Laboratory-rearing Techniques for Tephritid Fruit Flies in the South Pacific

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Abstract

Laboratory colonies of 15 economically important species of multi-host fruit flies (Diptera: Tephritidae) have been established in eight South Pacific island countries for the purpose of undertaking biological studies, particularly host status testing and research on quarantine treatments. Laboratory rearing techniques are based on the development of artificial diets for larvae consisting predominately of the pulp of locally available fruits including pawpaw, breadfruit and banana. The pawpaw diet is the standard diet and is used in seven countries for rearing 11 species. Diet ingredients are standard proportions of fruit pulp, hydrolysed protein and a bacterial and fungal inhibitor. The diet is particularly suitable for post-harvest treatment studies when larvae of known age are required. Another major development in the laboratory rearing system is the use of pure strains of *Enterobacteriaceae* bacterial cultures as important adult-feeding supplements. These bacterial cultures are dissected out of the crop of wild females, isolated by sub-culturing, and identified before supply to adults on peptone yeast extract agar plates. Most species are egged using thin, plastic receptacles perforated with 1 mm oviposition holes, with fruit juice or larval diet smeared internally as an oviposition stimulant. Laboratory rearing techniques have been standardised for all of the Pacific countries. Quality control monitoring is based on acceptable ranges in per cent egg hatch, pupal weight and pupal mortality. Colonies are rejuvenated every 6 to 12 months by crossing wild males with laboratory-reared females and vice versa. The standard rearing techniques, equipment and ingredients used in collecting, establishment, maintenance and quality control of these fruit fly species are detailed in this paper.

In laboratory rearing of tephritid fruit flies, the technical breakthrough that allowed high yields of pupae from larval diets was the introduction of dehydrated plant materials (i.e. carrot powder) and dry yeasts (Tsitsipis 1989). In Hawaii, the mass rearing programs for fruit flies have been based on the use of protein hydrolysate to promote egg production and on low cost, nutritious larval diets. These developments have made it possible to obtain high reproduction rates under controlled environments. However, in many instances insect quality has declined when increased numbers are produced (Vargas 1989), for example, when many millions of flies are required for eradication programs.

In 1990, the FAO/AusAID/UNDP/SPC Regional Fruit Fly Project (RFFP) commenced in the South Pacific. A major objective was to undertake biological studies on the economically important multi-host species of fruit flies (Diptera: Tephritidae), particularly research on host status testing and quarantine treatments for locally produced fruits and vegetables. For these studies, a prerequisite was the supply of good quality fruit fly of specific life stages.

In the past six years, laboratory colonies of 15 economically important species have been established (Table 1). One or more of these species are maintained in each of eight South Pacific island countries. Seven of these countries are participants in the RFFP-Cook Islands, Fiji, Tonga, Western Samoa, Solomon Islands, Vanuatu and Federated States of Micronesia (FSM). The other country involved in

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this type of research is New Caledonia. The emphasis while maintaining these colonies was low cost, simplicity and the use of local materials wherever possible. The basis of this has been the development of good quality artificial larval diets for which the major ingredient has been locally available fruits. The pawpaw diet is the standard diet and is used in seven countries for rearing 11 species. The diet is particularly suitable for post-harvest treatment studies when larvae of known age are required.

Table 1. Tephritid fruit fly species reared on artificial diets in the South Pacific.

Country	Fruit fly species	Artificial diet
Cook Islands	*Bactrocera melanotus* (Coquillett)	Pawpaw
Cook Islands	*B. xanthodes* (Broun)	Pawpaw
Fiji	*B. passiflorae* (Froggatt)	Pawpaw
Fiji	*B. xanthodes*	Pawpaw
Western Samoa	*B. kirki* (Froggatt)	Pawpaw
Western Samoa	*B. xanthodes*	Pawpaw
Tonga	*B. facialis* (Coquillett)	Pawpaw
Tonga	*B. kirki*	Pawpaw
Tonga	*B. xanthodes*	Pawpaw
New Caledonia	*B. tryoni* (Froggatt)	Banana
New Caledonia	*B. psidii* (Froggatt)	Banana
New Caledonia	*B. curvipennis* (Froggatt)	Banana
New Caledonia	*B. umbrosa* (Walker)	Potato/carrot
Federated States of Micronesia	*B. frauenfeldi* (Schiner)	Pawpaw
Solomon Islands	*B. cucurbitae* (Coquillett)	Pawpaw
Solomon Islands	*B. frauenfeldi*	Pawpaw
Solomon Islands	*B. umbrosa*	Breadfruit
Solomon Islands	*Dacus solomonensis*	Whole fruit
Vanuatu	*B. trilineola* (Drew)	Pawpaw
Vanuatu	*B. minuta* (Drew)	Pawpaw
Vanuatu	*B. paraxanthodes* (Drew)	Pawpaw
Vanuatu	*B. quadrisetosa* (Drew)	Pawpaw
Vanuatu	*B. umbrosa*	Breadfruit

Another major development in producing high quality fruit fly colonies in these countries is the use of pure strains of bacterial cultures (family *Enterobacteriaceae*) as important adult-feeding supplements. Drew and Lloyd (1989) consider that bacteria comprise proteinaceous food for adult fruit flies and probably larvae. The 'fruit fly type' bacteria are isolated by dissecting out the crops from wild females, sub-cultured and routinely fed to caged adults. These two new developments — the use of a larval diet consisting mainly of raw fruit pulp and *Enterobacteriaceae* as adult food — have proven to be extremely successful. Healthy colonies have been maintained in Fiji for more than five years with little difficulty. The colonies are rejuvenated every 6 to 12 months by the incorporation of wild flies to ensure laboratory colonies are genetically similar to wild populations.

Systematic measures of quality of the laboratory colonies have been implemented because, as Boller et al. (1981) comment, to produce the required quality of fly the average and variation in production and performance criteria must be monitored in the laboratory. Leppla (1989) maintains that the value of insect colonies depends on the conditions under which they are established and the precision with which they are managed.

This paper describes the rearing facilities and standard techniques that have been developed for the economically important fruit fly species in these subtropical, small island countries and are predominately based on work in Fiji and Cook Islands from 1990. The techniques include collecting, establishing, maintaining and monitoring the quality of these multi-host species for research when laboratory colonies capable of laying about 30 000 eggs in 5–6 hours are required.

Collecting

Vigorous fruit fly colonies of the economic species present in a region are best established by collecting as many individuals from as many different hosts and habitats as possible because, as Mackauer (1976) comments, the evolutionary potential of a laboratory population is essentially determined when the breeding stock is isolated from the field. This can be achieved by collecting as many ripe host fruits and vegetables as possible and setting up as for collection of pupae (see later) with drainage for wet fruit to prevent larvae from drowning. Collections at different times of the day and year are also recommended (Leppla 1989). Resultant pupae should be placed in a small emergence cage containing water and sugar and emerging adults identified and transferred to a separate culturing cage as they emerge. This process will eliminate other insects that may emerge from the fruit, including other fruit fly species and possible parasitoids. Every effort must be made to minimise mortality during this process.

Establishment

The major population bottleneck in laboratory rearing usually occurs within three to five generations of the collections being established, particularly the first generation (Leppla 1989). To preserve the genetic variability of the colony every effort must be made to minimise this mortality. High mortality can be avoided by not overcrowding the flies, which causes stress-related mortality, by providing excess food and by avoiding temperatures above 30 °C. In

the initial stages of establishment it is better to use small cages (about 20 × 35 × 25 cm) and combine as many adults from different hosts or habitats together, after identification of the fruit fly species, to maximise the number of males and females reproducing, yet avoiding overcrowding. Adults of similar age (within 2–3 weeks) should be combined in the same cage and as the numbers are increased into the 100s, all the adults should be transferred to larger cages (50 × 50 × 50 cm). Colonies should be increased to 5–6 large cages for each species required for research purposes, with each cage containing 5000–10 000 flies.

Whole fruit rearing

If a good diet has not yet been developed for the species being reared, or adult numbers collected from the field are low, whole fruit may be used to establish the colony. This alternative requires collecting preferred host fruit before it is infested (e.g. pawpaw before colour break) or protecting fruit to ensure it is not infested before use. The whole-fruit egging system requires spiking the fruit with a number of small 1 mm holes (number depends on the number of females and size of fruit) and ensuring sufficient fruit for each larva (2 g per larva). One female may oviposit 10–50 eggs in 24 hours so enough weight of fruit must be provided to sustain the possible number of larvae. Fruit should be set up with drainage to ensure larvae do not drown in liquid.

Maintenance

Maintaining viable fruit fly colonies is a matter of careful rearing, diligent monitoring of quality control, and periodic strain restoration or replacement (Leppla 1989). Leppla also notes that colonies evolve rapidly and then remain relatively stable if rearing techniques are not changed. Rossler (1975) considered that replenishment of a Medfly colony was possible and preferable to total replacement, while Leppla (1984) commented that the success of strain maintenance strategies (i.e. the frequency and degree of restoration or replacement) depended on the quality of the mass production system.

There is severe selection pressure during laboratory mass-rearing with consequent rapid adaptation of fruit flies to artificial rearing regimes. To ensure the colony remains biologically similar to the wild populations, the colonies are regularly rejuvenated by crossing wild males exclusively with laboratory-reared females and at the same time crossing the wild females with laboratory-reared males every 6 to 12 months. This is a labour intensive task but it is an integral part of rearing maintenance. Field collected host fruit are set up as for collecting and adult males aspirated out daily and placed in a cage containing only laboratory females and vice versa. Adult density is maximised to promote mating (without overcrowding). All new cages are set up with the progeny of these two cages with flies from older cages discarded once a healthy, 'new' colony is achieved (at about three cages, each with 5000 adult flies).

The details of technical aspects of laboratory rearing are described below and are based on methods mainly undertaken in Fiji but which are used for most of the fruit fly species listed in Table 1.

Requirements and Procedures for Different Life Cycle Stages

Rearing facilities

The maintenance of fruit fly laboratory colonies requires a secure area (room or building) with natural lighting although facilities should be supplemented with artificial lighting, usually in the form of 1 or 2 banks of fluorescent tube lighting. Artificial lighting should be attached to a time switch to ensure a 'natural' dawn and dusk, which is important for mating of most species. Thus, lights should turn on about one hour after dawn and turn off one hour before dusk. *B. kirki* in Tonga, however, responds better to longer days and very strong lighting. Some species mate during the day (e.g. *B. melanotus* and *B. psidii*) and perform better under natural light (G. Clare, pers. comm.).

The area must be secure from outside agents such as rats, and all efforts should be made to restrict ants and *Drosophila* species (vinegar flies) from the rearing room, thus fine insect screening should cover all windows and entrances. The internal area must have large benches that are ant-proof, which is usually achieved by placing water or oil traps under the bench stands. The whole area must be completely free of pesticide contamination or spray drift at all times. The area should have a temperature range of about 25–28 °C. This may require air conditioning especially if temperatures exceed 30 °C. Some countries require heating in winter to maintain an optimum temperature range.

Adults

Colonies should consist of 5–6 cages each containing 5000–10 000 adults of known age and quality. Eggs are laid after an initial pre-oviposition period of 2–4 weeks, depending on the species. After this period, eggs may be collected by allowing females to oviposit into fruit or artificial domes. At the peak of

production (weeks 4–7) good cages should produce 50 000 eggs in a 24-hour period and support egging three times weekly. These eggs must be produced without affecting normal colony maintenance requirements. Cages are discarded when there is significant mortality, usually after about 8–9 weeks.

Cages

Large colony cages (approx. 50 x 50 x 50 cm) can be made using frames of aluminium rod lengths with small, drilled plastic corners, or alternatively by using PVC water piping. These materials are relatively cheap, easy to clean and are also easy to dismantle and transport if required. Synthetic gauze used as a cage cover should have mesh holes small enough to keep ants and *Drosophila* out. The cage cover is sewn with an extended open sleeve on one side to allow easy access.

Adult food

Adult fruit flies can survive on sugar and water alone and this should be supplied in cages as the adults emerge. Water is supplied using a sealed container with a sponge wick and sugar is made available in granulated form on tissue which absorbs any excess moisture. At least two water and sugar sources should be continuously available in each cage. Water sources should be cleaned and replaced weekly.

Protein for adults

A few days after emergence, the females require a source of protein for egg maturation, and this is supplied in the form of enzymatic yeast hydrolysate. Hydrolysed yeast and sugar is normally mixed in a ratio of 1:3 with a minimum of water to make a thick paste which is plastered onto cards. A number of these cards are hung from the top of the cage, ensuring that the adults do not get trapped in the sticky mixture. Adults may perform better with extra nutrients and these can be supplied in the form of mineral (e.g. Wessons salts) and vitamin mixes (e.g. Vanderzant general insect vitamin mix) or added protein (e.g. dried egg yolk).

Bacteria for adults

Bactrocera species of fruit flies have a specific group of bacteria (family Enterobacteriaceae) associated with them which are important for both adult and larval development and are supplied to adult cages. A number of bacteria species are used in the region, but the one most commonly associated with the fruit fly species reared in the South Pacific is *Klebsiella oxytoca*. The bacterium is isolated by dissecting out the crop of wild female flies. Male flies are also a source of this bacterium, as are larvae in fruit which yielded a pure culture in Tonga (GPW, pers. observ.). Crop contents are smeared onto sterilised peptone yeast extract (PYE) agar plates and the bacteria sub-cultured until a pure culture is obtained (details on preparing agar plates and smearing techniques may be found in Walker and Hamacek 1992). This culture must be checked for purity and identified by a microbiologist before being sub-cultured again onto PYE agar plates for feeding to adult flies. The agar plates, with at least two days of bacterial growth, are supplied twice weekly to new cages by inverting the plates on top of the cages and loosening the agar so that it drops onto the cage cover. Adult dissections and bacterial smears should be undertaken in sterile conditions and utensils sterilised with ethyl alcohol or by flaming.

Eggs

Eggs are collected after the pre-oviposition period by placing artificial or fruit dome egging devices in cages where flies are about 3–7 weeks old.

Fruit domes

Fruit domes are produced by cutting the fruit in half and piercing the skin with a 1 mm diameter needle 30–100 times, depending on the size of the fruit and the number of females in the cage. The flesh is scooped out leaving as little flesh as possible on the skin. Domes are washed and sealed onto a petri dish or equivalent with moistened filter paper to prevent flies from entering the dome. Wet filter paper should be placed inside to keep the domes as moist as possible.

Artificial domes

Various artificial egging devices have been developed locally, but most consist of a thin plastic receptacle perforated with oviposition holes and containing a natural oviposition stimulant. Preparation of this device is a standard procedure. Small (1 mm) holes should be punctured in the sides of the receptacle. Host fruit pulp or larval diet should then be smeared onto the inside of the container. Juice is pushed through the holes and then excess material inside the container wiped away. The container should be sealed onto a petri dish with moist tissue. Wet tissue or sponge should be placed inside the artificial dome to maintain maximum humidity. The eggs must **never** become dry. Diluted host fruit juice (10:1, water:juice) saturated into cellulose sponge placed inside the dome may improve oviposition (Vargas et al. 1993).

Collecting and counting eggs

Eggs are collected by washing out the dome with water using a hand sprayer, or if using fruit domes, washing and then teasing egg bundles out of flesh with a scalpel or probe, or squeezing flesh gently with soft forceps. Egg numbers are estimated volumetrically by counting a measured sub-sample of eggs (e.g. one drop or a marked volume on a pipette) on moistened black filter paper.

Egg hatch test

An egg hatch test should be set up whenever egging is undertaken. At least 100 eggs are pipetted onto moistened black filter paper and held in a petri dish sealed in an air-tight container. Eggs must remain moist at all times. Percentage egg hatch is recorded after about three days.

Egg/diet ratio and seeding diet

Eggs should be dispensed onto diet at a known ratio of numbers of eggs to weight of diet to ensure an excess of food for the larvae. This ratio should be 2 eggs per 1 g of diet when establishing colonies, but the ratio may be adjusted after adequate studies to determine the optimum ratio. An optimum rate is one that does not affect the quality of flies while minimising underutilised (wasted) diet. Eggs can be pipetted directly onto the diet surface. The eggs must remain moist and they should be evenly distributed over the diet. Eggs with fruit residues from the domes placed on diet may lead to mould growth on the diet surface so eggs should be rinsed several times to remove contaminants (e.g. bits of fruit pulp). Eggs can be surface-sterilised in 0.025% NaClO for about 5 minutes, after which they must be immediately triple-rinsed in fresh water.

Larvae

Use of whole fruit

Whole fruit are used when the artificial diets available are not suitable for the particular species or when an artificial rearing system is still under development. Whole host fruit may be used when, and if, fly numbers are very low.

Artificial diets

It is essential to develop inexpensive diets that are nutritionally suitable for larval development, contain ingredients that are continuously available, are of known quality and free of any pollutants. The bulk of effort expended in improving tephritid fruit fly rearing world-wide has been directed at the development of larval diets. Virtually all artificial larval diets used world-wide have common characteristics. They normally include water, microbial inhibitors, sources of protein, carbohydrate and lipid, plus vitamins, salts, minerals and sterols. The two other ingredients usually added to fruit fly larval diets are a bulking agent and an agent for adjusting pH. In Hawaii, wheat millfeed standard diets have been used for *Bactrocera dorsalis* (Oriental fruit fly), *Ceratitis capitata* (Medfly) and *Bactrocera cucurbitae* (melon fly) and an improved diet based on bran has been developed for rearing *Bactrocera latifrons* (Vargas et al. 1993) (see Table 2).

Initial developments of the diets for the South Pacific were carried out in the Cook Islands by E. Hamacek and in Fiji by Hamacek and RFFP staff. The initial bulking agent used was cassava in the Cook Islands and then sugarcane bagasse in Fiji. Pawpaw is now the main ingredient used because it is generally available all year and, if picked at colour

Table 2: Artificial fruit fly diets used in the Pacific (ingredients in grams).

Ingredient	Orient/ Med	Melon fly	Latifrons	Dried carrot	Pawpaw/ cassava	Pawpaw/ bagasse	Pawpaw	Breadfruit	Banana
Fruit pulp					1500	1500	1000	1000	886
Mill feed	500	1027							
Bran			140						
Carrot powder			50	470					
Cooked cassava					1500				
Sugarcane bagasse						150			
Sugar	230	242	20						
Torula yeast	64	117	35	150	125	80	53	53	111
Nipagin	2	3.67	1.6	15	12.5	4	2.6	2.6	2.5
Sodium benzoate	4	3.67							
HCl (conc.)		6.67		31.5					
Citric acid			35						
Water (mL)	1200	2500	750	3000+	400	250+		1000	

break, is not infested by fruit flies. Another advantage of using fresh fruit in the diet is that the pH requires no adjustment. The pH is normally held at 4–5.5 to deter bacterial and fungal contamination. The other ingredients are Torula yeast and Nipagin, which are standard ingredients in insect diets. Torula yeast is a standard source of protein in larval diets although Brewer's yeast (*Saccharomyces* spp.) may also be used. Nipagin (methyl *p*-hydroxy-benzoate), or sodium benzoate at 0.1% of the diet, are added to stem both bacterial and fungal development, deleterious to fruit fly larvae.

Variations in the quality of the bulking agents, cassava and sugarcane bagasse used in the Cook Islands and Fiji led to unreliable data in larval developmental studies, and together with difficulties in extraction of larvae from these diets led to futher development work on the diet. Researchers working in the Cook Islands (RFFP staff and G. Clare) discovered that storing the other ingredients (pawpaw, Torula yeast and Nipagin) after mixing without the bulking agent at 4 °C for at least 24 hours led to gelling of the ingredients. Excess water produced in this gelling process could be decanted off, producing a diet from which larvae could be easily extracted using a sieve under running water. This, now standard diet has been further developed by substituting pawpaw with other available fruits, particularly breadfruit (used in Fiji, Solomon Islands and Vanuatu) and banana in New Caledonia (Lemontey and Mademba-Sy 1995).

The range of diets used in the eight different South Pacific island countries for various fruit fly species are given in Table 1 and ingredients are shown in Table 2. The standard diet, either with pawpaw, breadfruit or banana as the main ingredient, is used for all species in all countries except *B. umbrosa* in New Caledonia, which is reared on dried potato/carrot diet (G. Clare, pers. comm.). This species is reared on breadfruit diet in the Solomon Islands and Vanuatu. The standard diet is simple to make and stores well in the fridge but loses some quality if frozen (Clare, pers. comm.). The two main problems with these diets are the threat that the fruit may be contaminated by pesticides, or that they may contain wild fruit fly eggs or larvae. It is essential to ensure that fruit are not infested by picking them at the appropriate stage or protecting them by bagging. Pawpaw is picked at colour break and breadfruit and banana are picked at the mature green stage, about one week before use. All of the fruits are then carefully stored to prevent insect infestations and allow natural ripening.

Other standard diets may be used, and examples of these are given (Table 2). In New Caledonia researchers were having difficulty mass-rearing *B. umbrosa*. However, a suitable diet was developed by Clare based on potato and carrot. The use of carrot for fruit fly larval diets is well known, with dried carrot diet (Table 2) the most commonly used diet in small-scale rearing around the world. There is evidence that carotene (from carrot) is an important feeding stimulant which promotes growth, particularly during early larval development (Fay 1989). Water is also very important, both for minimising the effects of metabolic heat build-up during the final stages of larval development (Hooper 1978) and in affording greater access to nutrients. There are indications that *B. cucurbitae* needs a diet of higher moisture content than some other species. The standard diets used in the South Pacific that are based on natural fruit pulp have a naturally high water content but the addition of a little water is sometimes necessary for the right consistency when using fruit that are not fully ripe.

General preparation of fruit diets

All ingredients must be thoroughly mixed, particularly the Nipagin. This is achieved by mixing the Nipagin, dissolved in a little warm water if necessary, and yeast to small quantities of fruit. Use a blender if available. The diet is stored at 4 °C for at least 24 hours which allows the mixture to gel prior to decanting off excess water before use. Check pH is 4.0–5.5 with litmus paper. Concentrated hydrochloric acid (HCl) (0.5–3.55%) or citric acid can be used to increase acidity if required.

Larval trays and storage containers

Larvae may be reared in various shallow trays or dishes containing the diet. Diet thickness is an important factor as the greater the surface area to volume ratio the greater the likelihood of metabolic heat dissipation (Fay 1989). However, thin diet is prone to drying. Diet (500–800 g) is spread onto a shallow tray at a thickness of 3–5 cm. Diet trays with eggs are placed in plastic containers with tight-fitting lids sealed with tape and labelled. The lid must have a large ventilation hole covered with fine insect screen to keep *Drosophila* out. Ventilation is important for efficient gas and heat exchange. However, during the first 3–4 days the vent should be sealed to ensure high humidity for good egg hatch and prevent the entry of light which may promote excess yeast growth on the diet surface.

Pupae

Pupating substrate

Larvae enter a post-feeding stage and commence 'popping' or 'jumping' out of the diet trays. These larvae must be allowed to pupate in a moist substrate, normally a layer (>1 cm thick) of moistened, sterilised, untreated sawdust placed in the bottom of the holding container about 2 days before 'jumping' begins. Thoroughly washed sand or fine vermiculite may also be used as a pupating substrate. Before use, the sawdust should be sieved to retain only fine particles for easy pupal sorting. Sawdust or vermiculite must be heat-sterilised before use (120 °C for at least 2 hours).

Collection and storage conditions

Pupae are collected when at least a few days old by gentle sieving, and stored in moistened sawdust for eclosion. A small container should be layered with 1 cm of moist sawdust, pressed down. Pupae are then added and lightly covered with more moistened sawdust. Pupae should be held at about 25–28 °C or the same temperature as for larvae. Relative humidity should in the 70–80% range, high enough to prevent pupal water loss but not so high as to allow free water or inducement for mould. Pupae are normally placed in new cages 2–3 days before eclosion.

Quality Control and Recording Procedures

Quality control of mass-reared insects is divided commonly into two categories:

(1) production quality control where the parameters of rearing are addressed and which include such items as diet ingredients and environmental conditions; and
(2) product quality control where the insects produced are evaluated (Calkins 1989).

Production quality control in the South Pacific rearing system includes standardising the use and storage of materials and ingredients, monitoring production processes and ensuring facility and equipment maintenance. For monitoring purposes, all rearing cages are labelled with date, species and cage number, and all rearing containers with relevant informaton, e.g. weight of diet, number of eggs, diet type, date, and fruit fly species. Information on room temperatures is essential and relative humidity recording is also useful. All unusual observations should be noted along with any deviations from normal practice, e.g. diet wetness, growth on diet surface, abnormal condensation in larval diet container. These comments may help identify the source of any problems found later with quality of flies or pupae.

In the second category, product quality control, the quality of the fruit fly rearing system can normally be assessed at the pupal stage and can be correlated to pupal size (Fay 1989). However, pupal weight changes over time so that comparisons should be made with equal-aged pupae. Other simple tests of quality include percentage adult emergence (or percentage pupal mortality), percentage flight ability and percent survival (Boller et. al. 1981). Records should be kept of colony progress so that any anomalies associated with rearing processes can be identified and overcome (Fay 1989). Generation number, daily egg production, per cent egg hatch, ratio of numbers of eggs to weight of diet, larval duration and pupal recovery should all be recorded. Significant adult mortality in cages only 3–4 weeks old, or high numbers of flightless adults, are indicators that there is a problem with the laboratory colony.

Quality assurance monitoring in the South Pacific is based mainly on the pupal stage (weight and mortality) and egg hatch (see below). There are variations between individual fruit fly species in any country and between the same species in different countries but there is a range that indicates a colony is healthy. Checks on these variables are conducted continuously and recorded for future reference. Variations in performance in biological studies are normally preceded by indications in these regular checks that there is a problem in the rearing system. Details on guides to quality control and fault-finding guidelines can be found in Walker and Hamacek (1992).

Records used for quality assurance monitoring in the South Pacifc include:

- percentage egg hatch — should be >70%, 75–95% ideal (using >100 eggs);
- pupal weight — should be regular for same aged pupae (using 100 pupae);
- pupal mortality should be <10% (using 100 pupae);
- pupal recovery from number of eggs hatched should be >60%;
- adult recovery from eggs should be 45–50%.

Laboratory Hygiene

Rearing facilities should have a high standard of hygiene to minimise the risk of bacterial contamination. *Bacillus* spp. and *Serratia marcescens* are known pathogens of fruit flies. All cage covers, frames and all containers used to hold eggs, larvae,

pupae and implements should be disinfected in 0.2% sodium hypochlorite (NaClO) and then well rinsed in fresh water (preferably three times) before re-using. Benches should be regularly cleaned with water or wiped down with 75% ethyl alcohol.

Conclusion

Vargas et al. (1993) state that fruit fly rearing may be conducted with either a relatively small volume of adults to promote relatively high egg production, or a very high adult volume to compensate for reduced egg production per unit volume. The rearing system described in this paper uses a small 'volume' of flies to produce a relatively large number of eggs with the emphasis on quality rather than quantity for various life stage studies. Adult cages are discarded after a relatively short egging period and the colonies rejuvenated frequently. The adults are fed standard protein sources, but are supplemented with 'fruit fly type' bacteria, which has led to good egg production and egg hatch for most species in most countries. Efficient egging devices have been developed in the South Pacific, similar to devices used in Hawaii, and larvae are fed a 'natural' diet based on locally available fruit producing high quality fruit fly life stages.

The laboratory rearing system described in this paper requires a relatively small amount of labour input when compared with some other laboratory insect rearing systems, and most aspects of the work are quite simple. However, it does rely on staff who are highly committed to this work and who have access to continuously available, uncontaminated local produce to achieve research results that can be duplicated with confidence.

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