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Authors: Vera, Teresa, Abraham, Solana, Oviedo, Andrea, and Willink, Eduardo

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DEMOGRAPHIC AND QUALITY CONTROL PARAMETERS OF *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) MAINTAINED UNDER ARTIFICIAL REARING

TERESA VERA[1], SOLANA ABRAHAM, ANDREA OVIEDO AND EDUARDO WILLINK
Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombres
EEAOC, CC 9 (4101) Las Talitas, Tucumán, Argentina

[1]Member of Carrera de Investigador, CONICET, Argentina

ABSTRACT

The integration of the sterile insect technique (SIT) in the management of the South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) is a promising alternative to chemically-based control in those areas where it is sympatric with *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) or other tephritid species for which the SIT is being used. Implementation of the SIT requires the development of a cost effective mass-rearing protocol. In this work, we present demographic and quality control parameters for the *A. fraterculus* strain reared at the Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina. Considering the rearing cage as the reproduction unit, we observed that fecundity is optimal during the first 3 weeks after the onset of oviposition. Fertility was constant during this period. During 2003 and 2004, some improvements were made to the existing rearing protocol, which resulted in increased larval viability, pupal weight, and adult emergence. Current weekly egg production is 1 million per week. These eggs are used to maintain the colony and to assess quality parameters. Finally, research needs leading to improved yields and fly quality are discussed.

Key Words: *Anastrepha fraterculus*, sterile insect technique, mass-rearing, larval viability, fertility, fecundity

RESUMEN

La integración de la Técnica del Insecto Estéril (TIE) en el combate integrado de la mosca Sudamericana de la fruta, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), es una alternativa interesante para reemplazar al control químico en aquellas zonas donde esta especie es simpátrica con *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) u otros tefrítidos para los que ya se utiliza la TIE. La implementación de la TIE requiere del desarrollo de un protocolo de cría masiva que sea costo-efectivo. En este trabajo presentamos parámetros demográficos y de control de calidad de la cepa criada en la Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina. Considerando a la jaula de cría como unidad reproductiva, se observó que la fecundidad es óptima durante las tres primeras semanas de iniciada la oviposición y que la fertilidad se mantiene constante durante ese período. Durante 2003-2004 se implementaron mejoras en el protocolo de cría existente lo que resultó en un incremento de la viabilidad larvaria, del peso de pupas y del porcentaje de emergencia de adultos. La producción actual semanal es de un millón de huevos. Los mismos son utilizados para mantener la colonia y realizar distintos estudios de calidad de esta cepa. Por último, se sugieren necesidades de investigación para alcanzar mejores rendimientos.

Translation provided by the authors.

The South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) is a serious pest that occurs from the southern United States (Texas) to Argentina (Salles 1995; Steck 1998). It attacks over 80 species of plants, including major fruit crops, and represents a serious threat in some fruit production areas. In addition, its presence results in quarantine restrictions for fresh fruit exports by importing countries (Steck 1998). At present the only control method available is the use of bait sprays. This presents a problem in areas where it coexists with other fruit fly pests against which the sterile insect technique (SIT) is being used. Such is the case for some regions in Argentina, where *A. fraterculus* is sympatric with the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). In such situations, the application of the SIT against *A. fraterculus* can be considered an attractive alternative (Ortíz 1999).

One paramount prerequisite for SIT implementation is the development of cost-effective

mass-rearing protocols. Large-scale mass rearing has not been achieved for *A. fraterculus*. Efforts to colonize this species and to develop mass rearing methods have been reported in many countries (Ortíz 1999). Major constraints were related to oviposition and egg fertility. A preliminary mass-rearing strategy was developed by Jaldo et al. (2001). This procedure followed a simple egg collection method with minimal handling and high egg fertility. However, egg to pupae recovery was not optimal and rearing parameters reported were obtained from small-scale rearing in Petri dishes with egg densities lower than those used in the routine maintenance of the colony. Additional demographic parameters were published by Salles (1992) and Jaldo (2001).

Although individual females are responsible for total fecundity and fertility of the colony, under mass rearing conditions, demographic parameters such as egg production are more informative when the rearing cage is considered as the production unit.

In this work, we evaluate the fecundity and fertility of the *A. fraterculus* colony maintained at the Estación Experimental Agroindustrial Obispo Colombres in Tucumán, Argentina and provide rearing and quality control parameters.

Materials and Methods

Colony Maintenance

The colony established at the Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina, was derived from infested guavas collected in 1997 from the vicinity of Tafí Viejo, Tucumán province (northwestern Argentina). Since then, no wild material has been introduced into the colony. Rearing conditions were those proposed by Jaldo et al. (2001) with minor modifications, mainly related to humidity conditions in the rearing room and egg seeding density.

Adult colony cages were set up with 10,000 pupae and provided with water and adult food, which consisted of a mixture of hydrolyzed yeast, corn protein, and sugar (1:1:4) with a supply of vitamins and amino acids (Jaldo et al. 2001). Cages were held in a rearing room with 25 ± 1°C and a photoperiod of 12:12 (L:D). Humidity control was difficult during 2002-2003, and ranged from 50 to 90%, but normally it was at the lower values. From Jan 2004, with the addition of a new humidifier, the relative humidity in the rearing room was higher and more stable (80 ± 10% R.H.).

Egg collection began 1 week after adult emergence and eggs were collected 3 times per week. Females laid their eggs through one of the panels of the cage that was coated with a thin layer of silicon rubber (hereafter referred to as the oviposition panel). To avoid dehydration, moist foam rubber was applied to the outer side of the oviposition panel and held with clips to a transparent polycarbonate panel which was attached to the cage. After 24 h the foam rubber was removed and eggs were collected from the oviposition panel with the aid of a rubber sponge. The volume of eggs obtained for each cage was measured. Eggs were air bubbled in water within a plastic bottle (v/v ratio of 1:100) for 48 h and later seeded on larval diet (Salles 1992).

Seeding density decreased from 34 eggs/g of diet in 2003, to 22-15 eggs/g of diet during Jan-May 2004, to 11 eggs/g of diet from Jun 2004 onwards. Egg seeding density was reduced due to an increase in egg-pupae recovery and hence a need to avoid larval overcrowding. Approximately at d 7, larvae started leaving the diet and pupated in sand placed in a pan below the trays. Two d after pupation began; sand was sieved to remove pupae. The same pupal collection procedure was repeated twice. Pupae were placed in trays with a thin layer of sand and held 10-12 d for maturation. Two d before emergence, the total pupal production was weighted. Each week 2 batches of 120 g of pupae were prepared to set up 2 new adult holding cages. From Jun 2004, 2 batches of 45,000 eggs each were seeded per week to obtain enough pupae (around 60,000) to maintain the colony; any remaining eggs were discarded.

Demographic Analysis

Egg production was estimated by considering the total number of eggs collected during the lifespan of the production cages. Egg collection was carried out for the period of 4 weeks, for a total of 12 collections per cage. For each cage in each collection the volume of eggs collected was measured and the number of eggs obtained in each cage for each collection was determined by multiplying the volume of eggs (in mL) in each collection by 14,900, which was estimated as the number of eggs in 1 mL. In addition, from each cage in each egg collection, 3 samples of 100 eggs were placed in a Petri dish with moistened sponge to assess egg hatch over the oviposition period. This procedure was repeated in 7 cages from Oct to Nov 2003. Differences in mean values of egg hatch along the collections dates were tested by means of ANOVA with InfoStat (2004).

Quality Control Parameters

Several parameters related to the process of rearing and the quality of the pupae produced were estimated, including egg hatch, egg to pupae recovery, larval viability, number of eggs obtained per cage in each collection, number of eggs obtained per female in each collection, weekly egg and pupal production, pupal weight, adult emergence, and sex ratio. Egg hatch was determined 3 d after eggs were placed in Petri dishes containing a moistened cotton sponge. The number of unhatched eggs was counted

and the percentage of egg hatch was estimated. Egg to pupae recovery was estimated as the number of pupae recovered in each batch/eggs seeded × 100. For larval viability, the formula considered the number of viable eggs (obtained from the percentage of egg hatch). The number of eggs per female in each egg collection was determined by dividing the number of eggs obtained in each cage by the number of estimated females per cage. The number of females per cage was estimated from the amount of pupae used to set up the cage, the percentage of adult emergence, and the sex ratio. No mortality was assumed during this collection period.

Weekly egg production was determined by adding the number of eggs obtained for each cage in each of the 3 collections performed each week. Pupal weekly production was determined considering all the pupae obtained each week from the different batches. Pupal weight was assessed from 3 samples of 100 pupae weighed 2 d before adult emergence. These pupae were kept without food and water until all flies emerged and died. The number of emerged flies of each sex was recorded taking into account whether they were non-deformed, deformed, or partially emerged. Adult emergence and sex ratio were estimated from these figures as explained in the international fruit fly quality control manual (FAO/IAEA/USDA 2003). According to the changes in seeding density implemented from 2002 to the present, rearing and quality control parameters were estimated for 4 periods: 2002, 2003, Jan-May 2004 and Jun-Aug 2004. Egg hatch, egg to pupae recovery, and larval survival were determined for each pupal batch.

Results and Discussion

Fecundity and fertility for each egg collection during the period Oct-Nov 2003 is presented in Table 1. Fecundity over the 4-week period totaled 413,179 ± 53,026 eggs per cage. Considering the period in which this value was obtained, as well as the percentage of emergence, pupal weight, and sex ratio, the number of eggs per female along the complete oviposition period (i.e., until the cage was discarded) was 102 eggs. In other studies values from 394 eggs per female (Salles 1992) to 625 eggs per female (Jaldo et al. 2001) have been recorded. Our value is underestimated since it assumes no mortality during the collection period; and as mentioned before, values reported previously come from studies in which eggs were collected during the complete reproductive period of females confined in small cages and under relaxed, non-crowded, conditions. As such, they neither provide an estimate of the number of eggs to be collected per female or cage, nor an informative figure for the rearing facilities regarding the optimal time to discard the production cage. The eggs from the first 3 weeks of collection represented approximately 90% of the total collected (Table 1). During the fourth week the production dropped and it is expected that collecting for longer periods, where flies are more than 35 d old, would not increase overall egg production. This, and the fact that old cages are sources of fungal and mite infections in the rearing rooms, prompts us to suggest that 3 weeks of egg collection is optimal for a rearing facility, hence production cages should be discarded on d 28.

For *Anastrepha obliqua* (Macquart), *Anastrepha ludens* (Loew), and *Anastrepha serpentina* (Wiedemann), 41, 64, and 36 d, respectively, have been proposed as the life of the production cage (Liedo & Carey 1994). These periods take into account the amount of pupae that will be harvested in the facility to be released in the field and have been estimated from wild flies, which probably explains the higher values obtained (Liedo & Carey 1994). Our suggestion is more in agreement with

Table 1. *Anastrepha fraterculus* fertility and fecundity from Oct to Nov 2003: mean number of eggs (± SEM) collected in each egg collection and percentage of egg hatch.

Week	Collection	Number of eggs/cage[1]	Cumulative percentage	Egg hatch (%)
First	1	40,443 ± 9,437	9.8	76.0 ± 5.6
	2	46,214 ± 8,076	21.0	76.8 ± 6.7
	3	45,551 ± 8,321	32.0	85.8 ± 3.6
Second	1	46,829 ± 8,003	43.3	86.3 ± 1.8
	2	43,849 ± 5,067	53.9	87.9 ± 1.3
	3	43,423 ± 7,786	64.5	85.2 ± 3.0
Third	1	40,656 ± 11,025	74.3	87.2 ± 2.5
	2	38,101 ± 8,963	83.5	87.5 ± 2.6
	3	25,543 ± 5,775	89.7	88.4 ± 1.1
Fourth	1	17,241 ± 4,940	93.9	85.6 ± 3.2
	2	10,856 ± 2,383	96.5	82.2 ± 2.8
	3	14,474 ± 3,230	100.0	80.0 ± 4.3
	Mean	34,432 ± 3,895		84.1 ± 1.2

[1]Adult colony cages were set up with approximately 10,000 pupae which produced approximately 4,060 females.

values proposed by Carey & Vargas (1985), obtained from other tephritids, which were already adapted to mass rearing conditions, as well as the 21 and 17 d used in the Moscafrut Metapa facility in Mexico to mass rear *A. ludens* and *A. obliqua*, respectively, (Artiaga-López & Hernández, pers. comm.). More recent values obtained from Jun-Aug 2004 revealed that females laid approximately 135 eggs during the first 3 weeks of oviposition before the cage was discarded (Table 2). This value increased, compared to the one obtained during the trial in 2003, as a result of an improvement of some rearing parameters such as pupal weight, which resulted in larger and probably more fecund females (see below).

During the 4 weeks of egg collection from Oct to Nov 2003, egg hatch averaged 84.1 ± 1.2% (Table 1). Although for the first collections mean values were lower, probably due to oviposition of virgin females, there were no statistical differences among the collections (F = 1.39; df = 11,56; P > 0.05). Because the differences are not significant, and the production of eggs during the first week is important, it is not recommended to discard these eggs nor to start egg collection later.

Rearing and quality control data are presented in Table 2. The higher humidity in the rearing room from Jan 2004 may have been responsible for the higher egg to pupae recovery, because the larval diet did not dry out during the first days in which first instars require high humidity. This led to the need to reduce the seeding density from 34 eggs/g of diet to 22-15 eggs/g of diet. By Jun 2004, it was decided to seed eggs at an even lower density (11 eggs/g of diet). To prevent water loss from the diet, trays were covered with a polystyrene tray for the first 5 d of larval development. Covering rearing trays to prevent dehydration is a common practice in other insectaries and for other *Anastrepha* species (Pinsón et al. 1993). Subsequently, trays were uncovered and sugarcane bagasse was added to the diet to allow larvae to crawl outside the diet. Because the diet uses agar as a gelling agent (Salles 1992), we found that some larvae become stuck in the diet when trying to leave to pupate. The addition of sugarcane bagasse, which is produced locally as a by-product of the sugar industry, provides an adequate substrate for the larvae to crawl out of the diet and enhances larval recovery. In all, changes applied to the rearing conditions (higher and more stable relative humidity in the rearing room, lower seeding density and handling during the larval development), resulted in the increase of the viability, recovery, and quality control parameters (Table 2).

Egg to pupae recovery was higher than the 44% reported by Jaldo et al. (2001), even when those values were obtained from batches of 100 eggs seeded on Petri dishes under very relaxed conditions. Our values under such favorable conditions are approximately 83% recovery. Besides the increase in larval viability, the reduction in egg seeding density resulted in an increase in pupal weight and adult emergence. A gain in pupal weight may have resulted in larger and probably more fecund females. This may also explain the increase in the number of eggs collected per female as shown for other tephritids (Krainacker et al. 1989; Liedo et al. 1992).

Although larval viability improved, more improvement is still needed. The results obtained in the small-scale trials suggest that nutrients are not affecting the viability, but probably the structure of the diet. Finding the optimal moisture level and a good bulking agent to maximize intake of nutrients from the diet is an important goal to achieve. The present diet, which uses agar as a gelling agent, is too expensive for mass-rearing, and presents the added drawback of being difficult for larvae to leave and pupate. Sugarcane bagasse can be used to help the larvae leave the diet, it is locally available from the sugarcane in-

TABLE 2. REARING AND QUALITY CONTROL PARAMETERS OF THE *ANASTREPHA FRATERCULUS* COLONY MAINTAINED DURING 2002-2004 AT THE ESTACIÓN EXPERIMENTAL AGROINDUSTRIAL OBISPO COLOMBRES, TUCUMAN, ARGENTINA.

Parameter	2002	2003	Jan-May 2004	Jun-Aug 2004
Egg hatch (%)	74.1 ± 4.2	84.9 ± 1.3	83.1 ± 1.1	84.4 ± 0.9
Egg-pupae recovery (%)	—	29.3 ± 1.7	37.9 ± 2.1	56.3 ± 2.1
Larval viability (%)	—	33.8 ± 1.2	49.3 ± 3.8	66.6 ± 2.3
Eggs/cage/collection	—	28,221 ± 3,181	43,822 ± 3,099	63,323 ± 3,183
Eggs/female/collection	—	6 ± 1	11 ± 1	15 ± 1
Weekly egg production	—	83,531 ± 11,077	691,740 ± 41,580	1,074,425 ± 43,733
Weekly pupae production[1]	—	—	124,963 ± 17,754	55,894 ± 4,559
Pupal weight (mg)	10.9 ± 0.4	11.3 ± 0.4	12.0 ± 0.3	13.1 ± 0.2
Non-deformed adult emergence (%)	73.0 ± 2.5	77.3 ± 1.8	75.8 ± 3.2	85.0 ± 2.5
Adult emergence (%)	77.1 ± 2.3	81.6 ± 1.7	80.0 ± 3.1	88.6 ± 2.6
Male:female ratio	0.85 ± 0.03	0.95 ± 0.03	0.97 ± 0.04	0.93 ± 0.05

[1]From Jun 2004 onwards only 90,000 eggs per week were seeded; this explains the drop in the production of pupae.

dustry in the region, and it is free from insecticides. However, preliminary studies (unpublished data) have shown that yields with bagasse are not as good as those obtained with agar, indicating more improvement is still possible.

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