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PRODUCTION AND QUALITY ASSURANCE IN THE SIT AFRICA MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) REARING FACILITY IN SOUTH AFRICA

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ABSTRACT

A mass-rearing facility for Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) was commissioned in Stellenbosch in 1999 to produce sterile male fruit flies for a sterile insect technique (SIT) project in commercial fruit orchards and vineyards in the Western Cape province of South Africa. The mass-rearing procedure was largely based on systems developed by the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria. A number of genetic sexing strains were used to produce only males for release. Initial cramped rearing and quality management conditions were alleviated in 2001 with the construction of a new adult rearing room and quality control laboratory. In 2002 a comprehensive Quality Management System was implemented, and in 2003 an improved genetic sexing strain, VIENNA 8, was supplied by the FAO/IAEA Laboratory in Seibersdorf. For most of the first 3 years the facility was unable to supply the required number of sterile male Mediterranean fruit flies for the SIT program without importing sterile male pupae from another facility. From mid-2002, after the quality management system was implemented, both production and quality improved but remained below optimum. After the introduction of the VIENNA 8 genetic sexing strain, and together with an improvement in the climate control equipment, production stability, and quality assurance parameters improved substantially. The critical factors influencing production and quality were an inadequate rearing infrastructure, problems with the quality of the larval diet, and the initial absence of a quality management system. The results highlight the importance of effective quality management, the value of a stable and productive genetic sexing strain, and the necessity for a sound funding base for the mass-rearing facility.

Key Words: genetic sexing strain, mass rearing, Mediterranean fruit fly, sterile insect technique, quality management

RESUMEN

La facilidad para criar en masa la mosca mediterránea de la fruta, *Ceratitis capitata* (Wiedemann) fue comisionada en Stellenbosch en 1999 para producir machos estériles de moscas para el proyecto de la técnica del insecto estéril (TIE) en huertos de frutos y viñas comerciales en la provincia del Cabo Occidental del Sudáfrica. El procedimiento de criar en masa fue en su mayor parte basado en los sistemas desarrollados por el Laboratorio de Agricultura y Biotecnología de la FAO/IAEA, Seibersdorf, Austria. Un número de razas que separara los sexos genéticamente fueron utilizadas para producir solo machos para la liberación. La congestionada condición inicial para criar las moscas y su manejo de calidad fueron aliviadas en 2001 con la construcción de un nuevo cuarto de cría para adultos y un laboratorio de control de calidad. En 2002, un Sistema de Manejo de Calidad comprensivo fue implementado, y en 2003 una raza mejorada que separa los sexos genéticamente, VIENNA 8, fue proveído por el Laboratorio de la FAO/IAEA en Seibersdorf. En la mayor parte de los primeros 3 años la facilidad no pudo suplir el número requerido de machos estériles de la mosca mediterránea de la fruta para el programa de TIE sin la necesidad para importar machos estériles de otra facilidad. Desde medio del año de 2002, después que el sistema de manejo de calidad fue implementado, la producción y la calidad mejoraron pero aún quedaron por debajo del nivel óptimo. Después de la introducción de la raza VIENNA 8 que separa los sexos genéticamente, y junto con el equipo mejorado de control de clima, la estabilidad y los parámetros de seguridad de calidad mejoraron substancialmente. Los factores críticos que influyeron en la producción y la calidad fueron la infraestructura inadecuada para criar las moscas, problemas con la calidad de la dieta para las larvas y la ausencia inicial de un sistema de manejo de calidad. Los resultados muestran claramente la importancia de un manejo efectivo de la calidad, el valor de una raza productiva que separa los sexos genéticamente y la necesidad de contar con una base sólida de financiamiento para la infraestructura de una cría en masa.

The export deciduous fruit industry is of great economic importance to South Africa. Nearly 90 million cartons are exported annually, with total earnings of approximately US\$1 billion per annum. The Western Cape is the most important region for the production of deciduous fruit, with approximately 58,000 ha under cultivation (Optimal Agricultural Business Systems 2005).

The Western Cape is host to 2 species of tephritid fruit flies of economic importance, the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann), and the Natal fruit fly *Ceratitis rosa* (Karsch). Between them, they attack a wide variety of subtropical, tropical, and deciduous fruits (Annecke & Moran 1982). Both species are international quarantine pests with the potential to restrict international fruit trade with South Africa. Further details of their occurrence, behavior, and management in the Western Cape is given by Myburgh (1964) and Barnes (1994). It has been estimated that crop losses and control costs due to fruit flies in the Western Cape alone exceed US\$3.2 million per annum (Mumford & Tween 1997). While the economic impact of tephritid fruit flies country-wide has not been determined, the impact on the South African export fruit industry of a quarantine embargo on South African fruit due to the presence of fruit flies would be devastating. For the South African export fruit industry to remain viable, the creation of fruit fly-free or low prevalence areas is therefore an urgent necessity. The sterile insect technique (SIT), integrated with other measures, is widely regarded as the most practical and cost-effective means of establishing such areas.

A pilot project to suppress *C. capitata* in an isolated export table grape production area in the Western Cape, the Hex River Valley, with an SIT component was initiated in 1997. Sterile *C. capitata* were produced in a mass-rearing facility located at the Infruitec-Nietvoorbij Fruit, Vine and Wine Research Institute of the Agricultural Research Council (ARC) in Stellenbosch, with a number of different *temperature-sensitive lethal* (*tsl*) genetic sexing strains (Franz 2005). Production at the facility started in Apr 1999, with aerial releases of sterile males over 10,000 ha starting in Oct that year. Further details of the pilot project are described by Barnes et al. (2004). Aerial releases over the entire 10,000 ha were later replaced by ground releases due to the high cost of aerial releases in view of the relative small release area.

The SIT operations were not supported financially by the national government, although the provincial government sporadically funded the mass-rearing facility. As a result, the facility was initially financed through a formal SIT Partnership between the ARC, which coordinated the SIT component of the program, and the Deciduous Fruit Producer's Trust, a fruit-grower organiza-

tion. Continued lack of national support, together with the inability of the SIT Partnership to continue funding the mass-rearing facility, led to the commercialization of the production and distribution of sterile medflies via SIT Africa (Pty) Ltd. in 2003. The SIT programme has since expanded to 2 other production areas, and at the time of writing a total of 6 million sterile male medflies per week were being ground-released, specifically targeting backyards and host plants, over a total fruit production area of 15,600 ha.

Production volumes and quality parameters of sterile medflies produced by the rearing facility were well below optimum and varied a great deal during the course of the program. This article describes the rearing process, the genetic sexing strains used, and the production and quality parameters achieved over a period of 1 to 5 years, and discusses causes of the poor rearing performance and the factors that led to improved production and quality in the facility.

MATERIALS AND METHODS

Genetic Sexing Strains

A number of genetic sexing strains obtained from the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria, were used to produce sterile male medflies. In such strains, the females carry a *tsl* mutation that results in their mortality as embryos by heat treatment so that females can be eliminated before mass rearing of the males destined for sterilization and release (Franz et al. 1994). This results in greater cost-effectiveness as only the males are the active agent in the SIT (Hendrichs et al. 1995). In addition, the females are homozygous for the mutation *white pupae* (*wp*). This allows the integrity of the sexing system and the accuracy of the temperature treatment to be monitored and is required for a Filter Rearing System to manage the mother colony (Fisher & Cáceres 2000).

The genetic sexing strain VIENNA 7-97 was initially used when mass-rearing started in Apr 1999. This strain was replaced in Aug/Sep 1999 with a refreshed genetic sexing strain, VIENNA 7/Mix-99 (Fisher 1999) that was initially used for the first sterile male releases that started in Oct 1999. Due to genetic instability of this genetic sexing strain under local rearing conditions, especially in the absence of a filter rearing system, the colony strain was replaced three times between May 2000 and Dec 2001, with strains VIENNA 7/Tol 2000 in May 2000, VIENNA 7/Mix 2000 in Nov 2000, and VIENNA 7-D53/Mix 2001 in Dec 2001 (Robinson et al. 1999).

A filter rearing system to control the accumulation of genetic recombinants (Franz 2002) in the genetic sexing strain was set up in mid-2000. In

this system, recombinant individuals (females in brown pupae and males in white pupae; termed 'wrong sex') are removed from a mother colony maintained under more relaxed conditions. Production of sterile males with a filter rearing system comprised three reproductive 'streams'—the filter (or mother colony), amplification 1, and amplification 2. A fourth non-reproductive male-only (or release) stream produced all the males for sterilization and release (Fisher & Cáceres 2000).

In Sep 2003 a new genetic sexing strain with improved production and quality potential, VIENNA 8, was provided to the SIT Africa Facility by the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria. This facility was the first operational *C. capitata* facility to be provided with this strain.

Production and Release Procedure

Production. Initially, an old, disused building at the Pest Management Division of ARC Infruitec-Nietvoorbij was refurbished and used as a sterile fruit fly production facility. In Apr 2001, a new building was erected to house the adult colony and a quality control laboratory, alleviating cramped rearing conditions in the old building and providing for better management of quality control. This raised the maximum production potential to an estimated 10 million sterile males per week.

The mass-rearing procedure was largely developed by the FAO/IAEA Laboratory in Seibersdorf. Larvae were reared on the following artificial diet: digestive bran (28.75%), torula yeast (7.00%), white sugar (13.00%), sodium benzoate (0.25%), 30% hydrochloric acid (1.50%), formalin (0.08%) and water (49.43%). The pH of the diet was buffered to between 3.2 and 3.5. Five kg of diet were placed in each rearing tray, and 3.2 mL of eggs (for the colony stream) and 12.5 mL of eggs (male-only stream) were seeded per tray on an egg raft of toilet tissue.

After seeding, the trays were moved to Larvae Room #1 (25°C, 90-100% R.H.). After 3 d the trays were moved to Larvae Room #2 (22°C, 75-80% R.H.). After 3 d, trays were then moved to Larvae Room #3 (20°C, 65-70% R.H.), where mature larvae left the medium and were collected in water-filled gutters for either 5 d (colony production) or 2 d (male-only production). Each day's larval collection was mixed with fine vermiculite and kept at 20°C and 80-85% RH for pupation.

In the adult room, eggling cages measuring 0.74 m × 0.84 m in cross section and 2 m in height were surrounded on all 4 sides with fine mesh screen through which the females oviposited. The cages were mounted on wheels that ran on rails in a water bath. Each cage was loaded with 3.2 liters of pupae at a male:female ratio of 1:3, resulting in a total cage content of approximately 186,600

flies. Adult food consisted of a 1:3 mixture of enzymatic yeast hydrolysate (Separations, Johannesburg, South Africa) and sugar, and water was provided through soft cloths protruding through pipes filled with water. Eggs oviposited through the screen sides fell into the water bath that was drained once a day and the eggs were collected in a sieve. Each cage was kept in production for 12 d. Conditions in the adult room were maintained at 25°C and 60-65% R.H., with a photoperiod of 15.5:8.5 (L:D). All eggs were bubbled in water containing 0.1% sodium benzoate for 48 h under the same conditions. Eggs for the male-only stream were additionally heat-treated at 34°C for 16 h, which killed the female eggs.

Irradiation. Male pupae were sterilized 1 d before adult emergence (based on eye color; FAO/IAEA/USDA 2003) with 90 Gy from ⁶⁰Co in the ARC Infruitec-Nietvoorbij walk-in irradiator. It was equipped with a sealed point-source of ⁶⁰Co (Mayak Production Association, Ozyorsk, Russia) stored in a below-floor, lead-filled drum that was raised hydraulically to the required level when needed. The dose-rate was 5.5 Gy/min, initially determined with Fricke dosimeters and verified by Gafchromic dosimetry (IAEA 2004). Pupae packed in plastic bags (sample size = 180 mm × 150 mm; volume = 5 L) were irradiated under hypoxia on a rotating table (diameter 1.0 m; 60 s for 1 rotation) fixed around the vertical axis of the ⁶⁰Co source lifting rods. Eight rotating discs (diameter 0.2 m; 20 s for 1 rotation) were built into the table around its perimeter. The resulting dual rotation of the pupae facilitated optimum dose distribution throughout the sample. The dose was verified by Sterin® or RadTag® indicators in each container of pupae.

Irradiated pupae were dyed with Day-Glo® fluorescent dye (Radiant Color, Houthalen, Belgium) and were placed in paper bags (110 mL per bag) in Plastic Adult Release Containers ("PARC boxes"). During the period of aerial releases (VIENNA 7 genetic sexing strain) this yielded approximately 4,250 fliers/bag at 65% flight ability. During later ground releases (with VIENNA 8 genetic sexing strain) an improved flight ability of 75% yielded approximately 5,000 fliers/bag.

Food for the flies was provided by means of cakes of food grade agar plus sugar placed onto gauze vents on top of each PARC box for aerial releases, or for ground releases, by brown paper strips soaked in the agar and sugar mixture and placed into each bag. After poor performance of the food strips, food was later provided more effectively by means of small agar and sugar cakes in plastic containers (50 mm diameter, 20 mm deep) placed in the bottom of the bag.

Release. Details of aerial releases over the Hex River Valley are given by Barnes et al. (2004). These were replaced by ground releases in Jun 2003. For ground releases, bags of sterile flies

were transported to the release areas from 3 to 5 d after emergence and released the following day. All flies were released in fruit fly host plants in gardens and backyards, and in any other neglected fruit trees, at a density of 2,000 flies per hectare (Ortiz-Moreno 2002). This release system has resulted in effective suppression of *C. capitata* in the Hex River Valley at a reduced cost to the growers (I. Sutherland, SIT Africa [Pty] Ltd., Stellenbosch, South Africa, personal communication).

The targeted nature of ground releases resulted in a decrease in demand for sterile medflies in the Hex River Valley to 1.4 million sterile flies per week. In Dec 2003 releases of sterile flies started in a second area (Elgin, Grabouw, Vyeboom and Villiersdorp) increasing the demand to 4.2 million sterile flies per week. In Aug 2004 a third area (Riebeeck Valley) joined the SIT program, and the total requirement for releases was increased to 6 million sterile flies per week. The facility's production output goal was not reduced following the decrease in demand for sterile flies—the facility management decided rather to have an output safety 'cushion' to accommodate any unexpected decrease in production.

Production Performance and Quality Assurance

All rearing procedures and quality control measurements were carried out according to the international fruit fly quality control manual (FAO/IAEA/USDA 2003). Up to Dec 2001 the facility had no formal quality management system. Following consistent problems encountered with production volumes and sterile fly quality, a comprehensive Quality Management System was introduced in Jan 2002.

The quality management system covered two main aspects: production and quality assurance. A weekly review meeting evaluated each of these aspects according to set targets as follows: (1) Production (all reproductive streams)—number of adult cages set up, egg production (mL per day), number of trays seeded, number of pupae irradiated, number of sterile flies delivered; (2) Quality control (all streams)—egg hatch (%; 0 h and 48 h), egg to pupa efficiency (%); flight ability (%), genetic recombination (males in white pupae; %), females in the male-only stream (%), sterility of males for release (% fertility). Definitions of these parameters and the methods of assessment are given in FAO/IAEA/USDA (2003). Other quality parameters, e.g., of raw diet ingredients and water, were not at that point incorporated into the quality management system.

For the purposes of this article only the following production parameters are discussed: production—daily egg production, and number of pupae irradiated per week; quality assurance—egg hatch (48 h), egg to pupa efficiency in the male-only stream, flight ability of sterile males, and

percentage females in the male-only stream (as an indication of genetic recombination of the genetic sexing strain). Some records from the earlier part of the programme, before the new quality control laboratory was established, are incomplete and the data unreliable. Results are therefore given from as far back in each case as they were considered reliable. Data are presented as means \pm SD.

RESULTS

Egg Production

Daily egg production with the genetic sexing strains VIENNA 7/Mix 2000 and VIENNA 7-D53/Mix 2001 from Jan 2001, and with genetic sexing strain VIENNA 8 from Aug 2003 to Sep 2004, is illustrated in Fig. 1a and b. A target of 495 mL of eggs per day was initially set in order to achieve production levels of five million sterile males per week for aerial releases over the Hex River Valley. As illustrated by Fig. 1a between Jan 2001 and Aug 2002, this target was seldom achieved. From Aug 2002 the target was exceeded virtually without exception. After the introduction of VIENNA 8, the daily egg production target was reduced to 360 mL per day due to the better egg to pupa efficiency of this strain. Fig. 1b shows that with VIENNA 8, this target also was exceeded on all but one occasion (Feb to Mar 2004). Relatively wide fluctuations in egg production from Sep to Dec 2003 narrowed noticeably thereafter, probably as a result of adaptation by the new strain to local conditions.

Egg Hatch

Egg hatch after 48 h in the male-only stream is illustrated in Fig. 2a (VIENNA 7-D53/Mix 2001) and Fig. 2b (VIENNA 8). Except for a sharp drop in Sep/Oct 2002, egg hatch in the VIENNA 7 strain fluctuated (mean = $55.3 \pm 9.65\%$) for most of the reported period until Mar 2003, when it dropped an average of 5% below the target level. In the case of VIENNA 8, mean egg hatch was somewhat lower at $38.8 \pm 11.01\%$, but very close to the target of 40% for this strain (C. Cáceres, FAO/IAEA Biotechnology Laboratory, Seibersdorf, Austria, personal communication).

Egg to Pupa Efficiency

Egg to pupa efficiency in the male-only stream is illustrated in Fig. 3a (VIENNA 7D53/Mix 2001) and Fig. 3b (VIENNA 8). The target egg to pupa efficiency for the VIENNA 7 strain was 12%. Although the FAO/IAEA (2002) target for the VIENNA 8 strain is 20%, the rearing facility set an interim target of 16%. Efficiency in the VIENNA 7 strain fluctuated between 5 and 15%, with a

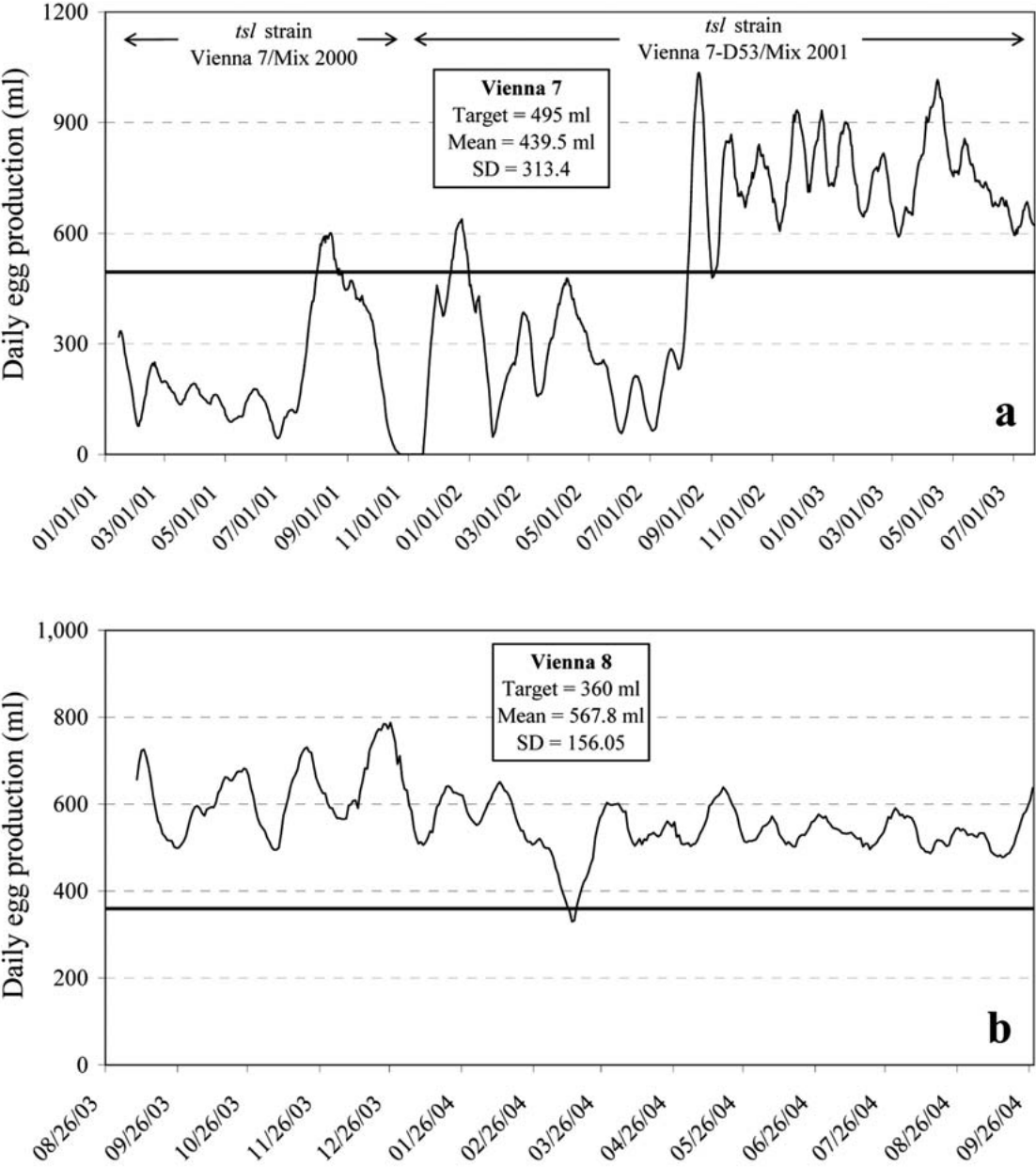


Fig. 1. Daily egg production (14-day moving average) by two genetic sexing strains of *C. capitata*; (a) by two strains of VIENNA 7 from Jan 2001 to Jul 2003, and (b) by VIENNA 8 from Aug 2003 to Sep 2004.

mean of $11.2 \pm 4.60\%$ and was characterized by three periods of substantial decreases in efficiency. There were only 2 periods of relative stability in efficiency, between May and Sep 2002 and Mar and Jul 2003. After the introduction of the VIENNA 8 strain, egg to pupa efficiency immediately improved to a mean of $16.8 \pm 4.18\%$. Two significant decreases in efficiency occurred

from Dec 2003 to Jan 2004 and from Mar to May 2004, both as a result of equipment malfunction.

Number of Pupae Irradiated per Week

The number of pupae irradiated per week from Oct 1999 to Sep 2004, as an illustration of production of sterile flies by the facility, is presented in

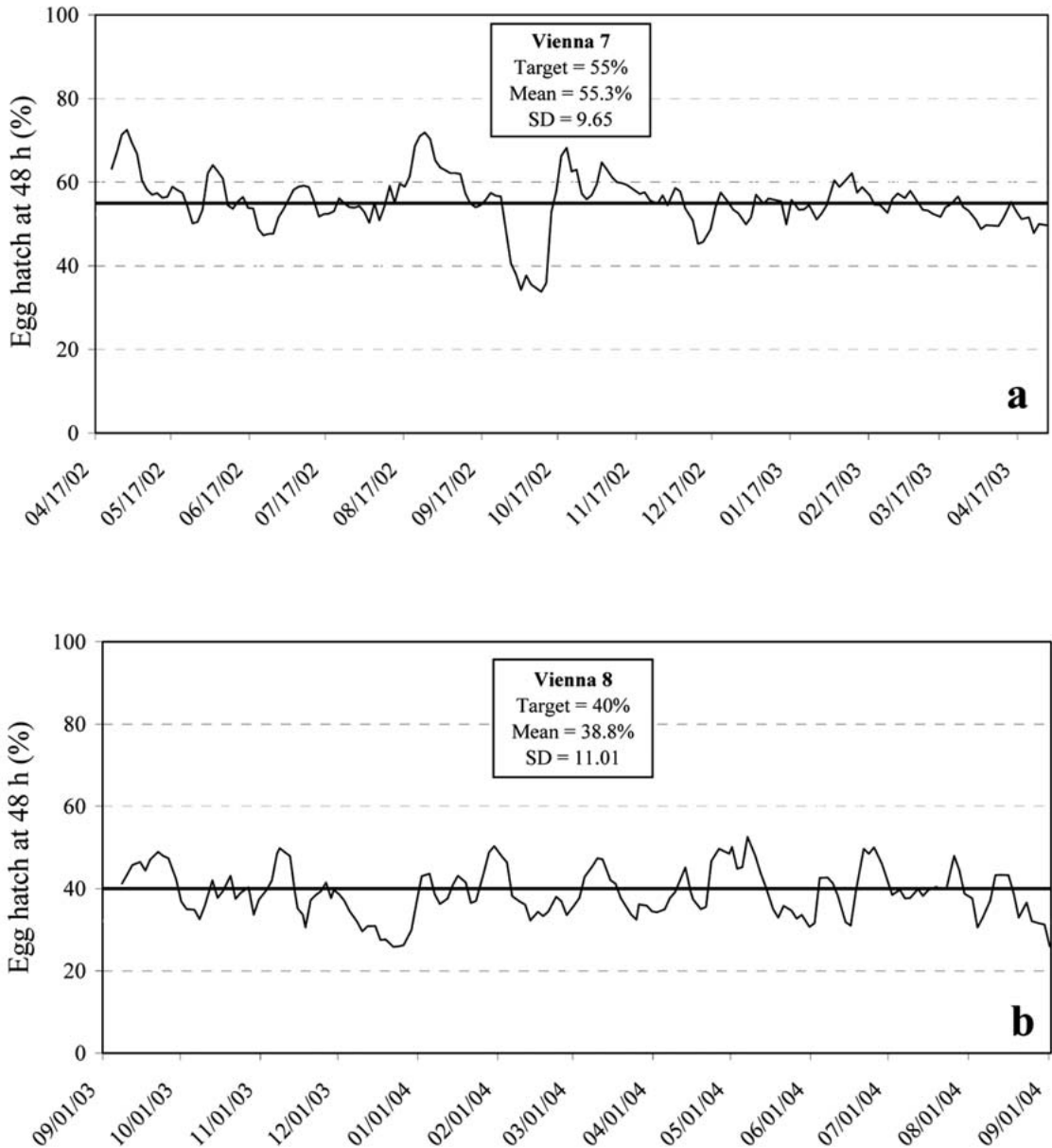


Fig. 2. Egg hatch at 48 h (7-day moving average) of two genetic sexing strains of *C. capitata*; (a) VIENNA 7-D53/Mix 2001, from Apr 2002 to Jul 2003, and (b) by VIENNA 8 from Sep 2003 to Sep 2004.

Fig. 4. With few exceptions, production by the facility was very poor and variable during the first two and a half years until mid-2002. On only three occasions, Jun and Aug 2001 and Nov/Dec 2002, did production match demand. During this period, sterile *C. capitata* pupae were frequently imported from the El Pino facility in Guatemala to supplement aerial releases. From Aug 2002, production steadily increased to more than 8 million sterilized

pupae per week for the next 10 months, albeit with 1 major slump in Jan and Feb 2003. In Jun 2003 production was deliberately decreased to approximately one million sterilized pupae per week when aerial releases were changed to ground releases. Production was then increased to 4 million and later 6 million sterilized pupae per week between Dec 2003 and Aug 2004 to accommodate additional fruit production areas implementing SIT.

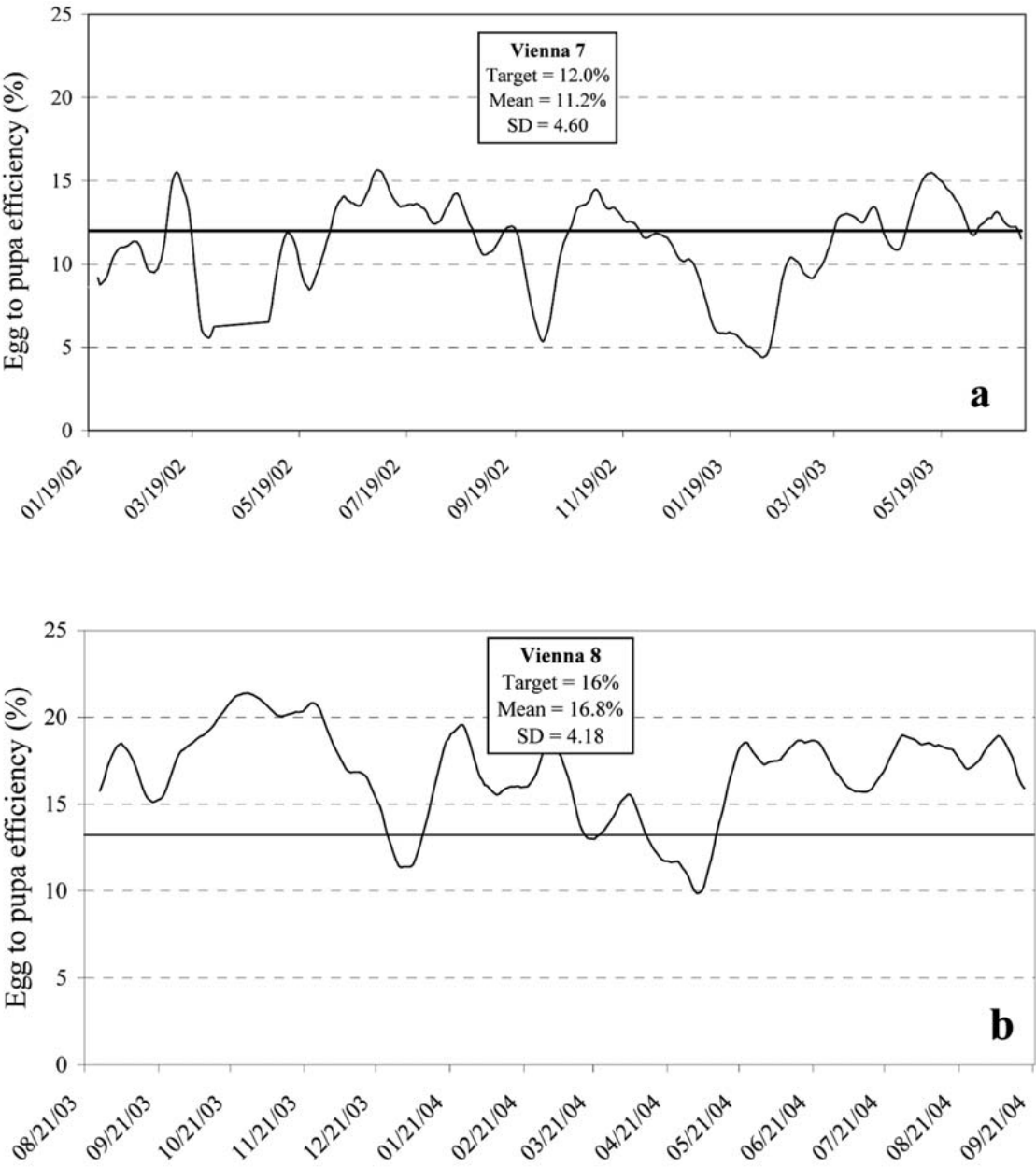


Fig. 3. Percentage egg to pupa efficiency (7-day moving average) in the male-only stream for (a) VIENNA 7-D53/Mix 2001 genetic sexing strain from Jan 2002 to Jul 2003, and (b) VIENNA 8 genetic sexing strain from Aug 2003 to Sep 2004.

Flight Ability of Sterile Males

Flight ability of sterile males is given in Fig. 5a (VIENNA 7-D53/Mix 2001) and Fig. 5b (VIENNA 8). The target flight ability for the VIENNA 7 and VIENNA 8 strains was 75% (C. Cáceres, Seibersdorf, Austria, personal communication). A target

of 82% for the VIENNA 8 strain is specified by FAO/IAEA (2002), but this is for the smaller colony reared by the IAEA at Seibersdorf. Flight ability for the VIENNA 7 strain fluctuated between 60 and 85%, with a mean of $73.0 \pm 10.92\%$. Flight ability improved with the VIENNA 8 strain, increasing to a mean of $78.3 \pm 7.37\%$.

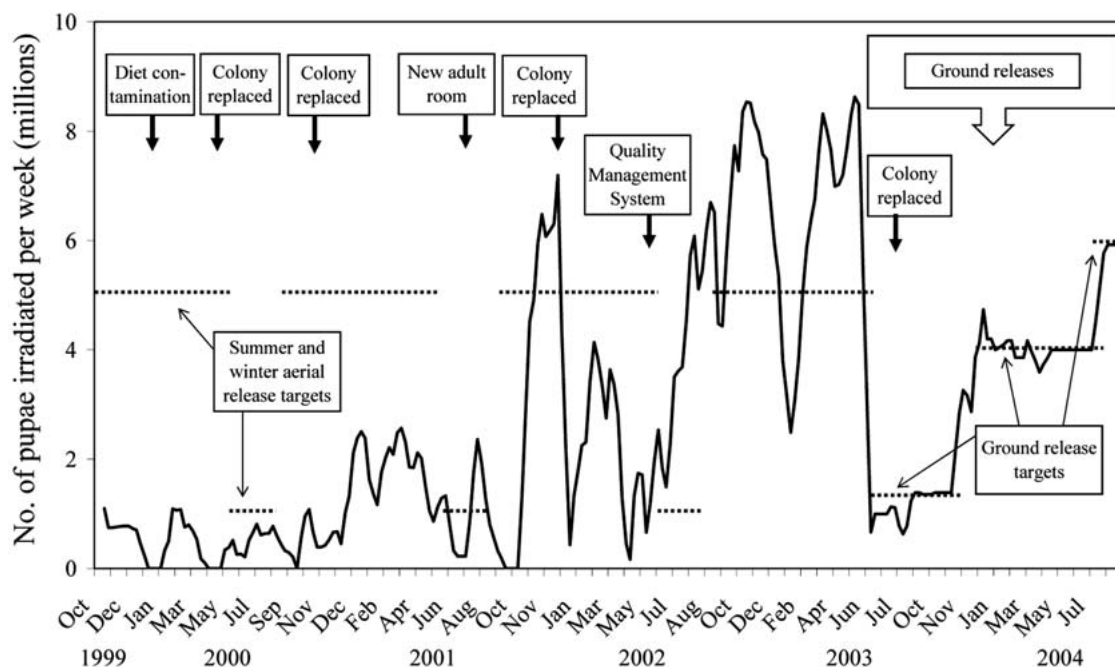


Fig. 4. Number in millions of *C. capitata* pupae irradiated from Oct 1999 to Sep 2004 (3-week moving average). The target number of sterile flies for release during different periods is indicated by dotted lines.

Females in Male-Only Stream

The occurrence of females in the male-only stream is summarized in Fig. 6a (VIENNA 7-D53/Mix 2001) and Fig. 6b (VIENNA 8). As sterile stings by females in commercial fruit can lead to infection by pathogens and secondary pests, a maximum level of 2% females in the male-only stream was set. From Dec 2002 to Apr 2003, the occurrence of females from the VIENNA 7 strain varied from about 1 to 3%, exceeding the maximum nearly 30% of the time. From Apr 2003, a steady increase in females of up to 11% was recorded until just before the introduction of the VIENNA 8 strain. The mean was $2.63 \pm 2.46\%$.

Following the introduction of VIENNA 8 in Aug 2004, the occurrence of females in the male-only stream dropped dramatically to a maximum of less than 0.4%, with females being recorded on only three occasions in 13 months. The mean was $0.02 \pm 0.09\%$.

DISCUSSION

During the period 1999 to mid-2002, production of sterile *C. capitata* by the facility was seldom sufficient to supply the requirements for aerial releases in the Hex River Valley, i.e., 5 million per week from Sep to May and 1 million per week from Jun to Aug. From mid-2002, after the positive effect of the implementation of the qual-

ity management system took effect, production was generally adequate with only one exception. Production and quality further improved and stabilized after the introduction of the VIENNA 8 strain in Aug 2003. Many factors contributed to the initial sub-standard production and quality parameters, the most important of which were the following:

- (1) Lack of adequate funding. In the absence of sustained government funding for operational expenses, and with an inadequate budget, the mass-rearing facility was constantly under financial duress. This negatively affected the integrity of the entire mass-rearing infrastructure, and consequently production and quality, and affected the overall success of the project.
- (2) Short start-up time for rearing facility. The rearing facility was required to supply 5 million sterile males per week to a fully operational SIT program within 7 months of starting up in a converted facility with new equipment and with new rearing staff with little experience. There was little opportunity for analyzing and solving initial problems common in a new mass-rearing operation. The rearing technicians had to gain most of their experience while the release program was in operation.
- (3) Cramped rearing conditions. Until mid-2001, all rearing took place in a small building that was converted into a rearing facility on a low budget. Inadequate space in both the adult

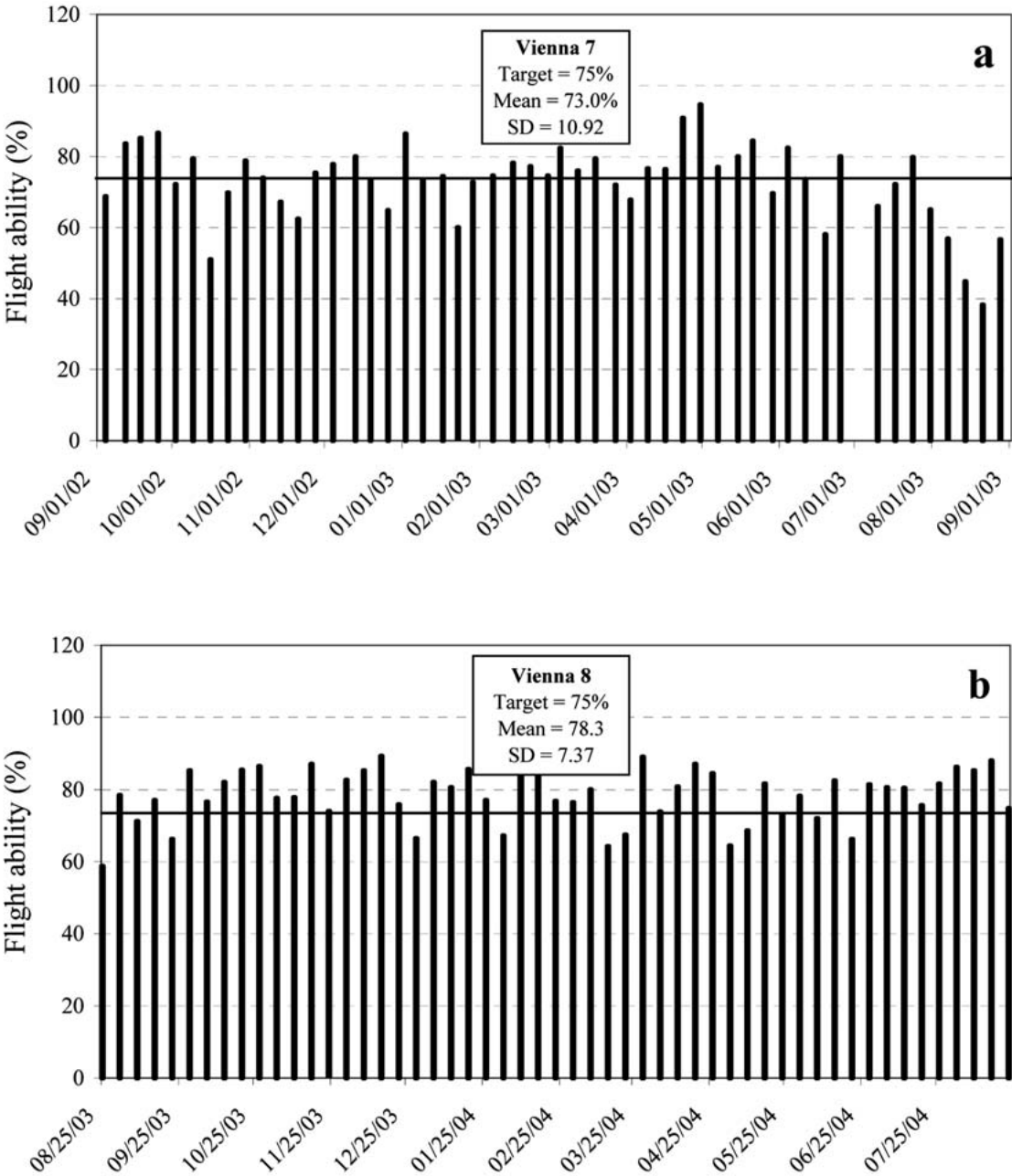


Fig. 5. Percentage flight ability of sterile males for (a) VIENNA 7-D53/Mix 2001 genetic sexing strain from Sep 2002 to Sep 2003, and (b) VIENNA 8 genetic sexing strain from Aug 2003 to Sep 2004.

and larval rooms led to poor egg production (e.g., Jan and Jun 2001) and poor larval production, which in turn resulted in failure to meet production targets. A new, spacious and well-equipped adult room was commissioned in Jun 2001, with a concomitant improvement in egg production thereafter.

- (4) Problems with larval diet. During late 1999, very poor larval production was ascribed to bran contaminated at source with the insecticide chlorpyrifos. This resulted in reduced numbers of pupae being produced between Dec 1999 and Jan 2000. During Nov 2002 to Feb 2003, a build up of rust in the larval diet

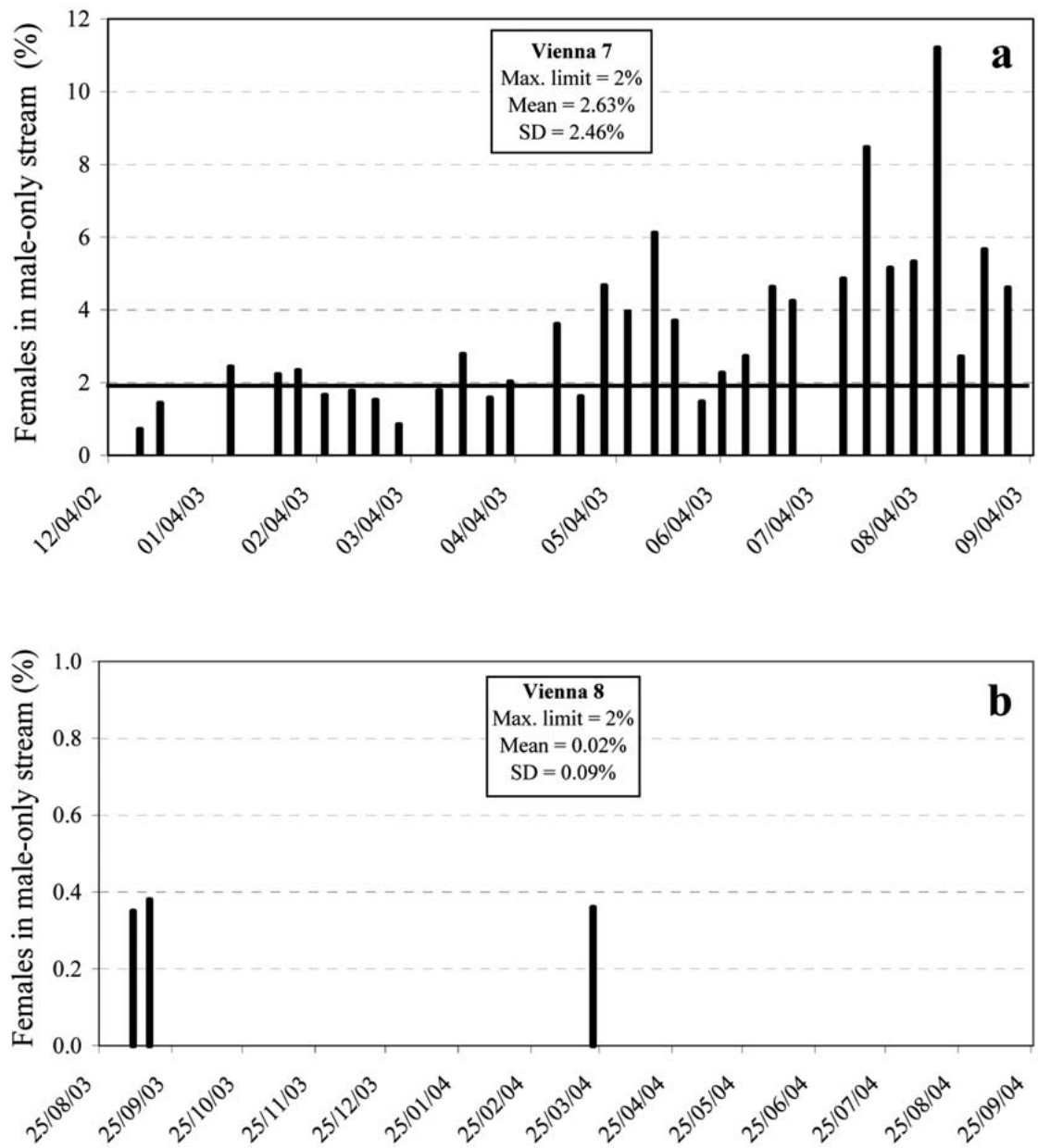


Fig. 6. Percentage females in the male-only stream for (a) VIENNA 7-D53/Mix 2001 genetic sexing strain from Dec 2002 to Sep 2003, and (b) VIENNA 8 genetic sexing strain from Aug 2003 to Sep 2004.

mixer resulted in the diet containing toxic levels of rust (C. Cáceres, FAO/IAEA Biotechnology Laboratory, Seibersdorf, Austria, personal communication), that reduced the egg to pupa efficiency and thus the number of pupae irradiated from Dec 2002 to Jan/Feb 2003. On a number of occasions, bran of varying consistency was delivered, being sometimes too fine and sometimes of mixed size grading. This resulted in sub-standard larval

- diet and egg to pupa efficiency (Mar to May 2002; Apr/May 2004) and in the decrease in the number of pupae irradiated during Mar to May 2002.
- (5) Colony replacements. The lack of an effective filter rearing system until mid-2001 contributed to unacceptable levels of genetic recombination of the genetic sexing strain and to consequent poor production and quality. As a result, the rearing colony had to be replaced 4

times during which production was severely affected (May 2000, Nov 2000, Oct/Nov, and July 2003). Production quantity and quality, and strain stability, started improving once a filter rearing system was introduced.

- (6) Equipment malfunction. Equipment installed in the new facility in 1999, in particular climate control equipment, broke down repeatedly. This was due mainly to budget restrictions precluding the purchase of higher-specification and higher-quality equipment, but also because high ambient temperatures during the hot summer months (Dec to Feb) raised temperatures in the larval rooms and put stress on the climate control equipment. Power failures also occurred. Production was negatively affected on each occasion.
- (7) Lack of a quality management system. Production and quality were generally poor in the absence of a quality management system. The benefit of the introduction of the quality management system in Jun 2002 can best be seen in daily egg production and number of pupae irradiated with the VIENNA 7 strains. Egg production increased from an average of 250 mL per day for 17 months pre-quality management system to 690 mL per day for 13 months post-quality management system. The average number of VIENNA 7 pupae irradiated increased from 1.4 million per week for 32 weeks pre-quality management system to 5.8 million per week for 13 months post-quality management system.

Performance of the VIENNA 8 Genetic Sexing Strain

The VIENNA 8 strain has been reported to show an approximate 20% improvement in performance relative to VIENNA 7-D53/Mix 2001 (Cáceres 2002). A comparison of data in Figs. 1a, 1b, 3a, 3b, 5a, 5b, 6a and 6b confirms the superiority of the VIENNA 8 strain. Egg production with VIENNA 8 was easily maintained at a high level and, once the strain had adapted to the new conditions, was relatively stable. Mean egg to pupa efficiency increased by 50% from 11.2% for VIENNA 7-D53/Mix 2001 to 16.8% for VIENNA 8. Mean flight ability increased by 7.3% from 73.0% with VIENNA 7-D53/Mix 2001 to 78.3% with VIENNA 8. The occurrence of females in the male-only stream decreased from a mean of 2.63% with VIENNA 7-D53/Mix 2001 to 0.02% with VIENNA 8, an improvement of 99.2%.

VIENNA 8 proved to be more genetically stable than the VIENNA 7 strains, exhibiting less genetic recombination (occurrence of 'wrong sex' pupae) following handling and environmental stress. After the introduction of VIENNA 8, equipment failure occurred on numerous occasions, yet, very low levels of recombination were recorded. Due to the better performance of VIENNA 8 it was possible to reduce the number of adult cages set up for the male-only stream from 12 per week to 8 per week without compromising

egg production. This in turn led to an estimated savings in production costs of 30%.

In conclusion, the experiences in South Africa have highlighted the importance for effective fruit fly SIT operations of the following factors: (a) sound rearing infrastructure with high quality equipment; (b) an adequate period of staff training and equipment testing before delivering sterile flies to an operational program; (c) an effective quality management system during the production of sterile insects; (d) a stable and productive genetic sexing strain; and (e) a sound funding base for the mass-rearing facility.

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REFERENCES CITED

- ANNECKE, D. P., AND V. C. MORAN. 1982. Insects and Mites of Cultivated Plants in South Africa. Butterworths & Co., Durban, South Africa, 383 pp.
- BARNES, B. N. 1994. Fruit fly management in pome and stone fruit orchards: Monitoring, bait application, cover sprays and management practices. *Deciduous Fruit Grower* 44: 244-249.
- BARNES, B. N., D. K. EYLES, AND G. FRANZ. 2004. South Africa's fruit fly SIT programme—the Hex River Valley pilot project and beyond, pp. 131-141 *In* B. N. Barnes [ed.], *Proc. 6th Int. Symp. Fruit Flies of Economic Importance*, Isteg Scientific Publications, Irene, South Africa. 510 pp.
- CÁCERES, C. 2002. Mass rearing of temperature sensitive genetic sexing strains in the Mediterranean fruit fly (*Ceratitis capitata*). *Genetica* 116: 107-116.
- FAO/IAEA. 2002. Annual Report. Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria.
- FAO/IAEA/USDA. 2003. Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies, Version 5.0. International Atomic Energy Agency, Vienna, Austria. 85 pp.
- FISHER, K. 1999. Medfly Mass Production in Stellenbosch, South Africa. Stage II; The establishment of VIENNA 7/Mix-99, a Temperature Sensitive Lethal Genetic Sexing Strain of Medfly. Expert Mission Report, 5-30 September 1999. International Atomic Energy Agency, Vienna, Austria. 26 pp.
- FISHER, K., AND C. CÁCERES. 2000. A filter rearing system for mass reared genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae), pp. 543-

- 550 In K. H. Tan [ed.], Area-Wide Control of Fruit Flies and Other Insect Pests. Penerbit Universiti Sains Malaysia, Penang, Malaysia. 782 pp.
- FRANZ, G., E. GENCHEVA, AND P. KERREMANS. 1994. Improved stability of genetic sex-separation strains for the Mediterranean fruit fly, *Ceratitis capitata*. *Genome* 37: 72-82.
- FRANZ, G. 2002. Recombination between homologous autosomes in medfly (*Ceratitis capitata*) males: type-1 recombination and the implications for the stability of genetic sexing strains. *Genetica* 116: 73-84.
- FRANZ, G. 2005. Genetic sexing strains amenable to large scale rearing as required for the sterile insect technique, pp. 427-452 In V. A. Dyck, J. Hendrichs, and A. S. Robinson [eds.], *The Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*. Springer, Dordrecht, The Netherlands. 787 pp.
- HENDRICHs, J., G. FRANZ, AND P. RENDÓN. 1995. Increased effectiveness and applicability of the sterile insect technique through male-only releases for control of Mediterranean fruit flies during fruiting seasons. *J. Appl. Entomol.* 119: 371-377.
- (IAEA) INTERNATIONAL ATOMIC ENERGY AGENCY. 2004. Dosimetry system for SIT: Standard operating procedure for Gafchromic film, International Atomic Energy Agency, Vienna, Austria. (http://www.iaea.org/programmes/nafa/d4/public/d4_pbl_5_2.html).
- MUMFORD, J., AND G. TWEEN. 1997. Economic feasibility study for the control of the Mediterranean and Natal fruit fly in the Western Cape Province. Expert Mission Report RU-7135, International Atomic Energy Agency, Vienna.
- MYBURGH, A. C. 1964. Orchard populations of the fruit fly, *Ceratitis capitata* (Wied.), in the Western Cape Province. *J. Entomol. Soc. Southern Africa* 26: 379-389.
- OPTIMAL AGRICULTURAL BUSINESS SYSTEMS 2005. Key deciduous fruit statistics 2005. Optimal Agricultural Business Systems, Paarl, South Africa.
- ORTÍZ-MORENO, G. 2002. Sterile insect technique integrated management of fruit fly (Phase II) SAF/5/002. Report on field operations review of the Medfly SIT control program at the Hex River Valley and buffer zones. Expert Mission Report IAEA-TCR-01550, 21 Oct-8 Nov 2002, International Atomic Energy Agency, Vienna, Austria. pp. 45.
- ROBINSON, A. S., G. FRANZ, AND K. FISHER. 1999. Genetic sexing strains in the medfly, *Ceratitis capitata*: Development, mass rearing and field application. *Trends in Entomol.* 2: 81-104.