Félix, R. National Institute of Nuclear Energy, Mexico City, Mexico. Multi-purpose medium for Drosophila cultures used in teaching or research.

The following procedure for Drosophila cultures has been employed with good results in our research laboratory. Its use is also recommended for small laboratories in schools and universities which use the fruit fly to compliment the theory in genetic and radiobiology courses.

1. Sterilize the polyurethane stoppers of the half-pint milk bottles, used for mass cultures, or those for the vials (see DIS 45:180, 1970) in an autoclave at 120°C and 20 lbs. pressure for 30 minutes.
2. Sterilize the flasks and vials in an oven at 70-80°C for 3 hours.
3. Place the following mixture on a gas stove boiling it for 20 minutes:

Distilled water	4,000 ml
Agar	40 gr
Cornmeal	250 gr
Sucrose	140 gr
Dextrose	100 gr

4. Remove the mixture from the heat and immediately add the mixture of brewer's yeast.
5. Prepare in advance the following mixture of yeast and water, stirring it for 2 minutes with an electric blender or a mechanical agitator (see DIS 45:178, 1970).

Brewer's yeast	120 gr
Distilled water	800 ml

6. After the above mixture is added to the medium, mix it for 3 minutes with the mechanical agitator.
7. Heat it once again; letting it boil for 20 minutes.
8. Remove it from the heat and cool, or chill it in a waterbath placed in the sink, stirring the prepared medium with a large spoon.
9. After the mixture has chilled in the waterbath or by exposure to room temperature, stir it again with the mechanical agitator. When the temperature drops below 60°C, add the two following compounds:

Propionic acid	20 ml
"Tegosept M"	20 ml

The two substances should be added in separate containers. The solution of "Tegosept M" (a trade name for methyl-p-hydroxybenzoate) is prepared by dissolving 12.5 gr in ethanol, equalling 100 ml with 96° ethanol. The propionic acid is added without dilution.

10. Upon reaching the above mentioned step, the milk bottles and vials are removed from the stove, so that they will be hot, at the appropriate temperature for emptying the medium.

This all-purpose food formula is recommended for obtaining a medium that neither will shake out, nor liquify in old overcrowded bottles and vial cultures.

Sometimes the stock cultures in our laboratory become contaminated with a reddish-brown bacterial growth that restrains larval development and kills the adults. Pembritin (ampicillin, Beecham) in a concentration of 0.065 mg/ml of food, effectively controls such contamination (see DIS 44:131, 1969).

However predatory mite infestations have been more difficult to control than the already well-known contaminations of molds or bacteria. The mites are introduced into the laboratory by the collected wild flies, or when Drosophila cultures received from other laboratories are not examined carefully before initiating new cultures. This pest is controlled by a treatment of benzly-benzoate (20%) in ethanol (see DIS 47:127, 1971).

Histiostoma sp. has a hypopus stage which attaches itself to Drosophila, as well as to other insects. The absence of long hairs distinguishes it from other less dangerous mites. In addition the predatory mite is characterized by a squatty body build, in contrast to the longer, thinner bodies of non-parasitic mites. Due to the fact that the female adults produce both male and female progeny, the existence of a single mite in the hypopus stage could infect the entire laboratory.

The small newly eclosed nymphs thrive on the culture medium, and in approximately a week metamorphosize into the migratory (hypopus) stage. These extremely active hypopi develop in large numbers in old, infested cultures. Their ability to penetrate tiny crevices facilitates the escape from the culture medium, thereby endangering other cultures, unless tightly stoppered. The migratory nymphs attach themselves to whatever insect they come in contact with, injecting the mouth parts into the insect. After 10 days they leave the host, growing to the adult stage on the surfact of the medium, where they reproduce.

A heavy infestation extending to the stock cultures occurred in our laboratory in 1970.

The mites entered among samples of wild flies collected at several trappings in Mexico City. In order to eliminate the mites, the infested cultures were treated in the following manner: all contaminated bottles and instruments were heated in a furnace before washing. The instruments used to manipulate flies, as well as the microscope surface, the exterior of bottles, and the table surfaces were repeatedly washed with a solution of benzyl-benzoate (20%) in 90° ethanol. Before starting new cultures all flies were examined under the microscope, and apparently mite-free adults selected for later use. As it is difficult to avoid contaminating the new medium, the adults were allowed to lay eggs on it only during a 24 hour period. When several small hypopus nymphs either from contaminated flies or from other cultures, were found in the new medium, its surface had to be covered with a solution of benzyl-benzoate (20%) in 96° ethanol. This treatment kills the nymphs without any apparent toxic effect on the Drosophila larvae, which then develop into the adult stage unimpeded by predatory mites. The newly emerged flies were transferred to new cultures every day in order to avoid the attachment of any mites which survived treatment.

In the heavily infested cultures all of the flies died; and a crowding of mite nymphs was found among the Drosophila larvae. In this case the application of a second treatment was necessary, thoroughly washing the larvae by immersion in a solution of benzyl-benzoate (20%) in ethanol. Following the 2-4 minute immersion in the benzyl-benzoate solution, the larvae were washed with Ringers solution and then transferred to fresh vials. The pest was controlled effectively after three weeks, adhering to the above steps.

Frankham, R. Macquarie University, Sydney, Australia. Instant mashed potato as a fly food.

The instant Drosophila media available commercially are expensive and must be imported if one lives in the antipodes. They are also unlikely to be available locally to people on field-collecting trips. Instant mashed potato added to water (in 1:2 proportions by volume) with a pinch of granulated live yeast on top provides a satisfactory instant media for culturing Drosophila. It is improved if powdered agar (10g to 160g instant mashed potato) and Nipagin (1.27g to the above) are added. Killed yeast may also be added to this powder. We have found differences among brands of instant mashed potato in their suitability for culturing Drosophila. This media has been used successfully by external students carrying out Drosophila experiments at home.

Merriam, J.R. and B. Howard. University of California, Los Angeles. Mite control with caffeine in Drosophila food.

During a recent set of experiments on behavioral responses to drugs we noticed that the flasks with caffeine were less susceptible to periodic mite infestations. We tested this conclusion more directly by comparing innoculations of flies from an old mite infested culture into vials made up with and without caffeine. The vials were placed in a pan containing mineral oil to prevent the mites from spreading. Visable mite appearance was delayed by about two weeks in the vials containing caffeine although there was no change evident in the times of larval, pupal or adult progeny appearance.

We now routinely add caffeine to about 0.02% of the final concentration of food. This comes to 3.4 g per 300 half pint bottle batch (17 l. H_2O). The caffeine is added after the food has cooked (along with the propionic acid mix) and is thoroughly mixed in. A neutral red food dye is used as marker. Anhydrous caffeine is purchased from the Sigma Co. ($10 for 500 gm).

We feel that this procedure has so far helped keep our lab relatively free of mites. However, caution should be exercised in determining the amount of caffeine added by other labs. Whereas a concentration of 0.02% does not visably affect the flies in our hands, we found that a concentration of 0.05% in vials kills about half the adults within 24 hours. It is interesting that 0.05% is probably about the same concentration as the caffeine in a "strong" cup of coffee. Yanders and Seaton (1962) did not find caffeine in these concentrations to be mutagenic in flies and we have no cause to disagree with their conclusion.