RESEARCH ARTICLE Agricultural Science Digest, Volume 44 Issue 4: 739-744 (August 2024)

# Comparison of Bisexual and Genetic Strains of *Ceratitis capitata* from Various Origins

Hasan Al-Khshemawee[1], Hassnen T. Kareem[1], Liqaa Mohammed Shiblawi[2]

10.18805/ag.DF-467


## ABSTRACT

**Background**: The sterile insect technique (SIT) of Mediterranean fruit fly *Ceratitis capitata* depends on the ability of male to compete successfully against wild male in obtaining matings with wild females.

**Methods**: To evaluate mass and wild reared the sexual performance and compatibility of medflies has been conducted in a series of field cages test. At the same time, this study has been done in the laboratory domestication as well. Three populations have been tested in this research: wild flies from of Perth Hills stone; Western Australia and Norwood road, Maida Vale, Perth; semi-wild flies after 7 to 10 generation in the lab. and a mass-reared and wild strains of flies were used in the study.

**Result**: All the field cages have been indicating that all population of medfly are all most the same. There is no significant difference in the number of egg and sex ratio of wild population. In the mass- reared males, the performed of males are significantly poorer than wild male in achieve mating with wild female. The study has been showed that mating performances are reduced in mass-reared and semi-wild fly after 8 to 11 generation in laboratory.


**Key words:** Genetic strains, Various origins.

## INTRODUCATION

Sterile Insect Technique (SIT) has been widely used to eradicate infestation of the Mediterranean fruit fly, *C. capitata* (Enkerlin, 2005, Al-Khsemawee *et al.,* 2017). The SIT involved the sterilization, production and released of huge number of *C. capitata* male into the field. Mating between sterile male and wild female results in the oviposition of infertile egg. Thus, causing decline in the wild populations. Insect from the same species may behave differently in different geographical areas depending on variations in selection pressures (Thornhill and Alcock, 1983). Recent studies with wild flies from different areas around the world (Australia, Argentina, Kenya, Crete, Guatemala, South Africa, Madeira-Portugal and Reunion-France) show that mating compatibility with each others (Robinson *et al*., 2002; Al-Khsemawee *et al.,* 2018). The United States Department of Agriculture (USDA) and the California Department of Food and Agriculture (CDFA) operated that the sterile insect technique of fruit fly, *C. capitata* means of depend on the ability of the released sterile flies to mate with the wild target population. There was evidence that releasing sterilized males reduces the efficiency of the sterile insect technique by distracting sterile males and damaging the fruit (Hendrichs *et al.,* 1995; Al-Khshemawee *et al.,* 2017; Ahmed *et al*., 2022). Eliminating males at early stages of insect (larvae or pupae) by obtaining genetically sexing strains could improve optimization of mass-rearing programs (Niyazi *et al.,* 2004), but, the courtship behaviour of these strains should be tested for compatibility with wild insects. Sterile insect technique requires a thorough knowledge of the monitor behaviour of the fruit flies. This program is effective only if the released, mass reared sterile male mate efficiently with wild insects under varied wild conditions. Thus, it is important to standardize way of quality control for the mass-reared lines (Calkins and Ashley, 1989). Measures of quality control of Medfly used the last 10 year ago include egg viability, relative mating speed, pupal weight, percentage of larvae which pupate, flight ability, longevity and mating behaviour.

In this study, flies tested the possibility of isolation among 10 generations of medfly populations from Perth Hills stone and down south of Western Australia. In addition, mating compatibility of the wild population with sterile flies and semi wild populations were studied. This research was conducted with the purpose of increasing the knowledge of sexual selection of medfly and comparing between wild and mass-reared of medfly. Wild and mass-reared strains were analysed in different cages and different stages of this insect. This research was conducted to determine the differences between wild and mass-reared strains and to understand the life history of these strains.

[1]Dapertmant of Plant Protection, College of Agriculture, Wasit University, Wasit, Iraq.
[2]College of Agriculture, Diyala University, Diyala, Iraq.

**Corresponding Author:** Hasan Al-Khshemawee, Dapertmant of Plant Protection, College of Agriculture, Wasit University, Wasit, Iraq. Email: hasanfaraj@uowasit.edu.iq.

**How to cite this article:** Al-Khshemawee, H., Kareem, H.T. and Shiblawi, L.M. (2024). Comparison of Bisexual and Genetic Strains of *Ceratitis capitata* from Various Origins. Agricultural Science Digest. 44(4): 739-744. doi: 10.18805/ag.DF-467.

**Submitted:** 15-03-2022 **Accepted:** 21-05-2024 **Online:** 06-06-2024

## MATERIALS AND METHODS

### Culture of insects

Ten generations population of medfly have been studied: (1) wild fruit flies from of Perth Hills stone; Western Australia (2), Australia. The experminet period was 1/2/2018 to 3/10/2019. Semi wild fruit flies after 8 to 11 generations in the lab. A mass-reared and wild strains of flies were used in the study. The wild strain was obtained from infested orange collected from local trees. The second generation of flies from wild strain were used in the study to obtain enough individuals for this experiment. The laboratory strain was started with flies collected from Department of Agriculture and Food Western Australia (DAFWA) and adapted to mass rearing during 2017. The laboratory strain has been reared in the Murdoch Laboratories for all generations. Temperature cabinet at 23±1°C, 75±4% relative humidity, 12:12 hours of D/L have been used for reared the flies. Carrot media has been used to rearing the eggs. After hatch, larvae were transferred to screen cage (40 $cm^3$). Pupae stage tooks 12 to 14 days for adults to emerge. Adults fed with a paste made from yeast hydrolysate, crystalline sugar (Bidvest, Australia) at the ratio of 4:1 mixed with water.

### Procedure of quality parameters

Some of quality parameters were collected separately. These parameters include egg hatch percentage, pupation percentage, pupal weight, adult's emergence, gender ratio and flight ability.

### Percentage of egg hatch

First step was collected eggs in fruit dome or cup for normal culturing. Then, count 300 eggs and place them on the black filter paper. The eggs were checked after 3 days for counting unhatched eggs.

### Percentage of pupation

After the larval developed, place the pupae in the box without the petri dish lid. It was after 14 days of egg collection.

**Table 1:** The viability of *C. capitata* for 2017 and 2018 from different origans.

| G | 2017 | | | | | 2018 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Egg no. | % AE | % females | % males | Viability % | % AE | % females | % males | Viability % |
| F1 | 100 | 81.81 | 40.74 | 59.25 | 54 | 95.08 | 53.44 | 46.55 | 58 |
| F2 | 100 | 81.01 | 45.31 | 54.68 | 64 | 80.00 | 46.42 | 53.57 | 56 |
| F3 | 100 | 84.61 | 51.51 | 48.48 | 66 | 80.82 | 57.62 | 42.37 | 59 |
| F4 | 100 | 73.23 | 48.07 | 51.92 | 52 | 76.05 | 55.55 | 44.44 | 54 |
| F5 | 100 | 74.07 | 47.50 | 52.50 | 40 | 95.34 | 58.53 | 41.46 | 41 |
| F6 | 100 | 88.00 | 50.00 | 50.00 | 66 | 98.48 | 50.76 | 49.23 | 65 |
| F7 | 100 | 95.31 | 52.45 | 47.54 | 61 | 95.65 | 53.03 | 48.48 | 66 |
| F8 | 100 | 84.21 | 51.5 | 48.43 | 64 | 90.78 | 55.07 | 44.92 | 69 |
| F9 | 100 | 85.48 | 60.37 | 39.62 | 53 | 95.31 | 57.37 | 42.62 | 61 |
| F10 | 100 | 92.00 | 57.97 | 42.02 | 69 | 95.89 | 58.57 | 41.42 | 70 |
| F11 | 100 | 77.27 | 50.98 | 49.01 | 51 | 88.05 | 49.15 | 50.84 | 59 |
| F12 | 100 | 77.77 | 52.38 | 47.61 | 42 | 89.09 | 44.89 | 55.10 | 49 |
| F13 | 100 | 81.81 | 55.55 | 44.44 | 63 | 80.26 | 59.01 | 47.54 | 61 |
| F14 | 100 | 91.56 | 52.63 | 47.36 | 76 | 90.41 | 46.96 | 53.03 | 66 |
| F15 | 100 | 81.96 | 52.00 | 48.00 | 50 | 91.89 | 51.47 | 48.52 | 68 |
| F16 | 100 | 83.09 | 55.93 | 44.06 | 59 | 87.32 | 50.00 | 50.00 | 62 |
| F17 | 100 | 85.18 | 41.30 | 58.69 | 46 | 90.90 | 47.50 | 52.50 | 40 |
| F18 | 100 | 84.61 | 60.00 | 40.00 | 55 | 78.48 | 53.22 | 46.77 | 62 |
| F19 | 100 | 79.72 | 49.15 | 50.84 | 59 | 94.44 | 45.58 | 54.41 | 68 |
| F20 | 100 | 85.91 | 52.45 | 47.54 | 61 | 94.28 | 53.03 | 46.96 | 66 |
| F21 | 100 | 84.12 | 58.49 | 41.50 | 53 | 95.38 | 53.22 | 46.77 | 62 |
| F22 | 100 | 75.75 | 62.00 | 38.00 | 50 | 92.18 | 49.15 | 50.84 | 59 |
| F23 | 100 | 77.77 | 48.97 | 51.02 | 49 | 90.16 | 47.27 | 49.09 | 55 |
| F24 | 100 | 59.42 | 51.21 | 48.78 | 41 | 90.76 | 50.84 | 49.15 | 59 |
| F25 | 100 | 87.03 | 42.55 | 57.44 | 47 | 87.03 | 42.55 | 57.44 | 47 |
| F26 | 100 | 71.83 | 33.33 | 66.66 | 51 | 85.89 | 47.76 | 52.20 | 67 |
| F27 | 100 | 74.28 | 44.23 | 55.76 | 52 | 90.78 | 50.72 | 49.27 | 69 |
| F28 | 100 | 78.26 | 55.55 | 44.44 | 54 | 90.54 | 49.25 | 50.74 | 67 |
| F29 | 100 | 83.60 | 52.94 | 47.05 | 51 | 94.23 | 57.14 | 42.85 | 49 |
| F30 | 100 | 70.90 | 61.53 | 38.46 | 39 | 93.84 | 52.45 | 47.54 | 61 |

Then, sieved the pupation media for collect the pupae. This experiment for determine % pupation from the number of eggs that hatched.

**Adult emergence and the gender ratio**

Place the pupae at optimum temperature and humidity and return them to the ventilated box. It was allowed the flies to emerge and die. After that, counted the number emerged and percentage of males and females. This step to determine the adult emergence from larvae that pupated and gender ratio. Three replicates were used from main culture to determine the gender ratio.

**Flight ability**

For flight ability test, samples of pupae have been taken from main culture to measure fight ability. Plastic dish lid (9 cm) containing black paper and glass tube (10 cm) light were used in this experiment. A hundred of pupae were put into the unit before adult emergence and they allowed for 10 days later. Calculate the flight ability index by:

$$FAI = \frac{\text{Number of flies left by flight}}{\text{Number of flies emerged}} \times 100$$

**Statistical analysis**

Data was analysed by SPSS 24 (2017) software. The data compare between wild and mass-reared strains. The parameters have been used to compare between these strains.

## RESULTS AND DISCUSSION

According to our observations, all of the successful courtships were composed of the following sequence of behaviors in males-calling, wing fanning, wing buzzing, peaceful attempt and copulation. Although the proportion

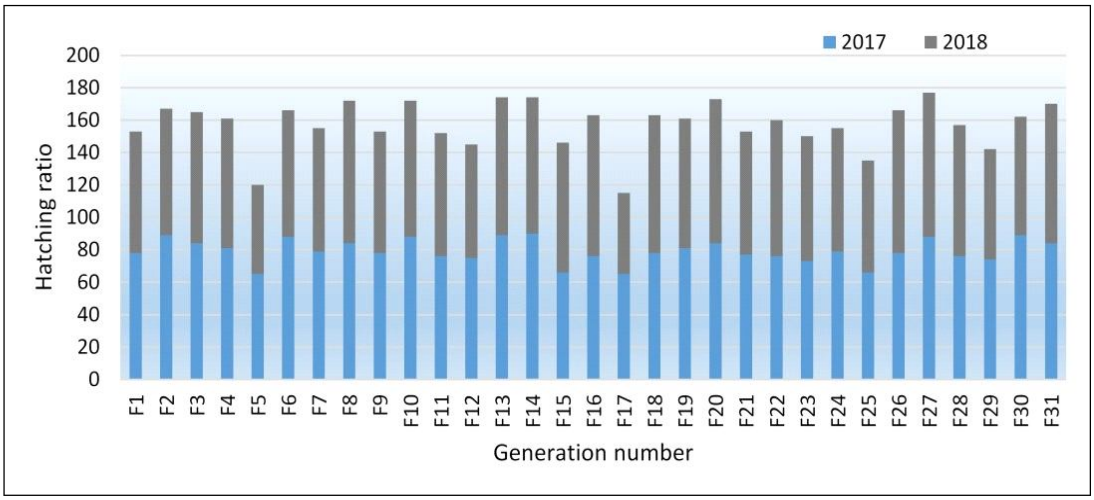



**Fig 1:** Egg hatchingof *C. capitata* for F31 (2 years).

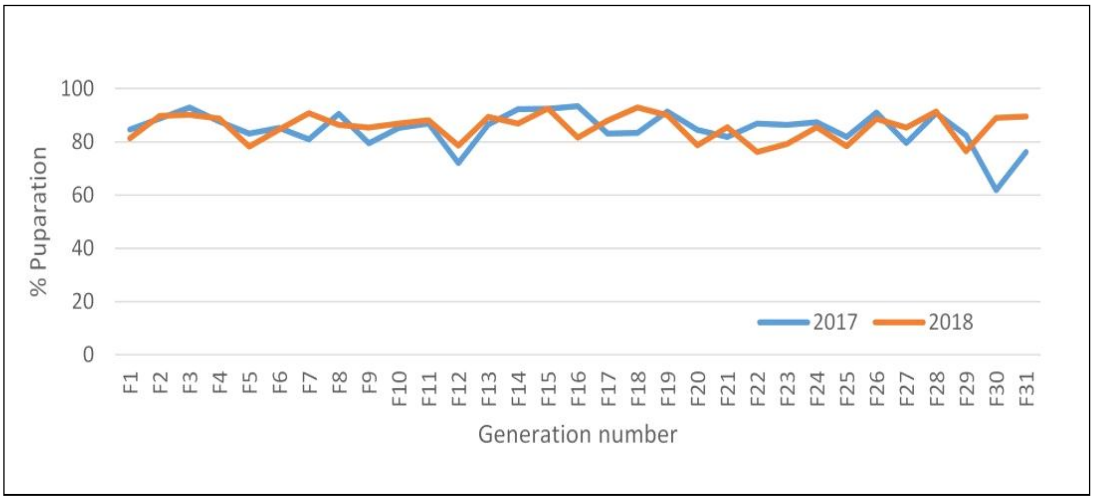



**Fig 2:** Puparation number of *C. capitata.*

of successful male did not differ significantly between strain, some differences have been observed when the times used for each activity were compared both between strains and between successful and unsuccessful males (Table 1). Unsuccessful males used significantly more time than successful males in several activities, particularly in stationary calling in both strains. However, successful males used more time in peaceful mating attempts, which quickly led to copulation. Some differences between successful and unsuccessful males also were observed between strains. For example, successful mass-reared males spent less time in mobile calling and fanning. In contrast, no significant difference was detected for these activities in the wild strain. There were marginally significant differences between strains (pooling successful and unsuccessful males) for some of the behavioral variables. Wild males spent more time in mobile, stationary and fighting activities than did mass-reared males, although these differences were not significant using a table wide P value of 0.05. Hence, copulation tends to be reached earlier by the mass-reared males. Almost all successful mass-reared males completed copulation within 20 min (Fig 1), while -GO% of the successful wild males had completed mating by this time.

Overall, these results suggest that the mass-reared males tended to use less time in activities which, under our experimental conditions, do not contribute to mating quickly. The results of the analysis using the number of occurrences of each activity are shown in (Fig 2). In general, wild males seem to be less sexually motivated, as they were busier in nonreproductive activities. For example, when a meeting was observed, wild males were less active in calling than mass-reared males. Adverse reactions during the meeting were more frequent in courtships involving wild males. Moreover, wild males were more likely to perform wing displays (signaling) during meetings. Differences between strains also were observed for the courtship steps (Fig 3). For example, wing fanning per

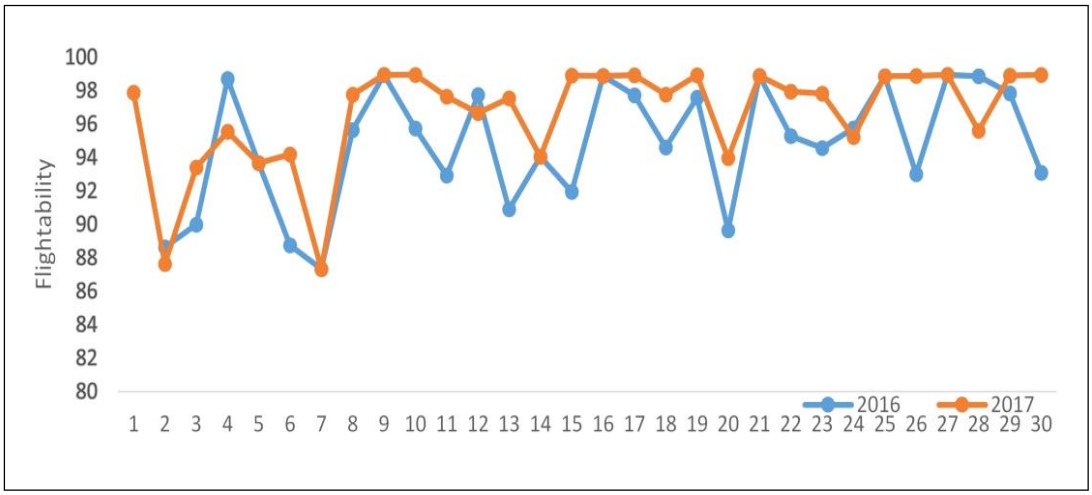



**Fig 3:** The flightability of *C. capitata* for 2017 and 2018.

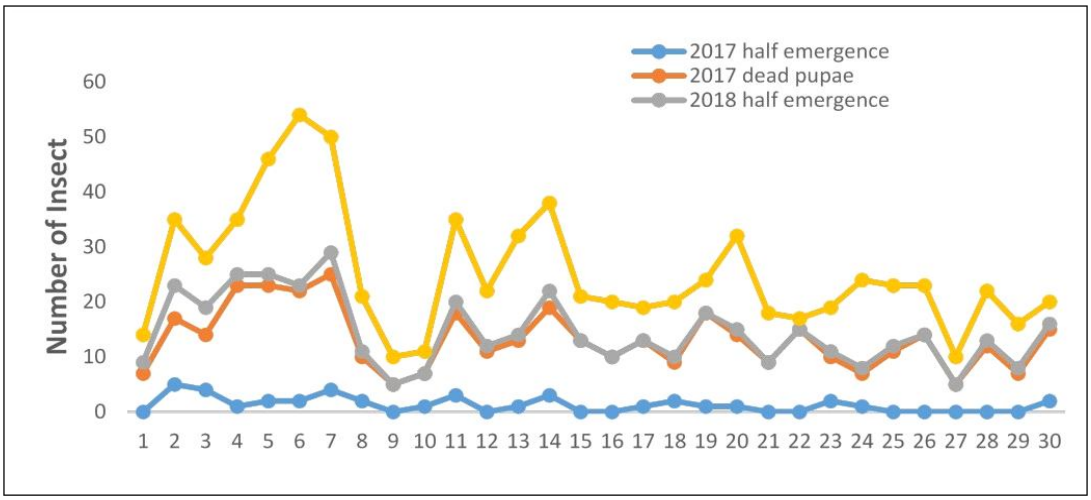



**Fig 4:** The number of insects develop to adults.

meeting was less frequent in wild than in mass-reared males. The same pattern of between strain variations also was observed for the buzzing behaviour (Fig 4).

The results of the first cage showed that there is no significant difference between the strains (Fig 1). The ration of this data was always at the minimum required (0.20) of data analysis. The wild males were successfully competing for the female that presented on Table (1). However, the mass reared flies showed the lower sexual success in the lab. The males in the experiments were against all females. The mass reared males were performed better when compete with wild females, while not compete for the female from their own strain. In overall, the data showed that there is no significant difference between wild strains. The number of hatched were different depends on the weather conditions (water, temperature, humidity and light), the hatch start with 81.81% (F1) and the (F30) was 70.90 (Table 1).

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 flies tested. These results were expected in accordance with the compatibility studies of by 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 (Cáceres *et al.,* 2002). 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 and Lopez, 1993). 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 (Fisher and Caceres, 1998; Al-khshemawee *et al*., 2019).

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 (Caceres, 2002; San Andrés *et al.,* 2007). For this reason, the development of a filter rearing system to manage mother colonies under rearing conditions (fly density, sex-ratio and physical features) more similar to conditions found in nature, as described by (Rendón *et al.,* 2004; Franz, 2005), is recommended. In conclusion, our data indicate no mating incompatibility among strains tested and 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 (Arredondo *et al.,* 2016).

## CONCLUSION

In this study, the experiments have been done the laboratory domestication. Three populations have been tested in this research: wild flies from of Perth Hills stone; Western Australia and Norwood road, Maida Vale, Perth; semi-wild flies after 7 to 10 generation in the lab. and a mass-reared and wild strains of flies were used in the study. All the field cages have been indicating that all population of medfly are all most the same. There is no significant difference in the number of egg and sex ratio of wild population. In the mass- reared males, the performed of males are significantly poorer than wild male in achieve mating with wild female. The study has been showed that mating performances are reduced in mass-reared and semi-wild fly after 8 to 11 generation in laboratory.

### Conflict of interest

The authors have declared that there is no conflict of interest.

## REFERENCES

Al-khshemawee, H., Manjree, A. and Yonglin, R. (2017). Detection of Mediterranean Fruit Fly larvae ceratitis capitata (Diptera: Tephritidae) in different types of fruit by HS-SPME GC-MS Method. Journal of Biosciences and Medicines. 5(1): 154-169.

Al-khshemawee, H., Manjree, A. and Yonglin, R. (2019). Stable isotope labelling of *Ceratitis capitata*. Plant Protection Science. 55 (1): 54-60.

Al-khshemawee, H., Xin, D., Manjree, A., Jeong, Y. and Yonglin, R. (2019). Application of direct immersion solid-phase microextraction (DI-SPME) for understanding biological changes of Mediterranean Fruit Fly (*Ceratitis capitata*) during mating Procedures. Molecules. 23(1): 2951.

Arredondo, J., Ruiz, L., Hernández, E., Montoya, P. and Díaz-Fleischer, F. (2016). Comparison of *Anastrepha ludens* (Diptera: Tephritidae) bisexual and genetic sexing (Tapachula-7) strains: effect of hypoxia, fly density, chilling period and food type on fly quality. Journal of Economic Entomology. 109(2): 572-579.

Cáceres C, J.P. Cayol, W. Enkerlin, G. Franz, J. Hendrichs, A.S. Robinson and B.N. Barnes. (2002). Comparison of Mediterranean fruit fly (*Ceratitis capitata*)(Tephritidae) bisexual and genetic sexing strains: development, evaluation and economics. In: Proceedings, Symposium: 6th International Symposium on Fruit Flies of Economic Importance. (pp. 6-10).

Caceres, C., (2002). Mass rearing of temperature sensitive genetic sexing strains in the Mediterranean fruit fly (*Ceratiti capitata*). Genetica. 116(1): 107-116.

Calkins, C. and Ashley, T. (1989). The impact of poor quality of mass reared Mediterranean fruit flies on the sterile insect technique used for eradication 1. Journal of Applied Entomology. 108: 401-408.

Cayol, J., Coronado, P. and Taher, M. (2002). Sexual compatibility in medfly (Diptera: Tephritidae) from different origins. Florida Entomologist. 85(1): 51-57.

Economopoulos, A. (1992). Adaptation of the Mediterranean fruit fly (Diptera: Tephritidae) to artificial rearing. Journal of Economic Entomology. 85: 753-758.

Enkerlin, W. (2005). Impact of fruit fly control programmes using the sterile insect technique. Sterile Insect Technique. 3(1): 651-676.

Fisher, K. and Caceres, A. (1998). A filter rearing system for mass reared genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae). In Proceedings of Area-wide control of fruit flies and other insect pests. Joint Proceedings of the International Conference on Area-wide control of Insect Pests, 28 May-2 June, 1998 and the Fifth International Symposium on Fruit Flies of Economic Importance, Penang, Malaysia, 1-5 June, 1998. pp. 543-550.

Franz, G. (2005). Genetic sexing strains in Mediterranean fruit fly, an example for other species amenable to large-scale rearing for the sterile insect technique. In: Sterile Insect Technique. Springer, Dordrecht. pp. 427-451.

Hendrichs, J., Franz, G. and Rendon, P. (1995). Increased effectiveness and applicability of the sterile insect technique through male only releases for control of Mediterranean fruit flies during fruiting seasons. Journal of Applied Entomology. 119(2): 371-377.

Niyazi, N., Lauzon, C.R. and Shelly, T.E. (2004). Effect of probiotic adult diets on fitness components of sterile male Mediterranean fruit flies (Diptera: Tephritidae) under laboratory and field cage conditions. Journal of Economic Entomology. 97: 1570-1580.

Orozco, D. and Lopez, R. (1993). Mating competitiveness of wild and laboratory mass-reared medflies: effect of male size. In Fruit Flies, Springer: 185-188.

Rendón, P., McInnis, D., Lance, D. and Stewart, J. (2004). Medfly (Diptera: Tephritidae) genetic sexing: Large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. Journal of Economic Entomology. 97(5): 1547-1553.

Robinson, A., Cayol, J. and Hendrichs, J. (2002). Recent findings on medfly sexual behavior: implications for SIT. Florida Entomologist. 85(1): 171-182.

San Andrés, V., Urbaneja, A., Sabater-Munoz, B. and Castanera, P. (2007). A novel molecular approach to assess mating success of sterile *Ceratitis capitata* (Diptera: Tephritidae) males in sterile insect technique programs. Journal of Economic Entomology. 100(4): 1444-1449.

Thornhill, R., Alcock, J. (1983). The Evolution of Insect Mating Systems; Harvard University Press.