

## MASS REARING OF *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE): A PRELIMINARY STRATEGY

H. E. JALDO<sup>1</sup>, M. C. GRAMAJO<sup>2</sup> AND E. WILLINK<sup>2</sup>

<sup>1</sup>Consejo Nac. de Invest. Científicas y Técnicas (CONICET)

Estación Experimental Agroindustrial Obispo Colombres (EEAOC), c.c.9, (4101) Las Talitas, Tucumán, Argentina

<sup>2</sup>Estación Experimental Agroindustrial Obispo Colombres (EEAOC), c.c.9, (4101) Las Talitas, Tucumán, Argentina

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), occurs from Mexico to Argentina and attacks some 80 species of host plants, including mango, citrus, guava, apple and coffee (Da Silva et al. 1996). In extensive fruit producing regions of Uruguay, Argentina and Peru, the only two fruit fly species of economic and quarantine importance are *A. fraterculus* and the introduced Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Manso & Lifchitz 1992). Unfortunately, there is as yet no environmentally friendly and effective strategy as Sterile Insect Technique (SIT) to use against *A. fraterculus*. If no reliable and economic methods were found, any possible benefits from *C. capitata* control in areas where it is sympatric with *A. fraterculus*, would be greatly reduced. The ability to mass-rear *A. fraterculus* is the key to development of SIT. At the *A. fraterculus* mass rearing Workshop held in 1996 at Viña del Mar, Chile, various participants from Argentina, Brazil, Colombia and Peru reported on their efforts to rear this fruit fly under laboratory conditions (Ortiz 1999). It was agreed that the main limiting factor to successful mass rearing was the need to develop a technique that would promote effective oviposition, facilitate collection of the eggs, and assure maximum fertility of the eggs (Salles 1992, 1999)

Here we describe a new method to potentially mass-rear *A. fraterculus* that produces high egg fertility and allows eggs to be collected easily with a minimum handling.

**REARING ROOM:** Rearing conditions were  $23 \pm 2^\circ\text{C}$  and 60-80% R.H., light intensity ranged from 4,000 to 5,000 lx, with a photoperiod of 12:12 (L:D).

**CAGES:** The colony was kept in iron-framed cages ( $0.96 \times 0.60 \times 0.30$  m), with a front and rear panel. The rear panel was covered with a fabric (voile) with 25 holes per linear centimeter. The front panel was made of the same fabric coated with a thin layer of transparent silicon rubber (0.5 mm thickness). This panel is very similar to that used for *A. obliqua* in Mexico (Dominguez, 1998). One or 2 days before emergence, 8,500 pupae were placed in each cage. After 10-14 days adults mated and begun oviposition. Adults were kept in the cages for 40 days.

**EGG COLLECTION:** Females laid their eggs through the oviposition panels onto foam rubber

sheets ( $0.01 \times 0.90 \times 0.60$  m), which were moistened with a mixture of water and peach juice (3:1) to avoid the dehydration and to attract females to oviposit. After 24 h the foam rubber sheets were taken out and washed in water to collect the eggs. The eggs were placed in a wet chamber (petri dishes with wet filter paper in the bottom) and kept at a temperature of  $23\text{-}26^\circ\text{C}$  until hatching. After 48 h the eggs began to hatch.

**LARVAL DEVELOPMENT:** The diet described by Salles (1992) was used with the addition of streptomycin sulfate at rates of 1 g per thousand to avoid bacterial contamination. Two hundred grams of diet was poured over the trays ( $18 \times 12$  cm) 2 cm deep. After 48 h the eggs were placed on the larval diet at a density of 8 to 10 eggs per gram of diet. The trays were placed in racks and covered with a fine voile mesh to prevent contamination by *Drosophila* spp. Wet sand was incorporated in the bottom of the racks. The larvae developed in the diet and, 16 days later, they crawled out of the trays and buried in the sand to pupate.

**PUPATION:** The pupae were collected and maintained, in a small container with sterilized wet sand. Fifteen days later they were introduced in cages to begin a new cycle.

**ADULTS:** Adults were maintained on a mixture of yeast hydrolyzed enzymatic 10 g; corn protein 10 g; sugar 40 g; water 50 ml; vitamins (Dayamineral, Abbott) 500 mg; aminoacids (Aminocefa 5%, Roux Ocefa) 1 ml. Water was also supplied to the adults.

This rearing has been carried out over 18 generation (F18) without problems.

**QUALITY CONTROL:** The quality of insects was assessed using some of standard quality control based in IAEA, USDA, FAO Quality Control publication (IAEA, USDA, FAO. 1998) and Orozco et al. (1983).

**REARING PARAMETERS:** Results of tests mentioned above are shown in Table 1. The main differences between our rearing technique and 3 previously published methods are shown in Table 2. The four rearing techniques used different oviposition devices. Nuñez & Guzman (1999) and Salles (1992, 1999) used colored hemispheres or domes to attract the female fruit flies and to stimulate oviposition, but they had to be placed inside the cage, which made handling difficult. Using

TABLE 1. REARING PARAMETERS OF THREE GENERATION TAKEN IN ACCOUNT TO EVALUATE REARING OF *ANASTREPHA FRATERCULUS*, TEST WITH EXPERIMENTAL DIET MENTIONED IN A TEXT.

Parameters	F3	F8	F12
Fertility (%)	84 ± 5.3*	75 ± 3.8	81 ± 2.4
Egg-pupa recovery (%)	44.9 ± 7.05	48.6 ± 3.0	46 ± 5.2
Weight of 100 pupae (g)	1.8 ± 0.2	1.5 ± 0.2	1.4 ± 0.3
Adult emergence (%)	68.5 ± 19.62	61 ± 15.9	65 ± 12.3
Male:female ratio	1:0.98	1:1.51	1:1.23

\*Average ± SD.

oviposition panels (as in Gonzalez et al. 1971, and our technique), allowed a much easier egg collection from outside the cages. In our case, collecting the eggs on rubber foam sheet kept the eggs hydrated, and improved egg hatch.

All adult diets used similar ingredients, a protein source plus sugar, but we found that the combination of hydrolyzed protein and corn protein was the best for female fecundity and egg hatch (Table 2).

The pupal weight obtained was slightly lower than the one obtained in Colombia by Nuñez & Guzman (1999).

Survival from pupa to adult was significantly better in Peru (Gonzalez et al. 1971) and Colombia (Nuñez et al. 1999).

The survival rate from egg through to adult obtained in our rearing was 44%. In Colombia survival to the adult stage was only 9.5%. In Peru 50.5% survival was achieved in small scale laboratory rearing but when insect were mass reared, survival dropped to only 5.3%.

This rearing methodology results in a more efficient egg collection with a good survival rate through all life history stages. Future studies will have the focus on refining and improving this new methodology, and larger scale testing, following small-scale test replication.

We gratefully acknowledge the excellent suggestions and review by Dr. Pablo Liedo Fernández from Ecosur, México; Dr. Pablo Cancino and Biol. Emilio Hernández from MOSCAFRUT, CONASAG-DGSV, Mexico.

## SUMMARY

A new technique for mass rearing *A. fraterculus* (Wiedemann) was developed. Use of silicon rubber on the cage wall encouraged oviposition and allowed and easy egg collection. When adults were reared on a diet of hydrolyzed enzymatic yeast and corn protein, females laid eggs with 83% successful development, which resulted in a feasible mass rearing process.

TABLE 2. COMPARISON BETWEEN OUR REARING TECHNIQUE AND THREE PREVIOUSLY PUBLISHED METHODS FOR *A. FRATERCULUS*.

	Salles (1992)	Gonzalez et al. (1971)	Nuñez and Guzman (1999)	Jaldo et al.
Oviposition devices	Fruit juices + agar covered with Parafilm	Red nylon cloth	Colored paraffin dome	Silicon white cloth
Adult diet	Hydrolyzed corn protein + brown sugar	Hydrolyzed brewers yeast + sugar	Hydrolyzed brewers yeast + Sugar	Hydrolyzed corn protein + sugar + hydrolyzed brewers yeast
Egg hatch %	20-70%	45%	66%	84%
Egg/Female	394	415	—	625
Larval diet basic ingredients	Brewers yeast wheat germ	Torula yeast carrot powder	Torula yeast wheat germ	Brewers yeast wheat germ
Pupal weight (100 pupae)	—	1.3 g	2.0 g	1.8 g
Pupal survival %	—	99.15%	76%	68.5%
Egg-Pupae Recovery %	—	5.3%	9.5%	44%

## REFERENCES CITED

- DA SILVA, N. M., S. SILVEIRA NETO, AND R. A. ZUCCHI. 1996. The natural host plant of *Anastrepha* in the state of Amazonas, Brasil. pp. 353-357. In B. A. McPherson and G. Steck (eds.). Fruit Fly Pest: A World Assessment of their Biology and Management. St. Lucia Press, Boca Raton, FL.
- GONZALEZ, J., C. VARGAS, AND B. JARA. 1971. Estudios sobre la aplicación de la técnica de machos estériles en el control de la mosca sudamericana de la fruta, *Anastrepha fraterculus* (Wied.). Revista Peruana de Entomología 14(1): 66-86.
- MANSO, F., AND LIFCHITZ. 1992. Nueva metodología genética para el mejoramiento de la eficiencia de la técnica del macho estéril en el control de la mosca del mediterráneo *Ceratitis capitata*. Ciencia e Investigación 44(4): 225-228.
- NÚÑEZ, L., AND R. GUZMAN. 1999. Avances sobre la cría artificial de *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) en Colombia. The South American fruit fly, *Anastrepha fraterculus* (Wied.); advances in artificial rearing, taxonomic status and biological studies. Proceedings of a workshop organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Viña del Mar, Chile, 1-2 November 1996. IAEA-TECDOC-1064: 85-94.
- ORTIZ, G. 1999. Introduction. The South American fruit fly, *Anastrepha fraterculus* (Wied.); advances in artificial rearing, taxonomic status and biological studies. Proceedings of a workshop organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Viña del Mar, Chile, 1-2 November 1996. IAEA-TECDOC-1064: 1-2.
- OROZCO, D. H. S., A. SCHWARZ, AND A. PEREZ. 1983. Manual de procedimientos de control de calidad. Programa Mosca del Mediterraneo. Secretaria de Agricultura y Recursos Hidráulicos, D.G.S.V. 137 pp.
- DOMÍNGUEZ, J. C. 1998. Métodos de cría masiva de moscas de la fruta. XII Curso Internacional sobre moscas de la fruta: 379-394.
- IAEA, USDA, FAO. 1998. Product Quality Control, Irradiation and Shipping procedures for Mass-reared Tephritid Fruit Flies for Sterile Insect Release Programs. 1-1 to 12-2.
- SALLES, L. A. B. 1992. Metodologia de criacao de *Anastrepha fraterculus* (Wied., 1830) (Diptera: Tephritidae) em dieta artificial em laboratorio. Anais da Sociedade Entomologica do Brasil 21(3): 479-486.
- SALLES, L. A. B. 1999. Rearing of *Anastrepha fraterculus* (Wiedemann). The South American fruit fly, *Anastrepha fraterculus* (Wied.); advances in artificial rearing, taxonomic status and biological studies. Proceedings of a workshop organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Viña del Mar, Chile, 1-2 November 1996. IAEA-TECDOC-1064: 95-100.