EFFECT OF ACCLIMATION TO OUTDOOR CONDITIONS ON THE SEXUAL PERFORMANCE OF MASS-PRODUCED MEDFLIES (DIPTERA: TEPHRITIDAE)

Authors: Pereira, Rui, Silva, Natalia, Quintal, Celio, Abreu, Ruben, Andrade, Jordan, et al.

Source: Florida Entomologist, 90(1) : 171-174

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2007)90[171:EOATOC]2.0.CO;2

EFFECT OF ACCLIMATION TO OUTDOOR CONDITIONS ON THE SEXUAL PERFORMANCE OF MASS-PRODUCED MEDFLIES (DIPTERA: TEPHRITIDAE)

RUI PEREIRA, NATALIA SILVA, CELIO QUINTAL, RUBEN ABREU, JORDAN ANDRADE AND LUIS DANTAS
Programa Madeira-Med, Estrada Eng. Abel Vieira, 262, 9135-260 Camacha, Madeira, Portugal

ABSTRACT

Application of the sterile insect technique (SIT) as part of integrated area-wide programs to control the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) require that the released males attract wild females and transfer sterile sperm. However, knowledge about male sexual performance after they are released is scarce. We conducted a study to evaluate male sexual performance in field cage tests, according to standard quality control procedures. Mass-reared 5-d-old sterile males from the genetic sexing strain VIENNA 7mix2000 were acclimated for 0, 1, and 3 d to outdoor conditions before competing with wild males for wild females. Although the proportion of mating (PM) in the test was satisfactory, the resulting relative sterility index (RSI) data showed no significant differences among the treatments. The data indicate that pre-conditioning males to outdoor conditions in Madeira did not confer an advantage in field cage sexual performance.

Key Words: acclimation, *Ceratitis capitata*, female mating, genetic sexing strains, relative sterility index, sexual success, SIT

RESUMEN

La aplicación de la técnica del insecto estéril (TIE) como parte de un programa integrado de amplio efecto para el control de la mosca mediterránea de la fruta *Ceratitis capitata* (Wiedemann) requiere que los machos liberados atraigan las hembras naturales y transfieran su esperma. Sin embargo, el conocimiento del desempeño sexual de los machos después de ser liberados es muy escaso. Nosotros realizamos un estudio para evaluar el desempeño sexual de los machos en pruebas usando jaulas del campo, según los procedimientos estandardizados de calidad. Machos estériles de 5 dias de edad de la raza que separa los sexos genéticamente VIENNA 7mix2000 criados en masa fueron aclimatados por 0, 1 y 3 días en condiciones de campo antes de competir con machos naturales para las hembras naturales. Aunque la proporción del apareamiento en la prueba fue satisfactorio, el índice relativo de esterilidad (IRS) resultante no mostró ninguna diferencia significativa entre los tratamientos. Los datos indicaron que al condicionar los machos anteriormente a las condiciones de campo en Madeira no confirió ventaja alguna en el desempeño sexual en la jaula de campo.

Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) control programs integrating the sterile insect technique (SIT) now use genetic sexing strains, which allow for the release of sterile males only (Franz 2005). Genetic sexing strains eliminate the sterile sting problem and mating between sterile insects, and they greatly increase the efficacy of the SIT (Hendrichs et al. 1995).

For effective control with the SIT, however, it is essential to produce sterile males that will compete successfully with wild males for copulations with wild females (Knipling 1955; Hendrichs et al. 2002). The compatibility (ability of sterile male flies to mate with wild females) and competitiveness (mating success in competition with the wild males) were in the past mainly tested in the laboratory (Fried 1971). However, laboratory tests cannot assess the full behavioral repertoire of mass-produced insects, and field cage tests are essential (Robinson et al. 2002).

Studies of survival and sexual performance in the open field after male release are scarce. Nevertheless, some studies were conducted in the field through mark release recapture studies to evaluate male dispersal and survival (Plant & Cunningham 1991; Hendrichs et al. 1993; Barbosa et al. 2000), fruit infestation (Cayol & Zarai 1999), and population suppression and induced sterility (McInnis et al. 1994). To study the behavior of sterile flies in the field, however, observations need to be done on field-caged host trees, where direct observations of behavior and evaluation of competitiveness can be done. Several such studies of male sexual performance (Prokopy & Hendrichs 1979; Wong et al. 1983; Cayol et al. 1999; Katsoyannos et al. 1999; Rendón et al. 2000; Calcagno et al. 2002; Economopoulos & Mavrikakis 2002) and mating compatibility (Cayol et al. 2002) have been conducted. The insects can be maintained as close as possible to natural conditions, with the advantage of being able to obtain fly performance measurements.

We conducted a field cage study in Madeira Island to evaluate the effects of acclimation in the field on sexual performance of mass reared

male medflies. Male medflies were acclimated to outdoor conditions for 0, 1, and 3 d before measuring their mating performance.

Materials and Methods

The medflies used in the study were (1) males of the genetic sexing strain (VIENNA 7mix2000) (Franz 2005) produced in the Madeira medfly factory (Pereira et al. 2000), and (2) Madeira wild flies collected from larval survey samples (mixed hosts). Mass-reared flies were irradiated under hypoxia in a Nordion ^{60}Co irradiator 24 to 48 h before emergence at a dose of 100 Gy. Pupae from both strains were placed in Plexiglas® cages (30 cm × 30 cm × 40 cm) until emergence. The wild flies were sexed within 24 h of emergence, and females were kept in separate rooms from males to avoid contact with the male pheromone before the tests. All flies were maintained at 24 ± 2°C, 65 ± 5% RH and daylight conditions, with water and optimal food (3 parts sugar and 1 part hydrolyzed yeast). Healthy flies were selected for the tests and marked with a dot of water-based paint on the thorax on the day before the test.

All tests were conducted in standard field cages (2.9 m diameter and 2.0 m high) (Calkins & Webb 1983) and followed the procedures outlined in the FAO/IAEA/USDA (2003) international quality control manual. Each field cage was placed over a citrus or mango tree (about 1.8 m tall). Pruning was sometimes necessary to facilitate observation in the field cage.

The testing period covered the time of maximum sexual activity of both wild and mass reared flies (sunrise to 12:00). Male flies were released 30 min before the females at dawn so that they could start forming leks (Prokopy & Hendrichs 1979). In each cage we released equal numbers of wild males (9-11 d old), mass-reared sterile males (5 d old), and wild, virgin females (10-12 d old). Mass-reared sterile males were submitted to the following 3 different treatments before the test: (1) zero days acclimation to outdoor conditions, (2) 1 d acclimation to outdoor conditions, and (3) 3 d acclimation to outdoor conditions.

Experiments were conducted in 3 sets, with different fly densities, (due to the availability of wild flies), but maintaining the ratio of (1:1:1). Experiment 1 was conducted in Aug and Sep, 2002 (30:30:30 flies per cage) and a total of 30 cages were run (10 per treatment). Experiment 2 was conducted in May, 2004 (45:45:45 flies per cage) with a total of 18 cages (6 per treatment), and experiment 3 was conducted in Jun, 2004 (40:40:40 flies per cage) with a total of 9 cages (3 per treatment).

The flies were observed by continuous census, and copulating pairs were collected in 20-mL vials. The proportion of females mating (PM) and relative sterility index (RSI) were determined according to the matings obtained. PM measures the suitability and sexual maturity of the flies, as well as an adequate environment for lek formation and mating. It represents the overall mating activity of the flies (McInnis et al. 1996; Cayol et al. 1999) and is defined as follows:

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

The RSI measures the proportion of mating achieved by sterile males when competing with wild males (McInnis et al. 1996) and is defined according the following formula where LW is the number of matings between laboratory sterile males and wild females, and WW the number of matings between wild males and wild females, as follows:

$$RSI = \frac{\text{LW}}{\text{LW} + \text{WW}}$$

The data were analyzed by analysis of variance (ANOVA) (Ott & Longnecker 2001). The significance value used in tests was 95% ($\alpha = 0.05$). Statistical analyses were performed with R software (version 2.1.0, www.r-project.org).

Results

Only the field cage tests that yielded a PM above 0.25 (more than 25% of the females mated) were considered in our data analysis (FAO/IAEA/USDA 2003). In the 57 field cage tests run, 51 met this requirement. The 6 cages having low PM were conducted on 2 days of heavy rain. Data were pooled to increase the statistical analysis power for comparisons among treatments since the ratio of the flies inside the cage was maintained and is corrected by the PM and RSI calculations. Data are presented in Table 1. No significant differences were found among the 3 treatments for either PM ($F_{2,}$ = 0.271; df = 2,48; P = 0.764) or RSI (F = 1.233; df = 2,48; P = 0.301). However, the RSI for all treatments is below 0.27,

Table 1. Proportion of females mating (PM) and relative sterility index (RSI) obtained in field cage tests for sterile males with different number of days of acclimatization to outdoor conditions. No differences were found for both parameters (P > 0.05).

Outdoor acclimatization	PM (± SD)	RSI (± SD)
0 d	0.45 ± 0.13	0.24 ± 0.11
1 d	0.43 ± 0.11	0.19 ± 0.12
3 d	0.42 ± 0.15	0.26 ± 0.18

which is a relatively low sexual success for these sterile males when interacting with wild males.

Discussion

When medfly sterile males are released into the field (2-4 d old) as part of SIT operations, we only indirectly know how they perform in the environment (Vreysen et al. 2005). As soon they are released, males need to be capable of participating in leks, where they have to compete with wild males (Prokopy & Hendrichs 1979). This aspect could be influenced by acclimatization to outdoor conditions. However, as we can conclude from our study, no advantages in sexual performance are achieved with acclimation to outdoor conditions, at least under the Madeira conditions tested.

Our question was do sterile males perform better in the field cage mating arenas after being exposed to outdoor conditions for 1 or 3 d? Our data show no advantage to acclimatizing the males to outdoor conditions. A few days of acclimatization to outdoor conditions was not sufficient for males to improve their sexual performance.

Even though outdoor conditioning plays apparently no role within field cages, in terms of sterile male survival in the open field, some release and recapture studies show an enormous reduction of recaptures in the first days (Hendrichs et al. 1993; Barbosa et al. 2000). These 2 studies conducted in 2 different geographical areas, as well as data from operational programs, show a very low percentage of recapture of sterile males after day four. After this, however, a relative constant number of sterile males are present. There are 2 possible explanations for this drastic mortality on the first days after releases as follows: (1) difficulties of male foraging outdoors to find food (Yuval et al. 1998), although Yuval et al. (this volume) confirm that sterile males are as capable as wild males in finding food when it is available, or (2) the sterile males suffered heavy predation and only a few manage to escape predation (Hendrichs & Hendrichs 1998; Hendrichs et al., 2007).

Another implication of this study involves fly emergence and handling facilities where flies are exposed to natural light. This is probably not required, since according to our data the sterile males gain no advantage when acclimated to outdoor conditions. However, ours is a preliminary study at the environmental conditions of Madeira. Further studies looking at other procedures and for longer periods of acclimatization, and in other geographical areas are recommended.

Acknowledgments

This research was financially supported by the International Atomic Energy Agency (IAEA) through the Research Contract 10842, and the Programa Madeira-Med.

References Cited

Barbosa, S., A. Mexia, and R. Pereira. 2000. Field study of the dispersal and survival of released Mediterranean fruit fly sterile "*tsl*" males, pp. 527-533 *In* T. K. Hong [ed.], Area-wide Control of Fruit Flies and Other Insect Pests. Universiti Sains Malaysia Press, Penang, Malaysia.

Calgagno, G. E., F. Manso, and J. C. Vilardi. 2002. Comparison of mating performance of medfly (Diptera: Tephritidae) genetic sexing and wild type strain: field cage and video recording experiments. Florida Entomol. 85: 41-50.

Calkins, C. O., and J. C. Webb. 1983. A cage and support framework for behavioral tests of fruit flies in the field. Florida Entomol. 66: 512-514.

Cayol, J. P., and M. Zarai. 1999. Field releases of two genetic sexing strains of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) in two isolated oases of Tozeur Governorate, Tunisia. J. Appl. Ent. 123: 613-619.

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 *Ceratitis capitata* (Diptera: Tephritidae) laboratory-reared strains under field cage conditions. J. Econ. Entomol. 92: 140-145.

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

Economopoulos, A. P., and P. G. Mavrikakis. 2002. Mating performance and spatial distribution of medfly (Diptera: Tephritidae) white pupae genetic sexing males under field cage conditions. Florida Entomol. 85: 58-62.

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, pp. 427-451 *In* V. A. Dyck, J. Hendrichs, and A. S. Robinson [eds.], Sterile Insect Technique: Principles and Practice in Area-wide Integrated Pest Management. Springer, Dordrecht, The Netherlands. 787 pp.

Fried, M. 1971. Determination of sterile insect competitiveness. J. Econ. Entomol. 64: 869-872.

FAO/IAEA/USDA. 2003. Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies. Version 5.0. International Atomic Energy Agency, Vienna, Austria.

Hendrichs, J., V. Wornoayporn, B. I. Katsoyannos, and K. Caggl. 1993. First field assessment of the dispersal and survival of mass reared sterile Mediterranean fruit fly males of an embryonal, temperature sensitive genetic sexing strain, pp. 453-462 *In* Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques. IAEA, Vienna, Austria.

Hendrichs, J., G. Franz, and P. Rendón. 1995. Increased effectiveness and applicability of the sterile insect technique through male-only releases for control of Mediterranean fruit flies during fruit seasons. J. Appl. Entomol. 119: 371-377.

Hendrichs, J., A. S. Robinson, J. P. Cayol, and W. Enkerlin. 2002. Medfly area-wide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. Florida Entomol. 85: 1-13.

Hendrichs, M. A., and J. Hendrichs. 1998. Perfumed to be killed: interception of Mediterranean fruit fly

(Diptera: Tephritidae) sexual signaling by predatory foraging wasps (Hymenoptera: Vespidae). Ann. Entomol. Soc. Am. 83: 228-234.

HENDRICHS, M. A., V. WORNOAYPORN, B. I. KATSOYANNOS, AND J. HENDRICHS. 2007 Quality control method to measure predator evasion in wild and mass-reared Mediterranean fruit fly (Diptera: Tephritidae). Florida Entomol. 90: 64-70.

KATSOYANNOS, B. I., N. T. PAPADOPOULOS, J. HENDRICHS, AND V. WORNOAYPORN. 1999. Comparative responses to citrus foliage and citrus fruit odour by wild and mass-reared sterile Mediterranean fruit fly males of a genetic sexing strain. J. Appl. Entomol. 123: 139-143.

KNIPLING, E. F. 1955. Possibilities of insect control or eradication through the use of sexual sterile males. J. Econ. Entomol. 48: 459-462.

MCINNIS D. O., S. Y. T. TAM, C. GRACE, AND D. MIYASHITA. 1994. Population suppression and sterility rates induced by variable sex ratio, sterile insect releases of *Ceratitis capitata* (Diptera: Tephritidae) in Hawaii. Ann. Entomol. Soc. Am. 87: 231-240.

MCINNIS D. O., D R. LANCE, AND C. G. JACKSON. 1996. Behavioral resistance to sterile insect technique by the Mediterranean fruit fly in Hawaii. Ann. Entomol. Soc. Am. 89: 739-744.

OTT, R. L., AND M. LONGNEAKER. 2001. An Introduction of Statistics Methods and Data Analyses, 5th ed. Duxbury Publishers, Pacific Grove, California, USA.

PEREIRA, R., A. BARBOSA, N. SILVA, L. DANTAS, J. CALDEIRA, AND J. PACHECO. 2000. Madeira-Med program, sterile insect technique against Mediterranean fruit fly in Madeira, Portugal, pp. 443-438 *In* T. K. Hong [ed.], Area-wide Control of Fruit Flies and Other Insect Pests. Universiti Sains Malaysia Press. Penang, Malaysia.

PLANT, R. E., AND R. T. CUNNINGHAM. 1991. Analyses of the dispersal of sterile Mediterranean fruit flies (Diptera: Tephritidae) released from a point source. Environ. Entomol. 20: 1493-1503.

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.

RENDÓN, P., D. O. MCINNIS, D. R. LANCE, AND J. STEWART. 2000. Comparison of medfly male-only and bisexual releases in large scale field trials, pp. 517-525 *In* T. K. Hong [ed.], Area-wide Control of Fruit Flies and Other Insect Pests. Universiti Sains Malaysia Press, Penang, Malaysia.

ROBINSON, A., J. P. CAYOL, AND J. HENDRICHS. 2002. Recent findings on medfly sexual behavior: implications for SIT. Florida Entomol. 85: 171-181.

VREYSEN, M. J. B. 2005. Monitoring sterile and wild insects in area-wide integrated pest management programmes, pp. 325-361 *In* V. A. Dyck, J. Hendrichs, and A. S. Robinson [eds.], Sterile Insect Technique: Principles and Practice in Area-wide Integrated Pest Management. Springer, Dordrecht, The Netherlands. 787 pp.

WONG, T. T. Y., J. I. NISHIMOTO, AND H. M. COUEY. 1983. Mediterranean fruit fly (Diptera: Tephritidae): further studies on selective mating response of wild and of unirradiated and irradiated, laboratory-reared flies in field cages. Ann. Entomol. Soc. Am. 76: 51-55.

YUVAL, B., R. KASPI, S. SHOLMIT, AND M. S. WARBURG. 1998. Nutritional reserves regulate male participation in Mediterranean fruit fly leks. Ecol. Entomol. 23: 211-215.

YUVAL, B., M. MAOR, K. LEVY, R. KASPI, P. TAYLOR, AND T. SHELLY. 2007. Breakfast of champions or kiss of death? Survival and sexual performance of protein-fed, sterile Mediterranean fruit flies (Diptera: Tephritidae). Florida Entomol. 90: 115-122.