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Mass Rearing and Quality Control Parameters for Tephritid Fruit Flies of Economic Importance in Africa

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1. Introduction

Tephritid fruit flies are recognized worldwide as the most important threat to horticulture (Allwood and Drew, 1997; Barnes, 2004; Ekesi and Billah, 2007). Sub-Saharan Africa (SSA) is the aboriginal home to 915 fruit fly species from 148 genera, out of which 299 species develop in either wild or cultivated fruit. They belong mainly to four genera: *Bactrocera, Ceratitis*, *Dacus,* and *Trirhithrum* (White and Elson-Harris, 1992). Most of the fruit fly species are highly polyphagous attacking several important fruits and vegetables including mango, citrus, guava, avocado, tomatoes, pepper, cucurbits etc. Female fruit flies that lay eggs under the skin of fruits and vegetables cause direct losses. The eggs hatch into larvae that feed in the decaying flesh of the crop. Infested fruits and vegetables quickly rot and become inedible or drop to the ground. Beside the direct damage to fruits, indirect losses is associated with quarantine restrictions because infestation and sometimes mere presence of the flies in a particular country could also restrict the free trade and export of fresh horticultural produce to large lucrative markets abroad. The introduction of uniform and strict Maximum Residue Levels (MRL) across Europe compounds the problem and further jeopardizes export. Of greater concern is the fact that even in countries where fruit fly management methods are undertaken, rejection by European markets is on the increase largely because with global trade and passenger trafficking, they are easily translocated and the risk of majority of African fruit flies as key and potential quarantine pests is becoming increasingly realized (OleMoiYoi and Lux, 2004).

Although fruit flies attack a variety of horticultural crops in SSA, most of the fruit fly R&D in SSA has concentrated largely on mango infesting species largely because of the recognition of this crop as an important source of food and nutritional security. Globally, mango is the second most internationally traded crop after pineapple providing much needed income to poor rural households and the national economy. In Africa, assessments by the *icipe*-led African Fruit Fly program (AFFP) has revealed that out of the 1.9 million tons of mangoes produced annually across Africa about 40% is lost to fruit fly infestation (Lux et al., 2003). Majority of fruit fly species in Africa are little known but rank high on quarantine list worldwide. Some of the most important species attacking mango include the

invasive fruit fly species *Bactrocera invadens* Drew Tsuruta & White and the native species; *Ceratitis cosyra* (Walker), *C. fasciventris* (Bezzi), and *C. rosa* (Karsch). The increasing awareness of the damage caused by these fruit flies to the fruit industry has created a demand for development of control measures against the fruit flies. Control methods to suppress or eradicate the insects are of utmost importance for the economy of developing countries whose income is highly dependent on agriculture. Pre-harvest control methods for fruit flies include methods such as the use of baiting and male annihilation techniques, Sterile Insect Technique (SIT), biological control (entomopathogens, parasitoids and predators), fruit bagging, early harvesting, and orchard sanitation (Allwood, 1997; Barnes, 2004; Mau, et al., 2007; Ekesi and Billah, 2007). To allow access to quarantine sensitive markets, pre-harvest methods are complemented with postharvest methods such as fumigation, heat treatment, cold treatments and irradiation (Armstrong, 1997; Ekesi and Billah, 2007). The success of majority of these control methods depends on the ability to establish a cost effective rearing methods. To conduct pre-requisite study on biology, response to attractants, and efficacy of the various biological control agents, a regular supply of good quality insects of pre-determined reproductive stages and age is necessary. Management methods that are especially based on the use of classical biological control, SIT, and post harvest treatment require the use of high quality developmental stages of various fruit fly species. To produce the required quality of flies, the average and variation in production, and quality control parameters must be established and closely monitored in mass rearing procedures. The quality of insect colonies also largely depends on the conditions under which they are maintained and the precision with which they are managed. Many aspects of mass-rearing environments directly affect the quality of fruit flies. Artificial larval and adult diets as well as rearing conditions (light intensity, photoperiod, insect density, temperature, humidity, etc.) can influence quality control parameters such as body size, survival, pupal weight, adult emergence, longevity, flight ability, fecundity, fertility and mating ability of the insects. Apart from *Ceratitis capitata* (Wiedemann), which has been reared for decades in different parts of the world (Vargas, 1989) with great levels of success, the mass rearing procedures for majority of economically important African fruit fly species such as *C. cosyra*, *C. fasciventris,* and *C. rosa* and the recently described invasive pest, *B. invadens* are little known and documented. Reliable methods for rearing large numbers of these insects have been developed by *icipe* scientists. In this chapter, the facilities and methodology for laboratory rearing of these species in relation to different quality control parameters is presented.

2. Rearing facilities

The fundamental and most essential requirement for sustainable fruit fly mass rearing is availability of well secured building. The entire building must be free from pesticide contamination and should be surrounded with a water trough at the ground level. The water trough prevents the entrance of crawling insects. Laboratory windows should be equipped with glass with the upper portion made up of fine wire mesh to ensure the provision of adequate natural light as well as to provide good ventilation. The fine mesh also safeguards against entry of lizard that may prey on the puparia and pupariating larvae. Proper lighting regime is vital for fruit fly behavior especially mating and additional artificial light which is controlled by a timer should be provided. For most of the mango

infesting fruit fly species photoperiod of L12:D12 have been found to be adequate. Heaters and or cooling systems should also be installed to ensure that temperature is kept at the optimum. At the *icipe* rearing facility, temperature is maintained at 26-28°C, humidity is kept at the range of 60-70 RH%. The inside area of the laboratory should be spacious enough to allow movement of trolleys with trays and plastic boxes containing the successive stages of the flies. On the laboratory benches the rearing cages should be suspended on small stands within a plastic bowl (12 cm dia and 5.5 depth) containing water and few drops of liquid soap to prevent ant from climbing into the rearing cages. Also a layer of grease should be applied to the stand of the laboratory bench. McPhail traps baited with 3% NuLure should be hung at the ceiling of the rearing room to trap *Drosophila* species and any other flies that may escape during the handling. Trays and plastic boxes with diet containing developing larvae should be covered with fine netting to prevent contamination with *Drosophila* species.

3. Laboratory hygiene

High hygienic standards must be maintained in the laboratories at all production stages. Whole fruit, fruit domes, and important rearing materials such as Perspex cages, trays, sponges, racks etc must be frequently rinsed with 0.025% NaClO followed by several rinses in sterile distilled water to eliminate bacterial and fungal infection. The benches must be disinfected by wiping with 70% ethanol every morning to prevent infestations by mite which are common causes for drastic reduction in adult emergence. Diet ingredients should be purchased from well reputable sources and kept in very clean, cool and dry storage room.

4. Handling procedure for different developmental stages

4.1 Eggs

Eggs are harvested using plastic bottle (8 cm dia, 13 cm length) oviposition device that have been tested and validated for this purpose (see section 7 of this chapter). The plastic bottles are exposed to sexually matured flies after pre-oviposition period of 5 and 10 days for *Ceratitis* spp. and *B. invadens* respectively. Plastic bottles are perforated with 500 oviposition holes (at 1 cm apart). Before introduction of the bottle into the adult rearing cage, a 3 x 3 x 9 cm cellulose sponge saturated with fruit juice of the fly's preferred host is placed inside the bottle to provide the necessary ovipoistion stimulus and prevent egg dessication. After an exposure period of 10 hours, the bottles are removed from the cage. Fruit domes are also an excellent egg collection device. Pesticide free mango fruit should be surface sterilized with 0.025% sodium hypochloride (NaClO) and then cut into two equal halves. Thereafter, the mango flesh and seed are scooped out, and the domes are pierced several times with entomological pin (00). The domes are then placed on Petri dish (9 cm diameter) lined with moistened filter paper, and the edge of the dome along the Petri dish is sealed with cellotape (1 cm width) to prevent flies entering inside the dome. The flies are then allowed to oviposit for 10 hours. Egg collection from the oviposition devices is done by washing with water using a hand sprayer (0.5 l). Freshly collected eggs should be washed with 0.025% NaClO followed by several rinses with sterile distilled water. The number of eggs are then estimated volumetrically and recorded.

4.2 Larvae

Larvae are reared on artificial diets that normally include sources of protein, carbohydrate, lipids, microbial inhibitors, vitamins, salts, minerals, water, sterols, bulking agent and substances for adjusting pH. Several artificial diets have been evaluated for African fruit fly species and results are presented in Sections 9 and 10 of this chapter. Diet ingredients must be thoroughly mixed. A blender is most appropriate for mixing and especially ensuring that microbial inhibitor in the diet is evenly distributed. Prior to use, solid-based diet can be stored at 4°C for at least 24 h to allow the mixture to gel before decanting excess water. Diet pH should range between 4.0-5.5. If necessary, concentrated hydrochloric acid (HCl) (0.5-3.55%) or citric acid can be used to increase acidity. Whenever required, diet (500-800 g or ml) are transferred into shallow trays which have been shown to be the most appropriate for larval rearing (Chang et al., 2004). The thickness (solid diet) and volume (liquid diet) can be an important factor in reducing mortality as the greater the surface area to volume ratio the greater the likelihood of metabolic heat dissipation (Fay, 1989; Chang et al., 2006). Trays with diet and egg should be placed in plastic containers with screened tight fitting lids (to keep out *Drosophila* flies) above sterilized sand. During the first 3-4 days, the screen on the lid should be sealed to ensure high humidity for egg hatch and thereafter ventilation becomes important for efficient gas and heat exchange.

4.3 Puparia

Sand that is used as pupariation medium should be thoroughly washed and sterilized at 120°C for at least 2 hours and thereafter cooled at ambient temperature before use. Sterilized sand should be placed at the bottom of rearing cages at about 2 days before the larvae commence popping or jumping out of the diet trays to puparíate in the moist sterile sand at the bottom of the diet tray. After 7 d in the sand, puparia should be separated from the pupation medium by gentle sifting. For all mango-infesting fruit fly species, the puparia are usually held at 26-28°C and RH of 60-70% which is adequate to prevent pupal water loss and minimize development of mould. The puparia should be transferred into new cages at 2-3 days before emergence.

4.4 Adult

At the *icipe* rearing facilities, adult cages are made from Perspex cages (50 x 50 x 50 cm). One side of the cages is provided with a screen with mesh holes small enough to keep ants and *Drosophila* species out but ample for cross ventilation. Another side of the cage is provided with a sleeve to facilitate access to the flies. Colonies consist of 7 cages labeled cages 0-6 (cage 0 contains one week old flies and cage 6 contains older flies at 7 weeks). Cages are discarded at 8 weeks old. At one week old, the cages would normally hold between 5,000 to 6,000 flies and the numbers decrease successively with the age due to natural mortality. Fruit flies diet consist of a volumetric mixture of 3:1 sugar and enzymatic yeast hydrolysate ultrapure (USB Corporation, Cleveland, OH) in a Petri dish (9 cm). In each cage and depending on the number of flies, 2 - 6 Petri dishes containing the diet are placed inside the cage to minimize overcrowding. Water is provided in Petri dish with a layer of pumice granules and replaced daily. Egg production commences after a pre-oviposition period of 5-10 days depending on the species, and eggs are collected using oviposition devices as described in section 4.1. Egging is carried out three times a week and at the peak of egg production and depending on the species, a good cage can produce up to 50,000 eggs in 24 h.

5. Quality control parameters and recording

Boller and Chambers (1977) defined quality control of mass reared insects as "The degree to which a product meets the requirements of the objective or expected function". Quality control provides a means of optimizing insect mass rearing by identifying and gradually correcting deficient production processes, thereby preserving the genetic variability of the strain (Leppla and Ashley (1989). Quality control therefore integrates methods development, colonization, maintenance and arrays of process control details that affect the production and use of insects for pest management purposes. It helps in reducing cost of insect maintenance by improving rearing techniques through careful monitoring of protocols and a minimal number of sensitive parameters. Leppla and Ashley (1989) categorized quality control of mass reared insects into three main interrelated elements:

1. Production quality control which manages the consistency, reliability and timeliness of the production output. At the *icipe* facility, production quality control include standardization of diet ingredients and the production process by ensuring that all cages are appropriately labeled with species, dates, cage number, and diet type. The use and efficient storage of all materials away from pesticide contamination and any other pollutants forms part of efficient production quality control.
2. Process quality control assures the performance of the production process so that unacceptable deviations do not occur in product quality. Here, logistical consideration such as strict compliance to rearing protocols and maintenance schedules are crucial. In addition, environmental conditions such as temperature, RH, photoperiod, light and atmospheric quality are monitored constantly to determine the conditions of the rearing operations. Process control is recommended even when production is unstable although the diagnosis and correction of problems is more complicated.
3. Product quality control regulates the conformity of the product to acceptable standards of quality and predicts the effectiveness of the product in performing its intended function. In this regard, biological parameters are evaluated to identify possible deficiencies and to predict insect quality. Production can immediately be improved through testing of key and sensitive parameters and feedback mechanisms. Tests should be practical, uncomplicated, efficient and reproducible. A minimum number of parameters using the smallest sample size are recommended. In fruit fly mass rearing, important parameters include pupal recovery, pupal weight, percent adult emergence, percent survival, flight ability, percent fecundity and percent egg hatch (Calkins, 1989; Walker et al., 1997; FAO/IAEA/USDA, 2003). Walker et al., (1997) noted that 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 that a colony is healthy. At the *icipe* facility, quality assurance are based on the following parameters: (1) pupal recovery from the number of eggs seeded should be >60%; (2) pupal weight, using 100 puparia should be regular for the same aged puparia; (3) adult emergence should be >70%; (4) percent fliers should be >80% and (5) percent egg hatch, using 100 eggs should >70%.

6. Colonization process

6.1 Fruit collection, handling, and processing

The evolutionary potential of a laboratory population is essentially determined when the breeding stock is isolated from the field (Mackauer, 1976). In this regard, all the mango

infesting fruit fly species that are currently reared at the *icipe* facility were started by collecting several cultivated and wild host fruit species. For example, *C. capitata* originated from coffee berries, *Coffea arabica* L. (Rubiaceae) collected from Ruiru, central highlands of Kenya, *C. rosa* from *Monodora grandidieri* Welw (Annonaceae), a wild host from Mombasa, coastal Kenya, *C. fasciventris* from coffee in Rurima, central highlands of Kenya, *C cosyra* from mango *Mangifera indica* L. (Anacardiaceae) from Rurima and marula, *Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae) from Nguruman, Rift Valley Kenya, *C. anonae* from *Antiaris toxicara* (Pers) Lesh (Moraceae), a wild host from Kakamega forest in western Kenya and *B. invadens* from mango, Nairobi, Kenya. Leppla (1989) also recommended collections at different times of the day and year and this was also practiced in our collection. Fruit samples of the different plant species were placed separately in sisal or polypropylene woven sacks and then transported to the rearing facility. At the fruit incubation room, each fruit type is counted, weighed and later placed in secure well-aerated transparent plastic containers with a layer of sterilized moistened sand at the bottom. The rim of the containers are covered with a fine netting material held in place by the cover of the containers of which the greater part of its inner area is removed. The sand in the cage and the plastic containers served both as the pupariation medium for the larvae that exited the fruit in addition to soaking up fruit juices. The cage and the container holding the fruits should be labeled with information regarding fruit type, date of collection, GPS reading of location, number and weight of fruits. Fruits are held at ambient conditions for three weeks, after which they are dissected and the larvae that fail to pop out are assisted using a pair of soft forceps. Fruit holding cages should be checked daily and resultant puparia picked from the sand with a pair of soft forceps, counted and placed in Petri dishes with moistened filter paper. The Petri dishes with puparia should then be held in small, ventilated, transparent cylindrical plastic cages (5.5 by 12.5 cm) until adult eclosion. Emerging tephritid flies should be provided with diet that consists of a volumetric mixture of 1:3 enzymatic yeast hydrolysate and sugar, and water in Petri dish with a layer of pumice granules. When flies develop full coloration (after 24-48 hr) they are identified, and each species kept in a separate cage and provided with food and water as described above.

6.2 Colony establishment and maintenance

When initiating a colony from wild population, the first 3 generations are crucial for successful establishment and extreme care is necessary to minimize mortality by avoiding stress related mortality resulting from overcrowding and exposure to suboptimal temperatures (>30 or <20°C, for tropical species). The starter colony should also not be based on smaller number of flies with very narrow genetic diversity. Use smaller cages of 30 x 30 x 30 cm at the initial establishment with 2-3 cages each with ~ 50-100 flies, of similar age. As the number of flies increase, they should be transferred to larger cages of 50 x 50 x 50 cm. Within several generations, laboratory colonies of fruit flies can undergo, considerable changes with respect to the conditions under which they are reared, through selection (Tsitsipis, 1983) followed by rapid adaptation. Genetic changes can also occurr within only 4 generations of laboratory rearing in artificial larval diet flies. Maintenance of reared flies in such a way that laboratory colonies are genetically similar with wild populations is a criterion for ensuring quality of the reared insects. However, Leppla and Ashley (1989) opined that maintenance strategies are intuitive primarily because criteria for deciding to change strains in colony have not been quantified. They suggested various options for retaining a strain, these include continuous rearing without altering the population

intentionally, rejuvenation by controlled selection, infusion with wild populations and hybridization of isolated lines. At the *icipe* facility, colonies are rejuvenated every 6-12 months by crossing wild males with laboratory reared females and at the same time crossing wild females with laboratory reared males. New cages are then set up with the progeny of the two cages. Therefore, the maintenance of vibrant colonies of fruit flies is a matter of careful rearing, diligent monitoring of quality control parameters and periodic strain restoration or replacement (Leppla, 1989). Although the process is laborious and costly, recent activities are exploring cryopreservation of embryos as described by Leopold (2007).

7. Evaluation of different oviposition devices for mass production of *Bactrocera invadens* and *Ceratitis cosyra*

Large quantities of eggs are usually required for mass production of fruit flies and an effective egg collection system is a prerequisite for mass rearing to reduce operational cost. In laboratory rearing, female fruit flies learn quickly to oviposit inside or through various substrates. To provide large number of flies for research and mass rearing of fruit fly parasitoids various egg collection systems were tested. The commonest egg collection device methods used for large-scale production of the most well known fruit fly, *C. Capitata,* is based on oviposition through screen into a trough containing water or moist blotter paper (Nadel, 1970). The process however requires genetic selection for screen-adapted flies which can take lengthy time period to adapt the insect to the laboratory procedure and Vargas (1984) opined that at mass production level, this may not be a straight forward process. Oviposition by *C. capitata* into perforated bottles and their subsequent removal from cages for egg collection has also been tested with significant levels of success (Tanaka 1965; Steiner and Mitchell 1966, Tanaka et al., 1970; Vargas 1984).

Using *B. invadens* and *C. cosyra*, we evaluated four egg collection devices namely mango dome, plastic bottle, funnel and plastic cup in order to select the most effective egg collection systems for mass rearing of these insects and other fruit fly species. Egg collection experiments were carried out using a 50 x 50 x 50 cm Plexiglas cages. Each device was perforated with 500 oviposition holes that were 1 cm apart. A 3 x 3 x 8 cm cellulose sponge saturated with 2% mango juice was placed in each perforated bottle to provide oviposition stimulus and prevent egg desiccation. Cages were then placed on a stand at ca. 1 m below two rows of 60-W fluorescent tubes. Oviposition cages were stocked with 500 ml of puparia. Four samples of 100 puparia were taken from each cage and held in small containers for determination of adult emergence and sex ratio. The results of which showed that in both species, adult emergence was > 85% and sex ratio was not different from the normal 1:1. The newly emerged adults were provided with water and a 3:1 volumetric mixture of sugar and enzymatic hydrolysate ultrapure. All cages were kept in a room maintained at 26- 28°C and 60 -70% RH under a photoperiod of L:D 12:12. The egging devices were exposed to sexually mature flies for a period of 4 hrs. Eggs were collected at 0900 h on days 8 through 17. The eggs were gently washed out of each egging device separately and the volume of eggs (ca. 12,000/ml for *B. invadens* and 13,000/ml for *C. cosyra*) obtained from each cage for each egging device was measured with a graduated cylinder. Three samples of 100 eggs were taken from the collection from each cage, spread with a Camel hair brush on moist blotting paper, and held in Petri dishes for determination of hatchability. This was replicated four times for each device. *Bactrocera invadens* readily accepted the 4 oviposition devices tested but to varying degrees. Over the 10-day standard egg collection period, the mean number of

eggs collected was significantly higher in the bottle and mango dome compared with the funnel and plastic cup devices (Fig. 1A). The mean percentage egg hatch was also marginally higher in the bottle compared with the other devices (Fig. 1B). In *C. cosyra*, the performance of the various devices followed the same pattern as in *B. invadens* with the flies significantly laying more eggs in bottles and mango dome than the other two devices although egg hatch was not affected by devices (Fig. 2A & B). The bottle egg collection device is adopted in Hawaii fruit fly mass rearing facility because of the protection it provides to eggs inside the saturated atmosphere of plastic containers with wet sponges (Tanaka, 1965; Tanaka et al., 1970). Vargas (1984) demonstrated that oviposition by *C. capitata* and egg hatchability was significantly higher in bottles than on the screen device. The Oriental fruit fly *Bactrocera dorsalis* (a close relative of *B. invadens*); and *B. cucurbitae* are also mass reared in Hawaii by use of bottle egg collection devices. At the *icipe* rearing facility, bottles have been found to be the best egg collection system and presently in use for mass rearing of the different fruit fly species. Increase female acceptance of this oviposition device has led to substantial reduction in labor and cost of mass rearing of the various fruit fly species.

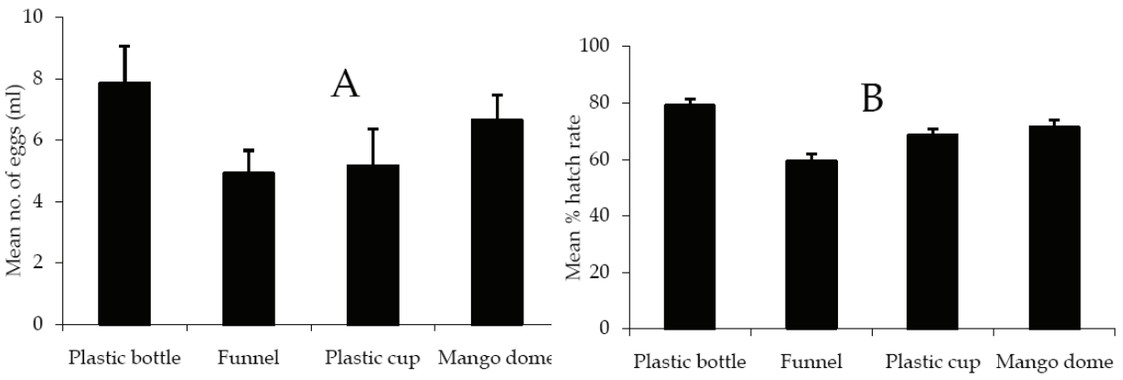

Fig. 1. Mean ± SE eggs collected (A) and percentage hatch rate (B) for four different devices in *Bactrocera invadens*

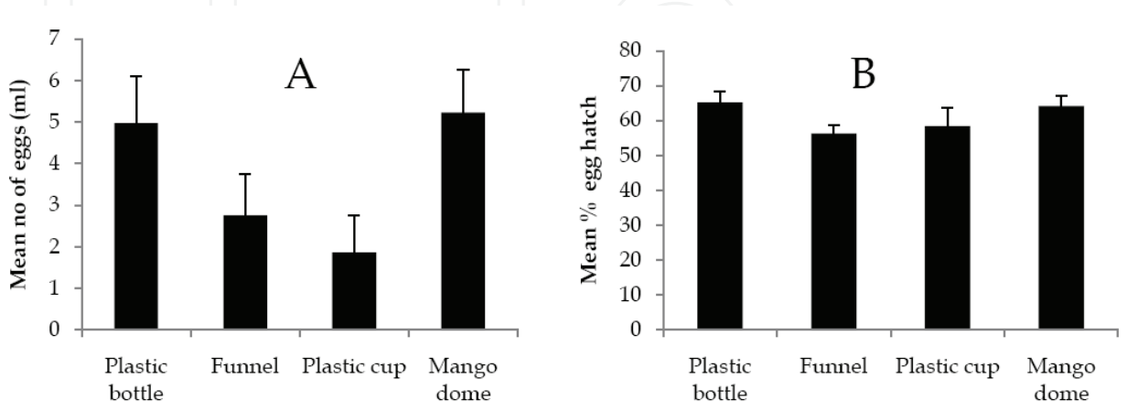

Fig. 2. Mean ± SE eggs collected (A) and percentage hatch rate (B) for four different devices in *Ceratitis cosyra*

8. Oviposition stimulant test

An important factor in the colonization process of insects is identification of a preferred oviposition stimulant which is important for egg deposition. Vargas et al., (1990) reported that fresh bell pepper juice stimulated oviposition by *Bactrocera latifrons*. We evaluated juices from different host plants of the target mango infesting fruit fly species for their ability to stimulate oviposition by the insects. Oviposition stimulant test was carried in a 50 x 50 x 50 cm Plexiglas cages. Plastic bottle similar to that used in the egg collection experiment above was used. A 3 x 3 x 8 cm cellulose sponge saturated with 100% juice of various hosts of the different fruit fly species as follows: *Bactrocera invadens* (mango, guava, *Terminalia catappa*, guava and marula), *C. cosyra* (mango, marula, guava and custard apple), *C. fasciventris* (mango, banana, guava, coffee, and citrus), and *C. rosa* (mango, *Monodora grandidieri, Uvaria lucida, Ludia mauritiana* and *Slacia elegans*). Sponges were placed in perforated bottle to provide oviposition stimulus and experimental procedures and egg collections were as previously described. In *B. invadens*, mean egg collection over a standard egg collection period of 14 days varied from 6.8-7.2 ml in mango, guava and marula and egg collection was 5.2 ml in *T. catappa*. When mango juice was compared at varying dilution rates of 1, 10, 50 and 100%, egg collection was at 3.5, 7.2, 5.8 and 7.0 ml, respectively. Vargas et al., (1993) showed that harvest of *B. latifrons* eggs from oviposition tubes that contained diluted papaya juices at 10% was 67% higher than from tubes that contained pure bell pepper juice which was the standard oviposition stimulant. They noted that with diluted juices, the humidity within the receptacle remained higher for a longer period of time compared with undiluted juices. In *C. cosyra*, juices of mango and marula at 100% elicited more egg laying (5.2 and 5.6 ml, respectively) compared with custard apple (2.1 ml) and dilution of custard apple juice had no effect on oviposition. Juices of mango, banana and coffee recorded 5.7, 6.1 and 5.8 ml of eggs, respectively in *C. fasciventris* compared with guava at 3.6 ml and citrus at 1.8 ml. Comparison of mango juice at varying dilution rates of 1, 10, 50 and 100%, resulted in *C. fascivntris* egg collection rates of 1.3, 5.8, 5.6 and 5.5 ml, respectively. Exposure of *C. rosa* adults to sponge cloth treated with juices of 4 different host plants in plastic bottles elicited oviposition but to varying degrees. Eggs laid in plastic bottles containing sponge cloth dipped in 100% juice of mango (6.2 ml), banana (5.8 ml) and *M. grandidieri* (3.9 ml) was higher followed by *U. lucida* (1.6 ml). Varying doses of mango had no effect on oviposition. In general, mango and banana juices seem to significantly elicit oviposition in all the target fruit flies perhaps not suprising given that these host plants are highly preferred by the target fruit fly species (Ekesi et al., 2006; Rwomushana et al., 2008; Mwatawala et al., 2010.). Fruits from different cultivars of both crops are also available all the year-round and banana is especially less expensive compared to mango and should serve as a readily available and convenient oviposition stimulants for mass production of the insects.

9. Adaptation of fruit flies to artificial rearing and quality control: case studies from *B. invadens* and other fruit flies

Conventional knowledge in fruit fly mass rearing demands that artificially reared flies produced for research should possess qualities and behavior nearly like insects in the wild (Calkins et al., 1994). However, laboratory colonization and mass production of fruit flies on artificial diet may require several generations for the insects to adapt to the artificial diet (Kamikado et al., 1987, Souza et al., 1988, Economopoulos 1992). In some cases, attempts to

raise a colony from wild populations on artificial diet have completely failed (Rössler 1975), but, if successful, long-term rearing on artificial diet may improve insect performance, e.g., through reduction in the pre-oviposition period and an increase in egg production (Vargas and Carey 1989). Ekesi et al., (2007) assessed the adaptation process of *B. invadens* to artificial diet when the flies were moved from rearing on whole mango fruits to a wheat-based artificial diet.

The insects that were used for the adaptation studies had originated from rotten mangoes collected at a local market in Nairobi, Kenya and maintained on whole mango fruits for five generations. Eggs of *B. invadens* were collected using a mango dome. The dome was placed into fly stock colonies for 1 h and eggs were carefully removed from the underside of the dome with a fine camel hair brush and then placed on a 9-cm diameter moist blotting paper. After 36 h, 100 newly emerged larvae from the above lots of eggs were gently introduced with a fine camel's hair brush onto the surface of 100 g of wheat-based diet (Table 1) in open 150 ml plastic cups or into artificially drilled holes on whole mango fruit. Each hole was perforated with an entomological pin and measured ~ 1 mm in diameter and 1 cm in depth. After inoculation with larvae, the fruit were placed in 200 ml plastic cups over a 1 cm deep layer of sand and the cups were covered with muselin cloth. Mature larvae exited the fruit and pupariated in the sand. Each of the artificial diet cups was placed in larger 250 ml plastic cups that contained a layer of heat-sterilized sand on the bottom as pupariation medium. Mature larvae left the artificial diet and entered the larger cups to pupariate. An experiment consisted of 4 cups of diet containing 100 larvae, with each group of 4 cups replicated 3 times or 4 fruits with the same number of larvae and replications. At every generation tested, records were kept on the following quality control parameters (1) adult survival expressed as % insect-days of 20 d (total possible d), (2) larval stage duration, (3) % puparia recovered, (4) weight of puparia, (5) percentage adult emergence, (6) flight ability, (7) fecundity over a 10-d period and (8) fertility. Adult survival was tested in 15 x 15 x 15 cm Plexiglas cages. Percent pupal recovery was calculated based on the initial number of larvae introduced into each container of rearing medium and pupal weight was based on four lots of 20 puparia from each replicate. Adult emergence was based on four lots of 20 puparia from each replicate that were placed in screened 12 cm diameter plastic containers and observed for a period of 21 d. The flight-ability test was conducted using flies emerging from four lots of 20 puparia from each replicate using the method of Boller et al., (1981). Fecundity and fertility were based on daily egg collections from 10 pairs of flies held after a pre-oviposition period of 7 d. Eggs were collected using a mango dome and egg hatchability was assessed after 72 h. In all experiments adults were fed on a diet consisting of 3 parts sugar and 1 part enzymatic yeast hydrolysate ultrapure, and water on pumice granules. All experiments were carried out in a room maintained at 26-28°C, 60 ± 70% RH with a photoperiod of 12:12 (L:D). Results showed that there were significant differences between the two rearing media tested for all quality control parameters evaluated. During the first three generations, survival of *B. invadens* was significantly lower on artificial diet compared with flies reared on whole mango fruits (Fig. 3A). Thereafter, no significant difference was observed between the media in the other generations. In *C. capitata* Leppla et al., (1983) reported that adult survival was reduced during the first three generations of colonization on artificial diet when a colony was initially kept on host fruits for the first five generations and then switched to artificial diet with a continuous decline in the following four generations, but recovering to normal levels thereafter. Economopoulos (1992) also observed

a reduction in adult survival of both sexes for three consecutive generations following colonization of new wild *C. capitata* colony with recovery to normal levels occurring in the fourth generation. Ekesi et al (2007) attributed the low survival to the reduced ability of flies to adapt to artificial diet and other rearing conditions, such as crowding. The authors did not observe any significant difference in larval developmental duration between the rearing media from the parent generation to generation F_3. However, in generation F_4 and F_5, development time was significantly shorter (7.8-8.2 d) on the artificial diet compared with that for the insects reared on whole mango (10.2-10.5 d) (Fig. 2B). With regard to pupal recovery, significantly less *B. invadens* were recovered on artificial diet between generations P to F_2 (Fig. 2C). From generation F_3 onward, the rate of pupal recovery was not significantly different between the two rearing media. At generations F_4 to F_5, pupal weight was higher on artificial diet compared with rearing on mango (Fig. 3D). Adult emergence and flight ability were not affected by the rearing media throughout the five generations tested (Fig. 3E &F). Over the generations, adult emergence ranged from 72-83% on artificial diet and 73 to 83% on mango. Flight ability varied from 75% to 85% on artificial diet and 72 to 82% on mango. Life history traits, including larval developmental period, are important quality control parameter in the evaluation of diet and the rearing process for fruit flies, and nutritional content of a diet can considerably affect development time, growth, and survival of fruit fly larvae (Krainacker et al., 1987, Vargas et al., 1994). With *B. invadens* Ekesi et al., (2007) observed a decrease in the length of larval development time in flies reared on artificial diet by generation F_4 and a corresponding increase in pupal weight at the same generation. Kaspi et al., (2002) demonstrated that the higher the protein level in larval diet the shorter the developmental time and the larger the size of individuals produced. The protein content in the artificial diet used in studies by Ekesi et al (2007) was higher than the quantity in the variety of mango used which could explain the shorter developmental time and higher pupal weight of *B. invadens*.

Bactrocera invadens fecundity over a 10-d period was found to be significantly lower (77 to 97 eggs) on artificial diet during generations P to F_2 compared with mango (101 to 124 eggs) (Fig. 3G). However, by generations F_3 to F_5, flies emerging from the artificial diet produced significantly more eggs (122 to 282 eggs) than insects reared on mango (109 to 117 eggs). During the first three generations, fertility was significantly reduced (34 to 61 %) on artificial diet compared with mango (70 to 84%). However, from generation F_3 onward, the egg hatchability did not differ significantly between the two rearing media (Fig. 3H). This study demonstrated that fecundity and fertility are affected during the adaptation process. It took four generations for the insects maintained on artificial diet to reach or surpass the level of fecundity recorded for flies reared on whole mango fruits. Economopoulos (1992) showed that even after nine generations, fecundity of wild *C. capitata* did not match the levels of laboratory-adapted flies. Ekesi et al., (2007) argued that the prolonged period of adaptation could be associated with poor mating, which the authors observed in flies maintained on artificial diet at the earlier stage of colonization. Previous studies with other fruit flies have demonstrated the importance of wheat and wheat-based products in fecundity and eclosion of larvae from eggs (Zumreoġlu et al., 1979, Vargas et al., 1994, Chang et al., 2006).

Leppla et al (1983) noted that the process of lab-adaptation of fruit flies is nonlinear with relative greater changes occurring initially and perhaps again later as the techniques are modified to increase rearing efficiency. Such 'crash-recovery cycle" was observed for *B. invadens* and it took three to four generations for the insect to adapt to artificial rearing

medium. The laboratory adaptation of any insect species is a function of their inherited ability to adapt to the new rearing media as well as the quality of the rearing effort during the colonization.

Souza et al., (1988) observed that at least 10 generations were needed for adaptation of *C. capitata* to artificial diet. In the olive fruit fly *Bactrocera oleae* (Gmelin), about three to four generations were required to adapt (Tsitsipis 1983), while in *B. cucurbitae* Coquillett, it took 14 generations to reach a permanent plateau (Kamikado et al., 1987). In the olive fruit fly, Loukas et al., (1985) found that colonization resulted in significant ADH (alcohol dehydrogenase), 6-PGD (6-phosphogluconate dehydrogenase) and HK (hexokinase) allozymes frequency changes within three generations of artificial rearing. Additionally, Economopoulos and Loukas (1985) reported that the selection factor responsible for the genetic changes was the artificial medium. It is therefore evident that several genetic, physiological and behavioural changes occur during colonization of wild populations of fruit flies and depending on the species could take several generations for the insect to fully adapt to the rearing medium (Economopoulos 1992).

10. Comparison of different solid-based artificial diet for larval rearing and quality control for different fruit flies

The identification of dehydrated plant material (i.e. carrot) and dry yeast as important ingredients for larval development has led to the production of viable colonies of different fruit fly species across the globe (Hooper, 1978; Tsitsipis, 1989; Walker et al., 1997). Evidence suggest that carotene (from carrot) is an important feeding stimulant that promotes growth, particularly during the early larval development (Fay 1989). Before the development of artificial larval diet for the various African fruit fly species under discussion, all the target insects were mass reared on whole fruits mainly mango, banana and coffee. In the whole fruit rearing process, flies are allowed to oviposit in the target fruits that are placed in Perspex cages. Infested fruits are transferred into Styrofoam boxes above a layer of sterilized sand, and puparia are separated from the sand by sifting. Mass production of fruit flies on fruits is expensive in addition to being labor-intensive and require a lot of space. Moreover, fruits are impractical to handle, difficult to standardize with respect to larval density, and prone to inconsistent rates of decomposition (Debouzie, 1978). Futhermore, unpredictability in fruit availability due to seasonal fluctuations can affect the whole production process hence the need to develop, test and adapt various artificial diets for mass rearing of fruit flies.

The wheat millfeed diet has been the standard diet of choice for fruit fly mass rearing in different parts of the world (Tanaka et al., 1965; Vargas et al., 1993; Walker et al., 1997). In addition to the millfeed as the bulking agent other ingredients includes yeast-based products, sugar, antimicrobial agents (nipagen, sodium benzoate, and streptomycin) and water (Tanaka et al., 1969). Water is particularly important for minimizing the effect of metabolic heat build-up during the final stage of larval development (Hooper, 1978). Fruit fly rearing and quality control parameters from millfeed diet have been satisfactory although the quality of millfeed can sometimes vary and adversely affect rearing process. A variety of factors such as the cultivar of wheat, spring or winter season, grain hardness, blends of bran and germ, and impurities can affect the quality of millfeed (Orth and Shellenberger 1988, Bass, 1988). Therefore, the need to test various bulking compounds in the mass rearing of fruit flies on artificial diet becomes important.

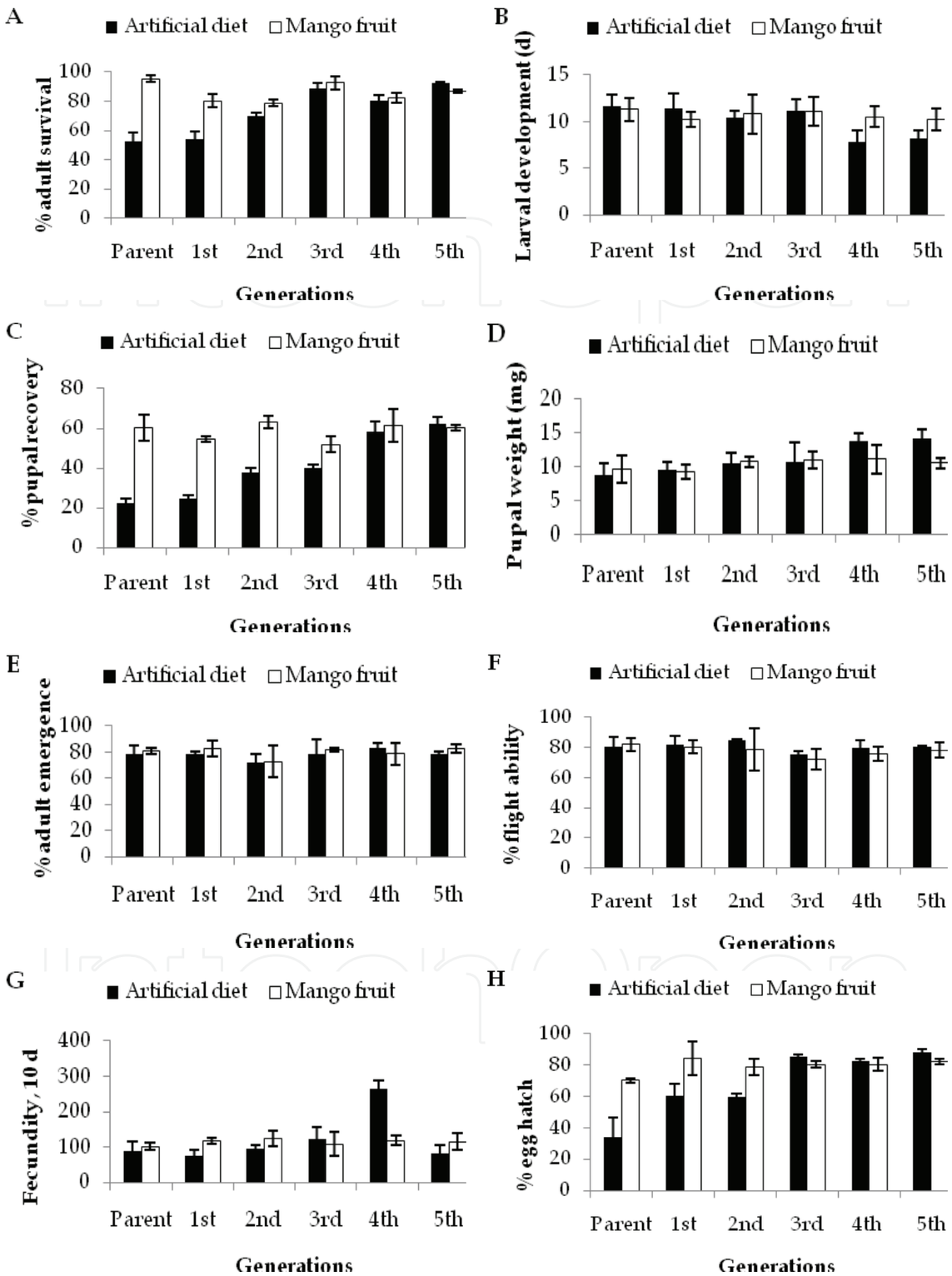

Fig. 3. Quality control parameters for *B. invadens* following adaptation to artificial rearing in the laboratory

Several bulking compounds including carrot, sugarcane bagasse, cassava, potato, corncob, paper, sawdust etc have been tested as alternative to millfeed in fruit fly mass rearing (Vargas et al., 1983; Minno and Holler, 1991; Stevens 1991, Vargas et al., 1994; Ekesi et al., 2007). These compounds absorb liquid when mixed in diets providing a uniform distribution of nutrients, physical consistency and texture, and serving as a substrate for larval feeding and development. In the Pacific Islands, the initial bulking agent used in the mass rearing of different *Bactrocera* species was cassava and sugarcane bagasse but fresh pawpaw has become the main ingredient in the mass production process because of its year-round availability (Walker et al., 1997). On the basis of these information, we explored different larval rearing media at the *icipe* mass rearing facility that are based on wheat, carrot, boiled cassava, sugarcane bagasse (*B. invadens, C. fasciventris* and *C. rosa*), and carrot supplemented with mango powder (*C. cosyra*) for mass rearing of the fruit fly species. The ingredients in the various diets are as presented in Table 1.

Ingredients	Solid-based						Liquid-based
	Wheat	Carrot alone	Carrot with mango A	Carrot with mango B	Sugarcane baggase	Cassava	
Torula yeast, g[a]	3.55	-	-	-	3.55	3.55	-
Brewer's yeast, g[b]	-	8.10	8.10	8.10	-	-	-
Lallemand yeast[c]	-	-	-	-	-	-	20.40
Methyl p-hydroxybenzoate, g[d]	0.11	0.20	0.20	0.20	0.20	0.20	0.20
Sodium benzoate, g[e]	0.11	-	-	-	-	-	0.20
Sugar, g[f]	7.83	16.20	16.20	16.20	16.20	16.20	12.18
Citric acid, g[g]	-	0.60	0.60	0.60	-	-	2.31
Mill feed, g[h]	31.20	-	-	-	-	-	-
Carrot, g[i]	-	24.20	21.20	18.20	-	-	-
Mango, g[i]	-	-	3.00	6.00	-	-	-
Sugarcane bagasse, g[f]	-	-	-	-	24.20	-	-
Cassava, g[i]	-	-	-	-	-	24.20	-
Wheat germ oil[j]	-	-	-	-	-	-	0.20
Water, ml	57.20	50.70	50.70	50.70	50.70	50.70	100

[a]ISCA Technologies, CA; [b]East African Breweries Limited, Nairobi; [c]Lallemand Inc. Bio-Ingredients (3 part whole cell yeast - LB12240 and 1 part hydrolyzed yeast -FNILS65); [d]Kobian Kenya Limited, Nairobi; [e]J.T. Baker Chemical Company, NJ; [f]Mumias Kenya Limited; [g]Chemoquip Limited, Nairobi; [h]Unga Limited, Nairobi; [i]Obtained as fresh fruit from hawkers market, Nairobi and then crushed, dried and blended into a powder; [i]Obtained as fresh roots from hawkers market, Nairobi and then boiled; [j]MP Biomedicals, Aurora, OH.

Table 1. Artificial larval diet ingredients used in the study

In this experiment, eggs (1 ml ~ 12, 000 eggs for *B. invadens* and ~ 13,000 eggs for *Ceratitis* spp.) collected within 1 hr after oviposition were seeded using a 1 ml transfer pipette onto a 5 x 5 cm strip of moist black cloth for each species separately. The cloth was placed on the center top of 1 kg of diet in 3 x 10 x 15 cm plastic trays. The experimental conditions were similar to the adaptation experiment described above. Upon hatching, larvae fed *ad libitum*

and the subsequent larval and pupal handling was the same as that described in section 9. All the mentioned quality control parameter above were also evaluated in this experiment and were measured in the same manner as described above.

Quality control parameters for the 4 diets tested and 4 fruit fly species are presented in Tables 2 & 3. For *B. invadens* pupal recovery was generally highest in wheat- (65%) followed by sugarcane bagasse- (55%) and carrot-based (54%) diets and lowest in the cassava-based diet (32%). Pupal weight was not significantly different among carrot-, wheat- and sugarcane bagasse-based diet and ranged from 13.6 to 14.1 mg. However, the pupal weight for flies reared on the cassava-based diet were significantly lighter (12.5 mg) compared to the other three media. Adult emergence was significantly higher in carrot- and wheat-based diet (80-86%) compared with sugarcane bagasse-based diet (70-77%) (Table 2). Fecundity over ten days period was also significantly higher in the carrot-, wheat- and sugarcane bagasse- (342-366 eggs per female) than in the cassava-based diet (233 eggs per female) (Table 2). In *C. fasciventris* and *C. rosa*, all quality control parameters from carrot and wheat outperformed those of sugarcane bagasse and cassava (Table 2). The cassava-based diet particularly did not support pupal recovery and egg production in both fly species.

Parameter	Carrot	Wheat	S/cane baggase	Cassava
Bactrocera invadens				
Pupal recovery (%)	54.2 ± 2.1b	65.4 ± 1.5a	55.4 ± 1.1b	32.1 ± 1.4c
Pupal weight (mg)	13.8 ± 1.1a	14.1 ± 1.3a	13.6 ± 1.3a	12.5 ± 1.6b
Adult emergence (%)	80.4 ± 1.7a	86.3 ± 2.3a	76.5 ± 1.2b	70.3 ± 2.2b
Flier (%)	82.2 ± 2.3a	81.3 ± 2.5a	76.8 ± 2.6a	80.4 ± 1.8a
Fecundity, 10-day	366.2 ± 11.2a	354.4 ± 12.7a	342.4 ± 14.1a	233.2 ± 7.8b
F_1 egg hatch (%)	80.2 ± 3.6a	74.4 ± 1.4a	70.3 ± 1.6b	58.4 ± 2.2c
Ceratitis fasciventris				
Pupal recovery (%)	38.5 ± 3.1a	40.8 ± 2.8a	20.2 ± 1.3b	11.1 ± 1.3c
Pupal weight (mg)	8.1 ± 0.6a	8.5 ± 0.1a	5.3 ± 1.4b	4.8 ± 0.9b
Adult emergence (%)	76.2 ± 1.0a	81.2 ± 4.1a	60.8 ± 2.5b	58.4 ± 1.7b
Flier (%)	78.8 ± 1.2a	80.8 ± 2.3a	64.2 ± 1.8b	65.5 ± 2.5b
Fecundity, 10-day	222.3 ± 26.2a	212.2 ± 18.7a	101.4 ± 13.8b	76.5 ± 10.4c
F_1 egg hatch (%)	72.4 ± 2.5a	68.8 ± 1.2a	51.2 ± 3.2b	48.1 ± 2.5b
Ceratitis rosa				
Pupal recovery (%)	54.4 ± 2.3a	50.2 ± 2.6a	22.4 ± 1.8b	13.3 ± 1.5c
Pupal weight (mg)	8.8 ± 1.2a	8.1 ± 1.1a	5.7 ± 0.9b	5.1 ± 1.1b
Adult emergence (%)	74.2 ± 1.4b	84.4 ± 2.6a	58.5 ± 2.2b	55.2 ± 3.1b
Flier (%)	81.2 ± 2.5a	80.0 ± 2.2a	70.1 ± 2.4b	68.9 ± 2.4b
Fecundity, 10-day	115.1 ± 10.5a	101.8 ± 12.1a	98.9 ± 8.6b	80.1 ± 12.3c
F_1 egg hatch (%)	64.5 ± 3.2a	60.8 ± 2.2a	40.2 ± 1.1b	40.5 ± 1.5b

Table 2. Effect of four artificial larval diets on pupal recovery, pupal weight, adult emergence, flier, fecundity, and egg hatch of *Bactrocera invadens, Ceratitis fasciventris* and *Ceratitis rosa*. Means within a row followed by the same letter do not differ significantly by Tukey HSD test (P=0.05)

Diets of carrot, carrot supplemented with mango and sugarcane bagasse also supported growth and reproduction by *C. cosyra* (Table 3). However supplementation of carrot with mango powder significantly increased pupal weight, egg production and to some extent egg hatchability compared to the other diets. Pupal weight was 10.5-11.7 mg in carrot supplemented with mango powder, 9.2 mg in carrot alone and 7.2 mg in sugarcane bagasse (Table 3). In fruit fly mass rearing, high pupal weight is considered a desirable characteristic in the production process, as it is a good indicator of flies' body size. Churchill-Stanland *et al.,* (1986) found that size of *C. capitata* was important in mating success and noted that 8 and 9 mg insects experienced greatest mating success followed by those that weighed 6 and 4 mg. Fly size is also a determinant factor of insect fertility and fecundity.

Overall, quality control parameters from carrot, carrot supplemented with mango and wheat were superior for mass rearing of *B. invadens, C. fasciventris, C. rosa* and *C. cosyra* compared with diets based on sugarcane bagasse and boiled cassava. In *B. invadens* the wheat-based diet seem to have an upper edge in terms of pupal recovery. In mass rearing of *B. latifrons*, Vargas and Mitchell (1987) found carrot to be less nutritious than wheat on the basis of female reproductive parameters. In the current study, apart from pupal recovery, all quality control parameters on both diets were uniform for the target insects. Based on these findings, *B. invadens*, *C. fasciventris* and *C. rosa* have successfully been maintained on a carrot-based artificial diet but in *C. cosyra* carrot is supplemented with mango to obtain appreciable quality control parameters. Although wheat millfeed is an equally effective bulking agent, a number of factors had favored the use of carrot over wheat. Firstly, carrot fruits are usually available all the year although prices can be exorbitant during the off-season period. Secondly, inconsistencies in the quality of wheat-based bulking agent associated with chemical pesticide residues have been observed. In most of the African countries, carrots are not treated with pesticides, thus free of residues. Thirdly, ingredients in the wheat-based diet are more expensive than those found in the carrot-based diet. Table 4 shows the weekly production data on carrot-based artificial diet for the 4 fruit fly species, and using the methodology described here healthy culture have been maintained at the *icipe* rearing facility.

Parameter	Carrot	Carrot supplemented with		Sugarcane bagasse
		3% mango	6% mango	
Pupal recovery (%)	37.8 ± 5.2a	40.4 ± 2.4a	48.4 ± 2.4a	20.1 ± 1.8b
Pupal weight (mg)	9.2 ± 1.8b	10.5 ± 3.2a	11.7 ± 1.3a	7.2 ± 1.3c
Adult emergence (%)	86.4 ± 4.4a	82.2 ± 6.2a	80.2 ± 3.6a	72.4 ± 2.3b
Flier (%)	84.2 ± 1.4a	80.5 ± 2.0a	80.4 ± 2.5a	82.8 ± 2.6a
Fecundity, 10-day	201.4 ± 10.2c	276.4 ± 22.2a	256.2 ± 31.4b	101.5 ± 24.8d
F_1 egg hatch (%)	62.4 ± 2.2b	68.2 ± 2.4b	75.2 ± 1.4a	54.2 ± 3.2c

Table 3. Effect of four artificial larval diets on pupal recovery, pupal weight, adult emergence, flier, fecundity, and egg hatch of *C. cosyra*. Means within a row followed by the same letter do not differ significantly by Tukey (HSD) test (P=0.05)

Parameter	*B. invadensa*	*C. cosyra**	*C. fasciventris*	*C. rosa*
Puparia	3012.1 ± 605.4	1134.5 ± 141.4	2561.8 ± 214.8	2984.2 ± 162.2
Pupal recovery (%)	55.3 ± 7.2	38.2 ± 4.8	38.8 ± 2.4	55.1 ± 4.2
Pupal weight (mg)	13.7 ± 2.2	10.8 ± 1.4	8.2 ± 1.1	8.4 ± 1.4
Adult emergence (%)	84.0 ± 2.2	72.2 ± 2.5	76.4 ± 1.8	75.8 ± 2.4

Each tray (3 x 10 x 15 cm) contained 500- g diet. *Supplemented with mango.

Table 4. Mean ± SE weekly production (n=28) of four fruit fly species per tray on carrot - based diets

11. Liquid-based artificial diet for larval rearing and quality control for four fruit fly species

Although we have been able to successfully rear all target fruit fly species on the solid-based artificial diets for over 100 generations, in recent years, the importance of liquid diet as an excellent substitute to solid based diet for fruit fly rearing is being promoted (Chang, 2009). The liquid larval diet is reported to have the following advantages over solid larval diet: (1) total consumption by flies if accurate proportion of diet volume to egg density are established thereby leaving minimal amount of spent diet (2) spent diet is water soluble, and can be simply rinsed off with a water gun (3) liquid-diet reared fruit fly larvae can develop in the same humidity/temperature controlled room, without the necessity of moving trays around thus providing more space for rearing (4) flies can be reared in smaller and shallower trays than those currently used for solid diet with each tray generating an equal amount of pupal production (5) lastly the most convenient inert bulking agent in the liquid which is the sponge cloth is mainly composed of natural cellulose and fiber. The sponge cloth is light weight with high water absorbance, and reusable, recyclable, biodegradable and also environmentally friendly. We evaluated the performance of our four target fruit fly species on liquid diet in comparison to the conventional solid based diet.

Liquid diet ingredients used were similar in composition to that described by Chang et al., (2006) for *Bactrocera dorsalis* (Hendel) larval rearing (Table 1). Each diet mixture was formulated by weighing all the ingredients and blending for 5 min in 1 litre electronic blender with the appropriate amount of water to ensure a homogenous mix of all the components. In the case of the liquid diet, plastic containers (3.2 x 13.5 x 20.6 cm) were rinsed with sterile distilled water and a piece of garden net (15 x 10 cm) was placed inside the plastic container. Sponge cloth (4 x 6 cm), the primary support matrix for larval feeding was rinsed 3 times with sterile distilled water, squeezed dry and placed above the garden net. The liquid diet mixture (150 ml) was then poured over the sponge cloth. In the case of the control solid diet, 500 g of diet was scooped from the blender and transferred into plastic trays of similar dimension as above. The initial pH at the end of the mixing and transfer of both diets was at 3.5.

Eggs (0.5 ml, ~ 6000 eggs in the case of *B. invadens* and 8000 eggs in the case of *Ceratitis* species) collected within 1 hr after oviposition were seeded using a 1 ml transfer pipette onto a 5 x 5 cm strip of moist black cloth. The cloth with the egg was placed in a tray on the center top of either a sponge cloth in liquid diet or the 500 g carrot-based diet. Upon

hatching the subsequent larval and pupal handling was the same as described in section 9 and quality control parameters measured as above.

Parameter	Carrot-based solid	Liquid-based
Bactrocera invadens		
Pupal recovery (%)	52.2 ± 2.1b	60.4 ± 7.3a
Pupal weight (mg)	12.8 ± 1.3b	13.8 ± 1.1a
Adult emergence (%)	76.5 ± 1.4b	92.4 ± 2.4a
Flier (%)	80.2 ± 2.5a	82.2 ± 1.2a
Fecundity, 10-day	209.8 ± 10.1b	214.2 ± 15.0a
F_1 egg hatch (%)	65.4 ± 1.4a	72.1 ± 4.1a
Ceratitis fasciventris		
Pupal recovery (%)	52.5 ± 5.1a	28.3 ± 2.4b
Pupal weight (mg)	7.3 ± 0.2a	6.3 ± 0.7b
Adult emergence (%)	70.8 ± 2.1a	70.2 ± 1.2a
Flier (%)	76.3 ± 2.4a	78.2 ± 2.1a
Fecundity, 10-day	98.5 ± 11.1a	65.8 ± 11.2b
F_1 egg hatch (%)	64.2 ± 1.6a	65.2 ± 2.2a
Ceratitis rosa		
Pupal recovery (%)	56.0 ± 2.8a	38.2 ± 1.2b
Pupal weight (mg)	8.5 ± 0.7a	6.8 ± 0.6b
Adult emergence (%)	72.2 ± 2.2a	65.6 ± 1.4b
Flier (%)	70.5 ± 1.3a	62.2 ± 1.4b
Fecundity, 10-day	95.6 ± 22.1a	58.4 ± 14.6b
F_1 egg hatch (%)	60.2 ± 1.4a	55.3 ± 1.8b
*Ceratitsi cosyra**		
Pupal recovery (%)	41.8 ± 1.2a	11.4 ± 1.4b
Pupal weight (mg)	11.7 ± 1.3a	5.5 ± 0.8b
Adult emergence (%)	76.4 ± 1.2a	56.5 ± 1.0b
Flier (%)	78.2 ± 1.4a	58.8 ± 2.6b
Fecundity, 10-day	142.2 ± 14.8a	77.9 ± 11.8b
F_1 egg hatch (%)	72.2 ± 1.6a	51.6 ± 1.4b

Table 5. Effect of solid and liquid artificial larval diets on pupal recovery, pupal weight, adult emergence, flier, fecundity, and egg hatch of *Bactrocera invadens, Ceratitis fasciventris* and *Ceratitis rosa.* Means within a row followed by the same letter do not differ significantly by *t* test (P=0.05). *Supplemented with 6% mango

Results showed that *B. invadens* reared on liquid diet had higher percent pupal recovery, heavier pupal weight, greater percent adult emergence and they were more fecund than those reared on solid carrot diet (Table 5). However, egg fertility and flight ability were not affected by diet. In the *Ceratitis* species in general, significant differences were observed between the liquid diet and the standard carrot-based solid diet especially with respect to pupal recovery, pupal weight and fecundity. *Ceratitis fasciventris* had a lower percent pupal recovery, lighter pupal weight, and lower fecundity when reared on liquid diet (Table 5). However, adult emergence, egg fertility and flight ability were not affected by diet type. In the other *Ceratitis* species, quality control parameters in the solid based diet were all superior to the liquid diet (Table 5). These results clearly indicate that the liquid diet can support the development of these insects but the insect probably require a longer period of adaptation to the diet to achieve the quality control parameters obtained from the solid diet. The rate of adaptation in laboratory rearing of insect depends on both the insect's ability to utilize the nutrients in the diet and also the quality of the rearing effort (Ekesi et al., 2007). The nutritional content of the liquid diet is quite high and has been found to be suitable for the development of other *Ceratitis* species such as *C. capitata* (Chang et al., 2007). Since fruit fly adaptation to artificial diet can vary with species (Souza et al., 1988; Tsisipis, 1983; Kamikado et al., 1987), it is likely that the *Ceratitis* species require a prolong period of adaptation to the liquid diet when compared with *B. invadens*.

12. Conclusions

Because artificially reared fruit flies used for research and management purposes must possess qualities and exhibit behaviours as close to that of wild insects as much as possible, quality control of mass reared insects is continuing to gain importance in mass rearing research. We have found that several oviposition stimulants are important for increasing egg deposition. Bottle egg collection system that have been used for producing related *Bactrocera* and *Ceratitis* species was identified as suitable for egg collection of all target fruit fly species. Using *B. invadens* as model insect to study adaptation of fruit flies to artificial diet, it was demonstrated that the process of adaptation for this insect, when moved from whole mango fruit rearing to artificial diet based on wheat-bran, took between three to five generations to reach the plateau of quality control parameters observed for rearing the insect on whole mango fruits. By comparing several solid-based artificial diets, it is concluded that diet based on carrot is the most suitable for mass rearing of all the target species based primarily on important quality control parameters (pupal recovery, pupal weight, adult emergence, flight ability and reproductive parameters), year-round availability of the bulking compound, absence of chemical impurities in carrot and low cost of other ingredients in the diet. The procedure allows for low-cost mass production system for all the target fruit fly species whereby 1135-3012 insects are produced per week for research and management purposes. *Bactrocera invadens* was also found to lend itself to mass rearing in liquid diet. Although the native *Ceratitis* species were able to successfully develop in the liquid diet, quality control parameters were consistently lower for all the species on liquid diet compared to the carrot-based solid diet. Long-term rearing on the diet may improve quality control parameters as the insect adapt to the liquid diet but additional research is required.

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Quality control is a standard which certainly has become a style of living. With the improvement of technology every day, we meet new and complicated devices and methods in different fields. Quality control explains the directed use of testing to measure the achievement of a specific standard. It is the process, procedures and authority used to accept or reject all components, drug product containers, closures, in-process materials, packaging material, labeling and drug products, and the authority to review production records to assure that no errors have occurred.The quality which is supposed to be achieved is not a concept which can be controlled by easy, numerical or other means, but it is the control over the intrinsic quality of a test facility and its studies. The aim of this book is to share useful and practical knowledge about quality control in several fields with the people who want to improve their knowledge.

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