

## The Artificial Culture of Fruit Flies and their Parasites<sup>1</sup>

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In the laboratory study of any insect it is necessary to maintain an adequate supply of the desired stages for experimental use. This is particularly true of the oriental fruit fly (*Dacus dorsalis* Hendel), which is difficult to obtain in quantity from natural infestations during the winter months, when host fruits are scarce and larval and adult populations of the fruit fly are at a low ebb. It has also become increasingly difficult to obtain for experimental purposes oriental fruit fly larvae reasonably free from natural parasitization by *Opius longicaudatus* (Ashmead) and *O. persulcatus* (Silvestri).

Studies were therefore undertaken to determine the practicability of culturing fruit flies (Tephritidae) and their parasites on agar-base media, as suggested by Mainland.<sup>2</sup>

**FRUIT FLY CULTURE**—Initial attempts to induce oviposition by *Dacus dorsalis* directly on agar media were largely unsuccessful, and larval development was found to be inhibited by undesirable molds introduced with the adult flies. Subsequent studies were conducted with known numbers of eggs or newly hatched larvae transferred to the media with a camel's-hair brush. Eggs were obtained from sections of orange peel fastened to glass plates with paraffin and placed in cages with gravid flies according to the techniques used in earlier studies on the Mediterranean fruit fly. When larvae were desired, the eggs were held on moist blotting paper until they hatched. The larvae were then reared in petri dishes containing about 1/2 inch of an agar medium. When the larvae were ready to pupate, the dishes were opened and placed at an angle over moist sand in battery jars. The puparia formed by the larvae entering the moist sand were screened out at intervals and held for emergence. It was also found that the agar media could be poured on sections of 1/4-inch-mesh hardware cloth, which allowed the mature larvae to fall directly on the sand.

Fifteen agar formulas were tried, all based on the standard Texas *Drosophila* formula.<sup>3</sup> Variations included the use of apple sauce, guava juice, and Spam in place of banana, differences in the amount of agar, some media without yeast and/or sucrose, some with double the amount of yeast, and others with only one of the mold inhibitors or with none at all.

<sup>1</sup> Approved by Avery S. Hoyt, Associate Chief, Bureau of Entomology and Plant Quarantine, for publication as Bureau manuscript No. 9673.

<sup>2</sup> Hawaii Agr. Expt. Sta. Rpt. 1946-48, p. 66.

<sup>3</sup> To make 1 liter use 760 cc. of water, 20 grams of bacto-agar, 220 cc. of banana pulp, 30 grams of dried yeast, 20 grams of sucrose with 3 cc. of propionic acid and 2.4 cc. (0.18%) of Moldex as mold inhibitors.

Although most of these tests were limited in scope, they clearly indicate the practicability of culturing *Dacus dorsalis* on agar-base media. On the complete Texas formula the larvae developed and pupated normally, producing larger than average puparia and adult flies normal in every respect. Survival was also high in the best series, as many as 80 to 100 per cent pupating and about 60 to 70 per cent of the introduced larvae reaching the adult stage.

It was found that yeast is essential for larval development. In fact, agar-media with yeast alone gave nearly as good results as the fruit-yeast mixtures, whereas very little development occurred on banana-agar without yeast, as shown in Table 1. In these tests the yeast and all other ingredients were boiled for 3 minutes. The results indicate that the protein furnished by dead yeasts is entirely adequate for larval nutrition. It appears likely that in nature fruits are merely the substrata for yeasts and other microorganisms from which the larvae derive most of their nourishment.

Table 1.—Development of *Dacus dorsalis* on Three Agar-Base Media<sup>4</sup>

| Medium                 | Per cent Pupating | Average Weight of Puparia (mg.) | Remarks   |
|------------------------|-------------------|---------------------------------|---|
| Complete Texas .....   | 91                | 15.8                            | Mostly subsurface feeding; development rapid.               |
| Yeast-agar-water ..... | 80                | 13.8                            | Mostly subsurface feeding; development rapid.               |
| Banana-agar-water .... | 28                | 6.0                             | Fed mostly on surface scum; development slow; puparia pale. |

<sup>4</sup> 200 newly hatched larvae (4 replicates containing 50 larvae) in each series.

A surface scum of unknown origin sometimes appeared on the cultures, occasionally causing high mortality to small first- and second-instar larvae but rarely to those of larger size. If the scum did not develop when the larvae were very small, it rarely became established, and it seemed to appear first on portions of the media on which they had not fed. It was most prevalent when larvae hatching from eggs taken from field-collected fruit were cultured, but it also occurred in laboratory cultures. With high larval populations (100 or more per dish) the surface scum caused little or no mortality. The crowded larvae may have been able to ingest the scum more rapidly than it could develop, or they may have secreted some antibiotic agent which checked its growth.

Quantitative studies have indicated that when more than 100 newly hatched larvae are placed in a 3½-inch petri dish containing about 30 grams of medium, the larval period is prolonged and the puparia are undersized. The optimum concentration is about 50 to 100 larvae per dish, or 2 to 4 larvae per gram of medium.

The larvae of *Dacus cucurbitae* Coquillett and *Ceratitis capitata* (Wiedemann) were also reared with equal facility on the same agar-base media, including the yeast-agar-water formula. In fact, they appeared to develop

more rapidly therein and to produce larger flies than in most field-collected fruits. This method therefore shows great promise for rearing all three species of fruit flies occurring in Hawaii. The present need, however, is for a mass-inoculation technique that will eliminate the individual transfer of eggs or larvae to the cultures. With this improvement quantity production in large-scale units should be entirely feasible.

**PROPAGATION OF PARASITES**—Numerous experiments were conducted to determine whether the parasite *Opius longicaudatus* can be propagated on *Dacus dorsalis* in agar-base media. Adult parasites of different ages and numbers were confined for varying periods in cylindrical glass cages over the culture dishes. At first the parasites became entangled in the wet media, but this difficulty was overcome by using a firmer agar (3 per cent) and a cheesecloth covering over the surface which enabled the parasites to oviposit through the meshes.

The oriental fruit fly cultures in agar-media are apparently unattractive to the parasite until the adults are at least 6 days old. After this time, however, the females oviposited very readily and produced a generally high degree of parasitization (Table 2), although the progeny were predominantly males. No female progeny were obtained from parasites more than 10 days of age. In these experiments three replicates of 10 newly hatched male and female *Opius longicaudatus* were caged over agar cultures containing 50 half-grown larvae, the same parasites being transferred to new cultures at 2-day intervals. At the end of 12 days the remaining 9 female and 7 male parasites were placed in a single cage, and they were transferred to new cultures at 2-day intervals until the 18th day, when only 2 of the original 30 females were still alive.

The addition of fruit juices and extracts and 0.5 per cent of pyridine to the cheesecloth patches over the agar did not increase the attractiveness of the cultures or the degree of parasitization.

Table 2.—Propagation of *Opius longicaudatus* on *Dacus dorsalis* in Agar-Base Media

| Age of Parasites,<br>Days | Per cent<br>Parasitized | Per cent of<br>Females Reared |
|---------------------------|-------------------------|-------------------------------|
| 0- 2                      | 0                       | .....                         |
| 3- 4                      | 0                       | .....                         |
| 5- 6                      | 0                       | .....                         |
| 7- 8                      | 51.3                    | 10.3                          |
| 9-10                      | 82.2                    | 36.8                          |
| 11-12                     | 76.3                    | 0                             |
| 13-14                     | 94.7                    | 0                             |
| 15-16                     | 54.5                    | 0                             |
| 17-18                     | 40.0                    | 0                             |

When cultures containing newly hatched first-instar oriental fruit fly larvae were exposed to *Opius longicaudatus*, parasitization was at least equal to that obtained from exposures of second and third instars. The smaller host larvae also produced a higher ratio of female parasites. In

other tests *O. longicaudatus* was found capable of parasitizing *Ceratitis capitata* in agar media.

The cynipid parasite *Trybliographa* sp. from Malaya and *Tetrastichus dacicida* Silvestri from Africa were also readily propagated on both *Dacus dorsalis* and *Ceratitis capitata* larvae developing in agar-base media. No progeny of these parasites were obtained from *D. cucurbitae* larvae, although they were attacked with equal readiness. These parasites are attracted to the media without delay and are able to work in and upon it without difficulty.