Technical adaptations of instant medium for *Drosophila.*

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Like all holometabolous insects, *Drosophila* occupy two very different habitats during their life cycle. Females lay their eggs on a soft substrate that is suitable for larval development. After the larval stage, the pupal stage sets in, inciting the imagos to emerge. The length of the life cycle varies from one species to another and is dependent on environmental factors such as temperature, type of substrate, and humidity (Powell, 1997).

The ease with which species can be cultured in the laboratory varies considerably. There is a clear correlation between the ease of culturing and the breadth of niche species, as well as with adaptation to be human commensals (Powell, 1997).

Domestic species, including the most studied model, *D. melanogaster*, have a broad range of habitat preferences and do not have particularly narrow nutritional requirements (Powell, 1997). However, there are species of this genus, that, due to the irrestricted ecology, have no reported studies concerning their biological and behavioral features, precisely because of the difficulty of keeping these species in a laboratory environment (Hofmann *et al*., 1984).

Drosophilids can live and forage on many types of substrates, such as flowers, fruit, leaves, sap, cactus, fungus, and also in decomposing organic matter. In a laboratory these substrates are replaced by culture medium in order to facilitate the maintenance and cut costs.

In species from the cardini group that occupy a narrow niche, the culture medium may be a reason for the limited population growth. The length of each genaration of the cardini group species is estimated to be 15 days (Markow, 2006). We were able to maintain our stock on standard agar-yeast-cornmeal medium; however, our strains of *D. polymorpha* did not develop properly, taking more than 25 days to complete their life cycle. Moreover, these conditions enabled some fungi to emerge, and the offspring also became considerably reduced, leading to the loss of many lines. Therefore, we decided to search for a culture medium more suitable for species from this group.

We reviewed the culture media used in stock centers and made some adjustments. We looked for an instant medium with a similar recipe to 4-24 from Carolina Biological. Therefore, we chose potato flakes, which are easily prepared because they do not require cooking, and only need the adding of the liquid portion. However, the liquid portion has to be enriched in order to ensure that the medium has all the essential components for the successful development of flies and also for yeast that eventually comes together with wild flies.

To this purpose, sugar, proteins, and minerals were added to the water, after which eventually an antifungal can beaded in. Because of the mixture's simplicity, independently we obtained a very similar recipe as proposed by Kliethermes *et al*. (2011).

We chose molasses as a source of sugar due to its greater diversity and quantity of sugar compared to regular sugar and honey. Yeasts were also added in, as a source of proteins and minerals. Propionic acid was chosen as an antifugal instead of nipagin (methylparaben), which is insoluble in water. The quantity of each ingredient is listed below:

400 ml water
20 g molasses
6 g yeast (*Saccharomyces cerevisiae*)
1 ml propionic acid

Firstly, we recommend dissolving the yeast and the molasses in a small quantity of water. This mixture needs to be boiled, thus ensuring the medium's sterilization. The remaining water and propionic acid can then be added and stirred. Finally, the liquid portion has to be added to the potato flakes, in a ratio of 2:1, or until it becomes a homogeneous mixture. Potato flakes will absorb the liquid portion in approximately 2 minutes after which the medium will be ready for use.

At first, we tried to use nipagin; however, only after we replaced it for propionic acid could we control the fungal contamination. The amount of propionic acid can be increased up to 4 ml if there are vestiges of fungi.

The medium was tested in *D. melanogaster*, and mainly in the species of the cardini group, such as *D. polymorpha*, *D. cardinoides*, and *D. cardini*, which reported a significantly shorter life cycle (approximately 2 weeks). Fungal contaminations were removed and the strains increased considerably. Species from other groups like annulimana, guarani, and tripunctata also developed successfully in this medium, and we are testing it with more species. We believe this can be an important step for the use of neotropical species as model organisms in genetic, ecological, and evolutionary studies.

References: Hoffmann, A.A., K. Nielsen, and P.A Parsons 1994, Devel. Genet. 4: 439; Kliethermes, C.L., 2011, Dros. Inf. Serv. 94: 132; Markow, T.A., and P.M. O'Grady 2006, Drosophila: *A Guide to Species Identification and Use*; Powell, J.R., 1997, *Progress and Prospects in Evolutionary Biology: The* Drosophila *Model*.

New versions of trap and bait for the collection of the fig-fly *Zaprionus indianus* Gupta 1970 (Diptera: Drosophilidae).

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Abstract

Zaprionus indianus was accidentally introduced in Brazil in 1999 and is characterized as pest in purple-fig plantations. It has caused serious damage to the marketing of this fruit in the last decade, since these fruits are used as breeding and feeding sites, whereas this contamination has been favored by fruit morphology. Several measures have been tested against *Z. indianus*; however, none of them showed very satisfactory results. As an alternative, traps have been used, usually from plastic material, with or without light contrasts, possessing appropriate locations for the placement of attractive baits. In order to improve the confection of these traps and their performance, this paper presents a new version with adaptations made in the trap first proposed by Tidon and Sene (1988) and later modified by Medeiros and Klaczko (1999), considering the environmental conditions found in