (see results). In this year, captures of blueberry maggot flies in funnel-traps baited with solid ammonium acetate were compared to captures on ammonium acetate-baited and unbaited 9-cm diameter green sticky sphere traps. These traps are the most effective method for capturing high numbers of blueberry maggot (Liburd *et al.*, 2000), although this sampling method kills the captured flies. Studies were conducted in both an abandoned site which has a very large blueberry maggot population and within a commercially maintained plantation with much lower fly incidence. As in the previous year, trap-types (treatments) were arranged in a randomized complete block design with five replicates. The sampling protocol was identical to that described for 2000.

In 2001, 160 (121 females and 39 males) of the captured blueberry maggot flies were collected for further analysis. Forty of the females were taken back to a laboratory and their ovaries were dissected to determine sexual maturity. The remainder of the females and all of the males were placed into 1 liter plastic cages containing a 15% sucrose solution in plastic cups with dental cotton wick protruding from their lids. The flies were maintained

Table 1. Captures of live blueberry maggot flies in funnel-traps baited with various formulations of ammonium acetate at the abandoned plantation, Fennville, Michigan, 7/16–8/12, 2000.

Bait formulation used with funnel-trap	Mean $\pm$ SEM no. flies captured per trap per week
Ammonium acetate (solid, 2.0 g)	34.4 $\pm$ 9.42 a
Ammonium acetate (liquid, 2.0 g)	26.1 $\pm$ 0.33 a
Unbaited	0.2 $\pm$ 0.1 b

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

at ca. 24°C under a 16:8 (L:D) photoperiod. Flies were checked daily to determine how long these wild-captured adults would remain alive under laboratory conditions.

#### Statistical Analysis

All field data were square-root transformed ( $x + 0.5$ ) and then subjected to a repeated measure ANOVA and means were separated by least significant difference (LSD) ( $P = 0.05$ ) (SAS Institute, 1989). The untransformed means and standard errors are presented in the tables.

#### Results

##### 2000

Funnel-traps baited with either solid or aqueous ammonium acetate captured significantly more blueberry maggot flies compared to unbaited funnel-traps (Table 1). There was no significant difference between the numbers of flies captured in funnel-traps baited with solid versus aqueous ammonium acetate (Table 1). Approximately 72% of the flies captured were females based on field determinations of sex.

##### 2001

In both the abandoned site and the commercial site, significantly more flies were captured on both ammonium-baited and unbaited green spheres than were caught in ammonium-baited funnel-traps (Tables 2 and 3). Captures of blueberry maggot flies in the funnel-traps were much higher in the highly infested abandoned block compared to the commercial site, in which very few blueberry maggot flies were captured in the funnel-traps. Funnel-traps captured ca. 6% of the mean number of flies captured by ammonium-baited sphere traps in the infested site and only ca. 0.04% of the mean number of flies captured by ammonium-baited spheres in the commercial site. Approximately 65.5% of the flies captured were females based on field determinations of sex.

Of the 40 wild-caught blueberry maggot females that were dissected over the course of the season, 38% were found to have sexually mature ovaries; the remaining flies were identified as sexually immature.

Wild-caught female blueberry maggot flies lived in plastic cups with sugar water for  $5.8 \pm 1.6$  (mean  $\pm$  SD) days while males flies survived for  $6.7 \pm 2.4$  days.

#### Discussion

We describe here a simple and effective method for capturing viable adult blueberry maggot flies in the field. This sampling technique is not intended to be an improvement upon current tools for monitoring blueberry maggot fly presence under integrated pest management regimes. In fact, this study proved that the green sticky

Table 2. Captures of live blueberry maggot flies in ammonium-baited funnel-traps relative to sticky sphere traps at the abandoned plantation, Fennville, Michigan, 7/25–8/20, 2001.

Trap-type	Mean $\pm$ SEM no. flies captured per trap per week
Ammonium-baited sphere	240.8 $\pm$ 40.6 a
Ammonium-baited funnel-trap	42.1 $\pm$ 9.42 c
Unbaited sphere	98.5 $\pm$ 31.2 b

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

Table 3. Captures of live blueberry maggot flies in ammonium-baited funnel-traps relative to sticky sphere traps at the commercial plantation, Fennville, Michigan, 7/25–8/20, 2001.

Trap-type	Mean $\pm$ SEM no. flies captured per trap per week
Ammonium-baited sphere	32.6 $\pm$ 7.6 a
Ammonium-baited funnel-trap	1.2 $\pm$ 1.0 c
Unbaited sphere	12.9 $\pm$ 4.2 b

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

sphere traps currently used for monitoring blueberry maggot (Liburd *et al.*, 2000) capture far more flies compared to the funnel-traps. Rather, this trap type is meant to serve the special function of capturing viable, live adults for further analyses and studies under controlled laboratory conditions. Using cone-type traps for capturing insects has been previously tested (Eckenrode and Chapman, 1971). Predominantly, cone or funnel-style traps are deployed in an inverted vertical position such that insects exhibiting negative geotaxis or attraction to light walk upwards into the funnel and are captured in a collection vial at the summit of the funnel. Eckenrode and Chapman (1971) deployed large funnel-traps (0.9 m diameter at base and 1.3 m high) approximately 8.0 cm above cabbage plants and effectively captured cabbage maggot, *Delia radicum*. It may be possible to deploy such large funnel-traps above lowbush blueberry plants to capture blueberry maggot flies. However, it would be much more difficult or impossible to use such large, inverted cones for capturing blueberry maggot in highbush blueberries or other *Rhagoletis* species that use fruit trees as host plants. In such cases, using a smaller funnel-trap that combines a powerfully attractive visual cue (yellow color) with a powerful chemical attractant (ammonia), such as described here, may prove more effective.

The results of this study indicate that it is possible to capture substantial numbers of blueberry maggot flies using the funnel-trap design described herein. However, the current funnel-trap prototype was less effective in an intensely-managed commercial blueberry plantation compared with an abandoned plantation, where the population density of blueberry maggot was substantially higher. Therefore, the use this trap design to capture live blueberry maggot flies may be more successful under heavier fly infestations.

The majority (65–72 %) of the flies collected were female. This finding is in line with multiple previous studies that have found blueberry maggot females (and other tephritid species) to exhibit greater attraction to ammonia than males (Liburd *et al.*, 1998a and citations within). Both sexes of flies survived under laboratory conditions for ca. one week. This duration should be ample time to carry out many experimental procedures with these wild-caught flies, such as toxicological studies, electrophysiological investigations, behavioral assays, or genetic analyses.

This technique may be useful for other tephritid species that exhibit strong attraction to ammonia-baits and yellow color (Rull and Prokopy, 2000; Liburd *et al.*, 2001). In addition, the use of attractive synthetic fruit baits instead of ammonium acetate lures may prove much more effective for other species such as the apple maggot fly, *R. pomonella* (Zhang *et al.*, 1999; Stelinski and Liburd, 2002). The funnel-trap prototype described herein is not meant to be the end-all model of this idea. We hope that inventive and interested fruit entomologists may improve-upon or modify this design to be effective in capturing live samples of the tephritid flies of their interest.

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