IMPROVING MATING PERFORMANCE OF MASS-REARED STERILE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) THROUGH CHANGES IN ADULT HOLDING CONDITIONS: DEMOGRAPHY AND MATING COMPETITIVENESS

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ABSTRACT

Mass rearing conditions affect the mating behavior of Mediterranean fruit flies (medflies) *Ceratitis capitata* (Wiedemann). We evaluated the effect of slight changes in the adult holding conditions of adult flies maintained for egg production on their mating performance. Colonization was initiated from wild flies collected as larvae from infested coffee berries (*Coffea arabica* L.). When pupae were close to adult emergence, they were randomly divided into 3 groups and the emerging adults were reared under the following conditions: (1) Metapa System (MS, control), consisting of $70 \times 45 \times 15$ cm aluminum frame, mesh covered cages, with a density of 2,200 flies per cage and a 1:1 initial sex ratio; (2) Insert System (IS), with the same type of cage, and the same fly density and sex ratio as in the MS treatment, but containing twelve Plexiglas® pieces (23×8.5 cm) to provide additional horizontal surface areas inside the cage; and (3) Sex-ratio System (SS), same as IS, but in this case the initial male: female ratio was 4:1. Three d later, newly emerged females were introduced, so the ratio became 3:1 and on the 6th d another group of newly emerged females was added to provide a 2:1 final sex ratio, at which the final density reached 1,675 flies per cage. The eggs collected from each of the 3 treatments were reared independently following standard procedures and the adults were held under the same experimental conditions. This process was repeated for over 10 to 13 generations (1 year). The experiment was repeated 3 times in 3 consecutive years, starting each replicate with a new collection of wild flies. Life tables were constructed for each treatment at the parental, 3rd, 6th, and 9th generations. Standard quality control parameters (pupation at 24 h, pupal weight, adult emergence, and flight ability), were estimated for each treatment every third generation in the third year. For the last generation each year, mating competitiveness was evaluated in field cage tests with wild flies. As colonization progressed, life expectancy and fecundity rates increased in the 3 rearing systems. There was no significant difference in standard quality control parameters among the 3 rearing systems. Wild males always achieved more matings than any of the mass reared males. Mating competitiveness of males from the IS, although surprisingly not from the SS, was significantly greater than that of males from the MS. Our results indicate that these slight changes in the adult holding conditions can significantly reduce the harmful effects of mass rearing on the mating performance of sterile flies.

Key Words: *Ceratitis capitata*, sterile insect technique, colonization, mating behavior, insect demography, mother colony

RESUMEN

Se ha demostrado que las condiciones de cría masiva afectan el comportamiento de apareamiento de la mosca del Mediterráneo *Ceratitis capitata* (Wiedemann). Nosotros evaluamos el efecto de ligeros cambios en las condiciones en las que los adultos son mantenidos para la producción de huevos, en el desempeño de apareamiento de las moscas estériles. La colonización se inició con moscas silvestres colectadas como larvas en cerezas de café (*Coffea arabica* L.) infestadas. Cuando las pupas estuvieron cerca de la emergencia de los adultos, se dividieron en tres grupos al azar y los adultos recién emergidos fueron criados en las siguientes condiciones: (1) Sistema Metapa (MS, testigo), consistente en jaulas con marco de aluminio de $70 \times 45 \times 15$ cm, cubiertas con malla, con una densidad de 2,200 moscas por jaula y una relación de sexos inicial de 1:1; (2); Sistema Insertos (IS), con el mismo tipo de jaula, densidad de moscas, y relación de sexos que en el MS, pero conteniendo 12 piezas de plexiglas (23×8.5 cm) para proporcionar superficie horizontal al interior de la jaula; y (3) Sistema de Relación de Sexos (SS), igual que el IS, pero en este caso la relación inicial macho: hembra fue de 4:1, tres días después se introdujeron hembras recién emergidas para tener una relación de 3:1 y en el 6° día se añadió otro grupo de hembras para tener una relación final de sexos de 2:1, que equivale a una densidad final de 1,675 moscas por jaula. Los huevos colectados de cada tratamiento fueron criados independientemente siguiendo los procedimientos estándares y los adultos fueron mantenidos en las mismas condiciones experimentales. Esto

se repitió por 10 a 13 generaciones (un año). El experimento se repitió en tres ocasiones en años consecutivos, iniciando cada repetición con una nueva colecta de moscas silvestres. Se construyeron tablas de vida de cada tratamiento en las generaciones parental, 3ª, 6ª y 9ª. Se estimaron los parametros estándares de calidad (pupación a las 24 h, peso de pupa, emergencia de adultos y habilidad de vuelo) para cada tratamiento, cada tercera generación en el tercer año. En la última generación de cada año, se evaluó la competitividad sexual en pruebas en jaulas de campo con moscas silvestres. Conforme avanzó la colonización, se encontró que la esperanza de vida y las tasas de fecundidad se incrementaron en los tres sistemas de cría. No hubo diferencia significativa en los parámetros estándar de control de calidad entre los tres sistemas. Los machos silvestres siempre lograron más apareamientos que los machos procedentes de cada sistema de cría masiva. La competitividad de los machos del sistema IS fue significativamente mayor que la de los machos del sistema MS. Nuestros resultados indican que estas ligeras modificaciones en las condiciones de la colonia de adultos reducen los efectos adversos de la cría masiva sobre el desempeño de apareamiento de los machos estériles.

Translation provided by the authors.

Since the early stages of the sterile insect technique (SIT), it was recognized that the mating competitiveness of the sterile insects was a critical factor for the successful application of the technique (Knipling 1955). Research results showed that the exposure to irradiation for sterilization affected the mating performance of the sterile fruit flies (Holbrook & Fujimoto 1970; Hooper 1971; Ohinata et al. 1977; Knipling 1979; Lux et al. 2002a). Later, it was found that both irradiation and the selection that occurs during colonization could adversely affect the mating performance of sterile flies (Rössler 1975; Wong & Nakahara 1978; Leppla et al. 1983; Wong et al. 1983; Harris et al. 1986).

In the case of the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) however, it has been shown that, despite a long time under mass rearing conditions, sterile males are still capable of locating hosts, mating arenas or leks, and mix and interact with their wild counterparts under natural conditions (Zapien et al. 1983; Whittier et al. 1992; Shelly & Whittier 1996; Katsoyannos et al. 1999). Also, it has been documented that the courtship patterns of flies from different geographical areas are sexually compatible (Cayol et al. 2002; Lux et al. 2002b). However, it has been shown that slight quantitative changes in the courtship displays of males might result in female rejection and that these changes could be attributed to the selection that occurs under mass rearing conditions. Male courtship behavior of mass reared flies tends to be less elaborate, and the degree to which it is affected was found to be associated with the time under mass rearing conditions (McInnis et al. 1996; Briceño & Eberhard 2002; Gaskin et al. 2002; Lux et al. 2002b; Robinson et al. 2002).

Harris et al. (1986) suggested that conditions for mass rearing select for fast mating. Since most flies in the rearing cages are of the same age and reach sexual maturity at nearly the same time, we speculated that the close to 1:1 "operational" sex ratio favored short male courtships and less choosy females, resulting in this fast mating behavior. Detailed observations of the mating behavior of flies in the mass rearing cages showed that male courtship was frequently interrupted (W. Eberhard & D. Briceño, personal communication).

Under natural conditions, this fast mating behavior results in less competitive sterile males in view of wild female mate choice, and therefore, less effective programs integrating the SIT. The goal of this study was to evaluate whether slight changes in the colony holding conditions, where adult flies are maintained for egg production, could reduce this selection for fast mating and thus produce more competitive flies. Two changes from the standard mass-rearing procedures (Schwarz et al. 1985) were tested: (1) horizontal clear inserts were introduced inside the rearing cages to increase the overall resting surface available and to imitate the undersurfaces of leaves where males usually perform their courtship under natural conditions (Prokopy & Hendrichs 1979), possibly reducing the frequency of courtship interruption; and (2) variation in the operational sex ratio by introducing the females into the cages at four different times, so the number of sexually mature males was always greater than the number of sexually mature females (Calkins 1989).

Materials and Methods

Biological Material

The study was initiated with wild flies collected from naturally infested coffee berries (*Coffea arabica* L.) in southwestern Guatemala. New collections were made in each of 3 consecutive years, each year being considered as a replicate of the whole experiment. The location, amount of coffee collected, and the approximate number of larvae and adults obtained for each collection are shown in Table 1.

TABLE 1. AMOUNT OF MATURE COFFEE BERRIES COLLECTED, APPROXIMATE NUMBER OF LARVAE AND ADULTS OBTAINED, AND LOCATION IN GUATEMALA OF COLLECTIONS.

Year of collection	Location	Coffee berries (kg)	Larvae recovered	Adults emerged
2000	Colomba	1,650	18,549	10,701
2001	Colomba	2,000	15,000	12,325
2002	Antigua	2,500	21,550	13,435

Rearing Systems

Experimental work was carried out at the Moscamed mass rearing facility in Metapa, Chiapas, Mexico. Standard rearing procedures and environmental conditions were used (Schwarz et al. 1985). 3 adult rearing systems were evaluated: (1) Metapa System (MS, control), which consisted of an aluminum frame, mesh covered cage ($70 \times 45 \times 15$ cm) with an initial density of 1,100 males and 1,100 females per cage, and an average surface area of 3.91 cm^2 per fly; (2) Insert System (IS), as above but with the addition of 12 pieces of clear plexiglas (polycarbonate) (23×8.5 cm) inside the cage as horizontal surface areas, resulting in a surface area of 5.85 cm^2 per fly; and (3) Sex-ratio System (SS), same as IS, but with an initial density of 1,100 males and 275 females (4:1 male: female ratio). Three d later 92 recently emerged virgin females were introduced to make a 3:1 ratio, and at the 6th day 183 recently emerged virgin females were introduced to make a 2:1 ratio, and a total of 1,100 males and 550 females. The surface area was 6.94 cm^2 per fly.

Adults were fed *ad libitum* with a mixture of enzymatic yeast hydrolysate (ICN Biomedical, Costa Mesa, CA) and sucrose (1:3). Water was provided in test tubes covered with cotton plugs. On both sides at the bottom of the cages, water channels were placed for egg collection. These eggs were reared following the standard procedures at the Metapa facility (Schwarz et al. 1985).

Demographic Analysis

To compute life tables, the number of dead flies and the volume of eggs collected were recorded daily from the cages. In addition, a sample of 30 pairs from each treatment, every third generation, was taken and placed in plastic cages (8 cm diameter by 15 cm long, one male and one female per cage) with food, water, and a 2-cm diameter agar sphere (3 L of water + 80 g of agar dyed with green food coloring and wrapped in Parafilm®) as an oviposition device (Boller 1968, Freeman & Carey 1990). These spheres were replaced every 24 h and the number of eggs laid were recorded. This was done until the last female in the cohort died.

Male Sexual Competitiveness

Each year, after 10 to 13 generations, field cage mating tests with host trees were conducted (FAO/IAEA/USDA 2003). In each cage, 50 wild females, 50 wild males, and 50 males of each rearing system were released. Wild flies were 9-13 d old and mass-reared sterile flies were 7-11 d old. These ages were selected following the results of Liedo et al. (2002). The tests were conducted at coffee plantations in Guatemala during 5 consecutive d. Each d, 3 replicates (field cages) were set up. The males were color marked on the thorax for treatment identification.

In the third year, in addition to these mating tests, the "Fried" field cage test was used (Fried 1971). In each cage, 50 wild females, 50 wild males, and 150 sterile mass-reared males were released (one field cage for each treatment), and 25 agar oviposition devices (as described above) were placed inside each cage. After 24 h, the agar devices were removed, and egg hatch was determined from the eggs obtained from these devices. One hundred wild flies (1:1 male: female ratio) were placed in a field control cage to collect eggs and determine egg hatch without sterile fly competition. Sterility induced was estimated from the difference between egg hatch in the control and egg hatch in competition. There were 3 cages per treatment, and the test was run during 2 d, making 6 replicates per adult holding system.

Standard Quality Control Tests

In the third year, standard quality control parameters (FAO/IAEA/USDA 2003) were determined for each treatment at the parental, 3rd, 6th, and 9th generations. The parameters evaluated were pupal weight, adult emergence, and flight ability.

Statistical Analysis

Life table demographic parameters used in this study are defined by Carey (1993). Laboratory and field tests followed the methods described in the international quality control manual for tephritid flies (FAO/IAEA/USDA 2003). Data from observed proportions were transformed as $\sqrt{x + 0.5}$, and

subjected to analysis of variance (ANOVA), followed by means separations by the Tukey test ($P \leq 0.05$) (SAS Institute 1992).

RESULTS

Demographic Analysis

Survival rapidly increased through colonization in the 3 treatments. Mean adult life expectancy increased significantly from the parental to the 3rd generation, then gradually increased or remained stable in the following generations, both in males and females. This trend was observed when the flies were evaluated individually (Fig. 1), although differences among generations were not significant ($F = 0.4355$, $P = 0.6556$ for males; $F = 2.9684$, $P = 0.0801$ for females). There were no significant differences among rearing systems ($F = 0.0790$, $P = 0.9244$ for males; $F = 0.1569$, $P = 0.8561$ for females) and there was no significant interaction between rearing systems and generations ($F = 0.2214$, $P = 0.9225$ for males; $F = 0.4244$, $P = 0.7888$ for females).

When survival data were taken directly from the rearing cages, there were highly significant differences among generations ($F = 7.7843$, $P = 0.0010$ for males; $F = 8.4050$, $P = 0.0006$). However, the differences among rearing systems were not significant ($F = 1.4461$, $P = 0.2570$ for males; $F = 0.5255$, $P = 0.5985$ for females) and there were also no interactions between generations and rearing systems ($F = 0.2589$; $P = 0.9502$ for males; $F = 0.1551$, $P = 0.9859$ for females) (Fig. 2).

Fecundity increased in a similar pattern. The number of eggs laid per female increased significantly from the parental generation to the 3rd generation in all treatments, then gradually increased every third generation. This was observed both in the data collected from single pairs (Fig. 3 top), as well as in those from the rearing cages (Fig. 3 bottom). There was wide variation in this parameter among treatments, particularly during the first 3 to 6 generations, but no statistical differences among treatments were found ($F = 2.2959$, $P = 0.1328$ for single pairs; $F = 0.0510$, $P = 0.9504$ for rearing cages). The difference among generations was highly significant in both cases, when the flies were obtained from single pairs ($F = 11.0348$, $P = 0.0009$), and when data were collected from the rearing cages ($F = 35.8365$, $P = 1.208 \times 10^{-8}$). The interaction between rearing systems and generations was not significant ($F = 0.2406$, $P = 0.9111$ for single pairs; $F = 0.4048$, $P = 0.8678$ for rearing cages). It is important to note the demographic implications of the significant

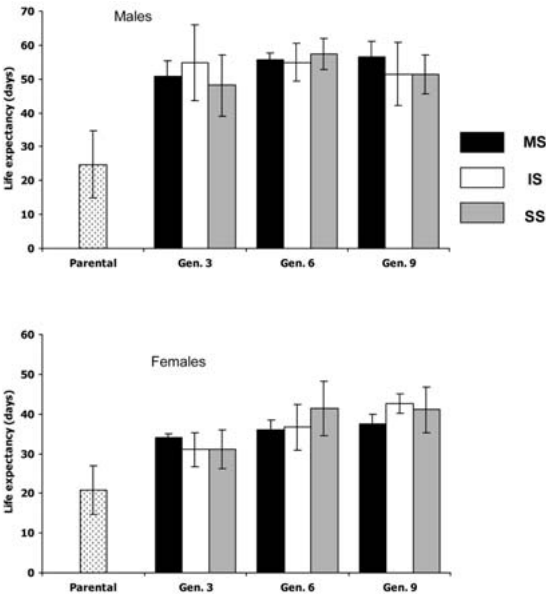

Fig. 1. Life expectancy (e_0) (days ± SE) of male (top) and female (bottom) Mediterranean fruit flies from 3 different adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System), estimated from single pair cages.

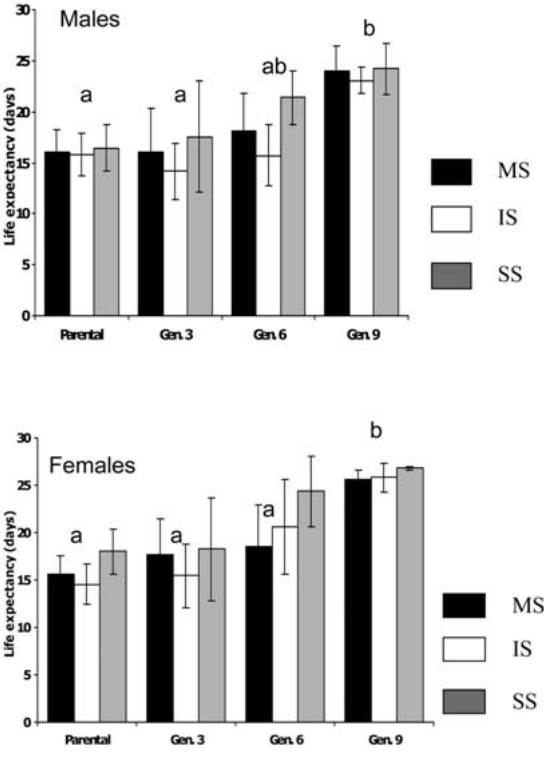

Fig. 2. Life expectancy (e_0) (days ± SE) of male (top) and female (bottom) Mediterranean fruit flies from 3 adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System), estimated from rearing cages.

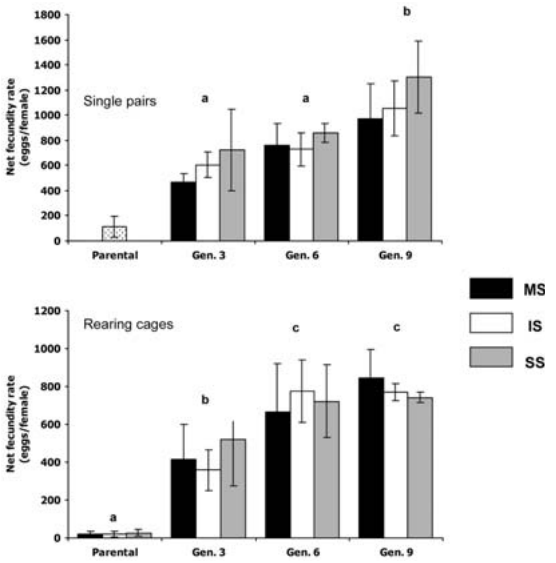

Fig. 3. Net fecundity rate ($\Sigma l_x m_x$) (eggs/female ± SE) of Mediterranean fruit fly females from 3 adult colony holding rearing systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System) at four different generations. Estimated from single pair cages (top) and from rearing cages (bottom).

differences between the parental and the 9th generations in both, survival and fecundity, in the 3 rearing systems.

Male Sexual Competitiveness

Results from the field cage mating tests during the 3 years were rather consistent. Wild males were always the most successful in terms of the average percent of matings achieved and the differences were statistically significant (F = 26.92; df = 3, 6; $P < 0.001$) (Fig. 4). Among the 3 rearing systems, there was a significant difference between the IS and the MS (control). The differences between the SS and the other 2 rearing systems were not significant.

The average mating index (± SE) estimated for each rearing system, according to the international quality control manual (FAO/IAEA/USDA 2003), also showed a significant difference between the IS and MS, and a non significant difference between the SS and the other 2 rearing systems (F = 35.08; df = 2, 6; P = 0.042) (Fig. 5).

Results from the Fried test showed that males from the IS were the ones that induced the greatest level of sterility (34%). Males from the SS and MS treatments only induced 18.3 and 16.3% sterility, respectively. However, differences among treatments were not statistically significant (F = 32.87; df = 2, 15; P = 0.101). Fig. 6 shows the average (± SE) level of sterility induced by each treatment. Natural sterility was 13.3%.

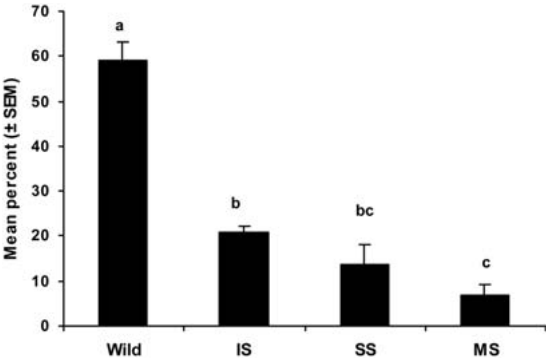

Fig. 4. Average percent of matings (%± SE) in field cage tests of Mediterranean fruit fly males reared under 3 different adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System) ($P < 0.05$).

Standard Quality Control Tests

The results of the standard quality control tests applied to the 3 rearing systems at the parental, 3rd, 6th, and 9th generations are shown in Fig. 7. All these values were within acceptable international ranges (FAO/IAEA/USDA 2003). There was a significant increase in pupal weight, from the parental flies to the mass reared flies. In the other 2 parameters, there were no significant differences among generations, although a similar pattern can be observed.

Pupal weight in the 3rd generation was greater in the SS compared with the other 2 treatments (F = 0.84; df = 2, 9; $P < 0.001$). There were no significant differences at the 6th generation (F = 1.36; df = 2, 9; P = 0.052). In the 9th generation, the IS produced the heaviest pupae (F = 1.62; df = 2, 9; P = 0.011).

Mean adult emergence was greater in the IS and SS than in the MS at the 3rd (F = 4.66; df =

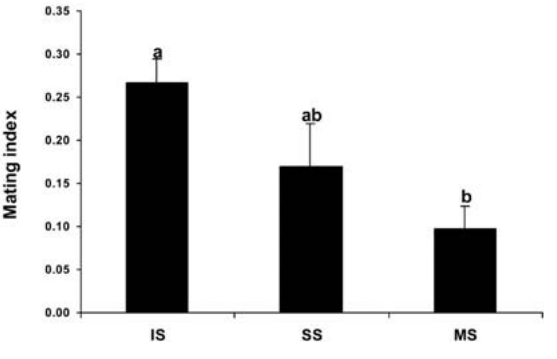

Fig. 5. Average mating index (± SE) for 3 adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System). This index was estimated following the quality control manual (FAO/IAEA/USDA 2003).

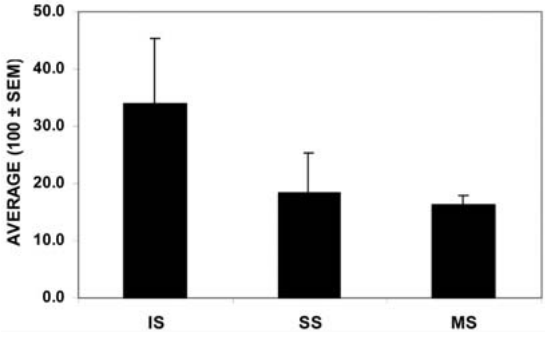

Fig. 6. Sterility levels (mean ± SE) induced by sterile Mediterranean fruit fly males reared under 3 different adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System) when competing with wild males in field cages (the natural sterility in the control was 13.3%).

2, 9; $P = 0.013$) and 6th generations ($F = 9.36$; $df = 2, 9$; $P = 0.021$). Differences in this parameter were not statistically significant at the 9th generation ($F = 9.08$; $df = 2, 9$; $P = 0.301$).

There were no significant differences among treatments in flight ability at the 3rd ($F = 2.43$; $df = 2, 9$; $P = 0.655$) and 9th ($F = 3.67$; $df = 2, 9$; $P = 0.231$) generations. At the 6th generation, flight ability was significantly greater in the IS than in the MS ($F = 5.64$; $df = 2, 9$; $P = 0.037$).

Discussion

Demographic data confirm that mass-reared flies have greater reproductive rates than wild flies (Liedo & Carey 1996) and show that colonization for mass-rearing is a selection process in which insects adapt to the new rearing conditions (Leppla et al. 1983; Leppla 1989). For mass rearing purposes, this is desirable and necessary in order to produce large number of insects in an efficient manner. However, this same selection process can result in negative effects on other biological attributes, such as mating behavior.

The results from the single pair cages and the rearing cages showed that the conditions in which flies are held affect the demographic parameters obtained, with greater values at the single pair cages than at the more stressful adult holding cages. However, in both cases, the general trends were similar, with mean expectation of life and net reproductive rates increasing with generations, as the flies gradually adapted to the crowded mass-rearing conditions. At the same time only very small or no differences were found among rearing systems.

Our results from the field cage mating tests corroborate that mass-rearing adversely affects the mating competitiveness of the reared insects compared to wild flies (Wong & Nakahara 1978; Wong et al. 1983; McInnis et al. 1996). The introduction of horizontal inserts in the rearing cages contributed to a significantly better mating performance of the IS mass-reared insects when compared to the standard-produced MS males. Although there were no significant differences in the level of sterility induced (Fried test), the pattern was similar (IS > SS > MS). This suggests that the number of matings recorded during the observation period in the field cage mating test is correlated with the induction of sterility in the wild population and that males from the IS were more competitive than males from the other 2 rearing systems.

The manipulation of the sex ratio did not have a significant effect on the mating performance of mass-reared flies. This result was unexpected. We

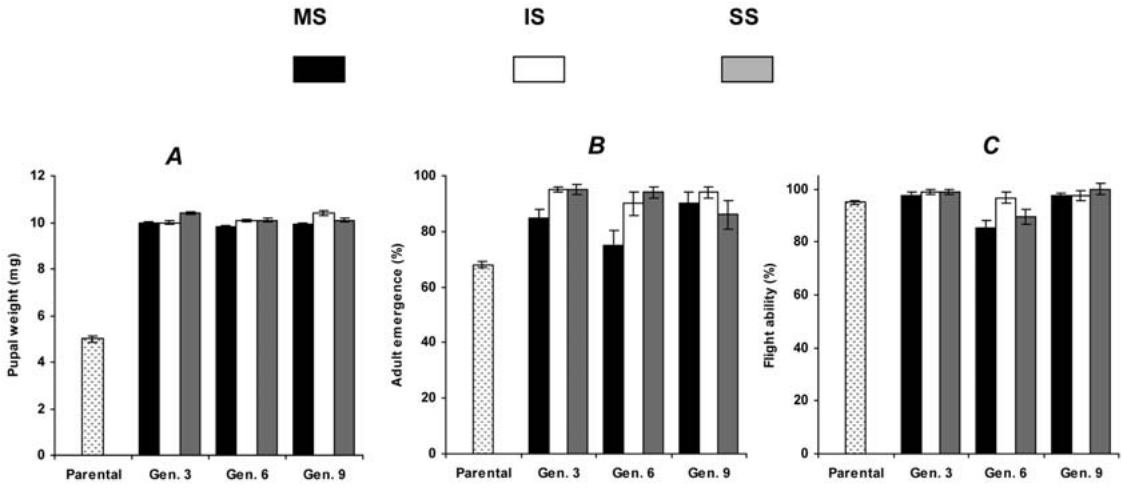

Fig. 7. Standard quality control tests: (A) pupal weight (mg), (B) adult emergence (%), (C) flight ability (%) of the 3 adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System).

were expecting that the biased sex ratio in favor of males would allow females to be more selective and result in more competitive males. One explanation for this could be the reduced offspring produced by the smaller number of females and as a result of harassment of ovipositing females by the excess of males in the cage; however, there are other potential causes that need to be investigated. The small number of offspring was particularly critical in the second year. Manipulation of operational sex ratio in adult holding cages is now feasible due to the current availability of genetic sexing strains. We believe that this research line, and the interaction with increased surface area in cages, should be further explored.

Data from the standard quality control tests demonstrate that the 3 colonization methods have no detrimental effect on most of these parameters. Pupal weight was the only attribute that significantly changed (increased) through colonization. These findings suggests that while the demographic and mating attributes, as well as pupal weight, were under selection pressure during colonization, this was not the case for attributes such as adult emergence and flight ability. This raises the question of whether other biological attributes could be under selection pressure during colonization (Harris 1988; Calkins 1989; Miyatake & Haraguchi 1996). Rodrigueiro et al. (2002) reported differences between wild and mass-reared medflies in some morphological traits. Lux et al. (2002b) found quantitative differences in the courtship behavior of wild and mass-reared Mediterranean fruit fly males. The biological attributes that show significant differences between wild and mass-reared flies deserve further research.

In the current study, we started all 3 treatments from wild collected flies. It will be interesting to investigate whether the introduction of inserts might have a reverse effect. Will a long term mass-reared strain increase its competitiveness if horizontal inserts are introduced to the mass rearing process, without starting a new colony from wild flies? Based on our results, the introduction of inserts in the rearing cages is strongly recommended, because its represent a minor change in the production process, with negligible costs, and important benefits in the application of the SIT.

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