Special care must be taken during construction of this apparatus to make sure all parts fit together tightly and exactly, taking into account any small irregularities in the egg crate material. In an experiment involving four simultaneous sets of (initially) 200 flies, no flies ever escaped or moved accidentally between cells in over 60 consecutive daily transfers.

Fluorescein dye as a tagging agent for *Drosophila* dispersal studies.

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Dispersal, or the unidirectional movement of organisms in search of improved conditions, is a commonly investigated and ecologically important aspect of plants, invertebrates, and vertebrates (Howe and Smallwood, 1982; Johnson and Gaines, 1990; Levin, 2006). This action is particularly important for organisms that are born into habitats with high population densities such as *Drosophila*. For these animals, rotting vegetation provides a nutritional resource for adults and larvae, as well as a location to meet mates and lay eggs. And while these conditions may support large populations, *Drosophila* are known to disperse (see Dobzhansky, 1973; Johnston and Heed, 1975; Grossfield, 1978). Of the studies that have empirically investigated dispersal outside the laboratory, many have relied on dusting flies with fluorescent microparticles to delineate the released population from wild individuals (see Crumpacker, 1974). This dusting can be performed actively in a "shake and bake" methodology, or through passive contact as the larvae emerge from their pupal stage when their media is doped with the particles. While dusting is a useful technique for examining dispersal, its possible influence on such behavior has been questioned (Turelli *et al*., 1985).

As part of a larger project to determine the dispersal and feeding range of the invasive fruitivorous *D. suzukii*, we investigated an alternative to dusting, namely the use of the fluorescent marker fluorescein sodium (C_{20}-H_{10}-O_5-Na_2). This common, relatively non-toxic water-soluble laboratory chemical is used in a number of applications including ophthalmological examinations of the cornea and as a tracer in ecohydrodynamic experiments (Maurice, 1967; Zimmer-Faust *et al*., 1995; Pesticide Action Network). Its ability to fluoresce green under UV or blue light makes it easy to visualize, while its safety and inexpensive nature make it a useful product for *Drosophila* work.

Methods and Results

Fluorescent techniques

Four application techniques were tested: dusting adults with fluorescein powder, spraying adults with a concentrated fluorescein solution (30 g/l), feeding adults media containing concentrated fluorescein solution (8 ml solution/vial), and feeding larvae the same media containing concentrated fluorescein solution. For the first two treatments (dusting and spraying), juvenile (<24 hours post-eclosion) flies from a laboratory population (Canton-S) of *Drosophila melanogaster* were collected and treated under CO_2 anesthesia and then placed (n = 15) in 5 replicate vials. Flourescein-fed-adults were collected after spending either 1 or 7 days on doped media, and flies from the flourescein-fed larval treatment were collected as emerging adults after spending their larval period in doped media. Pre-trial results determined that propionic acid, sometimes used when making the medium (Formula 4-24 Instant Media – Carolina Biological) to combat fungal infections, interacted with the dye decreasing or eliminating its fluorescence. Therefore, no propionic acid was added to the rearing media.

Effectiveness of all treatments was determined through visual comparison between treated flies and untreated control flies raised on the same media. Flies were observed at 30× using a dissecting scope while illuminated with a blue LED flashlight. Flies were qualitatively scored for their relative ease of discrimination when compared to untreated members of the same stock population. After initial observations were completed, the treated flies were all placed into vials containing standard media to determine how well the tagging held up over time, with observations taken again at 24, 48 and 72 hours.

Of the four techniques the best results were found in the groups in which flies or larvae had been fed media containing the fluorescein, both in terms of initial tagging and longevity of the tag. Dusting with fluorescein powder provided a discernable signal, but variation between individual flies, with respect to the amount of powder, was considerable and either through shedding of the particles or cleaning by the flies themselves the tag did not last long. Spraying adults with fluorescein made them visible, but this was attributed more to the dye mixing with the food and being ingested, rather than beading and sticking to the flies themselves. No discernable difference was seen between larvae raised on doped media and adults fed doped media for 24 hours. Fed flies possessed brightly fluorescing intestines (and feces) most easily seen in the abdominal region. Adults fed on doped media for a week, however, showed a noticeably greater level of fluorescence than all other treatments, primarily via increased fluorescence of the thorax. Observing the flies over the 72 hours following their initial inspection indicated that sprayed and dusted flies lost what fluorescence they had within 24 hours. In contrast, the fluorescein-fed flies still fluoresced at 72 hours, though it was diminished and primarily limited to the thoracic region. Fluorescent feces were noted in the vials containing fed flies, indicating that the gut residence time of the fluorescent food may be a determining factor on labeling longevity.

Mark and recapture

As proof of concept, a limited set of mark and recapture studies were undertaken on the lawn next to the laboratory. Bottles were placed every 2 m on either side of the release point and left in place for 12 hours after the release of ~800-1000 flies fed doped media for a week. Initial trials found extremely low recapture rates (< 0.1%) using yeasted fly medium. Subsequent trials delivered markedly higher recapture rates (up to 30%) using crushed fruit (bananas, peaches, raspberries, blueberries). While we were able to discriminate between fluorescing and non-fluorescing (wild) flies, the level of fluorescence was much lower than similarly treated flies that had not been released. This was likely due to the flies eating the fruit and therefore rapidly pushing out the labeled gut contents. As an alternative bait, a second set of bottles was deployed containing ~1 cm of cider vinegar. These were arranged in a line parallel and 4 m away from the fruit traps. While recapture rates were lower (6%) the fluorescence levels were much higher, likely due to the death of the flies due to drowning before they could feed.

Conclusions

For short-term dispersal studies, raising flies on fluorescein-doped media appears to be a viable alternative to more invasive dusting techniques. While we did not study the effects of the dye on the behavior of the flies, fluorescein is considered to be non-toxic and considering its short (< 72 hrs) residence time in the gut is not expected to have any acute or chronic effects. That same short residence time is also its limiting factor in terms of its use as a label. However, for studies such as ours, which seek to determine dispersal rates and food preferences for flies at time periods up to 24

hours, its use provides a cheap, easy way to tag flies in order to study ecologically important aspects of their biology such as feeding choices and dispersal distances *in-situ*.

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Fresh yeast media for *Drosophila* egg-collecting.

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Fertility and fecundity studies in *Drosophila* have described several egg-collecting techniques with different media as cornmeal agar or banana agar added with animal charcoal for contrast, sprayed with a fine yeast suspension (Spencer, 1937), with colored Carpenter's medium and fresh or dead yeast (Gupta, 1980) or yeast-agar with grape juice (Nichols and Pak, 1985). Egg-collecting media has been added to watch glasses (Delcour, 1969), to spoons (Gupta, 1980), or to Petri dishes with small drops of fresh yeast (Nüsslein-Volhard, 1977; Sabio *et al.*, 2010). For more contrasting of eggs some techniques include small drops of food coloring (Gupta, 1980) or vital stain (Acosta *et al*., 2000). Any kind of egg-collecting media and vials must have these requests: i) to allow collection of large quantities of eggs; ii) to make accessible microscopic observation and incubation; iii) to assure enough amount of food for parents and larvae (Acosta *et al*., 2000). In order to describe a method that combines the features described above, we present a simple method that allows a short preparation time, low cost, besides eggs-collecting, observation facilities and incubation efficiency. Centrifuge plastic tubes of 90 × 30 mm with removable plastic cap, and without thread, to which the conical base was cut (Acosta *et al*., 2000) to replace with a foam plug (Figure 1) were used for egg collecting. The removable cap of the tube is completely filled with fresh yeast, previously sucrose activated and incubated in a water bath at 39°C for 5 min in three successive periods. After placing the fresh yeast in the cap with a sharp object slits are made on the surface (Figure 2). It takes 400 g of fresh yeast to prepare 80 caps with this media. Couples of males and female virgins are placed in vials for 5-6 h without food to prevent egg-laying (Delcour, 1969). About 2 h after placing the media in the removable caps, couples are placed in the vials for egg-collecting, and the tubes are closed with the foam caps. Then the tubes are placed in an incubator at 25°C and 60% relative humidity for 24 h. This method has the following advantages: i) fresh yeast promotes egg-laying and gives parents and larvae enough food; ii) yeast color contrasts with the color of the eggs, preventing exposure to dyes (Figure 3); iii) the media remains with the characteristics required for 72 h; iv) it is easy to remove the cap for complete stereo microscope observation of the media´s surface; iv) caps can be replaced for continuous egg-collecting every 24 h, and caps with eggs can be incubated in new tubes.