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## SEXUAL PERFORMANCE OF MASS REARED AND WILD MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) FROM VARIOUS ORIGINS OF THE MADEIRA ISLANDS

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### ABSTRACT

The success of Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) control programs integrating the sterile insect technique (SIT) is based on the capacity of released sterile males to compete in the field for mates. The Islands of Madeira are composed of 2 populated islands (Madeira and Porto Santo) where the medfly is present. To evaluate the compatibility and sexual performance of sterile flies we conducted a series of field cage tests. At same time, the process of laboratory domestication was evaluated. 3 wild populations, one semi-wild strain, and 1 mass reared strain were evaluated: the wild populations of (1) Madeira Island (north coast), (2) Madeira Island (south coast), and (3) Porto Santo Island; (4) the semi-wild population after 7 to 10 generations of domestication in the laboratory (respectively, for first and second experiment); and (5) the genetic sexing strain in use at Madeira medfly facility (VIENNA 7mix2000). Field cage experiments showed that populations of all origins are mostly compatible. There were no significant differences among wild populations in sexual competitiveness. Semi-wild and mass-reared males performed significantly poorer in both experiments than wild males in achieving matings with wild females. The study indicates that there is no significant isolation among strains tested, although mating performance is reduced in mass-reared and semi-wild flies after 7 to 10 generations in the laboratory.

**Key Words:** *Ceratitis capitata*, sexual success, medfly domestication, medfly origin, proportion of mating, Madeira

### RESUMEN

El éxito de los programas de control de la mosca mediterránea de la fruta (*Ceratitis capitata* (Wiedemann) que integran la técnica del insecto estéril (TIE) esta basado en la capacidad de machos estériles para competir en el campo por sus parejas. Las Islas de Madeira consisten de 2 islas pobladas (Madeira y Porto Santo) donde la mosca mediterránea de la fruta esta presente. Para evaluar la compatibilidad y el funcionamiento sexual de moscas estériles nosotros realizamos una serie de pruebas de jaula en el campo. Al mismo tiempo, el proceso de la domesticación en el laboratorio fue evaluado. Tres poblaciones naturales, una población semi-natural y una población criada en masa fueron evaluadas: las poblaciones natural de (1) Isla de Madeira (costa norte), (2) Isla de Madeira (costa sur) y (3) Isla de Porto Santo; (4) una población semi-natural después de 7 a 10 generaciones de domesticación en el laboratorio (respectivamente, para el primero y segundo experimento); y (5) la raza para separar sexos genéticamente que es usada en el laboratorio de la mosca mediterránea de Madeira (VIENNA 7mix2000). Los experimentos usando jaulas en el campo mostraron que las poblaciones de diferentes orígenes fueron en su mayor parte compatibles. No hubo diferencias significativas en la capacidad para competir sexualmente entre las poblaciones naturales. Los machos semi-naturales y los machos criados en masa mostraron un desempeño significativamente bajo en ambos experimentos que los machos naturales en el logro de copula con las hembras naturales. Este estudio indica que no hay un aislamiento significativo entre las razas probadas, aunque el desempeño en el apareamiento fue reducido en las moscas criadas en masa y semi-naturales después de 7 a 10 generaciones en el laboratorio.

The Madeira Islands are located 980 km WSW from mainland Portugal (32°N and 17°W) and are composed by two populated islands. Porto Santo is small (about 50 km<sup>2</sup>) with topographic (maximum altitude 571 m) and temperature conditions favourable to the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann). However, poor soil and low rainfall (380 mm/year) do not permit an abundance of host fruits (the fig *Ficus carica* and *Opuntia* spp. are exceptions in terms of abun-

dance). The main island, Madeira (740 km<sup>2</sup>), is volcanic with very little level land suitable for large agricultural production. The north coast is cooler than the south coast (air temperature at sea level in the north corresponds to air temperature at 300 m elevation in the south) and the maximum elevation is 1881 m. The climate of Madeira Island, particularly below 600 m over sea level on the south coast and 400 m on the north coast, is favourable for the development of large medfly

populations. Conditions in Funchal (sea level in south coast), the capital city, and in low altitude areas are very favourable for medfly throughout the year. There are eight generations per year in the Funchal area (Vieira 1952).

Insects of the same species may behave differently in different geographical areas depending on variations in selection pressures (Thornhill & Alcock 1983). However, recent studies with wild medflies from different origins around the world (Argentina, Australia, Crete, Guatemala, Kenya, Madeira-Portugal, Reunion-France, and South Africa) show mating compatibility with each other (Cayol et al. 2002). The same authors tested wild flies against mass reared genetic sexing strains (VIENNA 4/Tol-94, VIENNA 7-97, SEIB 6-96, and AUSTRIA 6-96 (Franz 2005)) and no sexual isolation was found. However, the data showed that males from some origins perform better than others, including lower compatibility of Madeira flies with sexing strains tested.

Due to these findings, and because the Madeira-Med SIT program is currently ongoing in the Madeira Islands (Pereira et al. 2000), we performed additional studies to investigate in field cage tests the mating compatibility of locally mass produced sterile flies. A discrepancy in sterile male sexual performance observed in previous field cage tests conducted in Madeira (Dantas et al. 2004) underlined the need for this new study to confirm if there is some sexual isolation between sterile flies being released and wild flies from different origins in the Madeira Islands.

The evident difference in host structure and the isolation of the 2 islands (50 km apart), plus the semi-isolation between north and south coasts of Madeira Island provided a basis for comparison of the different populations. We tested the possibility of any isolation among these 3 medfly populations Porto Santo Island, north coast of Madeira, and south coast of Madeira. Additionally, the mating compatibility of these 3 wild populations with sterile flies and a semi-wild population was studied.

## MATERIALS AND METHODS

Five different populations of medfly were studied: (1) wild flies from south coast of Madeira Island (SC); (2) wild flies from north coast of Madeira Island (NC); (3) wild flies from Porto Santo Island (PS); (4) semi-wild flies after 7 to 10 generations in the laboratory (SW); and (5) sterile flies from Madeira-Med factory (L), strain VIENNA 7mix2000 (Franz 2005).

Two sets of field cage tests were conducted, each comparing males of 4 of the above 5 populations. The reason for this was due to wild fly availability (an incompatibility of fly abundance peak between wild medfly populations from Porto Santo Island and the north coast of Madeira Is-

land). In the first set (June and July, 2002) 17 cage tests were conducted (7 with PS females, 6 with SC females, and 4 with SW females). In the second set (October, 2002), 24 cage tests were carried out (8 with females from each of NC, SC, and SW).

Wild medflies were collected as pupae from larval survey samples (mixed hosts like peach, guava, apricot, and others). Semi-wild flies came from a small laboratory colony reared under low stress conditions. This colony was established with flies obtained from the fruit sampling of the Madeira Medfly program (Pereira 2001), i.e., from a mixture of hosts from the entire Madeira Island. Pupae of the mass-reared strain were irradiated with 100 Gy under hypoxia in a Gamma-cell <sup>60</sup>Co irradiator (Nordion, Canada) 24-48 h before emergence.

Pupae from all the strains were placed in a standard quality control plexiglas cage (30 cm × 30 cm × 40 cm) until emergence. In the first 24 h after emergence, the insects were sexed and females were kept in separate rooms from males to avoid contact with the male pheromone before the tests. In the case of the L (sterile) treatment, only males were used. All flies were maintained at 24 ± 2°C, 65 ± 5% RH and natural light (no artificial lighting was supplied) in 2-L plastic containers with water and adult food (1 part hydrolyzed yeast and 3 parts sugar) *ad libitum*.

At the time of release into field cages wild and semi-wild females were 11 to 13 d old, wild and semi-wild males 9 to 11 d old, and mass reared sterile males were 5 d old. Healthy flies were selected for the experiments and marked with a dot of water-based paint on the thorax the day before the field cage tests.

The field cages were cylindrical, with a flat floor and ceiling (2.9 m diameter and 2.0 m height) supported by a PVC frame (Calkins & Webb 1983). A potted 1.8-m high citrus tree was placed into each cage to serve as a substrate territory for sexual interactions.

The testing period covered the time of maximum sexual activity of medfly (sunrise to 12:00 h), and was conducted according to FAO/IAEA/USDA (2003). Male flies were released 30 min before the females so that they could start forming leks (Prokopy & Hendrichs 1979). In each cage, 60 males (15 from each of 4 treatments) were released to compete for 30 wild females.

Temperature, relative humidity, and light intensity in the field cages were recorded every 30 min. The mating pairs were recorded during a continuous census, and after initiation of mating, pairs were collected in 20-mL vials. The mating time, location on the tree, and leaf side were recorded. The observers had no prior information about which kind of males were in the cages.

In the field cage tests we measured the proportion of flies mating (PM) (McInnis et al. 1996), and a mating by origin index (MOI) that mea-

asures the sexual success of males from different origins. The former index was adapted from the relative sterility index (RSI) used in SIT operations to measure the mating success of sterile males when competing with wild males (McInnis et al. 1996). The PM measures the suitability of the flies and the environment for mating. It represents the overall mating activity of the flies, both wild and sterile, and is defined as follows:

PM = 
$$\frac{\text{Number of pairs collected}}{\text{Number of females released}}$$

The MOI was defined to measure the matings achieved by males from a certain origin in relation to the total matings. The expected index would be 0.25, since we used cages with 4 kinds of males evenly distributed (15 from each strain).

MOI = 
$$\frac{\text{Number of matings of a certain origin male}}{\text{Total matings}}$$

Data were analyzed by analysis of variance (ANOVA). If differences in means were detected, a complementary multiple comparisons of means test (Tukey's honest significant difference test) was performed (Ott & Longnecker 2001). The significance value was 95% ( $\alpha = 0.05$ ). Statistical analyses were performed with R software (version 2.1.0, [www.r-project.org](http://www.r-project.org)).

RESULTS

Results of the first set of field cage tests show no significant differences ( $F = 3.16$ ,  $df = 2,14$ ;  $P = 0.0737$ ) between the PM of the 3 strains of females used (Fig. 1). The PM was always above the minimum required (0.20) for data analysis (FAO/IAEA/USDA 2003). The MOI index that measures the relative male sexual success competing for a female is presented on Table 1, independently and pooled together. The data show significantly lower sexual success for mass-reared sterile males against all kinds of females in the experiment. Surprisingly, PS males performed significantly better when competing for SC females, but

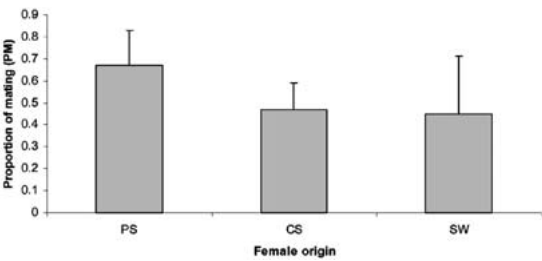


Fig. 1. Proportion of Mediterranean fruit fly females mating (PM  $\pm$  SD) from different origins (PS—Porto Santo Island, SC—south coast of Madeira Island, and SW—semi-wild after 7 generations in laboratory). The data show no significant differences ( $P = 0.0737$ ).

not when competing for females of their own strain (even with slightly higher PM) and the SW females. The overall data show no significant differences between the two wild strains (PS and SC). However, PS males performed significantly better than SW and L males, while SC males were only significantly better than L males.

The second set of field cage tests again showed no significant differences in PM ( $F = 1.768$ ;  $df = 2,21$ ;  $P = 0.1951$ ) among the females tested (SC, NC, and SW) (Fig. 2). As in the previous experiment, the PM is always above the minimum required (0.25) for data analysis (FAO/IAEA/USDA 2003). In terms of MOI (Table 2), the data are slightly different from the first set of experiments. Males from SW and L show a significantly lower MOI than the other males (NC and SC) when competing for NC and SW females. The same is true for the overall data. However, the MOI data show no significant differences among the 4 male treatments tested (SC, NC, SW, and L) when competing for SC females.

DISCUSSION

The data obtained in this study show the normal lower competitiveness of sterilized mass-reared males, but clearly no significant isolation in terms of mating compatibility among all the strains of

TABLE 1. MATING BY ORIGIN INDICES (MOI) FOR PORTO SANTO ISLAND (PS), SOUTH COAST OF MADEIRA ISLAND (SC), SEMI-WILD (SW) AND STERILE MASS-REARED (L) MEDITERRANEAN FRUIT FLY MALES IN THE PRESENCE OF PS, SC, SW FEMALES, AND POOLED RESULTS. ROWS WITH THE SAME LETTER PRESENT NO SIGNIFICANT DIFFERENCES AMONG MALES OF DIFFERENT ORIGIN (TUKEY'S HONESTLY SIGNIFICANT DIFFERENCE TEST,  $\alpha = 0.05$ ).

Female origin	Number of cages	Male origin			
		PS	SC	SW	L
PS	7	0.37 a	0.29 ab	0.25 b	0.09 c
SC	6	0.42 a	0.26 b	0.23 b	0.09 c
SW	4	0.39 a	0.28 a	0.27 a	0.06 b
Totals	17	0.39 a	0.28 ab	0.24 b	0.09 c

TABLE 2. MATING BY ORIGIN INDICES (MOI) FOR SOUTH COAST OF MADEIRA ISLAND (SC), NORTH COAST OF MADEIRA ISLAND (NC), SEMI-WILD (SW) AND STERILE MASS-REARED (L) MEDITERRANEAN FRUIT FLY MALES WHEN IN PRESENCE OF SC, NC, SW FEMALES, AND POOLED RESULTS. ROWS WITH THE SAME LETTER PRESENT NO SIGNIFICANT DIFFERENCES AMONG MALES OF DIFFERENT ORIGIN (TUKEY'S HONESTLY SIGNIFICANT DIFFERENCE TEST,  $\alpha = 0.05$ ).

Female origin	Number of cages	Male origin			
		SC	NC	SW	L
SC	8	0.30 a	0.28 a	0.25 a	0.17 a
NC	8	0.34 a	0.37 a	0.16 b	0.13 b
SW	8	0.34 a	0.34 a	0.19 b	0.13 b
Totals	24	0.33 a	0.32 a	0.20 b	0.15 b

flies tested. These results were expected in accordance with the compatibility studies of Cayol et al. (2002) for several medfly strains from many regions of the world, including Madeira Island.

Important results were obtained when recently domesticated male medflies were tested in the field cages. These semi-wild males performed significantly worse compared to the best wild male treatment in each of the experiments. However, the semi-wild males performed better than sterilized mass-reared males. The phenomenon of rapid decrease in mating sexual performance soon after strains of flies are adapted to mass-rearing conditions is well documented (Economopoulos 1992; Orozco & López 1993; Cayol 2000). The loss of sexual competitiveness of recently domesticated flies (only 7 to 10 generations from the wild) even under low stress conditions, i.e., low adult fly and larval density, respectively, in adult cages and in larval diet, is likely a result of high selection pressure that laboratory conditions impose on the insects.

The phenomenon of rapid strain deterioration after colonization is likely more evident when under the high stress of mass-rearing conditions as is common in the medfly factories around the world. For this reason, the development of a filter rearing system (Fisher & Cáceres 2000) to manage mother colonies under rearing conditions (fly

density, sex-ratio, and physical features) more similar to conditions found in nature, as described by Robinson et al. (2002), is recommended.

In conclusion, our data indicate no mating incompatibility among strains tested and also support the need to improve sterile male competitiveness by instituting in medfly mass rearing facilities filter rearing systems to manage adult colonies under less stressful conditions.

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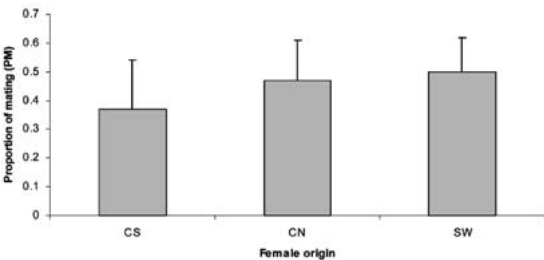


Fig 2. Proportion of Mediterranean fruit fly females mating (PM  $\pm$  SD) from different origins (SC—south coast and NC—north coast of Madeira Island, and SW—semi-wild after 7 generations in the laboratory). The data show no significant differences ( $P = 0.1951$ ).

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