

Caribbean Fruit Fly, *Anastrepha suspensa* (Diptera: Tephritidae): Life History and Laboratory Rearing Methods

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Introduction

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), poses an economic threat to citrus, guava, and other subtropical fruits in south Florida. Adult females have well-developed ovipositors, inserting their eggs beneath the skin of host fruits. Crop damage results from larval feeding and development within the pulp (Fig. 1). Consequently, larval infestation is difficult to detect.

A. suspensa has been the subject of much research aimed at lessening its impact on Florida agriculture, and at preventing introduction of other *Anastrepha* species which currently threaten the state. Research at the USDA-ARS-SHRS is facilitated by mass rearing of *A. suspensa* on an artificial diet. This poster outlines those rearing procedures and illustrates the developmental stages of this pest.



Infested guava (*Psidium guajava*)

Laboratory Colony

The Caribbean fruit fly colony at the USDA-ARS-SHRS in Miami, FL was established in 1968. Larvae are reared on a nutrient-rich, semi-solid agar medium; adult flies are given water (liberated from agar blocks) and fed a 4:1 mixture of refined cane sugar and protein hydrolysate. The colony is maintained at $25 \pm 1^\circ\text{C}$, 80% RH, and a photoperiod of 12:12 hr (L:D). Under these conditions, it takes 37 days to cycle from initial egg collection to the first harvest of eggs from the next generation (Table 1). Currently, the colony produces approximately 120,000 flies per week.

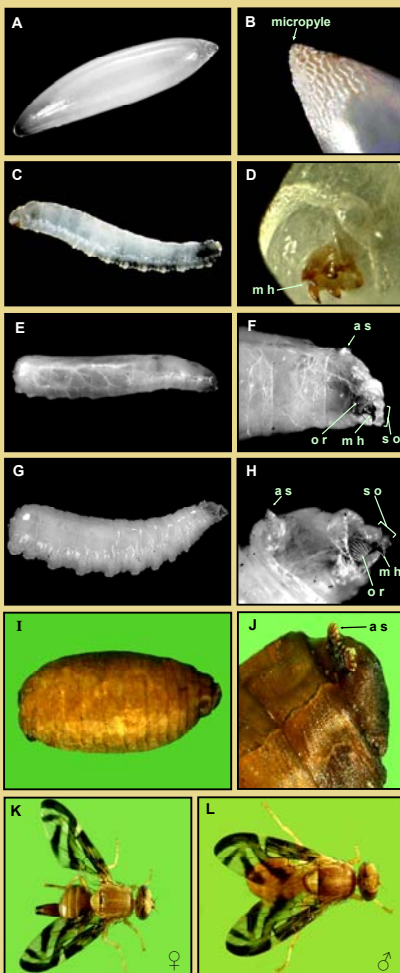
Table 1

1d	2d	4d	12d	12-13d	24d	27-29d	34-36d
Discard old eggs	Collect new eggs (<24hr)	Set eggs on diet	Wash larvae (3 rd instars)	Larvae pupate	Puparia sifted	Adult flies eclose	Adults lay 1 st eggs

Life History

Development in *A. suspensa* is holometabolous, consisting of four distinct stages (Fig. 2): egg, larva, puparium, and adult fly. The larval stage is comprised of three instars, which can be distinguished by size and examination of morphological characters on the head and prothoracic segment, including the mouth hooks (mh), anterior spiracles (as), oral ridges (or) and several types of sensory organs (so).

Fig. 2



Photographs taken with a Leica MZ16 Microscope and Image-Pro MC 5.1 software. Focus stacking, a digital imaging technique, was used to achieve enhanced depth of field.

EGG. Eggs (2A) are elliptical, pale to glistening white. The anterior end (2B) bears the micropyle and a characteristic polygonal pattern in surface architecture of the chorion.

LARVA. Larvae are vermiform maggots, wedge-shaped and tapering toward the anterior end.

1st instar larvae (2C) are clear to pale white. Mouth hooks (2D) are the dominant feature, consisting of two sets of light brown, weakly-sclerotized hooks on the ventral surface. The upper set of hooks will continue to develop through the later instars.

2nd instar larvae (2E) are white to pale cream, with better developed anterior structures (2F), including the anterior spiracles, small oral ridges, and black mouth hooks.

3rd instar larvae (2G) are cream to pale yellow with well-defined anterior morphology (2H). Mouth hooks consist of a single pair of sharply pointed teeth, black and heavily sclerotized, and oral ridges have strong serrations. Overall body surface is roughly textured, with better developed creeping welts than in 2nd instar larvae.

PUPARIUM. Pupae are exarate and encapsulated in a puparium (2I), the hardened skin of the 3rd larval instar. Puparia are ovoid, golden to reddish brown with visible anterior spiracles (2J).

ADULT. Adult females (2K) can be distinguished from males (2L) by the prominent ovipositor. A mature female can lay ~200 eggs over a two month lifespan.

Fig. 3



REARING PROCEDURES

Egg Collection. On Day 1, twenty-four hours prior to harvesting viable eggs, old eggs are brushed off the cage's waxed-cheesecloth panel and discarded. On Day 2, newly laid eggs (<24 hr old) are washed from the panel with water (Fig. 3A), sifted, and treated with 5% bleach solution (3B). Eggs are then suspended in cell-u-wet solution (1:9 ml), pipetted onto Masslin strips (3C), and incubated for 48 hours (3D).

Diet and Larval Development. Diet ingredients (3E) consisting mainly of wheat germ, sugar, and torula yeast are mixed with a hot agar solution to make larval diet-medium (20 L) (3F). Two liters of diet-medium is poured into shallow plastic trays (3G) and allowed to solidify. Once the medium has set, egg strips are placed face-down on the surface (Day 4) (3H). Trays are stacked and left undisturbed to permit larval development up through the late 3rd instar. On Day 12, larvae are separated from the medium by rinsing them with water and collecting them in a sieve. (3I). The washed larvae are mixed with vermiculite (1kg:4L) (3J) and placed in trays for pupation (3K), which occurs between Day 12-13.

Puparia and Adult Flies. Puparia are sifted from the vermiculite on Day 24 and placed inside screen cages (2 L per cage) (3L). Adult flies, which eclose from Day 27-29, are provisioned with water and food *ad libitum*. By Day 34-36, females begin ovipositing on the waxed-panel of the cage (3M).

The procedure supra is repeated on a continuous schedule.