

## SEXUAL COMPETITIVENESS AND COMPATIBILITY BETWEEN MASS-REARED STERILE FLIES AND WILD POPULATIONS OF *ANASTREPHA LUDENS* (DIPTERA: TEPHRITIDAE) FROM DIFFERENT REGIONS IN MEXICO

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### ABSTRACT

The mass-reared colony of *Anastrepha ludens* (Loew) currently used in Mexico for suppression of the Mexican fruit fly has been in use for over 10 years. Sterile flies are released into a wide range of environmental conditions as part of an integrated area-wide approach to suppress diverse populations of this pest in the Mexican Republic. This paper assesses the performance of the sterile flies interacting with wild populations from the different environments. We investigated the sexual compatibility and competitiveness of the sterile flies when competing with wild populations from 6 representative Mexican states: Nuevo León, Tamaulipas, Sinaloa, Nayarit, Michoacán, and Chiapas. Results show that the males of the wild populations differed in the time to the onset and peak of sexual activity. Nevertheless, the index of sexual isolation (ISI) reflected sexual compatibility between the populations and the mass-reared strain, indicating that the sterile individuals mate satisfactorily with the wild populations from the 6 states. The male relative performance index (MRPI) showed that the sterile male is as effective in copulating as the wild males. The female relative performance index (FRPI) reflected a general tendency for wild females to copulate in greater proportion than the sterile females, except for the strains from Tamaulipas and Chiapas. In general, the lower participation of the sterile females in copulation increases the possibilities of sterile males to mate with wild females. The relative sterility index (RSI) showed that the acceptance by wild females of the sterile males (25-55%) was similar to that of wild males. Females of the Chiapas strain showed the lowest acceptance of sterile males. Finally, the results obtained in the Fried test (which measures induced sterility in eggs) showed a competitiveness coefficient ranging from 0.2 to 0.5. This suggests that sterile males successfully compete and are compatible with flies from different geographic origins.

Key Words: *Anastrepha ludens*, Tephritidae, SIT, sexual compatibility, competitiveness, Mexico

### RESUMEN

La colonia actualmente usada para controlar la mosca mexicana de la fruta, *Anastrepha ludens* (Loew), en México tiene más de 10 años en cría masiva. Los insectos estériles son liberados en una gran variedad de condiciones ambientales como parte de un control integrado para suprimir diversas poblaciones de esta plaga dentro de la República Mexicana. El objetivo de este documento está dirigido a revisar el desempeño de las moscas estériles frente a poblaciones silvestres procedentes de diferentes ambientes y para esto se realizaron comparaciones de compatibilidad y competitividad sexual de las moscas estériles contra poblaciones silvestres de seis estados representativos de la República Mexicana: Nuevo León, Tamaulipas, Sinaloa, Nayarit, Michoacán y Chiapas. Los resultados obtenidos manifiestan diferencias en el horario de inicio de llamado y mayor actividad sexual del macho entre las moscas provenientes de cada estado. Sin embargo el índice de aislamiento (ISI) reflejó compatibilidad sexual entre la cepa de laboratorio y todas las poblaciones analizadas, indicando que los individuos estériles pueden aparearse satisfactoriamente con las poblaciones silvestres de los seis estados. El índice de efectividad de apareamiento del macho (MRPI) reflejó de manera global que los machos estériles son tan efectivos para copular como los silvestres. El índice de efectividad de apareamiento de la hembra (FRPI) reflejó que en la mayoría de los estados las hembras silvestres copularon en mayor proporción que las hembras estériles, excepto para las poblaciones de Tamaulipas y Chiapas. En general, la baja participación de las hembras estériles en el campo permitió al macho estéril ampliar su probabilidad de apareamiento con las hembras silvestres. En cuanto al índice de esterilidad relativa (RSI), observamos que la aceptación de las hembras silvestres al macho estéril (25-55%) fue similar a la de los machos silvestres. Las hembras de la población de Chiapas registró la menor aceptación. Finalmente, los resultados obtenidos en la prueba de Fried, la cual determina la esterilidad inducida presentaron un coeficiente de competitividad entre 0.2 y 0.5. Esto sugiere que los machos estériles compliten exitosamente y son compatibles con moscas de diferentes orígenes geográficos.

Translation provided by the authors.

The extraordinary capacity of *Anastrepha ludens* (Loew) (Diptera: Tephritidae) to adapt to diverse environments allows its proliferation in semitropical, tropical, and desert climates, and it is considered a pest throughout Mexico (Aluja 1994). The effectiveness of the sterile insect technique (SIT) applied as part of area-wide integrated pest management (AW-IPM) programs depends on the efficient transfer of sperm carrying dominant lethal mutations from sterile males to wild females (Knipling 1955). Thus, the success or failure of a sterile insect release is critically dependent on the quality and the ability of sterile males to search for and copulate effectively with wild females.

Mass rearing requires a broad and deep knowledge of the pest insect's biology and ecology in order to produce large numbers of insects without compromising insect quality. In most mass-rearing facilities there is a tendency to maintain the same strain for long periods of time (Roessler 1975). As a consequence, and after a certain number of generations of mass-rearing, insect quality tends to deteriorate (Partridge 1996).

Research has been conducted with field caged host trees and different tephritids to assess the changes that occur in the sexual behavior of mass-reared sterile fruit flies in comparison with wild populations. It has been found that the high densities at which adult flies are commonly kept in mass-rearing may be selecting for traits such as males with simpler courting sequences, changes in sexual competitiveness, shorter copulation, and less discriminating females (Calkins 1984; Harris et al. 1986; Boake et al. 1996; Iwahashi 1996; Briceño & Eberhard 1998). One of the ways to counteract this development is to regularly replace the colony. Nonetheless, one of the main problems observed during colonization of a new strain is the production bottleneck that occurs in the initial phase of colonization, where only a fraction of the individuals survive and reproduce (Leppla et al. 1983; Leppla 1989). This increases the time required to achieve the required colony size and reduces the initial gene pool of the new strain. Over the medium term, this reduction may cause deviations in the behavior of laboratory flies, such as strain incompatibility and sexual isolation, with respect to the wild flies.

To monitor mating compatibility and competitiveness changes, quality control field cage tests have to be conducted (FAO/IAEA/USDA 2003). For this study, mating compatibility refers to randomness of mating between sterile mass-reared insects and their wild counterparts. The competitiveness tests measure the ability of sterile males to achieve copulations with wild females and the degree of sterility of the eggs produced by wild females when wild and sterile males compete to mate with them.

Our goal was to determine the mating compatibility and competitiveness of sterilized mass-

reared *A. ludens* flies of a strain that has been in use for over 10 years in comparison with wild flies from different regions of México, where the SIT is currently applied as a component of area-wide campaigns to suppress this pest.

## MATERIALS AND METHODS

### Origin of the Biological Material

Wild pupae from the Tamaulipas region were obtained from the townships of Guemez, Hidalgo, Padilla, and Ciudad Victoria, where they were collected from yellow sapote fruits (*Sargentia greggi*). In Nuevo León, pupae were obtained from Linares, El Cercado, Monterrey, and Guadalupe, also from yellow sapote fruits. In the Sinaloa region collections covered the townships of Badiraguato, Mocorito, and Culiacán, where sapote fruit (*Casimiroa eudilis*) were the hosts. In Nayarit pupae were obtained from bitter orange (*Citrus aurantis*), white sapote (*Casimiroa edulesleve*), matasano (*Pouteria campechiana*), and black sapote (*Diospyros digynajaca*), collected in Miravalles, Compostela, Xalisco, Testarazo, Aquiles Serdán, Emiliano Zapata, Tepic, Libertad, Lo de García, Cuachisnes, San Blas, Acajoneta, Tuxpan, Pantanales, and San Pedro. In Michoacán the pupae were obtained from bitter orange (*Citrus aurantis*) collected in Uruapan, and in Chiapas from this same fruit collected in the Soconusco region.

Fruit was collected directly from the host plant and from the ground and taken to the laboratory where it was placed in containers to let the larvae mature. Once the larvae had matured, the fruit was dissected and the larvae and/or pupae were transferred to a pupation substrate (slightly damp vermiculite). The pupae obtained were kept for approximately 20 days in a room at a temperature of  $25 \pm 1^\circ\text{C}$  and  $75 \pm 5\%$  RH.

Sterile pupae were obtained directly from the *A. ludens* production line at the Moscafrut mass rearing facility in Metapa de Dominguez, Chiapas, México (Dominguez Gordillo 1996). The original colony is a mixture of an old colony from Mission, Texas, and wild material collected from different regions in Chiapas; the Mission colony had been mass-reared for more than 10 years.

### Size and Weight of the Pupae

Due to the influence of adult size on successful mating (Burk & Webb 1983; Churchill-Standland et al. 1986; Orozco & López 1993), 2 days prior to emergence, and when the pupal eye color was dark brownish-green, the pupation substrate was withdrawn and the weight and size-distribution of the pupae were obtained with aid of a pupal sizing and separating machine (FAO/IAEA/USDA 2003). This equipment was used to distribute the

sterile and wild pupae into 10 different size groups (with #1 being the smallest and #10 the largest class, from 1.30 to 2.90 mm, respectively). The wild and sterile pupae obtained in the size categories 7 (2.30-2.45 mm), 8 (2.45-2.60 mm) and 9 (2.60-2.75 mm) were placed into containers, and these containers were placed into 30 × 30 × 30-cm cages in a room at 25 ± 1°C temperature and 75 ± 5% RH. After emergence, the flies were sorted by sex.

#### Field Cages

Six field cages, measuring 3 m in diameter and 2 m in height, and supported by a metal frame (Chambers et al. 1983; Calkins & Webb 1983) were used. Potted host mango and citrus trees were placed alternately around the inside circumference and central section of each cage. The cages were set up in a mango (*Ataulfo* cv) plantation in the hills of the municipality of Tapachula, at an altitude of 137 m above sea level. The tests were conducted in random blocks with a minimum of 6 replicates

#### Male Calling

The numbers of calling males were recorded in 30-min periods. The required characteristics for confirmation of male calling were vigorous wing flapping, everted prostiger, and puffed pleural glands. Observations were carried out from 15:00 to 19:30 h (summer schedule), since this is the time when sexual activity in *A. ludens* is the greatest (Aluja et al. 1983).

#### Sexual Compatibility

In each cage 20 males and 20 females of the tested wild populations and 20 sterile males and 20 sterile females of the mass reared strain were released. Wild flies were 16-21 d old while sterile flies were 10 d old (Orozco et al. 2001). In order to identify the individual flies, a small piece of paper with a number was stuck to each fly's dorsal side by white glue. Throughout the observation period the number and type of matings was recorded as wild male and female (WW), sterile male and female (SS), wild male and sterile female (WS), and sterile male and wild female (SW).

#### Sexual Competitiveness (Induced Sterility)

For each wild population 5 field cages were set up as follows: (1) "wild control" cage into which 32 wild males were released along with 8 wild females; (2) "sterile control" cage into which 32 sterile males were released along with 8 sterile females; and (3) three "competitiveness" cages into each of which 24 sterile males, 8 wild males and 8 wild females were released. Each cage contained

3 feeding (sugar and hydrolyzed protein in a 3:1 ratio) and watering areas, and 8 artificial host fruits, placed into each cage in order to collect the eggs to measure the induced sterility. The flies were left in the cages for 5 d; after the second d, the host fruits were changed daily to estimate fecundity and fertility of the females.

#### Data Analysis

To estimate sexual compatibility, the index of sexual isolation (ISI), male and female relative performance indices MRPI and FRPI (Cayol et al. 1999), and the relative sterility index (RSI) (McInnis 1996) were calculated. We used the 0.25 value as variance limit for equal mating propensity in ISI, MRPI, and FRPI, and for equal competitiveness in the RSI. The overall competitiveness value C of sterile males, as indicated by the reduction in egg hatch, was estimated by the Fried formula (Fried 1971). Indices between populations were compared by an ANOVA and Fisher's PLSD test with StatView software ver. 5.0.

## RESULTS

All the evaluations were carried out during summer, which corresponds to the rainy season in Mexico. Humidity ranged between 88 and 99% and during the tests (at 17:00 h) it was usually cloudy and rainy. The maximal light intensity recorded was 1440 lux and the minimal 0 lux was at 19:30 h. The temperatures recorded ranged between 24 to 32°C. The only exception was with the Chiapas strain that was evaluated during spring, which corresponds to the hot season without rain. In this case the temperature range was higher but the relative humidity was significantly lower, fluctuating between 40 and 60%.

Male calling and mating activity during the sexual activity period are presented in Fig. 1. Some differences in the sexual activity patterns were detected. Males from the Nayarit area began their sexual activity at 16:00 h and reached a mating peak at 16:30 h. Males from Sinaloa, Tamaulipas, and Michoacán initiated their sexual activity at 16:30 h and reached their maximum level at 19:00 h, 18:30 h and 17:30 h, respectively. Chiapas and Nuevo León initiated sexual activity at 17:00 h and reached a maximum at 18:30 h.

The results obtained from the mating compatibility test are shown in Table 1. The propensity for mating (PM) indicates the overall percentage of the couples that mated. All the PM values were larger than 0.20, indicating that the conditions under which the tests were run were satisfactory (FAO/IAEA/USDA 2003). The index of sexual isolation (ISI) is a measure of mating compatibility between populations. The index considers the number of couples obtained for each possible mating combination, with values range from -1 (com-

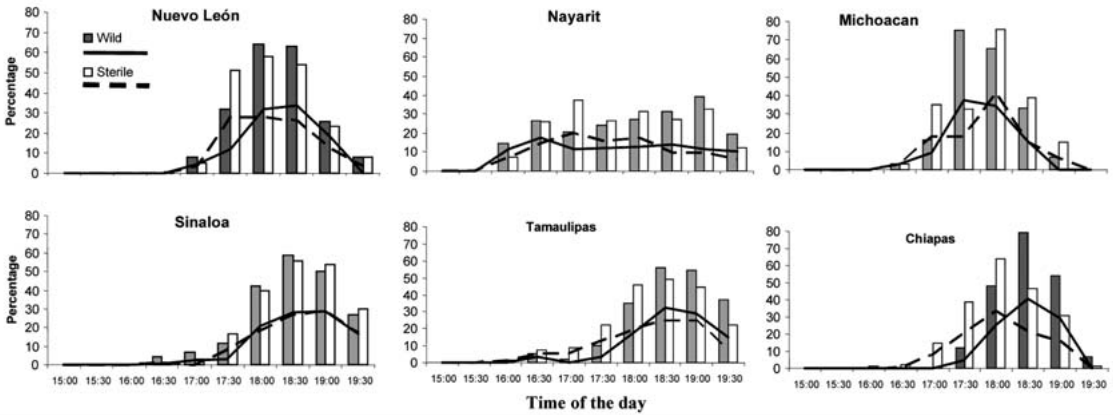


Fig. 1. Distribution of matings (bars) and males calling (lines) during the sexual activity period (the matings are with both wild and sterile females).

plete negative assortative mating, that is, all mating are with members of the opposite population) through 0 (random mating) to +1 (complete positive assortative mating, that is, total mating isolation of the two populations). The ISI values (Fig. 2) show satisfactory levels of compatibility between the sterile insects and the different wild populations, and there was no significant difference among populations ( $F = 1.159; df = 4,26; P = 0.3514$ ).

The male relative performance index (MRPI) is a measure of the propensity of sterile males to mate with wild females, with values ranking from -1 to +1. A value of -1 indicates that all matings were carried out by wild males, while a value of +1 indicates that all matings were carried out by sterile males. Zero indicates that males from both populations participated equally in matings. Fig. 3 shows that the sterile males were as effective at

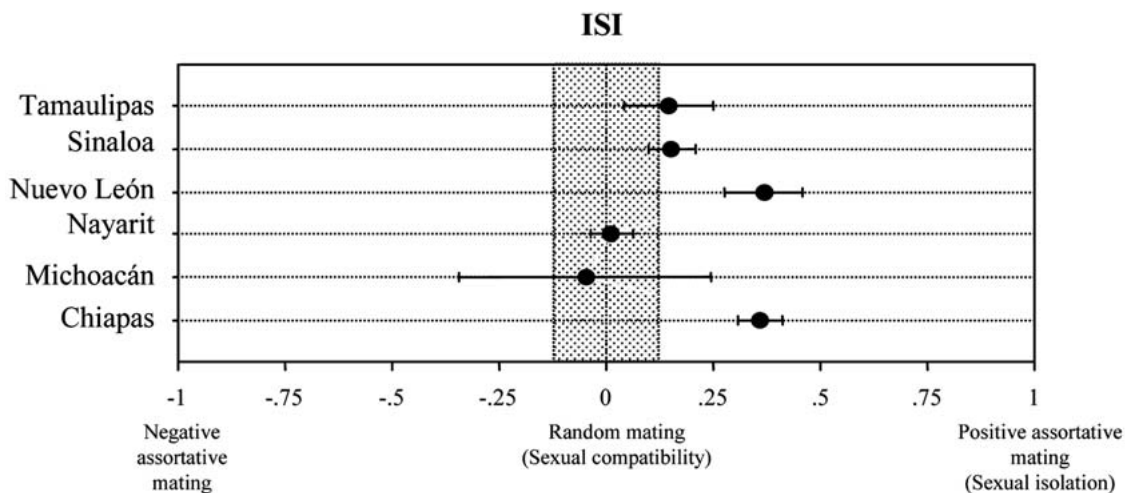
obtaining mates as the wild males and there was no overall differences between the populations ( $F = 3.699; df = 4,26; P = 0.1702$ ). Nevertheless, between individual populations there was a significant difference with the Tamaulipas population ( $F = 3.699; df = 4,26; P = 0.0164$ ). This suggests that the sterile males were more effective when competing against wild flies of the Tamaulipas populations.

The female relative performance index (FRPI) is a measure of the propensity of sterile females to mate with wild males, with values ranking from -1 to +1. A value of -1 indicates that all matings were carried out by wild females, while a value of +1 indicates that all matings were carried out by sterile females. Zero indicates that females from both populations participated equally in mating. In most regions, the wild females copulated more than the sterile females (Fig. 4), with the excep-

TABLE 1. PROPENSITY OF MATING (PM), SEXUAL COMPATIBILITY (ISI) AND COMPETITIVENESS INDICES OBTAINED IN FIELD CAGES FROM INTERACTIONS BETWEEN 6 WILD MEXICAN FRUIT FLY POPULATIONS FROM MEXICO AND A MASS-REARED STRAIN.

State	Compatibility and Competitiveness Indices					n
	PM	ISI	MRPI	FRPI	RSI	
Tamaulipas	0.65	0.147 ± 0.103 ab	0.200 ± 0.076 c	0.148 ± 0.083 c	0.530 ± 0.061 d	6
Sinaloa	0.66	0.152 ± 0.055 ab	-0.114 ± 0.058 ab	-0.232 ± 0.062 ab	0.390 ± 0.024 bc	9
Nuevo León	0.40	0.368 ± 0.092 a	-0.131 ± 0.064 a	-0.383 ± 0.105 a	0.331 ± 0.042 ac	11
Nayarit	0.76	0.013 ± 0.051 b	-0.094 ± 0.059 ab	-0.198 ± 0.045 b	0.457 ± 0.039 bd	7
Michoacán	0.55	-0.049 ± 0.295 b	0.030 ± 0.041 ab	-0.424 ± 0.118 ab	0.532 ± 0.124 bd	3
Chiapas	0.57	0.361 ± 0.052 a	-0.011 ± 0.032 b	0.244 ± 0.055 c	0.240 ± 0.053 a	12

Propensity of mating (PM) = No. of pairs collected/No. of females released.  
 Isolation index (ISI) = (SS+WW)-(SW+WS) / (SS+WW+SW+WS).  
 Male relative performance index (MRPI) = (SS+SW)-(WS+WW) / (SS+WW+SW+WS).  
 Female relative performance index (FRPI) = (SS+WS)-(SW+WW) / (SS+SW+WS+WW).  
 Relative sterile index (RSI) = (SW) / (SW+WW).  
 n = Number of replicates performed for each wild population.



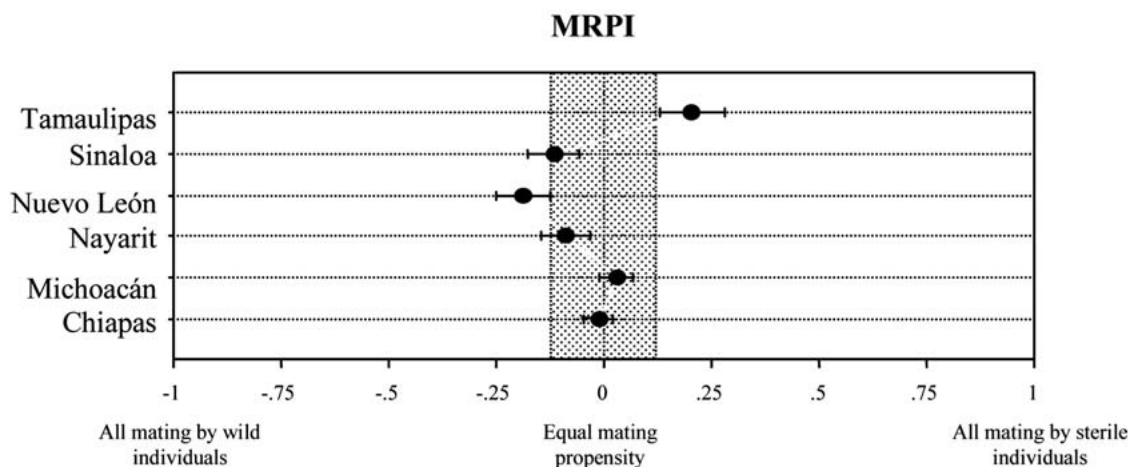
$$ISI = (SS+WW)-(SW+WS) / (SS+WW+SW+WS)$$

Fig. 2. Index of sexual isolation comparing the compatibility of the sterile strain with the wild strain from each region.

tion of the Tamaulipas and Chiapas strains ( $F = 8.285$ ;  $df = 4,26$ ;  $P = 0.0002$ ), for which a significant difference was found in comparison with the other regions.

The relative sterility index (RSI) indicates the sexual competitiveness between two strains. Val-

ues range between 0 and +1. Zero means that wild females mate only with wild males; a value of +0.5 indicate that wild females mate indiscriminately with wild or sterile males; a value of +1 indicate that wild females mate only with sterile males. The RSI in most cases reflected the prefer-



$$MRPI = (SS+SW)-(WS+WW) / (SS+WW+SW+WS)$$

Fig. 3. Male relative performance index between each regional wild strain with the sterile strain.

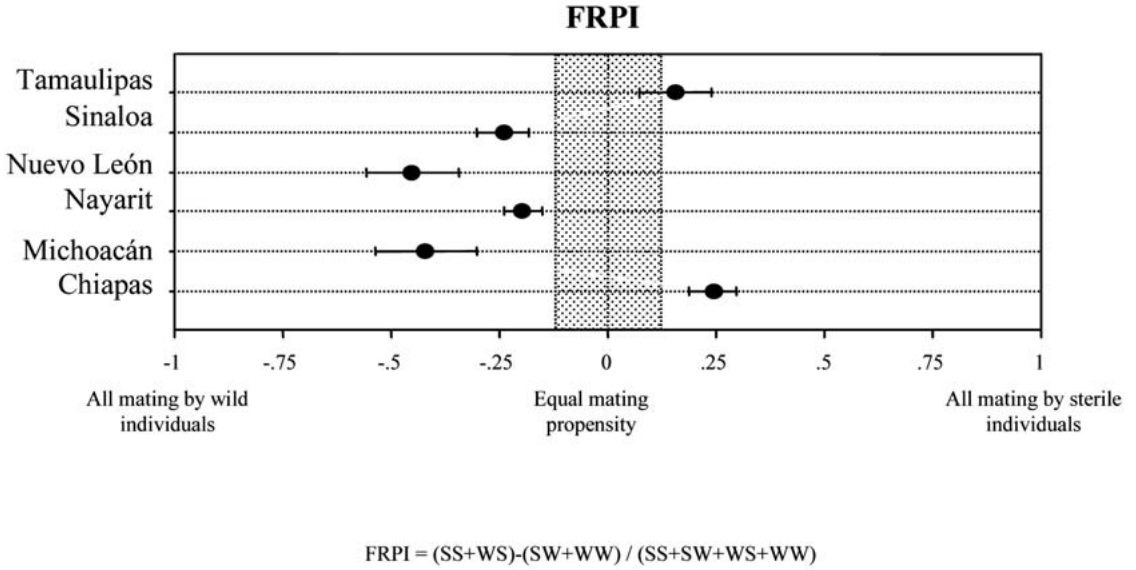


Fig. 4. Female relative performance index between each regional wild strain with the sterile strain.

ence of wild females for wild males over sterile males. There was, however, no significant difference for the strains from Michoacán and Tamaulipas ( $F = 2.422$ ;  $df = 4,26$ ;  $P = 0.0136$ ), for which the wild males were found to be less competitive (Fig. 5).

Values for the Fried's competitiveness coefficient range from 1 to 0. Values of 1 indicate an equivalent level of competitiveness between the two types of males, while values close to zero indicate superior competitiveness of the wild male (Fried 1971). The values obtained ranged from 0.23 to 0.56 (Table 2).

DISCUSSION AND CONCLUSIONS

Mating competitiveness and sexual compatibility are important quality control parameters that affect the performance of released sterile insects. The present study analyzed the sexual competitiveness and compatibility of sterile insects from the Moscafrut mass-rearing facility with wild populations of *A. ludens* coming from different regions from México. Unlike the wild populations, which are exposed to the natural environmental conditions, strains reared under laboratory conditions are normally exposed to fairly sta-

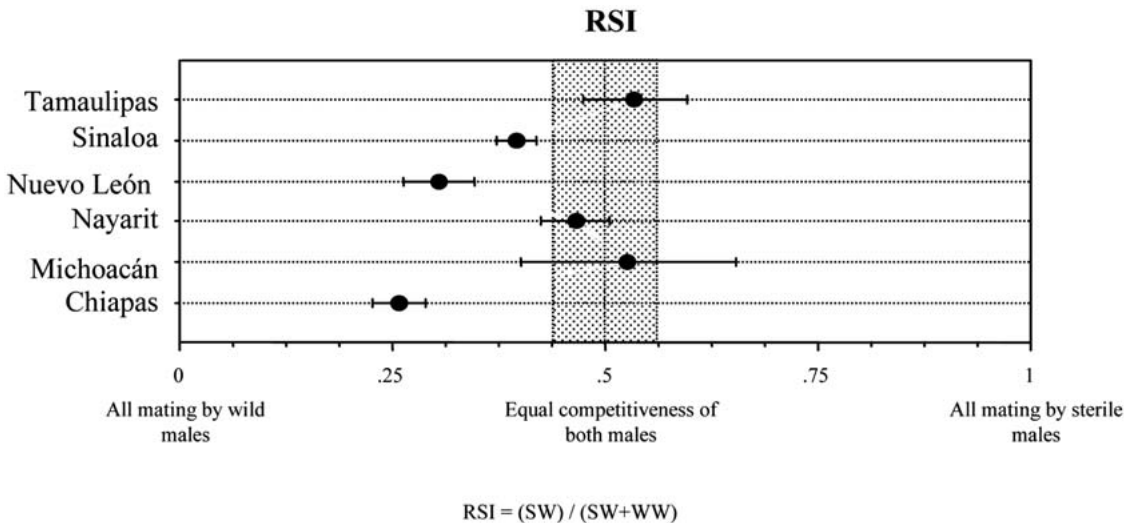


Fig. 5. Relative sterility index for the sterile strain with each of the regional wild strains.

TABLE 2. RESULTS FROM THE COMPETITIVENESS TEST (FRIED) CARRIED OUT IN FIELD CAGES BETWEEN 6 WILD MEXICAN FRUIT FLY POPULATIONS FROM MEXICO AND STERILE FLIES FROM A MASS-REARED STRAIN.

State	Percent egg hatch			Fried competitiveness value (C)
	Wild control cage	Sterile control cage	Competitiveness cage	
Tamaulipas	57.73	0.00	21.50	0.36
Sinaloa	88.31	0.00	46.28	0.39
Nuevo León	73.60	0.00	43.07	0.29
Nayarit	76.73	0.00	32.67	0.45
Michoacán	28.06	0.00	16.71	0.23
Chiapas	57.82	0.00	19.71	0.56

Fried competitiveness value (C) = (W/S) X (Hw - Hc) / (Hc - Hs).

W = Number of wild males.

S = Number of sterile males.

Hw = Egg hatch from wild females in the wild control cage.

Hc = Egg hatch from wild females in the competitiveness cage.

Hs = Egg hatch from lab females in the sterile control cage.

ble environmental conditions. This may lead to a change in the behavior of the mass-reared adults.

In general there was no evidence of any incompatibility between the different wild populations and the mass reared insects, even though the results obtained from male pheromone-calling activity revealed differences in the time of the onset of male calling among the wild populations. The flies from Nayarit began calling earliest in the day, while the flies from Nuevo León began the latest, even though both states have relatively similar latitudes (respectively, 22 and 26 degrees north) in north-western and north-eastern Mexico, and thus benefit from approximately the same daylight h. This independence of calling time initiation from the latitude is also evident from the fact that the onset of pheromone-calling in the southern-most population from Chiapas (16 degrees north), was similar to the Nuevo León population in the northeast.

The combined data of the different indices (ISI, MRPI, FRPI, and RSI) provide a complete and reliable picture of the sexual compatibility between the wild populations and the mass reared sterile flies, as well as their relative competitiveness. The ISI demonstrated good sexual compatibility between the wild populations and the mass-reared strain, indicating that the sterile individuals mate satisfactorily with the wild populations from the 6 states.

The MRPI showed that the sterile males mass-produced at the Moscafrut facility are as effective in copulating with wild females as the wild males. The FRPI reflected a general tendency for wild females to copulate in greater proportion than the sterile females, except for the populations from Tamaulipas and Chiapas. In general, the lower participation of the sterile females suggests that the sterile males have greater possibilities of mating with wild females.

The RSI results show that wild female acceptance of the sterile males was high (25-55%). The results obtained from the Fried test that measures induced sterility, indicate a competitiveness coefficient ranging from 0.2 to 0.5 and suggest that sterile males successfully competed with flies from different geographic origin. This outcome supports the results found in the compatibility tests.

Compatibility and competitiveness are regular quality control tests that are used to determine if a particular mass-rearing strain needs to be replaced (FAO/IAEA/USDA 2003). Previous studies have shown that long periods under mass-rearing conditions adversely can affect the performance of sterile fruit flies (McInnis 1996). Other studies have shown that the geographic origin of different strain might result in sexual incompatibility (Vera et al. 2006). Our current work demonstrates that the mass-reared *A. ludens* strain currently being produced at the Moscafrut and used over the last 10 years in different geographic regions for Mexican fruit fly control programs is still suitable for this purpose. Our data are very similar to those recently published (Rull et al. 2005), although they arrived at a somewhat different conclusion due to the fact that a different analysis was carried out. Continued careful monitoring of the performance of this mass-reared strain under semi-natural or natural is required.

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