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Abstract

The use of the Sterile Insect Technique to control and/or eradicate insect pest populations has been extensively applied to medfly. However, patented differences in sexual compatibility between populations or strains from different origins has been a serious concern to a wider use of sterile flies, and in particular sterile males of genetic sexing strains (GSS). In the present experiments, the sexual compatibility and mating performance of flies from 9 countries representing 5 continents and 4 GSS were measured. It is demonstrated that, from a qualitative standpoint, wild medfly populations world-wide have not yet evolved specific sexual behaviors indicative of incipient pre-mating isolation mechanisms under local natural selection. Wild medfly populations are as sexually compatible with GSS as they are with other wild populations. On that basis, the same mass reared strain can now be used worldwide, as long as it fulfills the standard quality control requirements.

Key Words: medfly, *Ceratitis capitata*, sexual compatibility, comparison, wild population, genetic sexing strain

Resumen

El uso de la técnica del insecto estéril para controlar y o erradicar poblaciones de plagas insectiles ha sido aplicado extensamente a la mosca del Mediterráneo. Sin embargo, diferencias en compatibilidad sexual entre poblaciones o razas de diferentes orígenes ha sido una seria preocupación para un uso mas amplio de las moscas estériles y en particular machos estériles de Cepas genéticamente sexadas (CGS). En los siguientes experimentos, la compatibilidad sexual y la capacidad de apareamiento de las moscas de 9 países, representando 5 continentes y 4 RGS fueron evaluadas. Se ha demostrado que, desde un punto de vista cualitativo, poblaciones salvajes a nivel mundial de la mosca mediterránea aun no han evolucionado bajo selección natural local comportamientos sexuales específicos indicativos de incipientes mecanismos de aislamiento anteriores al apareamiento. Poblaciones salvajes de moscas mediterráneas son tan sexualmente compatibles con CGS que con otras poblaciones salvajes. Sobre esas bases, la misma cepa criada en masa se puede utilizar ahora a nivel mundial con la condición de que cumpla con los requerimientos estándares de control de calidad.

The Mediterranean fruit fly (medfly), *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), is often referred to as the most important agricultural pest in the world (Liquido et al. 1990) and this "title" is widely justified. From its origin in Eastern Africa (Silvestri 1913, Bezzi 1918), the pest efficiently conquered new countries and new hosts. If medfly was present in North African and almost all European Mediterranean countries by the mid-19th century, its introduction in North, Central and Latin America occurred nearly 100 years later (Dridi 1990). Following the development of fruit and vegetable trade worldwide, and the increasing number of international, including intercontinental, airway connections, medfly successfully spread over five continents in less than 150 years, and is found developing, to date, in more than 350 wild and cultivated host plants of various families (Liquido et al. 1990). Such a threat for agriculture represented by a single species turned medfly into one of the main targets of pest control programs, including the Sterile Insect Technique (SIT) described by Knipling (1953).

The use of SIT requires that rearing facilities be developed to produce large numbers of insects for sterile fly releases. In the early stages of medfly control using SIT, mass reared strains were established by colonizing wild insects collected from, or in the vicinity of, the target area. Such strains have been reared in Mexico, Chile, Hawaii and Guatemala rearing facilities. More recently, with the increasing demand for sterile medflies

and the limited number of mass rearing facilities available worldwide, some of these facilities began to export sterilized medflies to other countries. Eight facilities have now reached production levels, which allow them to export sterile insects (Fisher & Caceres 2000) on a regional or inter-regional basis. When this procedure is used, the flies released have to compete with wild flies of a different geographic origin.

The increasing use of medfly genetic sexing strains (GSS) has also resulted in the same strain being used in different countries. To date, five rearing facilities in the world produce GSS (Fisher & Caceres 2000). Since GSS are assembled from specific components, it is impossible to "colonize" them from each country where sterile GSS flies are needed. The GSS are sometimes outcrossed with insects from the target population to increase the genetic variability (Franz et al. 1996), although in some cases this presents problems (G. Franz, IPCS, FAO/IAEA, Vienna, unpublished data). In practice, a single wild population is used as a basis for the synthesis of the GSS. Consequently, the same GSS based on the same wild genetic material may be used in various countries/continents and the question was raised concerning the sexual compatibility of these strains with wild medfly populations in different countries.

In the present work, the sexual compatibility of wild populations originating from nine countries, representing five continents, was measured in pairwise comparisons under semi-natural field cage conditions. In a second series of experiments, flies from four GSS were evaluated.

MATERIALS AND METHODS

Wild Material

Wild insects were collected as pupae from infested fruits in their country of origin. Pupae were shipped by express air mail to Seibersdorf, Austria (or hand-carried), except for field cage tests run in Argentina where wild flies were tested on site (Cayol et al. 1999). Wild insects originating from Argentina (Patagonia region), Australia (Perth), France (Reunion Island), Greece (Crete Island), Guatemala (Antigua), Israel (near Tel Aviv and from the Arava Valley), Kenya (near Nairobi), Portugal (Madeira Island) and South Africa (Western Cape Province) were tested. Their host of origin was guava (Israel, both locations; Portugal; South Africa), coffee (Guatemala, Kenya), orange (Australia, Greece), fig and peach (Argentina) and milkwood (France). Upon reception of a shipment, pupae were weighed and counted. On emergence, flies were sexed and kept in separate ventilated Plexiglas cages ($11 \times 15.5 \times 11$ cm) until tested and provided with adult food (sugar and yeast in 3:1 ratio) and water.

Genetic Sexing Strains

Flies of several genetic sexing strains (GSS) were obtained as pupae from the FAO/IAEA facility at Seibersdorf for green house tests. In the field cage tests in Argentina, GSS flies were provided by the KM8 facility in Mendoza (Cayol et al. 1999). The following four GSS were tested. SEIB 6-96 is a GSS carrying a white pupa (*wp*) mutation (Rössler 1979) in combination with the translocation T(Y;5) 2-22 (Franz et al. 1994). VIENNA 4/TOL-94 is a GSS carrying *wp* and temperature sensitive lethal (*tsl*) mutations in combination with the translocation T(Y;5) 1-61 (Franz et al. 1994). VIENNA 7-97 is a GSS carrying *wp* and *tsl* mutations in combination with the translocation T(Y;5) 3-129 (Kerremans & Franz 1995). AUSTRIA 6-97 is a triple mutant strain carrying *wp*, *tsl* and yellow body (*y*) (Rössler & Rosenthal 1992) selectable markers in combination with the translocation T(Y;5) 2-22 (G. Franz, IPCS, FAO/IAEA, Vienna, unpublished data). The genetic background of SEIB 6-96, VIENNA 7-97 and AUSTRIA 6-96 GSS originates from Egypt. The genetic background of VIENNA 4/TOL-94 originates from Guatemala highlands (Lake Atitlan), following an outcrossing of the original strain (Franz et al. 1996). After sexing on emergence, GSS flies were maintained under the same conditions as wild flies.

Testing Cage

Flies were tested in a greenhouse located at the FAO/IAEA Agriculture and Biotechnology Laboratory (Seibersdorf, Austria). The green house was temperature monitored (temperature ranging between 24 and 32 degrees Celsius). A cage made of netting material was placed inside the greenhouse. The cage contained 6 potted citrus trees (up to 1.8 meter height) in a total volume of 15 m^3. In Argentina, flies were tested in outdoor field cages (Chambers et al. 1983) containing a single planted citrus tree (Cayol et al. 1999). In both greenhouse and field cage tests, the cages were covered with a shading cloth filtering 85% of sunlight to avoid any "greenhouse effect".

The strains were tested in pair-wise comparisons. Depending on the availability of biological material, two types of tests could be run: (i) wild-wild comparisons, where wild flies from two different geographic origins were tested and (ii) wild-GSS comparisons, where wild flies originating from one country were tested against GSS flies. In both types of test, the protocol described by Cayol et al. (1999) for "bisexual" type test was applied. Two days before being tested, active and flying flies were selected. Males and females, from alternatively one of the two populations were marked with a dot of water-based paint on the notum for identification during the course of the

tests. On the day of the test, 30 flies of each sex and each strain were released into the cages at dawn. Males were released 30 minutes before females to give them time to establish a territory and start forming leks (Prokopy & Hendrichs 1979). The number of calling males and the environmental conditions (temperature, relative humidity, light intensity and air pressure) were checked every half-hour. The number and type of mating pairs were checked on a continuous basis, and 5 minutes after initiation of mating, the pairs were collected and placed in vials (50 ml volume) to monitor mating duration. The mated flies were not replaced or released back into the cage after separation (Chambers et al. 1983). Tests lasted for 7-8 consecutive hours. Tests were performed from March 1997 until September 1998, whenever flies were available. A total of 19 combinations were tested as shown in Table 1. Due to the availability of flies from different origins, the number of replications for each combination was variable.

Statistical Analysis

Raw data were transformed following an ARC-SIN $[ASIN(\sqrt{(X/100)}*180/PI)]$ transformation to stabilize variance.

For each of the parameters measured and whenever it was relevant, data were first pooled according to the type of combination tested "wild versus wild" or "wild versus GSS" (later called "wild/wild and wild/GSS comparisons"). As a second step

TABLE 1. TYPE OF MATING COMBINATIONS TESTED.

Wild population	Tested against	
	Wild	GSS
Argentina[a]		Seib 6-96
Australia	Crete Israel	Vienna 4/Tol-94
Crete	Australia	Seib 6-96
Guatemala	Israel Kenya Madeira	Vienna 4/Tol-94
Israel	Australia Guatemala Madeira Reunion	Vienna 4/Tol-94 Austria 6-96 Vienna 7-97
Kenya	Guatemala Madeira	Vienna 4/Tol-94
Madeira	Guatemala Israel Kenya	Vienna 4/Tol-94 Vienna 7-97
Reunion	Israel	
South Africa		Vienna 7-97

[a]Tested in field cages in San Miguel de Tucuman (Argentina) (Cayol et al. 1999).

of the analysis, data were pooled according to the origin of the strain (Madeira, Argentina, Vienna 7-97, etc.) (later called "strain comparison").

In both cases, data were analyzed using Systat 9.0 (Systat, 1999) for analysis of variance (ANOVA), followed by Tukey's HSD test.

RESULTS

Participation of Flies in Mating

This measures the suitability of the flies and the environmental conditions of the tests for mating. It represents the overall mating activity of the flies (Table 2). If PM < 0.20 (proportion of mating) then the results of the test must be rejected (IAEA 1997).

Wild/Wild and Wild/GSS Comparison. The mean PM values obtained in comparing wild/wild and wild/GSS combinations confirmed that the test conditions were suitable for mating, as about 40% of the possible matings were achieved. However, there was a highly significant difference between the two mean PM values, 0.407 (wild) and 0.484 (GSS), ($F = 7.530$; df=1,71; $P = 0.008$) showing that somewhat more matings took place when GSS flies were involved in the test (Table 3).

Strain Comparison. When comparing the PM values obtained for each strain tested, even though the overall mating activity was satisfactory in each case (PM > 0.20), some significant differences can be found among the strains ($F = 2.789$; df = 12,134; $P = 0.002$). Significantly more matings were achieved in tests involving wild flies from Australia (PM = 0.554) than in tests involving wild flies from Kenya, Madeira or Austria 6-96 GSS flies (PM values 0.349, 0.386 and 0.345 respectively). Those differences might reflect various adaptations to the test conditions or a generally higher mating activity of Australian flies.

Sexual Compatibility

In all of the 19 comparisons involving any of the wild populations or GSS tested, each of the four possible types of mating was encountered confirming that there was no absolute behavioral incompatibility among these populations. The sexual compatibility among the flies from different origins was assessed using the Isolation Index (ISI) (Cayol et al. 1999) as described in Table 2. The ISI ranges from -1 ("negative assortative mating", i.e. flies only mate with a "foreign" partner) to +1 ("positive assortative mating" or total sexual isolation, i.e. flies only mate with partner of the same origin), through an equilibrium at 0 (uniform sexual compatibility, i.e. no mating preferences).

Wild/Wild and Wild/GSS Comparison. There was no significant difference between the overall mean value of ISI obtained when comparing wild versus wild populations and wild versus GSS ($F = $

TABLE 2. INDICES USED TO MEASURE SEXUAL COMPATIBILITY OF MEDFLY STRAINS FROM DIFFERENT ORIGINS.[a]

Trait measured	Index formula[b]	
Participation in mating	PM =	$\dfrac{\text{No. of pairs collected}}{\text{No. of females released}}$
Sexual isolation	ISI =	$\dfrac{(aa + bb) - (ab + ba)}{\text{Total no. of matings}}$
Male relative performance	MRPI =	$\dfrac{(aa + ab) - (bb + ba)}{\text{Total no. of matings}}$
Female relative performance	FRPI =	$\dfrac{(aa + ba) - (bb + ab)}{\text{Total no. of matings}}$
Male mating competitivenessa[c]	RSI =	$\dfrac{\text{LW}}{\text{LW} + \text{WW}}$

[a]After Cayol et al. (1999) and McInnis et al. 1996.
[b]"ab": number of matings of "a" males with "b" females.
[c]In RSI: "L" for mass reared males and "W" for wild flies (males or females).

0.030; df 1,71; P = 0.864). Even though the two mean ISI values showed a tendency for homologous (male and female of the same origin) mating (Table 3), there was certainly no evidence of sexual isolation. Of utmost importance, these results show that wild flies did not discriminate against GSS flies more than wild flies originating from a different area or continent. In other words, wild populations are as behaviorally compatible with GSS as they are with other wild populations from various geographic origins.

Strain Comparison. The mean ISI values obtained for the 9 wild populations and the 4 GSS did not differ significantly (F = 1.499; df 12,134; P = 0.132) (Table 4). This confirms that, even if there are some minor differences among the various wild populations and GSS tested, none of them developed, to date, a significant behavioral isolation (ISI > 0.50).

Male and female Relative Mating Performance

Two other indices which look at the relative mating performance of males (MRPI) and females (FRPI) of the two strains, regardless of their mating partners, were measured (Cayol et al. 1999). These indices range between -1 (all matings achieved by one type of male (MRPI) or female (FRPI)) and +1 (all matings achieved by the other type of male (MRPI) or female (FRPI)) through an equilibrium at 0 (equal mating performance of males or females of the two strains) (Table 2). These indices complement the ISI value by better describing the role played by males and females of the two strains compared.

Wild/Wild and Wild/GSS Comparison. The male relative mating performance is significantly higher when comparing wild versus wild populations than it is when comparing wild populations versus GSS (F = 4.693; df 1,71; P = 0.034) (Table 3). This demonstrates that, when two types of wild males of different geographic origin are present in the same cage, one of the two types of males mates more than the other. However, when wild and GSS males are present, the relative performance is more "balanced", i.e. both types of males mate in a similar proportion (regardless of

TABLE 3. SEXUAL COMPATIBILITY AND PERFORMANCE MEASURED WHEN TESTING MEDFLY WILD POPULATIONS AGAINST WILD OR GSS.

| Parameter measured | Combination tested | | |
	Wild/wild	Wild/GSS	
PM	0.407 b ± 0.020	0.484 a ± 0.018	F = 7.530; df = 1,71; P = 0.008
ISI	0.233 a ± 0.057	0.221 a ± 0.034	F = 0.030; df = 1,71; P = 0.864
MRPI[a]	0.375 a ± 0.042	0.265 b ± 0.030	F = 4.693; df = 1,71; P = 0.034
FRPI[a]	0.288 a ± 0.033	0.345 a ± 0.032	F = 1.330; df = 1,71; P = 0.253

[a]Based on absolute values.
[b]Data are presented as mean ± SEM. Data followed by the same letter on the same row do not differ significantly according to Tukey's HSD test (P > 0.05).

TABLE 4. Sexual compatibility and performance of wild populations and GSS.[b]

| Origin of the flies | Parameter measured | | | |
	PM	ISI	MRPI[a]	FRPI[a]
Argentina	0.488 ab ± 0.032	0.309 a ± 0.062	0.360 b ± 0.023	0.332 ab ± 0.051
Australia	0.554 a ± 0.029	0.069 a ± 0.104	0.480 abc ± 0.069	0.496 a ± 0.075
Crete	0.508 ab ± 0.025	0.108 a ± 0.123	0.586 ab ± 0.066	0.300 ab ± 0.114
Guatemala	0.462 ab ± 0.043	0.188 a ± 0.069	0.462 abc ± 0.072	0.442 a ± 0.085
Israel	0.419 ab ± 0.021	0.200 a ± 0.056	0.316 bc ± 0.043	0.491 a ± 0.047
Kenya	0.349 b ± 0.039	0.319 a ± 0.161	0.171 c ± 0.056	0.420 ab ± 0.102
Madeira	0.386 b ± 0.022	0.196 a ± 0.079	0.600 a ± 0.053	0.156 b ± 0.028
Reunion	0.389 ab ± 0.053	0.377 a ± 0.085	0.278 c ± 0.056	0.361 ab ± 0.081
South Africa	0.477 ab ± 0.043	0.259 a ± 0.064	0.421 abc ± 0.054	0.299 ab ± 0.083
Vienna 4/tol-94	0.522 ab ± 0.038	0.235 a ± 0.070	0.456 abc ± 0.061	0.335 ab ± 0.042
Vienna 7-97	0.457 ab ± 0.029	0.092 a ± 0.084	0.236 c ± 0.058	0.563 a ± 0.061
Seib 6-96	0.494 ab ± 0.028	0.300 a ± 0.038	0.313 bc ± 0.049	0.396 ab ± 0.030
Austria 6-96	0.345 b ± 0.029	0.104 a ± 0.021	0.200 c ± 0.200	0.470 ab ± 0.004
	$F = 2.789$ $df = 12,134$ $P = 0.002$	$F = 1.499$ $df = 12,134$ $P = 0.132$	$F = 4.563$ $df = 12,134$ $P = 0.000$	$F = 3.985$ $df = 12,134$ $P = 0.000$

[a]Based on absolute values.
[b]Data are presented as mean ± SEM. Data followed by the same letter in the same column do not differ significantly according to Tukey's HSD test ($P > 0.05$).

the type of female). No significant difference was found in the female relative mating performance ($F = 1.330$; $df = 1,71$; $P = 0.253$) (Table 3). In both cases (wild/wild and wild/GSS), there is a slight tendency for one of the two types of females to outcompete the other. In wild/GSS comparisons, the GSS females often mate more than their wild counterparts (regardless of the type of males).

Strain Comparison. The MRPI value of the Madeira wild population is significantly higher than that of the Kenya and the Reunion wild populations, and that of the Vienna 7-97 and the Austria 6-96 GSS ($F = 4.563$; $df = 12,134$; $P < 0.000$) (Table 4). Whatever strain they were compared to, Madeira males very often, and by far, outcompeted the other type of males for mates. To the contrary, and under similar conditions, Kenya, Reunion, Vienna 7-97 and Austria 6-96 males were outcompeted by any other type of males they were compared to, even with their own female counterparts. There was a significant difference between the higher FRPI value of the Australia, Guatemala and Israel wild populations and the Vienna 7-97 GSS and that of the Madeira wild population ($F = 3.985$; $df = 12,134$; $P < 0.000$) (Table 4). This shows that these 4 types of females were more prone to mate than were wild Madeira females. This would indicate that Madeira females were more "selective" in choosing a mate than were the other strains of females.

Mating Competitiveness of GSS Males

The mating competitiveness of GSS males with wild males for wild female mates was mea-

sured by the Relative Sterility Index (RSI) (McInnis et al. 1996) described in Table 2. When RSI = 0.5, wild and GSS males are equally competitive. The mean RSI value has been compared for the 4 GSS tested and results are shown in Table 5.

The analysis showed that, even though all the GSS males did compete with wild males for wild female mates, Vienna 4/Tol-94 males were about twice as competitive as Vienna 7-97 males ($F = 2.967$; $df = 3,48$; $P = 0.041$) (Table 5). This result confirms a poor relative mating performance for Vienna 7-97 males, which has been previously demonstrated by the relatively low MRPI value.

Duration of Mating

Time spent in copula (duration of mating) was measured and compared for the homologous type of mating (male and female of the same origin) for each GSS and wild population tested and these results are shown in Table 6.

TABLE 5. Mating competitiveness of males from the different GSS.[a]

Strain	Relative Sterility Index[b]
Vienna 4/Tol-94	0.448 a ± 0.059
Vienna 7-97	0.217 b ± 0.045
Seib 6-96	0.302 ab ± 0.049
Austria 6-96	0.250 ab ± 0.087

[a]Data are presented as mean ± SEM.
[b]Data followed by the same letter do not differ significantly according to Tukey's HSD test ($F = 2.967$; $df = 3,48$; $P = 0.041$).

TABLE 6. DURATION OF HOMOLOGOUS MATING (MALE AND FEMALE OF THE SAME ORIGIN) FOR THE DIFFERENT WILD POPULATIONS AND GSS.

Origin of the flies	Duration of homologous pairing (min)[a,b]
Argentina	164.466 cd ± 4.777
Australia	204.920 ab ± 5.962
Crete	195.500 ad ± 6.859
Guatemala	156.970 d ± 4.180
Israel	157.418 d ± 4.728
Kenya	198.464 ac ± 8.412
Madeira	178.095 cd ± 4.559
Reunion	178.538 bcd ± 9.047
South Africa	227.310 a ± 6.241
Vienna 4/Tol-94	164.983 cd ± 3.593
Vienna 7-97	158.072 d ± 4.225
Seib 6-96	124.035 e ± 3.700
Austria 6-96	164.278 cde ± 8.664

[a]Data are presented as mean ± SEM.
[b]Data followed by the same letter do not differ significantly according to Tukey's HSD test ($F = 28.710$; df = 12,1157; $P = 0.000$).

A large and significant variation in mating duration has been found ($F = 28.710$; df = 12,1157; $P < 0.000$) (Table 6). Average time spent in copula can vary from 2 hours for the Seib 6-96 GSS up to nearly 4 hours for the South Africa wild population. However, this duration seems to be relatively stable for each strain, as demonstrated by the low standard error values. In addition, the GSS flies tend to mate for a relatively shorter period (2 to 3 hours) than do wild flies (3 to 4 hours).

DISCUSSION

These data demonstrate that, from a qualitative standpoint, wild medfly populations worldwide have not yet evolved specific sexual behaviors indicative of incipient pre-mating isolation mechanisms under local natural selection. In addition, it was shown that wild populations are as sexually compatible with GSS as they are with other wild populations.

However, some quantitative differences have been measured among wild populations or GSS, such as a lower or higher male or female relative performance. In the case of Madeira for example, the high male performance, and the relative selectiveness of the females as shown in the present experiments could, in a long run, result in a lower mating acceptance of relatively poorly competitive mass-reared males, such as Vienna 7-97, which could affect the effectiveness of SIT. Additional tests have been run to look at this specific case which tends to show that Madeira females do discriminate against Vienna 7-97 males more than other wild or GSS males (J. P. C., unpublished data).

The high importance of mating behavior studies to the SIT has encouraged the Insect Pest Control Section of the International Atomic Energy Agency to investigate this subject. The coordinated research program started in 1994 by the IAEA examined details of male courtship behavior in wild populations from nine countries (FAO/IAEA 1994) from both qualitative and quantitative standpoints, using slow motion video recording. Some minor differences have been found among the wild populations, as demonstrated by Briceño et al. (2002). When comparing wild flies from Costa Rica and Argentina, the authors showed that some significant differences of the courtship songs could be identified and measured. In addition, it was shown that long term rearing could affect significantly the duration of the mass-reared male courtship (Eberhard & Briceño 1996, Briceño & Eberhard 2002) and love songs (Briceño & Eberhard 1997). The present findings tend to show that copula duration is also shortened in mass rearing. Those differences in mating duration warrant further study in relation to post-mating isolation. Post-mating isolation could affect the efficacy of SIT due to remating of wild females, shortly after a first mating with a sterile GSS male.

Concerns about the sexual compatibility among medflies from different origins represented somewhat of a threat to the shipment of sterile flies from one country to another to support SIT programs. These concerns become more pronounced when a GSS was proposed to be used in many different SIT programs. The findings of the present experiments support the potential use of the same GSS anywhere in the world. Out of the 4 GSS tested in the present experiments, the only one, which was outcrossed with a wild population (Vienna 4/Tol-94), did show the highest mating competitiveness. This strongly supported the idea of building-up a new GSS based on mixing wild populations from various origins. This new and very promising GSS has now been developed and is currently under testing (G. Franz, IPCS, FAO/IAEA, Vienna, unpublished data).

Gasparich et al. (1997) showed that the mitochondrial DNA of medfly populations from 100 different origins was indeed variable and that it probably reflected the colonization pattern of medfly from its origin in Eastern Africa about 200 years ago. However there was no evidence that substantial genetic differentiation had occurred. When a medfly outbreak occurs, program managers sometimes worry that the sterile flies released might not be from the same geographic origin and hence would not mate. A second concern is that the "foreign" flies might introduce new genetic material into the country. The fear is that "foreign" fertile flies would establish a new population with its own genetic and behavioral characteristics. However, the present work based on populations representative of five continents,

clearly demonstrates that there are no significant population specific mating behavior traits. These observations together with the genetic data suggest that the risk of introducing a more virulent form of medfly into a specific country is remote.

In conclusion, strains to be used in SIT programs in any country must be selected to maximize the quality of the flies produced, rather than based on the geographic origin of the strain.

REFERENCES CITED

BEZZI, M. 1918. Notes on the Ethiopian fruit flies of the family *Trypaneidae*, other than *Dacus* (S.L.) with descriptions of new genera and species. Bull. Entomol. Res. 7: 215-251.

BRICEÑO, R. D., AND W. EBERHARD. 1997. Comparisons of the songs of successful and unsuccessful males of different genetic sexing strains of medfly, *Ceratitis capitata* (Diptera: Tephritidae). *In* IAEA [ed.] Medfly mating behaviour studies under field cage conditions: Report of the Third Research Co-ordination Meeting—Tel Aviv (Israel) 15-19 Sep. 1997. Vienna. 34 pp.

BRICEÑO, R. D., W. G. EBERHARD, J. C. VILARDI, AND P. LIEDO. 2002. Geographic variation in the intermittent buzzing songs of wild and mass-reared male medflies (Diptera: Tephritidae: *Ceratitis capitata*). Florida Entomol. 85: 14-31.

BRICEÑO, R. D., AND W. G. EBERHARD. 2002. Decisions during courtship by male and female medflies, *Ceratitis capitata* (Diptera, Tephritidae): coordinated changes in male behavior and female acceptance criteria in mass-reared flies. Florida Entomol. 85: 32-40.

CAYOL, J. P., J. VILARDI, E. RIAL, AND M. T. VERA. 1999. New indices and method to measure the sexual compatibility and mating performance of medfly (Diptera: Tephritidae) laboratory reared strains under field cage conditions. J. Econ. Entomol. 92(1): 140-145.

CHAMBERS, D. L., C. O. CALKINS, E. F. BOLLER, Y. ITÔ, AND R. T. CUNNINGHAM. 1983. Measuring, monitoring, and improving the quality of mass-reared Mediterranean fruit flies, *Ceratitis capitata* Wied. 2. Field tests for confirming and extending laboratory results. Z. Angew. Entomol. 95: 285-303.

DRIDI, B. 1990. Etude de quelques aspects de la biologie de la mouche méditerranéenne des fruits: *Ceratitis capitata* Wiedemann (Diptère, Trypetidae). Différenciation entre souche d'élevage et population sauvage d'Algérie. Ph.D. dissertation, Fac. Sci. et Tech. St. Jérôme Univ. d'Aix-Marseille 3, Marseille, France. 133 pp.

EBERHARD, W., AND R. D. BRICEÑO. 1996. Genetic assimilation of the effects of crowding on the duration of premounting courtship behaviour of male medflies (Diptera: Tephritidae). *In* IAEA [ed.] Medfly mating behaviour studies under field cage conditions: Report of the Second Research Co-ordination Meeting—Tapachula (Mexico) 19-23 Feb. 1996. Vienna. 53 pp.

FISHER, K., AND C. CACERES. 2000. A filter rearing system for mass reared medfly. pp. 543-550. *In* T. K. Hong [ed.] Area-wide management of fruit flies and other major insect pests. Universiti Sains Malaysia Press. Penang, Malaysia.

FRANZ, G., E. GENCHEVA, AND PH. KERREMANS. 1994. Improved stability of sex-separation strains for the Mediterranean fruit fly, *Ceratitis capitata*. Genome 37: 72-82.

FRANZ, G., PH. KERREMANS, P. RENDON, AND J. HENDRICHS. 1996. Development and application of genetic sexing systems for the mediterranean fruit fly based on a temperature sensitive lethal. pp. 185-191. *In* B. A. McPheron and G. J. Steck [eds.], Fruit fly pests: a world assessment of their biology and management. St. Lucie Press, Delray Beach.

GASPARICH, G. E., J. C. SILVA, H. Y. HAN, B. A. MCPHERON, G. J. STECK, AND W. S. SHEPPARD. 1997. Population genetic structure of Mediterranean fruit fly (Diptera: Tephritidae) and implications for world-wide colonization patterns. Ann. Entomol. Soc. Am. 90(6): 790-797.

INTERNATIONAL ATOMIC ENERGY AGENCY (FAO/IAEA). 1994. Medfly mating behaviour studies under field cage conditions: Report of the First Research Co-ordination Meeting—Vienna (Austria) 4-7 Oct. 1994. IAEA, Vienna. 13 pp.

INTERNATIONAL ATOMIC ENERGY AGENCY (FAO/IAEA). 1997. Medfly mating behaviour studies under field cage conditions: Report of the Third Research Co-ordination Meeting—Tel Aviv (Israel) 15-19 Sep. 1997. IAEA, Vienna. 36 pp.

KERREMANS, PH., AND G. FRANZ. 1995. Isolation and cytogenetic analyses of genetic sexing strains for the medfly, *Ceratitis capitata*. Theor. Appl. Genet. 91: 255-261.

KNIPLING, E. F. 1953. Possibilities of insect control or eradication through the use of sexually sterile males. J. Econ. Entomol. 48(4): 459-462.

LIQUIDO, N. J., R. T. CUNNINGHAM, AND S. NAKAGAWA. 1990. Hosts plants of the mediterranean fruit fly (Diptera: Tephritidae) on the Island of Hawaii (1949-1985 survey). J. Econ. Entomol. 83: 1863-1868.

MCINNIS, D. O., D. R. LANCE, AND C. G. JACKSON. 1996. Behavioral resistance to the Sterile Insect Technique by Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. Ann. Entomol. Soc. Am. 89(5): 739-744.

PROKOPY, R. J., AND J. HENDRICHS. 1979. Mating behavior of *Ceratitis capitata* on a field caged host tree. Ann. Entomol. Soc. Am. 72: 642-648.

RÖSSLER, Y. 1979. The genetics of the Mediterranean fruit fly: white pupae mutant. Ann. Entomol. Soc. Am. 72: 583-585.

RÖSSLER, Y., AND J. ROSENTHAL. 1992. Genetics of the Mediterranean fruit fly (Diptera: Tephritidae): Morphological mutants on chromosome 5. Ann. Entomol. Soc. Am. 85(4): 525-531.

SILVESTRI, F. 1913. Viaggio in Agrica per cercare parassiti di mosche dei frutti. Boll. Lab. Zool. Agric. Portici. 8: 1-164.

SYSTAT, INC. 1999. Systat for Windows: statistics, Version 9 Edition. Evanston, I1: SYSTAT, Inc., 1999.