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Capture of melon flies, *Zeugodacus cucurbitae* (Diptera: Tephritidae), in a food-baited Multilure trap: Influence of distance, diet, and sex

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ABSTRACT

Many countries operate trapping programs to detect invasions of pestiferous fruit fly species (Diptera: Tephritidae). Surveillance relies heavily on traps baited with male lures, which, while highly attractive, have limited effectiveness, because (i) they are sex-specific and (ii) males of some species do not respond to the lures currently in use. For these reasons, detection programs also include food-baited traps that are neither sex- nor species-specific. Compared to male lure-baited traps, however, few studies have measured the attractiveness of food-based traps. The present study describes a mark-release-recapture study conducted in a fruit orchard in Hawaii that measured the attractiveness of a liquid protein hydrolysate-based (torula yeast/borax slurry) trap to adults of the melon fly *Zeugodacus cucurbitae* (Coquillett). Multiple release points were used at varying distances from a single, central trap to generate estimates of distance-dependent capture probabilities. The potential influences of sex and pre-release diet on capture probability were also examined. Flies were released at 14 d of age and were maintained on one of four dietary regimes that offered a protein hydrolysate-rich diet for varying intervals (i.e., 0, 3, 7, or 14 d, respectively). Recapture rates were similar between the sexes and over both sexes and all diets averaged 3.6%, 3.2%, and 0.6% for release distances of 10, 25, and 50 m, respectively. Pre-release diet had a significant effect on recapture probability for releases at 10 and 25 m: flies fed sugar only or protein hydrolysate-rich diet for only 3 d were captured more frequently than flies that had longer access to yeast extract prior to release.

Introduction

A number of true fruit fly species (Diptera: Tephritidae) are important pests of fruits and vegetables and cause serious economic losses both domestically through direct damage and subsequent unmarketability of crops and internationally through trade restrictions on crops with perceived risks of infestation (White and Elson-Harris, 1992). As a result, many fruit fly-free countries operate continuous and large-scale trapping programs to detect and monitor incipient invasions (e.g., Gonzalez and Troncoso, 2007; Jessup et al., 2007). These programs rely heavily on so-called male lures, which are natural or synthetic compounds that are attractive to males of many important pest tephritids (Tan et al., 2014). While highly attractive, male lures have limited effectiveness, because they are sex-specific, and males of several important pest species do not respond to the lures currently in use (Drew and Hooper, 1981; Royer, 2015). For these reasons, detection programs also include food-baited traps that are neither sex- nor species-specific. Generally, such traps are baited with a liquid protein hydrolysate-based solution (e.g., torula yeast/borax slurry) or protein-based synthetic lures (e.g., ammonium acetate, putrescine and trimethylamine) (Epsky et al., 2014).

Despite the importance of trapping programs in the detection and control of pest tephritids, relatively few studies have measured the attractiveness or efficiency (i.e., distance-dependent capture probability) of traps baited with either male lures or protein-derived odors in the field. Moreover, most of these studies have focused on male lures (Cunningham and Couey, 1986; Lance and Gates, 1994; Shelly et al., 2010; Manoukis and Gayle, 2016). In contrast, although food-baited traps have been employed in studies of dispersal and longevity (e.g., Baker et al., 1986; Thomas and Loera-Gallardo, 1998; Kovaleski et al., 1999), little attention has been given to estimating their attractiveness. Two recent studies (Epsky et al., 2010; Kendra et al., 2010) included mark-recapture experiments within grids of food-based traps (baited with synthetic protein-based lures) to estimate their effective sampling range, which was defined as the area around a trap that accounted for 90% of the recaptures. Epsky et al. (2010) estimated the effective sampling range as 28 m for the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), in a mango (*Mangifera indica* L.) orchard in

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Honduras. Experimenting with feral *Anastrepha suspensa* (Loew), Kendra et al. (2010) reported a similar range of 30 m in a small grove of guava (*Psidium guajava* L.) trees in Florida. To our knowledge, these studies provide the first field estimates of the attractiveness of protein-based traps for any tephritid species.

The present study similarly describes a mark-release-recapture study conducted in Hawaii that measured the attractiveness of a liquid protein hydrolysate (torula yeast/borax slurry) trap to adults of the melon fly *Zeugodacus cucurbitae* (Coquillett) (formerly, in the genus *Bactrocera*; Virgilio et al., 2015). However, while the two aforementioned projects used a single release point embedded within a grid of traps, this study adopted the opposite approach, i.e., multiple release points were used at varying distances from a single, central trap to generate estimates of distance-dependent capture probabilities. In addition to considering distance, the present work examined the potential influences of sex and pre-release diet on the capture probabilities of melon flies. As described below, flies were provided protein hydrolysate-containing or sugar-only diets for varying intervals prior to their release. Based on previous laboratory (Miller et al., 2004) and field cage (Piñero et al., 2011) studies on *Z. cucurbitae*, protein-deprived flies, particularly females, were expected to respond more strongly to the food odor and thus show higher recapture rates.

Materials and methods

Insects

All flies used in this study were obtained from a laboratory colony started with 300 to 400 adults reared from zucchini (*Cucurbita pepo* L.) collected in commercial fields near Kapolei, Oahu, HI, U.S.A. The colony was maintained in a screen cage (60 × 40 × 30 cm) and provided a food mixture of sugar and yeast hydrolysate (a material with high amino acid content, Fanson and Taylor, 2012; 5:1 v:v; hereafter referred to as full diet) ad libitum and water. Zucchinis were supplied for oviposition, and the infested vegetables were placed in opaque, plastic boxes on wire-mesh screening over a layer of vermiculite for pupation. Pupae were sifted and placed in paper bags for emergence. To obtain flies for the experiments, adults were separated by sex using an aspirator within 2 d of emergence and placed in cubical screen cages (30 cm per side; ≈250 flies per cage) with food and water as described below. Flies were held at 23–27 °C, 50–80% relative humidity, and a natural photoperiod (≈12:12 L:D). When used in this study, flies were 11 to 13 generations removed from the wild.

To distinguish between different treatments released at the same time, flies were marked using fluorescent dye following procedures used in the Sterile Insect Technique (FAO/IAEA/USDA, 2014). Pupae were coated with fluorescent orange or green dye (blaze orange and signal green, respectively, DayGlo Corporation, Cleveland, OH). Upon adult emergence, dye was usually visible on the face and under the wings with a dissecting microscope under UV (black) light. However, where external dye was not conspicuous, the head was crushed with forceps to examine the collapsed ptilinum, which picks up dye particles upon emergence from the puparium.

Study site

Field work was conducted at the Urban Garden of the University of Hawaii at Manoa located in Pearl City, Oahu. The site is surrounded by residential and commercial development and hence isolated from agricultural fields. The site is just above sea level (5 m in elevation) and is generally dry and sunny. The study was conducted during December 2016–April 2017, and the average daily maximum and minimum temperatures during this period were 27.8 °C and 20.6 °C, respectively (www.wunderground.com). All releases were made in a mixed orchard, which contained common fig (*Ficus carica* L.), avocado (*Persea americana* Mill.), jack fruit (*Artocarpus heterophyllus* Lamk.), mango (*Mangifera indica* L.), and star fruit (*Averrhoa carambola* L.), with trees spaced 3 to 4 m apart within and between rows. As described below in more detail, releases were made from concentric circles around a single, central Multilure trap (Better World Manufacturing, Fresno, California) placed in the same fig tree over the course of the entire study.

Dietary treatments

Upon emergence, flies were subject to 4 different dietary regimes: full diet for days 1 to 3 and granular sugar for days 4 to 14, full diet for days 1 to 7 and then granular sugar for days 8 to 14, full diet for days 1 to 14, or granular sugar only for days 1 to 14. For shorthand, these different treatments are hereafter referred to as the F3, F7, F14, and S diets, respectively. Water was supplied to all treatments, and flies in all treatments were released when 14 d old (the approximate age of sexual maturation; Shelly, unpublished data).

Release-recapture protocol

Releases were made at 3 to 5 d intervals and involved flies from 1 to 2 diet treatments. Typically, flies from two diets, marked with different dye colors, were released per day at different distances from the trap, with the diet treatments and distances selected randomly. Preliminary trapping indicated that flies were unlikely to be captured 2–3 days after release, and consequently captured flies marked a particular color were assumed to have derived from the most recent release using that color. While this assumption may not always have been true, the possibility of "misidentifications" was considered negligible given the high dispersal ability of *Z. cucurbitae* (Nakamori and Soemori, 1981) and offset by the high release rate allowed, which enabled a relatively high rate of data collection for multiple dietary regimes and release distances.

On the day of release, groups of 50 flies were counted and transferred from the screen holding cages to clear plastic containers (1 L) with screen lids for transport to the study site. Fifty females and 50 males were released at the appropriate distance along each cardinal direction (i.e., north, south, east, and west) from the trap. Thus, a total of 200 flies were released per sex per replicate for a given diet x distance combination. Flies were released 10, 25, and 50 m from a central Multilure trap by opening the plastic containers at the base of trees and allowing the flies to exit freely. The same 12 release sites (=3 distances × 4 directions) were used over the entire study.

The central Multilure trap was suspended from a shaded branch of a fig tree about 2 m above ground and was baited with 300 mL of standard torula yeast/borax slurry (1 yeast/borax pellet [Scentry Biologicals Inc., Billings, Montana] per 100 mL of water) that had been aged 1–2 d prior to use. The Multilure trap has a McPhail design and is a 2-piece plastic cylinder, with a transparent top and an open, yellow invaginated base through which flies enter the trap (FAO/IAEA, 2013). The slurry served both as an attractant and a kill mechanism as attracted flies drown in the liquid. No toxicant or additional preservative was added to the slurry. Flies were collected 3 to 4 d after release by pouring the liquid through a sieve. Retained material was examined in the laboratory, and marked melon flies were scored by sex and dye color using a black light and a dissecting microscope. Used food slurry was discarded, and the trap was baited with a new batch of liquid (aged 1–2 d as described above) immediately prior to a release. Eight releases (replicates) of both males and females were performed for each diet x distance combination (i.e., 4 diets × 3 distances × 8 replicates = 96 total releases).

Data analysis

Catch data were not normally distributed, so a Generalized Linear Model (GLM) and non-parametric Wilcoxon rank sum test were used to test the effect of sex, diet, and distance on recapture. Statistical analysis was conducted using R version 3.3.2 (R Core Team, 2017) with **α** was