MARKING BLUEBERRY MAGGOT FLIES (DIPTERA: TEPHRITIDAE) WITH FLUORESCENT DIET FOR RECAPTURE STUDIES

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Mark, release, and recapture techniques have been used since the late 1890s when C. G. J. Petersen used them to study growth and migration of flatfish populations (Bailey 1952). Lincoln (1930) formalized mark, release, and recapture techniques for population size estimation with the development of the "Lincoln index", which estimates the size of a population based on the relationship between marked, released, and recaptured individuals. Subsequently, the mark, release, and recapture technique became an important ecological tool to quantify population size and dispersal behavior (Turchin 1998). The techniques employed to mark insects have included the use of tags, paints or dyes, mutilation, and more recently genetic markers (Hagler & Jackson 2001). Some of these techniques can have a negative effect on the survival of marked organisms, or cause rejection of marked individuals by the colony in the case of social insects (Porter & Jorgensen 1980). Other techniques such as protein and genetic markers are expensive and time consuming (Hagler & Jackson 2001). However, new techniques using enzyme-linked immunosorbent assay (ELISA) protocols also are being used to mark populations located in a field. This technique is an inexpensive way to monitor insect movement from fields that have been sprayed with a know protein. (Jones et al. 2006). We assessed a novel technique using fluorescent dye added to diet in the laboratory to mark blueberry maggot flies (BMF), *Rhagoletis mendax* Curan. This technique was initially developed and tested with the European red ant, *Myrmica rubra* L. (Arévalo & Groden, unpublished data).

Pupae of BMF were collected from infested blueberry fields in Jonesboro, ME during 2006. Emerging adults were marked by being fed a 1-mMol solution mixture of Fluorescent Brightener 28 (Sigma Aldrich, St. Louis, MO) in honey. Honey was heated slightly to thin and facilitate mixing with the brightener. The mixture was presented to flies in soaked cotton balls to prevent drowning.

Initial laboratory experiments compared survival of treated and untreated flies and fluorescence units between marked and unmarked flies. Individuals were kept in the laboratory in separate plastic cages with and without the florescence diet for 5 d. Marked flies were fed Fluorescent Brightener 28: honey mixture, while control (unmarked) flies where fed honey. At the end of 5 d, live and dead flies were tallied in both treatments. All individuals were rinsed with distilled water and 70% ethanol to reduce external contaminants and then were ground with pestle pellets in a 1500-µL micro-tubes (Fisher Scientific, Pittsburgh, PA) with 60 µL of deionized water. The micro-tubes were vortex-mixed at low speed for 2 min. Solutions (50 µL) from each fly (microtube) were pipetted into a labeled well within a black 96-well plate (Nunc™ Rochester, NY). The plates were scanned in a microplate fluorometer (Fluoroskan Ascent SL,Thermo Electron Co., Waltham, MA), with a 355-nm excitation filter, and a 460-nm emission filter. The result was measured by the flourometer in fluorescent units, a function of the amount of fluorescent brightener found in the sample.

The number of fluorescent units per individual fly were analyzed by ANOVA with a completely randomized 2×2, unbalanced factorial design in which the 2 main factors were (1) treated—untreated and (2) live—dead. There was no differences in fluorescence between dead (52.75 ± 9.63) and live flies (49.67 ± 4.55) ($F = 0.28$; $df = 1, 9$; $P = 0.62$), but fluorescence was significantly higher in treated (60.49 ± 5.37) compared with untreated flies (41.31 ± 3.62) ($F = 11.19$; $df = 1, 9$; $P = 0.015$). An analysis of the dye treatment x survival interaction was not significant ($F = 3.93$; $df = 1, 9$; $P = 0.095$); least square means (LSM) comparison analysis, $\alpha = 0.05$) (SAS Institute Inc. 2002).

In field experiments, adults of *R. mendax* were allowed to feed on the honey/brightener solution for 1 week in the laboratory before release. One hundred marked flies were placed in an open cage that placed 1.5 m from the ground on a tree located 3.0 m from the edge of a fruit-bearing blueberry field (Jonesboro, ME). Additional flies were fed honey-only diet for 1week to be used as untreated controls (scutum marked with Testor® paint) and released in the field with the dye-fed flies.

To recapture the marked BMF, 3 sets of 7 yellow, Pherocon® AM traps (Great Lakes IPM, Vestaburg, MI) were placed in 3 transects running into the blueberry field from the release site. For each transect, 1 trap was placed at the field edge, 0 m, and additional traps were placed at 3.0, 7.6, 15.2, 30.5, 61.0, and 91.4 m from the release point. Traps were serviced at 2, 4, and 7 d after release. Blueberry maggot flies captured were re-

corded, removed from the trap, and placed in kerosene for 24 h to remove sticky residue. Captured flies were rinsed 3 times, alternating deionized water and 70% ethanol to reduce external contaminants (trap glue and kerosene used as glue solvent) that could bias the measurements obtained from the fluorometer. Individuals were air-dried on filter paper at room temperature and frozen until processed. Flies were considered positive (marked) when the number of fluorescent units observed was higher than the upper 99% confidence interval limit of the number of fluorescent units observed for control flies (>61 fluorescent units). Marked individuals represented an unusually high percentage of the recaptured flies, indicating a low natural population of BMF in the field at the time of the recapture (Table 1). The low natural population may have been due to the timing of the experiments, which was late in the season when the natural population was in decline, after the period of the fly's maximum occurrence.

To determine the persistence of the mark, and whether the dye is a reliable marker we calculated the ratio of negative to positive flies over time. We used 95% confidence intervals of each of the days when samples were taken. These intervals were estimated by bootstrapping (n = 5000 repetitions) and winsorize trimming (Huber 2002) on the data for the first day due to a very large variation from the mean. There was no significant variation among days, which suggests that the brightener persisted for at least the first 7 d (Fig. 1).

This mark, release, and recapture method is inexpensive, accurate and does not require extensive labor. The use of this technique might open several possibilities for the study of population dynamics, dispersion, and management alternatives for the blueberry maggot fly, which is considered to be a major pest for blueberries throughout eastern North America. The success of this technique in other applications will depend on the sensitivity of the test-insects to the brightener used and the ability to mix the diet and the brightener in a homogenous manner. However, a significant factor that affects the success of this technique, and which can be difficult to control, is quantity of diet consumed. Enough treated food needs to be consumed to increase the concentration of brightener to a level above natural fluorescence so it can be detected by the fluorometer, and differentiated from control individuals. Unpublished observations by the authors in other taxa such as ants, suggest the possibility of using this technique on a diversity of other insects.

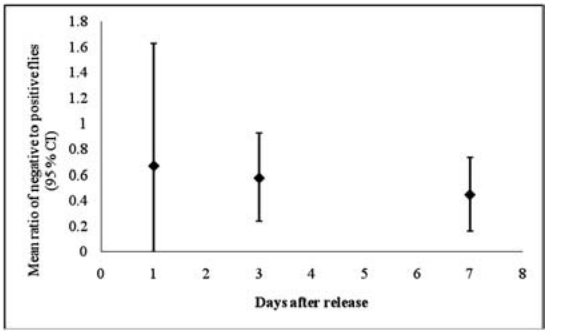

Fig. 1. 95% CI of the mean ratio between flies testing negative to the brightner relative to the number of flies testing positive.

TABLE 1. PERCENTAGE RECAPTURED FLIES CONSIDERED TO BE POSITIVE FOR THE PRESENCE OF FLUORESCENT BRIGHTENER 28 (MARKED) AT VARIOUS DISTANCES FROM THE RELEASE POINT FOR SEVERAL DAYS AFTER RELEASE.

	Days after release		
Distance (m)	2	4	7
0	89% (9)[1]	80% (5)	100% (1)
3	0% (1)	40% (5)	100% (1)
7.6	100% (3)	50% (6)	50% (4)
15.2	100% (10)	90% (10)	100% (9)
30	20% (2)	89% (9)	50% (2)
61	100% (1)	—	100% (5)
91.4	100% (2)	100% (1)	50% (2)
control	0% (6)		

[1]The number in parenthesis represents the total number, n of flies captured for that specific sample.

SUMMARY

We present a novel technique to mark blueberry maggot flies, *Rhagoletis mendax* Curan, using fluorescent markers incorporated into the diet of the flies previous to the release. Our results indicate that the marking is reliable for at least 7 d and can be detected equally in live or dead flies.

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