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A simple and cost-effective mass rearing technique for the tephritid fruit fly, *Bactrocera dorsalis* (Hendel)

Fruit flies belonging to the families Tephritidae and Drosophilidae are major subjects for basic scientific investigations. The genus *Drosophila* of the latter family has been a subject of genetic and molecular studies on account of its easy rearing and quick adaptability to laboratory conditions. According to Wood *et al.*[1], the tephritids also have many qualities of a good genetic subject. Ecologists and geneticists are becoming increasingly aware of the diversity and rapidity of evolutionary changes taking place in sibling species complexes of tephritid fruit flies. This has been a fascinating subject of study with great economic implications, and has led to investigations in genetic studies like genome mapping, on these species. Such studies help to understand the significance of evolutionary changes, biosystematics, ecotypes, and biotechnological and molecular clues as basis for advancing tephritid management. Spanos *et al.*[2] studied the complete sequence of the mitochondrial genome of *Ceratitis capitata* Weidemann, that is potentially useful for the development of diagnostic tools for population analysis applications, as for example, determining the source of new introductions. Orchando and Reyes[3] studied the genetic population structure, gene flow and patterns of geographic distribution in olive fly, *Bactrocera oleae* (Gmelin) and found that natural selection, probably due to agricultural practices, may be the major factor responsible for the pattern of genetic variability observed in that species.

The potential of tephritids, though good laboratory material for investigations, has been under-exploited. One of the main reasons is due to problems related to the easiness of culturing the insects. A pre-requisite for a successful study of the genetics or for management applications like male sterile techniques of these fruit flies, is the improvement of its rearing technique. For this study, we selected the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Tephritidae: Diptera) which has been extensively studied by evolutionary and population geneticists, and its host races and sibling species form a classical example of presumed sympatric speciation[4–6]. It is widely distributed in the Oriental region[6] from Australia and Hawaii to Pakistan; hence it is also called Oriental fruit fly. It has also been reported from California, USA[7] in 1974, but was subsequently eradicated[8]. Further, it is a major pest of mango (*Mangifera indica* L.) in several parts of the world[7].

The artificial diet first recommended for rearing the Oriental fruit fly, *B. dorsalis* consisted of fresh carrots as a major ingredient supplemented with brewer's yeast and inhibitors against mould and bacterial growth[9]. Mitchell *et al.*[10] described a rearing medium for *B. dorsalis*, consisting of dehydrated carrot powder (in the place of fresh carrots) with brewer's yeast, sodium benzoate and hydrochloric acid. Later, Rejesus[11] mass-reared *B. dorsalis* on an artificial diet containing cooked yellow sweet potato (*Ipomea batatus* L.), rice bran, brewer's yeast, sugar, sodium benzoate, water and hydrochloric acid. Tanaka *et al.*[12] developed a low-cost rearing medium for *B. dorsalis* using methyl ρ-hydroxybenzoate, sodium yeast type 200, concentrated hydrochloric acid, wheat shorts, wheat middlings, gelgard M and water. The main problem encountered using carrots in artificial diets is the microbial contamination of diets and consequent high price of ingredients such as brewer's yeast and dehydrated carrot powder. Since the goal of mass rearing is to produce maximum number of healthy insects economically, a need was felt to make an attempt to mass-rear fruit flies on cheaper fruits easily available throughout the year, in countries distributed in the Orient. In any mass rearing, artificial diets are resorted to, only if obtaining and maintenance of natural host is difficult. For example, rearing mealybug on cucurbits with long shelf-life (e.g. pumpkin) is cheaper and easily viable than on arti-

ficial diets. Therefore, the objective of the present study was to find a simple and low-cost rearing method for the Oriental fruit fly, *B. dorsalis* on natural hosts hitherto not popularized.

The study was conducted in the insectary of the Fruit Entomology Laboratory at the Indian Institute of Horticultural Research, Bangalore. Three fruit hosts, viz. banana (cvs *Robusta* and *Elakki*), guava and papaya, were used. Mango, a natural host was used as standard for comparison. Of these, banana is regarded as an unusual host for Oriental fruit fly[6] and according to Armstrong[13], Oriental fruit flies do not infest bananas when unripe. However, under laboratory conditions, we found fruit flies infesting mature, pre-ripened bananas, and therefore, the idea to try it as a lab host emerged.

The sequence of mass rearing was as follows. A parental stock (F_0) of *B. dorsalis* was reared in the laboratory from infested mango fruits (cv. *Banganpalli*) in June 2000. The emerged adult flies were held in a cage (1 m × 1 m × 1 m). Flies were maintained in the room at 28 ± 1°C under the natural light phase. A mixture of sugar, yeast and water was placed in a Petri dish as a food supplement in the cage. Further, regular changing of cotton swabs with water was essential to avoid microbial contamination, especially mould development.

Two hundred pairs of fruit flies from this stock culture were sexed and released into a cage containing banana (cvs *Elakki* and *Robusta*), papaya (cv. *Solo*), guava (cv. *Allahabad Safed*) and mango (cv. *Banganpalli*) for oviposition after determining the weight of each fruit. The fruits were exposed to the females for 24 h. The sexually mature female *B. dorsalis* successfully laid eggs on these fruits and then each fruit was kept separately in plastic jars containing fine sand at the bottom (for maggots to pupate) covered with blotting paper (to absorb excess moisture). The maggots, which developed in the fruits, exited out to pupate in the sand. Puparia were collected and the number of puparia that emerged from each fruit was counted. These treatments were replicated five times. Each cage consisting of four fruits each of banana, papaya, guava and mango constitutes a replicate. Observations were recorded fruit-wise on incubation, larval and puparial periods, colour, weight, length and time taken for adult emergence and sex ratio for each fruit. The data were tabulated and analysed using analysis of variance, with *F* and *t* tests as criteria.

The results are presented in Table 1. Of all the fruits, banana had the highest puparial recovery per 100 g of fruit. The two banana varieties *Robusta* and *Elakki* with 77.79 puparia and 69.17 puparia respectively, were not significantly different (*F* value < table value). Papaya had significantly low puparial recovery (9.19 pupae). *F* values for mango (35.49) and guava (45.13) were significantly lower compared to banana (*F* value 2.18, > table value with CD, 9.58 @ $P = 0.05$). From the puparial recovery point of view, banana was the best host for fruit fly mass-rearing in the laboratory.

The cost/100 g fruit was lesser for banana (*Robusta* Rs 1.10; *Elakki* Rs 0.80) compared to other fruits, making it the most economical laboratory host for the tephritid, *B. dorsalis*. Though the cost for guava and papaya was less (Rs 1.00 and Rs 1.20/100 g fruit respectively), the puparial recovery was not satisfactory (45.13 and 9.19/100 g fruit respectively). Mango was found to be costly (2.50/100 g fruit) with lower puparial yield (35.49/100 g fruit) (Figure 1). In mango the maggots took longer duration for development before pupating (Table 1).

In mango, the maggots were able to make exit holes for pupation, whereas in green banana (cv. *Robusta*) which has thick, fibrous outer rind, the maggots remained inside the fruit even after completion of final instar. It was found that the fruit rind needed a little peeling to enable the maggots to exit for pupation. But in case of *Elakki*, the maggots entered the soil without any peeling because of its thin outer skin. In case of guava, even though the outer skin is thin, the maggots needed help to exit and to enter the soil for pupation. This may be because of fibrous veins and hard seeds present in the pulp, which hinder the movement of maggots.

Of all host fruits tried, duration of lifecycle was lowest on papaya with high mortality of late instar maggots. This is because of quick depletion of food material due to faster ripening and subsequent spoilage of papaya, hastening metamorphosis. Hence, the maggots were found prematurely entering pupation, and failing to form healthy pupae and adults.

Table 1. Development of *B. dorsalis* on different hosts

Host fruit used for culturing	Duration in days			Puparial colour	Sex ratio (♂ : ♀)	Remarks
	Oviposition to pupation	Pupation to adult emergence	Mean lifecycle (egg to emergence)			
Banana						
Robusta	13–16	8–11	19.00	Golden yellowish-brown	1 : 0.92	Little peeling is needed to help maggots to exit and enter sand for pupation
Elakki	11–14	8–10	19.00	Light yellowish-brown	1 : 1.09	No help is needed for maggots to exit, but pupal size is small
Guava	16–19	8–10	22.75	Light brown	1 : 1.10	Development was slow; Late instar maggots should be helped to enter the sand
Mango	18–21	10–12	25.50	Dark honey-brown	1 : 1.70	Development was slow
Papaya	11–14	8–10	18.50	Pale yellowish-brown	1 : 1.00	Forced pupation and mortality during larval–pupal interface was observed due to quick spoilage of fruit

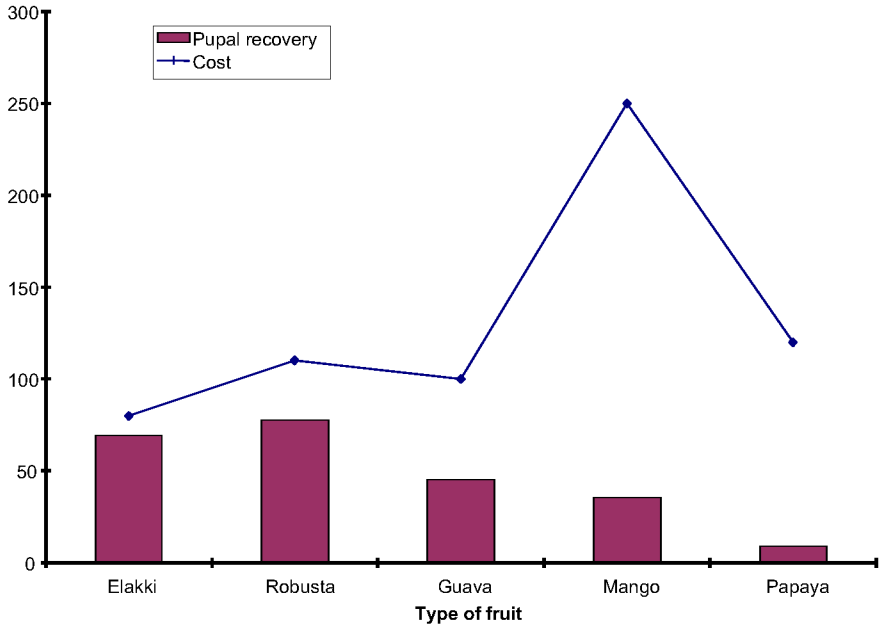

Figure 1. Puparial recovery and cost involved per 100 g fruit.

The puparial colour ranged from light golden-yellow to dark honey-brown. In case of sex ratio, among all the fruits tried, mango supported a female-biased sex ratio of 1♂ : 1.70♀ followed by guava (Table 1). On banana it was 1♂ : 1.09♀ (in *Elakki*) and 1♂ : 0.92♀ (in *Robusta*), which was ideal to sustain several generations.

All host fruits were sufficient enough to support the maggots till the final instars, except papaya, due to poor keeping quality. After examining the puparial yield, and round the year availability of the fruit, banana was selected for further rearing. The F_1 generation and the succeeding generations were also able to sustain on banana. We were able to continue stable mass-rearing of *B. dorsalis* on a large scale for ten generations.

Further, the following problems have to be circumvented for the successful rearing of *B. dorsalis*. Dirty or unsanitary laboratory conditions will inevitably lead to contamination from other dipteran flies. One has to be vigilant about predators like house lizards, spiders and ants, which will rapidly destroy the whole colony. Arthropod pests such as spiders, drosophilids and ants can be discouraged by regular cleaning of the cages and removing corpses and rotting food. Ants can be kept off by placing the legs of the cages in small plastic dishes filled with water. Care should be taken to avoid over-crowding in the cages. Regular cleaning of the laboratory with an antiseptic solution or water containing neem oil 5%-soap emulsion is necessary, to ensure hygiene for continuous culturing of *B. dorsalis*.

Thus, it was found that banana (cvs *Elakki* or *Robusta*) is suitable as a laboratory host for mass rearing of *B. dorsalis*. This fruit is comparatively cheap and easily available throughout the year. Rearing fruit flies on it circumvents the need for semi-synthetic/artificial diet and the cost and labour involved. *B. dorsalis*, being Oriental in distribution, is an ideal subject to investigate and understand the ecological, biological and evolutionary processes in the Oriental region. This will further give useful biotechnological and molecular clues in the management of economically important tephritids.

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