Proceedings of an FAO/IAEA Coordinated Research Project on Quality Assurance of Mass-reared and Released Fruit Flies for Use in SIT Programs

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EDITOR'S NOTE:

The first 24 papers, beginning with Cáceres et al. and ending with Briceno et al., comprise the Proceedings of a 5-year FAO/IAEA Coordinated Research Project on Fruit Fly Quality, Control, and Behavior. Guest editors for these papers were C. Cáceres, J. Hendrichs, E. B. Jang, D. O. McInnis, A. S. Robinson, and T. E. Shelly.
QUALITY MANAGEMENT SYSTEMS FOR FRUIT FLY (DIPTERA: TEPHRITIDAE) STERILE INSECT TECHNIQUE

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ABSTRACT

The papers presented in this issue are focused on developing and validating procedures to improve the overall quality of sterile fruit flies for use in area-wide integrated pest management (AW-IPM) programs with a sterile insect technique (SIT) component. The group was coordinated and partially funded by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria, under a five-year Coordinated Research Project (CRP) on “Quality Assurance in Mass-Reared and Released Fruit Flies for Use in SIT Programmes”. Participants in the CRP from 16 countries came from both basic and applied fields of expertise to ensure that appropriate and relevant procedures were developed. A variety of studies was undertaken to develop protocols to assess strain compatibility and to improve colonization procedures and strain management. Specific studies addressed issues related to insect nutrition, irradiation protocols, field dispersal and survival, field cage behavior assessments, and enhancement of mating competitiveness. The main objective was to increase the efficiency of operational fruit fly programs using sterile insects and to reduce their cost. Many of the protocols developed or improved during the CRP will be incorporated into the international quality control manual for sterile tephritid fruit flies, standardizing key components of the production, sterilization, shipment, handling, and release of sterile insects.

Key Words: quality control, Tephritidae, fruit flies, behavior, SIT, sterile insects

RESUMEN

Los artículos presentados en este número se enfocan en el desarrollo y la validación de procedimientos para mejorar la calidad total de moscas de las frutas estériles para su uso en programas de manejo integrado de plagas en donde la técnica del insecto estéril (TIE) es uno de los componentes clave. El grupo fue coordinado y parcialmente financiado por la División Conjunta de Técnicas Nucleares para la Alimentación y la Agricultura de la FAO/OIEA, Viena, Austria, por un periodo de cinco años bajo el proyecto de Investigación Coordinada (PIC) sobre “el Aseguramiento de la Calidad de Moscas de las Frutas Criadas y Liberadas para su Uso en Programas de TIE”. Los participantes en el PIC representan 16 países con experiencia en campos de investigación básica y aplicada. Para asegurar que los procedimientos desarrollados fueran apropiados y pertinentes, se realizaron una variedad de estudios para el desarrollo de protocolos para evaluar la compatibilidad y para mejorar los procedimientos de colonización y manejo de cepas salvajes. Estudios específicos trataron asuntos relacionados con la nutrición de insectos, los protocolos de irradiación, la dispersión y supervivencia en el campo, evaluación del comportamiento en jaulas de campo, y el mejoramiento de la competitividad sexual. Los objetivos fundamentales fueron el aumentar la eficiencia y reducir los costos de los programas operacionales de control de moscas de las frutas donde TIE es utilizada. Muchos de los protocolos desarrollados o mejorados durante el PIC serán incorporados en el Manual Internacional de Control de Calidad para Moscas Estériles de la familia Tephritidae, para estandarizar componentes claves como la producción, esterilización, envío, manejo y liberación de insectos estériles.
The sterile insect technique (SIT) is rapidly becoming a major component of many area-wide integrated pest management (AW-IPM) programs for fruit fly control (Dyck et al. 2005). For use in these programs, sterile insects are produced in large numbers, sterilized, and then released systematically into the field. In all of these processes, quality management systems can be used to monitor or improve the overall effectiveness of sterile insects in the field and to reduce variability in cases where production fluctuates widely in quantity and quality. The insect production facility must be a reliable source of high quality sterile insects either for local use or shipment to programs elsewhere.

There are generally two sets of conditions that favor the long-distance or even trans-boundary shipment of sterile insects (Enkerlin & Quinlan 2004). First, shipment may be necessary to areas such as California and Florida, which are typically pest-free (thus precluding the establishment of a local fly production facility) but have repeated introductions of fruit flies that demand a preventive area-wide control program (Dowell et al. 2000). Second, shipping sterile insects is economical for small SIT programs, when the costs of insect production locally would be prohibitively high (Dowell et al. 2005). In the past, mass rearing facilities were developed with public funds; however, the private sector has recently shown interest in commercializing sterile insect production, e.g., Israel (Bassi 2005) and South Africa (Barnes et al. this volume). This interest has been encouraged by the wider use of sterile insects in suppression programs (Hendrichs et al. 1995), rather than only in eradication programs.

The International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations (FAO) through their Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture have played a leading role in the development and implementation of SIT technology. One mechanism used to further this technology is through Coordinated Research Projects (CRPs) (www.iaea.org/programmes/ri/uc_infoi.html) under the IAEA Research Contract Programme (IAEA 2006). This issue of Florida Entomologist summarizes the results of the five-year CRP on “Quality Assurance in Mass-Reared and Released Fruit Flies for Use in SIT Programmes” together with several invited papers. The papers in this volume identify components or steps in the SIT process that can be changed or improved, such as reducing the negative impact of mass rearing and sterilization on the efficiency of sterile insects in the field and establishing new standards and quality control protocols for updating and inclusion in the international manual on “Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies” (FAO/IAEA/USDA 2003).

This introductory paper highlights the relevance of the papers to a quality management system for fruit fly production, sterilization, shipment, emergence, feeding, holding, and release, and identifies gaps and areas for future research. The papers included in this volume do not represent the total spectrum of a quality management system as it relates to fruit fly mass rearing, but reflect the breadth of subjects that are related to typical quality management systems as reported in the CRP.

**Strain Compatibility**

Assessing the mating compatibility of a strain to be released with its potential target population remains a critical activity. In the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann), mass-reared strains have shown no significant mating incompatibilities with any of the medfly populations assessed to date (Cayol et al. 2002; Lux et al. 2002), except for 2 exceptional island cases. The first involved the use of a 38-yr old mass-production strain from Hawaii (McInnis et al. 1996), and the second involved the use of a tsl genetic sexing strain (Franz 2005) against a wild Madeira strain. However, Pereira et al. (2007a) show in recently conducted field cage assessments a low but acceptable level of compatibility between the tsl mass-reared genetic sexing strain, VIENNA 7 (Franz 2005), and wild populations from different islands in Madeira. Briceno et al. (2007a) present interesting data that may explain some of the observed mating incompatibilities in Madeira. Wild Madeira males appear to have significantly longer durations of some courtship components (e.g., head rocking and wing buzzing). Therefore, Pereira et al. (2007a) stress the need for incorporation of Madeira genetic background into the genetic sexing strain that is used for field releases in order to overcome the low level of compatibility and improve efficiency in the Madeira SIT program.

For the Mexican fruit fly Anastrepha ludens (Loew), no mating incompatibilities were observed between mass-reared flies and populations from 6 different locations in Mexico, where programs with an SIT component have been established (Orozco et al. 2007). This finding is very important because sterile insects are produced in a single rearing facility in southern Mexico. This facility supplies all the SIT control programs in Mexico plus the preventive release program in southern California. In the case of the South American fruit fly Anastrepha fraterculus (Wiedemann), previous studies (Petit-Marty et al. 2004) have shown good mating compatibility among populations across Argentina. Allinghi et al. (2007a) report that sterile insects from a candidate strain for mass production and SIT releases are reasonably compatible with wild flies from Argentina and southern
Brazil. However, in studies of wild populations of *A. fraterculus* from different regions of South America (Argentina, Brazil, Peru, Colombia), there was evidence of substantial isolation between certain populations (Vera et al. 2006).

In a majority of cases, strain compatibility is not a constraint for the application of the SIT; e.g., for the Oriental fruit fly *Bactrocera dorsalis* (Hendel), no mating incompatibility between the pupal color genetic sexing strain from Hawaii and the wild population from Thailand was observed (McInnis; personal communication), and for melon fly, *Bactrocera cucurbitae* (Coquillett) between mass reared flies from the Okinawa strain and wild insects from Taiwan (Matsuyama & Kuba 2004). Compatibility tests, however, remain an essential component of SIT feasibility studies.

**Colonization and Strain Management**

One of the main problems faced by producers of sterile insects is how best to colonize and mass rear a strain while at the same time minimizing the impact on the intrinsic characteristics of the strain. Filter rearing systems for genetic sexing strains (Fisher & Cáceres 2000), or mother colonies for bisexual strains, are a way to solve part of this problem (Calkins & Parker 2005). In the case of genetic sexing strains, a filter rearing system is used for the additional purpose of maintaining strain genetic stability.

Liedo et al. (2007) show that the use of inserts to increase the surface resting area within adult cages in the mother colony, as well as the use of low adult cage density during colonization and mass rearing, resulted in strains with increased mating competitiveness as demonstrated in standard field cage mating tests.

**Quality Management System**

New mass rearing production indices and quality standards developed during the CRP have been used to update the international quality control manual (FAO/IAEA/USDA 2003), contributing to the development of a new quality management system. Such a quality management system increases end user confidence and facility reliability, improves work processes, SIT efficiency, and integration with other control methods. A quality management system can be realized through the adoption of ISO 9000 standards, developed and published by the International Standardization Organization (ISO 1996) that defines, establishes, and maintains an effective quality assurance system for manufacturing and service industries. The ISO 9000 standard is the most widely known and has perhaps had the most impact of the standards published by ISO (ISO 1996). An organization can receive ISO 9000 certification if an external audit recognizes successful implementation and performance of standard production protocols.

Leppla & Fisher (1989) suggested the adoption of industrial quality assurance protocols by mass rearing facilities, and the medfly mass-rearing facility in Mendoza, Argentina, became the first facility to receive ISO 9000 certification in 1999 (G. Taret, personal communication). Barnes et al. (2007) stress the importance of a quality management system in maintaining high quality and predictable insect production at the Infruitec medfly mass-rearing facility in Stellenbosch, S. Africa. A quality management system can be extended to all other steps of the SIT, including packing and fly emergence and handling at release centers.

**Standardization of Mass Production and Quality Control Procedures**

Routine Quality Control Procedures

Standard procedures and established threshold values are available and summarized in the international quality control manual (FAO/IAEA/USDA 2003) used by most fruit fly production facilities, which routinely keep records of the quality of their production. Barnes et al. (2007), provide a good example of how results from routine quality control tests can be analyzed and utilized as a feedback to help facility managers identify quality problems and stabilize production. The establishment of demographic and quality control parameters for mass rearing are required in order to develop benefit/cost scenarios that can be used to determine the feasibility of the SIT implementation in integrated control programs. For *A. fraterculus*, significant improvements in the quality control and mass rearing protocols are reported for all developmental stages (Vera et al. 2007). Similar developments are reported in the Philippines for *Bactrocera philippinensis* (Drew & Hancock) (Resilva et al. 2007).

Hendrichs et al. (2007) describe a simple quality control test that might be useful to measure predator evasion in the medfly. They showed that current adult colony management and mass-rearing procedures significantly reduce the ability of sterile males to escape predation in comparison to wild males, resulting in the rapid elimination due to predation of a large proportion of the released sterile male population. They stress the importance of measuring this parameter and propose that simple quality control tests be developed to routinely measure the evasive ability of sterile males of the different mass reared strains.

**Pupal Age and Development Synchronization**

Synchronization and exposure of pupae to the irradiation treatment at an optimal age is crucial to obtain a desirable level of sterility with mini-
nal effect on insect quality. Day degree models to determine the correct physiological age for irradiation of a bisexual medfly strain and its relationship with pupal eye color have been calculated (Ruhm & Calkins 1981). Based on these earlier studies, Resilva et al. (2007) present a method to determine the relationship of pupal eye color with physiological development of B. philippinensis held under different temperature regimes. This method helped determine the optimal stage for pupal irradiation and is now being extended to other fruit fly species.

Nestel et al. (2007a) tried to correlate respiratory rate in pupae with digital recordings of eye color. Their study describes the relationship between a digitized image of pupal eye color and the respiratory rate of pupae. This may enable irradiation to be performed with greater precision. However, the practicality of the tests under operational conditions needs to be evaluated.

Dosimetry and Irradiation Doses

X-rays, electron beam generators, or gamma irradiators with $^{60}$Co or $^{137}$Cs can be used to sterilize insects. It is important to minimize the somatic effects induced by radiation during the sterilization process (Bakri et al. 2005). A standard dosimetry system (Gafchromic system) has been developed and adopted, and a standard operating procedure (SOP) for the implementation of this system as part of the quality management system in production facilities has been compiled (Parker & Mehta 2007; Bakri et al. 2005). For A. fraterculus (Allinghi et al. 2007b) and B. philippinensis (Resilva et al. 2007), optimal doses for sterilization have been determined. However, in choosing an optimal dose for sterilization, a balance needs to be reached between the levels of sterility and fly competitiveness (Toledo et al. 2004). Unfortunately it appears that many of the current operational programs applying the SIT are not achieving an appropriate balance. Parker & Mehta (2007) describe a mathematical model that can be used by these programs to derive such an optimal dose. The authors stress that the model still requires extensive validation.

Atmosphere During Irradiation

Irradiation in the presence of nitrogen or low oxygen atmospheres can improve insect quality (Ashraf et al. 1975; Ohinata et al. 1977; Fisher 1997). Nestel et al. (2007b) assessed the effect of irradiation under different decreased oxygen levels, and the results indicated that mating competitiveness drastically decreases when pupae are irradiated under full O$_2$ conditions, when the packing bags remained open before irradiation. Hypoxic oxygen concentrations of 2% and 10% in sealed bags at the beginning of irradiation did not affect the mating competitiveness of males compared to males irradiated under maximal hypoxia (anoxia). Current practices in mass-rearing facilities are discussed by Nestel et al. (2007b) in light of these results.

Shipments of Biological Material

Shipments of sterile pupae or fertile eggs from production facilities are routinely performed (Enkerlin & Quinlan 2004). A specific insulated pupal shipping container is already in use and described in the international quality control manual (FAO/IAEA/USDA 2003). A protocol for long distance shipment of medfly eggs has been developed (Cáceres et al. 2007; Mamán & Cáceres 2007) and a long distance shipment protocol for sterile B. philippinensis pupae is described by Resilva et al. (2007).

**Nutrition**

Recent laboratory studies have led to the development of chemically defined larval and adult diets for the medfly (Chang et al. 2001). Nutritional larval diet manipulations have been demonstrated to affect the physiological traits of mass-reared medfly males. In this regard, Nestel et al. (2004) have shown that lipid and protein levels in pupating medfly larvae were affected and correlated with routine quality control parameters and growth factors. Nevertheless, there is still no clear understanding of how larval diet affects male competitiveness.

In contrast to larval nutrition, there is now increasing evidence that post-teneral diet can play an important role in sterile male competitiveness (Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi & Yuval 2000; Kaspi et al. 2000; Yuval & Hendrichs 2000; Maor et al. 2004; Niyazi et al. 2004). Yuval et al. (2007) discuss the effect of post-teneral feeding of sterile medfly males and, more specifically, whether the addition of protein to the adult diet enhances mating success. The main conclusion is that the addition of protein to the adult diet increases male sexual performance, but in some cases this can be associated with increased susceptibility to starvation. However, when there is sufficient nutrition available in the field, protein fed and protein deprived males have equal ability finding nutrients in the field. The authors suggest that the best strategy could be to release protein fed sterile males, which, though relatively short lived if nutrients are not found in the field, are highly competitive, rather than protein-deprived insects which may live longer but mate less successfully.

**Dispersal and Survival**

Dispersal and survival tests have been developed and validated, but there is still need for
standardization. The release recapture method has been used as a quality control test and was used to evaluate the dispersal ability and survival of mass-reared fruit flies (Shaw et al. 1967; Baker & Chan 1991). Hernández et al. (2007) present valuable information on longevity and dispersal in the field for mass-reared *A. ludens* and *A. obliqua*. They show that a majority of released sterile males do not survive the first 3 to 4 d after release in the field, and hence never reach the sexual maturity required to inseminate wild females. This information is very important for operational programs releasing sterile insects in order to modify the adult holding and release procedures, and to establish the frequency of releases and distance between release points.

Meats (2007) presented both actual field data from *Bactrocera tryoni* (Froggatt) sterile fly releases in Australia and data from simulated modeling studies comparing patterns of fly dispersion for sterile and wild flies in the field. The more similar the dispersion patterns, the more effective will be the sterile fly release, and the lower will be the expected rate of population increase for the wild flies. The author provides quantitative estimates of the rate of wild fly increase based on specific levels of mismatch between sterile and wild fly dispersal patterns. A solution for large mismatches in dispersal is closer flight lanes for aerial release, or the use of additional sterile fly releases in the areas surrounding traps containing the lowest sterile to wild fly ratios.

Gómez et al. (2007) report data on the longevity of mass-reared irradiated and non-irradiated *A. fraterculus* in field cages. Their study showed that within protected field cages laboratory-reared flies lived longer than wild flies. Therefore, they conclude that there was no adverse effect of irradiation on longevity.

**Behavior Assessments**

Segura et al. (2007) describe a field cage study that correlates *A. fraterculus* male mating success with pheromone calling activity, perching location within the tree canopy, presence in leks, and morphological characters. The data showed that some components of the sexual behavior and some morphological traits were associated with mating success. Mating success was higher for males grouped in a region of the tree characterized by the highest light intensity during the first 2 h of the morning. Highest mating success was for pheromone calling males inside a lek.

Sciurano et al. (2007) investigated the significance of morphological differences between successful and unsuccessful males of *A. fraterculus* in field cage mating competitiveness tests. Morphometric analyses were used to determine the relationship between phenotype and copulatory success. The authors conclude that no linear association existed between expected fitness and morphological traits (wing width and thorax length) that were suggested as targets of sexual selection. Nevertheless, they suggest that sexual selection could be affected by other morphological characters in *A. fraterculus*.

Pereira et al. (2007b) compare the mating success of mass-reared, sterile medfly males after being held for different periods of time in outdoor conditions. However, no positive effect of the treatment could be demonstrated. Briceño et al. (2007a) compared the duration of certain courtship elements of mass-reared males courting wild females. Courtship was filmed in the laboratory, and videotapes were analyzed. The authors report comparative data on male courtship from 4 wild populations of medfly. The results document differences of behavior between strains based on the interaction between males and females of the same strain. No inter-strain mating comparisons were carried out.

**Enhancement of Copulatory Success**

Exposure of sterile male medflies to ginger root oil or citrus peel oils significantly improves their mating success (Shelly 2001; Katsyoyannos et al. 2004) and also for Oriental fruit flies exposed to methyl eugenol (Shelly & Nishida 2004). Progress has been made in large-scale application of aromatherapy for medfly and chemotherapy for the Oriental fruit fly. Incorporating ginger root oil into medfly sterile release programs may increase the effectiveness of the SIT and allow a reduction in the number of sterile flies released (Barry et al. 2003).

Briceño et al. (2007b) analyzed courtship behavior of medfly males exposed to ginger root oil. The results show no clear effect on courtship between treated and untreated males, though a small sample of wild females did accept aromatized males faster than control males. Thus, further studies are needed to identify the components that are responsible for the enhanced male sexual performance of males treated with ginger root oil.

**Conclusions**

Increasing the efficiency of the SIT is of cardinal importance, both to ensure the success of such operations and to reduce cost. The CRP has identified components that may improve quality management systems and quality control protocols for SIT. While some reports suggest obvious improvements that could be realized from implementation of changes identified, others will require more research before they can be fully implemented into a total quality management system for fruit fly rearing, sterilization, shipment, holding, and release. It is clear, however, that this project has succeeded in bringing together an in-
ternational group of researchers to take a critical look at what factors will contribute to improve mass rearing and handling of sterile flies for improved SIT implementation. In addition, the CRP fostered networking and collaboration among the community of basic and applied fruit fly investigators.

The major outcome of this research network can be summarized as follows:

- promotion and implementation of quality management systems and quality control protocols
- promotion and implementation of colonization techniques to enhance and maintain strain quality and genetic stability
- promotion and dissemination of the use of precise standards for measuring sterile male compatibility and competitiveness under field conditions
- identification of nutritional factors affecting sterile male performance
- formulation of protocols for evaluating longevity and dispersal of sterile fruit flies and identification of survival problems of sexually immature sterile males in the field that need to be addressed
- development and testing of strategies for enhancing sterile male performance
- identification of optimal doses of irradiation in relation to mating competitiveness
- development of SIT-specific dosimetry procedures to allow precise and comparative measurements of the applied radiation dose
- development of a model to optimize radiation dose, maximizing male competitiveness and minimizing somatic damage
- development of packaging and long-distance shipping procedures for fertile eggs or sterile pupae
- revision of the international quality control manual for fruit flies (FAO/IAEA/USDA 2003) and addition of new quality control protocols.

Furthermore, it is important that operational programs implementing the SIT continue routinely revising and updating all protocols to ensure increased efficiency. Two-way feedback is essential between the mass rearing facility and field operations in order to be able to correct errors during all aspects of the process. Continued interaction between different SIT programs is recommended to exchange information and to further standardize operational procedures for all SIT components. However, defining new and better methods to measure and improve the quality of mass reared insects remains an elusive but overarching goal of any program implementing the SIT.

Finally, it is necessary to point out that this series of studies did not cover all the aspects of total quality management for fruit fly rearing, sterilization, shipment, holding, and release as it relates to the SIT application. Therefore, it is essential to continue conducting additional R&D in priority areas (many identified in this issue) to improve overall efficiency of operational SIT programs and to facilitate the establishment of new programs for fruit fly species for which the technology is not available.

Specific priority areas identified for further R&D are (1) improving strain selection and colony management, especially adult holding conditions and a better implementation of the filter rearing system, (2) identifying the optimal radiation dose in terms of induced sterility and competitiveness, (3) identifying the optimal stage for radiation related to the development of the reproductive system in both sexes, (4) addressing the low survival of released mass-reared flies to the age of sexual maturity, including the use of hormones for accelerating sexual maturation (Teal 2000), and increasing the low predator evasion capacity, (5) expanding and validating the use of nutritional, microbiological, and semiochemical supplements in the teneral adult diet to enhance male sexual performance, and (6) evaluating the effect of pre-release feeding, storage, and chilling conditions on sterile insect quality.

To support rapid progress in some of the identified R&D areas, 2 new 5-year FAO/IAEA CRPs have been initiated, involving scientists from the world’s main fruit fly research centers and major fruit fly SIT programs. In 2004 a CRP was initiated on “Improving Sterile Male Performance in Fruit Fly Programmes”, and in 2005 a second one was started on “Development of Mass Rearing for New World (Anastrepha) and Asian (Bactrocera) Fruit Fly Pests”.

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

RUI PEREIRA, NATALIA SILVA, CELIO QUINTAL, RUBEN ABREU, JORDAN ANDRADE AND LUIS DANTAS

ABSTRACT

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

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

RESUMEN

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

The Madeira Islands are located 980 km WSW from mainland Portugal (32°N and 17°W) and are composed by two populated islands. Porto Santo is small (about 50 km²) with topographic (maximum altitude 571 m) and temperature conditions favourable to the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann). However, poor soil and low rainfall (380 mm/year) do not permit an abundance of host fruits (the fig Ficus carica and Opuntia spp. are exceptions in terms of abundance). The main island, Madeira (740 km²), is volcanic with very little level land suitable for large agricultural production. The north coast is cooler than the south coast (air temperature at sea level in the north corresponds to air temperature at 300 m elevation in the south) and the maximum elevation is 1881 m. The climate of Madeira Island, particularly below 600 m over sea level on the south coast and 400 m on the north coast, is favourable for the development of large medfly
populations. Conditions in Funchal (sea level in south coast), the capital city, and in low altitude areas are very favourable for medfly throughout the year. There are eight generations per year in the Funchal area (Vieira 1952).

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

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

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

**MATERIALS AND METHODS**

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

Two sets of field cage tests were conducted, each comparing males of 4 of the above 5 populations. The reason for this was due to wild fly availability (an incompatibility of fly abundance peak between wild medfly populations from Porto Santo Island and the north coast of Madeira Island). In the first set (June and July, 2002) 17 cage tests were conducted (7 with PS females, 6 with SC females, and 4 with SW females). In the second set (October, 2002), 24 cage tests were carried out (8 with females from each of NC, SC, and SW).

Wild medflies were collected as pupae from larval survey samples (mixed hosts like peach, guava, apricot, and others). Semi-wild flies came from a small laboratory colony reared under low stress conditions. This colony was established with flies obtained from the fruit sampling of the Madeira Medfly program (Pereira 2001), i.e., from a mixture of hosts from the entire Madeira Island. Pupae of the mass-reared strain were irradiated with 100 Gy under hypoxia in a Gamma-cell 70 irradiator (Nordion, Canada) 24-48 h before emergence.

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

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

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

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

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

In the field cage tests we measured the proportion of flies mating (PM) (McInnis et al. 1996), and a mating by origin index (MOI) that mea-
sures the sexual success of males from different origins. The former index was adapted from the relative sterility index (RSI) used in SIT operations to measure the mating success of sterile males when competing with wild males (McInnis et al. 1996). The PM measures the suitability of the flies and the environment for mating. It represents the overall mating activity of the flies, both wild and sterile, and is defined as follows:

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

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

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

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

RESULTS

Results of the first set of field cage tests show no significant differences \((F = 3.16, df = 2.14; P = 0.0737)\) between the PM of the 3 strains of females used (Fig. 1). The PM was always above the minimum required \((0.20)\) for data analysis (FAO/IAEA/USDA 2003). The MOI index that measures the relative male sexual success competing for a female is presented on Table 1, independently and pooled together. The data show significantly lower sexual success for mass-reared sterile males against all kinds of females in the experiment. Surprisingly, PS males performed significantly better when competing for SC females, but not when competing for females of their own strain (even with slightly higher PM) and the SW females. The overall data show no significant differences between the two wild strains (PS and SC). However, PS males performed significantly better than SW and L males, while SC males were only significantly better than L males.

The second set of field cage tests again showed no significant differences in PM \((F = 1.768; df = 2.21; P = 0.1951)\) among the females tested (SC, NC, and SW) (Fig. 2). As in the previous experiment, the PM is always above the minimum required \((0.25)\) for data analysis (FAO/IAEA/USDA 2003). In terms of MOI (Table 2), the data are slightly different from the first set of experiments. Males from SW and L show a significantly lower MOI than the other males (NC and SC) when competing for NC and SW females. The same is true for the overall data. However, the MOI data show no significant differences among the 4 male treatments tested (SC, NC, SW, and L) when competing for SC females.

DISCUSSION

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

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

<table>
<thead>
<tr>
<th>Female origin</th>
<th>Number of cages</th>
<th>PS</th>
<th>SC</th>
<th>SW</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>7</td>
<td>0.37 a</td>
<td>0.29 ab</td>
<td>0.25 b</td>
<td>0.09 c</td>
</tr>
<tr>
<td>SC</td>
<td>6</td>
<td>0.42 a</td>
<td>0.26 b</td>
<td>0.23 b</td>
<td>0.09 c</td>
</tr>
<tr>
<td>SW</td>
<td>4</td>
<td>0.39 a</td>
<td>0.28 a</td>
<td>0.27 a</td>
<td>0.06 b</td>
</tr>
<tr>
<td>Totals</td>
<td>17</td>
<td>0.39 a</td>
<td>0.28 ab</td>
<td>0.24 b</td>
<td>0.09 c</td>
</tr>
</tbody>
</table>
flies tested. These results were expected in accordance with the compatibility studies of Cayol et al. (2002) for several medfly strains from many regions of the world, including Madeira Island.

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

The phenomenon of rapid strain deterioration after colonization is likely more evident when under the high stress of mass-rearing conditions as is common in the medfly factories around the world. For this reason, the development of a filter rearing system (Fisher & Cáceres 2000) to manage mother colonies under rearing conditions (fly density, sex-ratio, and physical features) more similar to conditions found in nature, as described by Robinson et al. (2002), is recommended.

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

**ACKNOWLEDGMENTS**

This research was financially supported by the International Atomic Energy Agency (IAEA), through research contract POR 10842, and the Madeira-Med SIT Program.

**REFERENCES CITED**


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

<table>
<thead>
<tr>
<th>Female origin</th>
<th>Number of cages</th>
<th>SC</th>
<th>NC</th>
<th>SW</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>8</td>
<td>0.30 a</td>
<td>0.28 a</td>
<td>0.25 a</td>
<td>0.17 a</td>
</tr>
<tr>
<td>NC</td>
<td>8</td>
<td>0.34 a</td>
<td>0.37 a</td>
<td>0.16 b</td>
<td>0.13 b</td>
</tr>
<tr>
<td>SW</td>
<td>8</td>
<td>0.34 a</td>
<td>0.34 a</td>
<td>0.19 b</td>
<td>0.13 b</td>
</tr>
<tr>
<td>Totals</td>
<td>24</td>
<td>0.33 a</td>
<td>0.32 a</td>
<td>0.20 b</td>
<td>0.15 b</td>
</tr>
</tbody>
</table>

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


COURTSHIP BEHAVIOR OF DIFFERENT WILD STRAINS
OF CERATITIS CAPITATA (DIPTERA: TEPHRITIDAE)

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ABSTRACT

This study documents differences in the courtship behavior of wild strains of Ceratitis capitata (Wiedemann) from Madeira (Portugal), Hawaii (U.S.A.), Costa Rica, and Patagonia (Argentina). Some traits showed large variations and others substantial overlaps. The angle at which the male faced toward the female at the moment of transition from continuous wing vibration and intermittent buzzing changed very little during the course of courtship in all strains, but males from Madeira tended to face more directly toward the female than other males. Females tended to look more, and more directly, toward the males as courtship progressed in all strains. The distance between male and female tended to decrease as courtship proceeded in all strains, but the distances at which males initiated continuous vibration, intermittent buzzing, and jumped onto the female were relatively less variable between strains, except for the strain from Costa Rica. Flies of Madeira courted for longer and the male moved his head and buzzed his wings longer than the other strains.

Key Words: courtship behavior, wild flies, medfly, geographic differences, Madeira, Costa Rica, Argentina, Hawaii

RESUMEN

Este estudio documenta diferencias en el comportamiento de cortejo de cepas silvestres de Ceratitis capitata (Wied.) provenientes de Madeira (Portugal), Hawaii (Estados Unidos de Norte América), Costa Rica y Patagonia (Argentina). Algunas características mostraron grandes variaciones y traslapes substanciales. Los ángulos a los cuales los machos miraron hacia las hembras cambiaron muy poco en el momento de la transición de la vibración continua al zumbido intermitente durante el curso del cortejo en todas las cepas. Pero los machos de Madeira tendieron a enfrentarse más directamente a la hembra que otros machos. Los ángulos de las hembras disminuyeron claramente durante el cortejo en todas las cepas. La distancia entre el macho y la hembra tendió a disminuir conforme el cortejo continuaba en todas las cepas, pero las distancias a las cuales los machos iniciaron la vibración continua, el zumbido intermitente, y el salto sobre la hembra eran relativamente menos variables entre cepas excepto la cepa de Costa Rica. Moscas de Madeira cortejaron más tiempo y el macho movió su cabeza y zumbaba sus alas mas prolongadamente que las otras cepas.

Translation provided by the author.

The use of sterile males for the integrated control populations of Ceratitis capitata (Wiedemann) makes it economically important to understand which male stimuli induce females to mate, in order to design appropriate quality control measures for mass-reared males (FAO/IAEA/USDA 2003; Calkins & Parker 2005). Because it is difficult to induce wild flies to reproduce in the laboratory (Rössler 1975), some strains have been maintained under mass-rearing conditions for many years. These conditions differ from those in the wild in a number of respects (Cayol 2000). Briceño & Eberhard (1998) found that males from mass-reared strains court for shorter periods before attempting to mount the female, apparently due to the crowded conditions in mass rearing cages which result in frequent interruptions of courtships. There are at least five differences between the sexual behavior of mass-reared males and wild males (Briceño & Eberhard 2002a).
Mass-reared males are generally less able to induce wild females to copulate than wild males. Although several aspects of male courtship behavior are known to have changed in at least some mass-reared strains (Zapien et al. 1983; Limatainen et al. 1997; Briceño & Eberhard 1998; Calcagno et al. 1999; Briceño et al. 2001), it is not clear whether these or other male traits are more important in producing this inferiority (Eberhard 2000). Such differences in male behavior may result in partial reproductive isolation between strains (Lux et al. 2002).

This paper explores the possibility that there are differences in courtship behavior among four wild *C. capitata* populations from Costa Rica, Patagonia (Argentina), Hawaii (USA) and Madeira (Portugal). Differences between wild strains may have important implications for development strategies for SIT implementation (Dyck et al. 2005).

**Materials and Methods**

Flies of each strain were separated by sex within 48 h of emergence as adults, and kept in buckets topped with screen, with *ad libitum* access to water and hydrolyzed yeast and sugar (1:3). Wild flies from Costa Rica were raised from larvae that emerged from infested tangerines collected at the Estación Experimental Fabio Baudrit near Alajuela, Costa Rica. Wild flies from Argentina were a laboratory F₁ derivative from flies raised from fruit collected in the field in the Alto Valle region of Patagonia. Wild flies from Hawaii were raised from larvae collected from coffee fruit on Kauai. Wild flies from Madeira were collected from infested mixed hosts.

Flies in Costa Rica and Hawaii were videotaped in plastic chambers that were 13.7 cm in diameter and 1.8 cm tall. They were videotaped from below through a transparent glass table (Briceño & Eberhard 1998) with a Sony Hi8 camcorder equipped with +6 close-up lenses. Pairs from Patagonia and Madeira wild flies were videotaped in a clear plastic cylinder 7.3 cm high and 9.0 cm in diameter. Each morning a fresh leaf from a citrus tree was attached to the ceiling of the cage, and a male was released in the cage (or mating chamber). Five min after the male began emitting pheromone, a female was released into the cage, and the behavior of the 2 flies was recorded for 30 min or until they copulated. Flies in mating trials were sexually mature, 10 days old, and each fly was used only once.

Measurements of different aspects of courtship behavior that led to a mounting attempt by the male were made from frame by frame analyses of videotapes. Only a single courtship was analyzed for each male to avoid pseudoreplication. Durations of the following male behaviors were analyzed: (1) continuous wing vibration (wings directed postero-laterally and vibrated rapidly dorso-ventrally); (2) intermittent buzzing (wings moved back and forth from being directed postero-laterally to the abdomen to anteriorly and also vibrated rapidly); for detailed descriptions of both these wing movements, see Briceño & Eberhard (2000b); (3) head rocking (head was rotated from side to side and turned and laterally just before intermittent buzzing began); (4) the total time the female remained immobile (no walking) before the male launched his mounting attempt; (5) and total courtship duration from the start of continuous vibration until the mounting attempt.

The directions the 2 flies were facing with respect to the midpoint of the other fly’s prothorax and the distances between them were determined at 3 stages of the courtship (initiation of continuous wing vibration; initiation of intermittent buzzing; and launch of the male’s jump onto the female) with 0° indicated that one fly was facing directly toward the other. The “male angle” was the angle between the direction the male faced and the orientation directly toward the female; the “female angle” was the equivalent for the female.

In Madeira and Hawaii strains the time during which the male’s aristae touched those of the female was measured because contact with the male’s sexually dimorphic aristae during head rocking and buzzing appears to be an important part of medfly courtship (Briceño & Eberhard 2002b). The number of bouts of wing buzzing was counted in 2 strains. All means are followed by + SD. Statistical tests were Mann-Whitney *U* Tests unless otherwise specified.

**Results**

Data in Table 1 show that there were differences between at least 1 pair of geographic strains in 12 of the 14 variables measured (the male angle when the male jumped, and the amount of time the female was quiet before the male jumped, are exceptions). There were also large differences in most variables (especially males vibrating and wing buzzing), and substantial overlaps between different strains in most behavioral traits. Madeira males rocked their heads and buzzed their wings significantly longer, and their total courtship was also longer.

The male angles at the moment of transition between wing vibration and wing buzzing changed very little during the course of courtship in all strains, but males from Madeira tended to face more directly toward the female than other males. Female angles clearly decreased during courtship in all strains. The distance between the male and female tended to decrease as courtship proceeded in all strains. The distances at which males initiated continuous vibration, intermittent buzzing, and jumped onto the female were...
relatively less variable among strains, except for the strain from Costa Rica.

**DISCUSSION**

Our results confirm several conclusions from previous studies regarding possible female acceptance variables. The gradual reduction in the distance between male and female, the increase in the female's tendency to look more directly toward the male, and her relative immobility prior to the male's jump are in accordance with the idea that one result of successful male courtship behavior is to induce the female to approach him, to look directly toward him, and to remain immobile (Briceño & Eberhard 2002a).

Lux et al. (2002) measured average duration of vibration and buzzing in 3 wild populations, and reported that in flies from Israel and Patagonia these activities lasted longer than in flies from Kenya (likely to be more similar to the original ancestor of this African species) but failed to present data or statistical tests. The values for wing vibration in the Kenyan populations we studied were much lower, i.e., 8.6-17.2 compared to 57.9 (Lux et al. 2002). One behavior (head rocking) that was absent in one of the wild strains (Israel) studied by Lux et al. (2002) was present in all the strains we studied.

There are several possible reasons for geographic differences in courtship behavior, including founder effects and divergent sexual selection.

**TABLE 1. MEANS AND STANDARD DEVIATIONS OF COURTSHIP BEHAVIOR OF CERATITIS CAPITATA FLIES FROM DIFFERENT GEOGRAPHIC AREAS, AND SIGNIFICANCE DIFFERENCES WITH MANN-WHITNEY U TESTS. VALUES IN THE SAME ROW FOLLOWED BY THE SAME LETTER AND NUMBER ARE SIGNIFICANTLY DIFFERENT (A = p < 0.05; B = p < 0.01; C = p < 0.001, NS = NO SIGNIFICANT DIFFERENCE).**

<table>
<thead>
<tr>
<th></th>
<th>Madeira</th>
<th>Costa Rica</th>
<th>Hawaii</th>
<th>Patagonia</th>
</tr>
</thead>
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<tr>
<td><strong>Angles (°)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>contmal</td>
<td>1.8 ± 2.6 b&lt;sub&gt;a&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6.8 ± 9.1 b&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.8 ± 0.9 b&lt;sub&gt;a&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.9 ± 4.5 a&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>confem</td>
<td>22.1 ± 29.0 c&lt;sub&gt;1&lt;/sub&gt;</td>
<td>43.9 ± 31.4 a&lt;sub&gt;c&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5.4 ± 3.2 a&lt;sub&gt;b&lt;/sub&gt;</td>
<td>36.5 ± 39.9 c&lt;sub&gt;b&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
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<td>2.7 ± 4.6</td>
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<td>2.7 ± 5.2 c&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>1.6 ± 2.1 a&lt;sub&gt;1&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.7 ± 3.7 a&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>distinter</td>
<td>1.71 ± 0.9 a&lt;sub&gt;c&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.3 ± 0.1 a&lt;sub&gt;c&lt;/sub&gt;&lt;sub&gt;1&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.0 ± 0.9 a&lt;sub&gt;b&lt;/sub&gt;&lt;sub&gt;c&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.8 ± 1.1 a&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>distjump</td>
<td>0.09 ± 0.03 ns</td>
<td>0.1 ± 0.3 ns</td>
<td>0.15 ± 0.4 ns</td>
<td>0.10 ± 0.03 ns</td>
</tr>
<tr>
<td>dihead</td>
<td>3.3 ± 1.8 ns</td>
<td>3.1 ± 1.0 ns</td>
<td>3.1 ± 1.0 ns</td>
<td>3.1 ± 1.0 ns</td>
</tr>
<tr>
<td><strong>Duration (seconds)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>femquiet</td>
<td>8.0 ± 6.3 ns</td>
<td>5.9 ± 3.7 ns</td>
<td>5.8 ± 4.9 ns</td>
<td>5.9 ± 5.6 ns</td>
</tr>
<tr>
<td>buzz</td>
<td>18.1 ± 19.6 b&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.6 ± 8.3 b&lt;sub&gt;1&lt;/sub&gt;</td>
<td>12.3 ± 10.6</td>
<td>8.2 ± 6.2 b&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>vibrate</td>
<td>17.2 ± 20.7 ns</td>
<td>14.8 ± 19.8 ns</td>
<td>5.7 ± 8.2 c&lt;sub&gt;2&lt;/sub&gt;</td>
<td>8.6 ± 6.8 c&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>head rocking</td>
<td>3.9 ± 4.6 c&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.77 ± 0.45 c&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.3 ± 4.8 c&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.4 ± 1.3 c&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>court</td>
<td>29.4 ± 27.1 c&lt;sub&gt;1&lt;/sub&gt;</td>
<td>19.8 ± 20.3 c&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15.7 ± 115.3 c&lt;sub&gt;1&lt;/sub&gt;</td>
<td>16.8 ± 10.6</td>
</tr>
<tr>
<td>antenna touches</td>
<td>9.4 ± 8.7 ns</td>
<td>5.6 ± 4.3 ns</td>
<td>5.6 ± 4.3 ns</td>
<td>5.6 ± 4.3 ns</td>
</tr>
<tr>
<td>number buzzes</td>
<td>21.3 ± 13.7 ns</td>
<td>5.6 ± 13.7 ns</td>
<td>21.3 ± 13.7 ns</td>
<td>28.7 ± 16.2 ns</td>
</tr>
</tbody>
</table>

contmal = male angle when continuous vibration began
confem = female angle when continuous vibration began
intermal = male angle when intermittent buzzing began
interfem = female angle when intermittent buzzing began
jumpmal = male angle when male jumped onto female
jumpfem = female angle when male jumped onto female
distcont = distance in cm between flies when male began continuous vibration
distinter = distance in cm between flies when male began intermittent buzzing
distjump = distance in cm between flies when male jumped onto female
dihead = distance in cm between flies when male began head rocking
defemquiet = time in s female was motionless prior to the male’s jump
buzz = duration in s of continuous wing vibration
court = duration in s of entire courtship
vibrate = duration in s of intermittent buzzing
antenna touches = duration in s of the antenna touches by the male during intermittent buzzing
head rocking = duration in s for head rocking
in different populations. The behavioral differences between wild flies indicate that there is appreciable genetic variation for these male courtship traits in field populations. The question of whether variation exists in male traits under sexual selection in natural populations has been controversial. Our results are in accord with the trend for genetic variation seen in other groups (Anderson 1994). On a practical level, the variance we found means that relatively large samples of courtships are needed to test for significant differences among strains. The differences between strains documented here involved males interacting with females of the same strain. Given the probable effects of the behavior of one sex on that of the other (Briceño & Eberhard 2002b), it is not possible to attribute differences to one sex or the other until cross-strain pairs are studied.

There was an apparent tendency of the wild Madeira males to rock their heads and buzz their wings significantly longer, and to court for longer before mounting. Because it appears that Madeira females are the “choosiest” among populations studied (Cayol et al. 2002), this could suggest that males with this suite of behaviors would be good candidates for medfly SIT operations world-wide.

ACKNOWLEDGMENTS

RDB and WGE thank Eddy Camacho for technical help, Bernal Burgos and Hernán Camacho for specimens of wild Costa Rican flies, Jorge Lobo and Federico Bolaños for help with statistics, and the International Atomic Energy Agency, and Smithsonian Tropical Research and Vicerrectoría of the Universidad de Costa Rica for financial support. A BARD grant (project No IS-2689-SGR) awarded to B. Yuval and T. E. Shelly financed the trip of RDB to Hawaii to film flies.

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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 representatives 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 mas 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 esta 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) reflejo 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) reflejo 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 compiten 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 (Knipping 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 (*Sargenttia 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 edulis*) were the hosts. In Yaritán pupae were obtained from bitter orange (*Citrus aurantiifolia*), white sapote (*Casimiroa edulelle*), matasano (*Pouteria campechiana*), and black sapote (*Diospyros digynajaca*), collected in Miravalles, Compostela, Xalisco, Testarazo, Aguiles Sérdan, Emiliano Zapata, Tepic, Libertad, Lerdo de García, Cuachisnes, San Blas, Acaponeta, Túxpan, Pantanales, and San Pedro. In Michoacán the pupae were obtained from bitter orange (*Citrus aurantiifolia*) collected in Uruapan, and in Chihuapas from this same fruit collected in the Soconuca 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 ± 1°C and 75 ± 5% RH.

Sterile pupae were obtained directly from the *A. ludens* production line at the Moscafrut mass rearing facility in Metapa de Dominguez, Chihuapas, México (Domínguez Gordillo 1996). The original colony is a mixture of an old colony from Mission, Texas, and wild material collected from different regions in Chihuapas; 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 record 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 16:00 h and reached a 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-
complete 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.

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

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+WW+SW+WS).
Relative sterile index (RSI) = (SW) / (SW+WW).

n = Number of replicates performed for each wild population.

Fig. 1. Distribution of matings (bars) and males calling (lines) during the sexual activity period (the matings are with both wild and sterile females).
Orozco et al.: *Anastrepha ludens* Sexual Competitiveness and Compatibility

**ISI**

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

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

\[ \text{ISI} = \frac{(SS+WW)-(SW+WS)}{(SS+WW+SW+WS)} \]

**MRPI**

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

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

\[ \text{MRPI} = \frac{(SS+SW)-(WS+WW)}{(SS+WW+SW+WS)} \]
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-
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.

<table>
<thead>
<tr>
<th>State</th>
<th>Percent egg hatch</th>
<th>Fried competitiveness value (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild control cage</td>
<td>Sterile control cage</td>
</tr>
<tr>
<td>Tamaulipas</td>
<td>57.73</td>
<td>0.00</td>
</tr>
<tr>
<td>Sinaloa</td>
<td>88.31</td>
<td>0.00</td>
</tr>
<tr>
<td>Nuevo León</td>
<td>73.60</td>
<td>0.00</td>
</tr>
<tr>
<td>Nayarit</td>
<td>76.73</td>
<td>0.00</td>
</tr>
<tr>
<td>Michoacán</td>
<td>28.06</td>
<td>0.00</td>
</tr>
<tr>
<td>Chiapas</td>
<td>57.82</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Fried competitiveness value (C) = \((W/S) \times (H_w - H_c) / (H_c - H_s)\).

W = Number of wild males.
S = Number of sterile males.
H_w = Egg hatch from wild females in the wild control cage.
H_c = Egg hatch from wild females in the competitiveness cage.
H_s = Egg hatch from lab females in the sterile control cage.

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.

Acknowledgments

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COMPATIBILITY AND COMPETITIVENESS OF A LABORATORY STRAIN OF ANASTREPHA FRATERCULUS (DIPTERA: TEPHRITIDAE) AFTER IRRADIATION TREATMENT

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ABSTRACT

We evaluated under semi-natural field cage conditions sexual compatibility and competitiveness of a laboratory strain (LAB) compared to a wild population (TUC) of Anastrepha fraterculus (Wiedemann). The LAB strain is produced under semi-mass rearing conditions at the Estación Experimental Agroindustrial Obispo Colombres facility (Tucumán, Argentina). Wild flies were obtained at Horco Molle (Tucumán, Argentina) from infested guava fruits. LAB pupae were irradiated (60Co) 48 h before adult emergence. The tested doses were 0 (control), 40, 70, and 100 Gy. Twenty-five males and 25 females each of TUC and LAB were released into cages and mating pairs collected. Only 1 irradiation dose was considered at a time. Females were separated and allowed to lay eggs into artificial fruits to estimate induced sterility from the corresponding hatching rate. Copulation start time did not differ significantly between strains nor among irradiation treatments. Copulation duration showed highly significant differences among irradiation doses, but no differences between strains. The index of sexual isolation (ISI) and the relative sterility index (RSI) indices indicated that LAB and TUC are fully compatible, males from TUC and LAB did not differ in mating competitiveness, and irradiation within the range tested did not affect these indices. Non-irradiated LAB females exhibited higher mating propensity than TUC ones. However, a significant reduction in the female relative performance index (FRPI) index was observed with increasing irradiation dose. The analysis of induced sterility indicated that treatment with 40 Gy reduces male fertility from about 80% to 0.75%, and higher doses produce total sterility. In females, the 40 Gy dose reduces fertility to about 2% and higher doses prevent egg laying.

Key Words: mating compatibility, Anastrepha fraterculus, Irradiation, mating indices, fruit fly, Tephritidae

RESUMEN

Se evaluó bajo condiciones semi-naturales en jaulas de campo la compatibilidad y la competitividad sexual de una línea de laboratorio (LAB) con respecto a una población salvaje (TUC) de Anastrepha fraterculus (Wiedemann). La línea de laboratorio se produce en condiciones de cría semi-masiva en las instalaciones de la Estación Experimental Agroindustrial Obispo Colombres (Tucumán, Argentina). Las moscas salvajes se obtuvieron de frutas infestadas de guayabos en Horco Molle (Tucumán, Argentina). Las pupas de laboratorio fueron irradiadas (60Co) 48 horas antes de la emergencia del adulto. Las dosis utilizadas fueron 0 (control), 40, 70, y 100 Gy. Se liberaron 25 machos y 25 hembras de TUC y LAB dentro de las jaulas y se recolectaron las parejas formadas. Sólo se considero 1 dosis de irradiación por vez. Las hembras apareadas fueron separadas y se les permitió poner huevos en frutas artificiales para estimar la esterilidad inducida a través del porcentaje de eclosión. La hora de inicio de la cópula no difirió significativamente entre poblaciones ni entre los tratamientos de irradiación. La duración de la cópula mostró grandes diferencias entre dosis de irradiación pero no entre cepas. Los índices ISI (aislamiento) y el RSI (esterilidad relativa) indican que LAB y TUC son totalmente compatibles, los machos de TUC y LAB no difieren en su competitividad y la irradiación dentro del rango de dosis utilizadas tampoco afectó este índice. Las hembras LAB no irradiadas muestran una mayor propensión para el apareamiento que las hembras de TUC. Sin embargo se observó una reducción significativa del índice FRPI (actuación relativa de hembras) a medida que se aumenta la dosis de irradiación. El análisis de la esterilidad inducida indica que con dosis de 40 Gy la fertilidad disminuye del 80% al
Anastrepha fraterculus (Wiedemann) the South American fruit fly (Stone 1942) is an important pest of fruit production in Argentina and the species is abundant in the northwestern and northeastern regions (Vergani 1956). The range of A. fraterculus overlaps at least partially with that of the Mediterranean fruit fly Ceratitis capitata (Wiedemann). The programs of suppression or eradication of the latter species, integrating the Sterile Insect Technique (SIT) in this country, have shown a remarkable success (SENASA 1997, http://www.senasa.gov.ar/vegetal/mosca1.php), and point to the necessity of developing and applying similar control strategies for A. fraterculus (Guillén & Sanchez 2007).

We analyzed under laboratory conditions the optimal irradiation dose and pupal age at the moment of irradiation to induce sterility in A. fraterculus (Allinghi et al. 2007). Irradiated males were able to transfer sperm and exhibited apparently minimal effects, if any, of the irradiation on their performance in comparison with non-irradiated males. However, the sine qua non condition for the SIT is sexual compatibility between sterilized and released laboratory reared flies and wild flies. Therefore, it is necessary to evaluate under quasi-natural conditions the mating performance of laboratory males when competing with wild males for wild females.

In the present work we evaluated, on field-caged host trees the sexual compatibility and competitiveness of a laboratory strain (LAB) in relation to a wild population from Tucumán (Argentina) (TUC). We also analyzed the effects of different radiation doses on mating competitiveness, strain compatibility, fertility, copulation duration, and copulation start time.

MATERIALS AND METHODS

The LAB strain used in this study was produced under semi-mass rearing conditions (Jaldo et al. 2001) since 1997 at the Estación Experimental Agroindustrial Obispo Colombes facility (Tucumán, Argentina). Wild flies were obtained from fruiting guava trees Psidium guajava L. (Myrtaceae) at Horco Molle (26°48’S, 65°20’W) from Tucumán, Argentina. The LAB strain and the collected fruits were sent to the Laboratory of Insects, Instituto Nacional de Tecnología Agropecuaria (INTA), in Castelar, Argentina. The collected fruits were placed on plastic trays over a layer of sand to allow pupation. The sand was periodically sifted to obtain pupae, which were then placed in plastic 1-L flasks. The LAB and the TUC pupae were maintained under controlled conditions (25 ± 1°C, 80 ± 5% rh and a photoperiod of 12:12 L:D) until adult emergence.

LAB pupae were irradiated 48 h before adult emergence (Allinghi et al. 2007) at the Centro Atómico Ezeiza facility (Comisión Nacional de Energía Atómica, Argentina) in a Gammacell 220 (MDS Nordion, Canada) irradiator (60Co source) with a dose rate of 1.4 Gy/min. Lots of 500 pupae were held in 20-mL ventilated glass containers during the exposures of 40, 70, and 100 Gy in normal atmosphere. After irradiation, the pupae were placed in 3-L glass containers. Flies of the control group were subjected to all of the same handling procedures except irradiation. Emerging adults were removed from the flasks every 24 h. To facilitate sorting by sex, flies were anaesthetized by exposure to a temperature of 0°C for 10 min. Fifty individuals of each sex were placed in separate 3-L glass containers and supplied with water and adult food. The food consisted of a 2:1 dry mixture of brown sugar: hydrolyzed corn protein (R. M. SAIC). Manso (1998) showed that laboratory strain adults fed this diet developed to sexual maturity. Adults were kept under laboratory conditions (25 ± 2°C, 60 ± 20% r.h.), and a photoperiod of 12:12 (L:D) until sexually mature. De Lima et al. (1994) reported flies under such conditions reach sexual maturity in 16 d. In a pilot field cage test, we found an increasing proportion of mating with fly age; however, mortality also increased with age. The age of 20 ± 1 d after emergence was found to be the best compromise between maturity and viability.

Three d prior to each experiment, flies were labeled to identify their origin. This was done by placing approximately 10 flies in a mesh bag (1 mm mesh diameter) where, one at a time, they were gently immobilized and painted on the thorax with a dot of water-based paint (Tempera Alba, Alba, Inc., Argentina). Colors green, red, white, and yellow were interchanged sequentially each day. After labeling, 25 flies were placed in 1-L containers with food and water and held under laboratory conditions until required. Outdoor nylon screened cages (2.9 m tall × 3 m diameter) were erected over rooted 1.5 m tall, 4-year-old tangerine trees, Citrus reticulata Blanco ( Rutaceae). Field cages were identified by number, and each day treatments and observers were randomly assigned to them. In field cages, 25 males and 25 females each of TUC and LAB strains were released. For each radiation dose, 6 replicates were made. Only 1 irradiation dose was considered at a time.

Translation provided by the authors.
Because mating occurs mainly in the morning (Malavasi et al. 1983; Morgante et al. 1983; De Lima et al. 1994; Petit-Marty et al. 2004), the observation period was from 08.00 h to 13.00 h. Males were released 15 min before females to allow establishment in the cage. Only healthy marked flies were released, while non-active or dead flies were replaced. For each mating pair, the following data were recorded: copulation start time, copulation location (fruit, net, ground, stem, abaxial-adaxial side of a leaf, height in the tree), and male and female colors. The pairs were gently induced to walk into 20-mL plastic vials and placed in the shade until the mating couple disengaged. This moment was recorded as the copulation end-time. These field cage tests were performed at INTA Castelar (Buenos Aires Province) between April 4 and 16, 2002. Temperatures, relative humidity, and sunshine records during this period were favorable for fly requirements. Copulation start time and copulation duration were compared among laboratory irradiated flies by one-way analysis of variance.

Sexual compatibility was estimated by means of the index of sexual isolation (ISI) (Cayol et al. 1999) and the relative sterile index (RSI) (McInnis et al. 1996). Male and female competitiveness was evaluated respectively through male (MRPI) and female relative performance (FRPI) indices (Cayol et al. 1999). The statistical significance of any departure from random mating or equal performance of each sex was tested, following Petit-Marty et al. (2004), by means of an independence chi squared test taking into account the total number of mated and unmated males (MRPI) or females (FRPI), of each population. Compatibility and relative performance analyses were based only on those trials where the percentage of mating was sufficiently high (>20% of mated females). Matings occurring on the cage screen or on the floor were not included, following the international fruit fly quality control manual (FAO/IAEA/USDA 2003).

For each treatment, induced sterility was evaluated from the percent of egg hatching. At the end of the experiments in the field cages, females were separated according to radiation treatment and male origin and transferred to 3-L flasks. They were allowed to lay eggs into artificial fruits (Manso 1998). Eggs were collected and incubated in Petri dishes, and the hatching rate was recorded.

### RESULTS

For both LAB and TUC flies, most matings occurred on the lower side of peripheral leaves at an intermediate canopy height. Copulation start time (Table 1) did not differ significantly between strains and the irradiation treatment did not show any effect on this variable ($F = 0.23$, $P = 0.63$ and $F = 3.16$, $P = 0.08$ for males and females, respectively). Copulation duration (Table 1) showed highly significant differences among treatments ($F = 4.97$, $P < 10^{-4}$ and $F = 10.08$, $P < 10^{-4}$ for males and females, respectively). These differences are totally attributable to the irradiation treatment. Indeed, TUC and non-irradiated LAB flies did not differ significantly in copulation duration ($P = 0.88$ and $P = 0.41$ for males and females, respectively), but if these two classes are grouped and compared with irradiated flies the differences are highly significant ($F = 18.71$, $P < 10^{-4}$ and $F = 40.08$, $P < 10^{-9}$ for males and females, respectively).

The analysis of mating compatibility by means of the ISI indicated that LAB and TUC are fully compatible. The estimated values did not depart significantly from that expected for random mating, and no effect of irradiation was observed (Table 2). Males from TUC and LAB did not differ in mating competitiveness, and irradiation did not affect this index. Non-irradiated LAB females exhibited higher mating propensity (FRPI signifi-

<table>
<thead>
<tr>
<th>Strain/dose</th>
<th>Start time (Avg: SE)</th>
<th>Duration (Avg: SE)</th>
<th>n</th>
<th>Start time (Avg: SE)</th>
<th>Duration (Avg: SE)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUC</td>
<td>9:11:0:42</td>
<td>1:14:0:37</td>
<td>554</td>
<td>9:13:0:42</td>
<td>1:15:0:36</td>
<td>523</td>
</tr>
<tr>
<td>LAB/0</td>
<td>9:16:0:46</td>
<td>1:19:0:41</td>
<td>136</td>
<td>9:14:0:46</td>
<td>1:21:0:43</td>
<td>154</td>
</tr>
<tr>
<td>LAB/40</td>
<td>9:11:0:41</td>
<td>1:07:0:35</td>
<td>134</td>
<td>9:07:0:42</td>
<td>1:04:0:34</td>
<td>144</td>
</tr>
<tr>
<td>LAB/70</td>
<td>9:11:0:43</td>
<td>1:02:0:30</td>
<td>135</td>
<td>9:12:0:40</td>
<td>1:03:0:33</td>
<td>144</td>
</tr>
<tr>
<td>LAB/100</td>
<td>9:19:0:45</td>
<td>1:05:0:33</td>
<td>128</td>
<td>9:13:0:44</td>
<td>1:02:0:32</td>
<td>122</td>
</tr>
</tbody>
</table>

$^a$TUC = wild flies from Tucumán obtained from guava fruits; non irradiated controls.

$^b$LAB = laboratory strain reared at the Estación Experimental Agroindustrial Obispo Colombres.

### Table 1. Copulation Start Times and Mating Duration (h:min) of TUC$^a$ and LAB$^b$ Flies with Different Irradiation Doses.
cantly higher than 0) than TUC females. However, a significant reduction in the FRPI was observed as irradiation dose was increased (r = -0.98; P = 0.014). The mating competitiveness of irradiated LAB males with TUC males for TUC female mates was measured by the RSI. The estimated RSI values approached 0.5, showing that at all radiation doses LAB males competed efficiently with TUC males for mating with TUC females (Table 2).

The analysis of induced sterility as a function of irradiation dose was based on the proportion of eggs hatching for the TUC strain. Egg hatch rate was estimated from all reciprocal crosses in all tests with the exception of the 70 Gy TUC-LAB test, in which the data collection was missed (Table 3). In each case 4 egg collections were obtained during a 12 d period. The results indicated that a treatment with 40 Gy reduces male fertility from about 80% to 0.75% and higher doses produce total sterility. In females, the 40 Gy dose reduces fertility to about 2% and higher doses prevent egg laying. No differences were observed among egg collection dates, indicating that fertility is not recovered after the irradiation.

**DISCUSSION**

The adaptation of insects to laboratory conditions, mass rearing, and sterilizing by irradiation is known to produce genetic and physiological effects in strains (Shelly et al. 1994; Lance et al. 2000; Alphay 2002; Benedict & Robinson 2003). These factors can influence the efficiency of mass reared and sterilized flies once they are released into the field in support of control programs integrating the sterile insect technique. Males of the Mexican fruit fly *Anastrepha ludens* (Loew) produced in bio-factories, for example, start their sexual activity well before wild ones. This may pose a problem in conventional strains involving the release of both sterile males and females, as these may mate among

<table>
<thead>
<tr>
<th>Dose</th>
<th>ISI</th>
<th>P</th>
<th>MRPI</th>
<th>P</th>
<th>FRPI</th>
<th>P</th>
<th>RSI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-0.029</td>
<td>0.238</td>
<td>0.046</td>
<td>0.477</td>
<td>0.121</td>
<td>0.061</td>
<td>0.543</td>
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</tr>
<tr>
<td>40</td>
<td>0.008</td>
<td>0.909</td>
<td>-0.023</td>
<td>0.709</td>
<td>0.034</td>
<td>0.534</td>
<td>0.484</td>
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</tr>
<tr>
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<td>0.067</td>
<td>0.585</td>
<td>-0.052</td>
<td>0.392</td>
<td>0</td>
<td>1</td>
<td>0.440</td>
<td></td>
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<tr>
<td>100</td>
<td>-0.004</td>
<td>0.751</td>
<td>0.004</td>
<td>0.949</td>
<td>-0.050</td>
<td>0.273</td>
<td>0.504</td>
<td></td>
</tr>
</tbody>
</table>

P = probability of obtaining the observed results assuming random mating.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mating (male-female)</th>
<th>No. pairs</th>
<th>Hatched eggs</th>
<th>Total eggs</th>
<th>% hatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>LAB-TUC</td>
<td>57</td>
<td>351</td>
<td>437</td>
<td>80.32</td>
</tr>
<tr>
<td></td>
<td>TUC-LAB</td>
<td>66</td>
<td>506</td>
<td>547</td>
<td>92.50</td>
</tr>
<tr>
<td></td>
<td>TUC-TUC</td>
<td>48</td>
<td>336</td>
<td>422</td>
<td>79.62</td>
</tr>
<tr>
<td></td>
<td>LAB-LAB</td>
<td>68</td>
<td>349</td>
<td>459</td>
<td>76.03</td>
</tr>
<tr>
<td>40 Gy</td>
<td>LAB-TUC</td>
<td>62</td>
<td>4</td>
<td>536</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>TUC-LAB</td>
<td>68</td>
<td>1</td>
<td>46</td>
<td>2.17</td>
</tr>
<tr>
<td></td>
<td>TUC-TUC</td>
<td>64</td>
<td>425</td>
<td>460</td>
<td>92.39</td>
</tr>
<tr>
<td></td>
<td>LAB-LAB</td>
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<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>70 Gy</td>
<td>LAB-TUC</td>
<td>59</td>
<td>0</td>
<td>464</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>TUC-LAB</td>
<td>66</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>TUC-TUC</td>
<td>75</td>
<td>291</td>
<td>336</td>
<td>86.61</td>
</tr>
<tr>
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<td>LAB-LAB</td>
<td>68</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
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<tr>
<td>100 Gy</td>
<td>LAB-TUC</td>
<td>65</td>
<td>0</td>
<td>625</td>
<td>0.00</td>
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<tr>
<td></td>
<td>TUC-TUC</td>
<td>64</td>
<td>316</td>
<td>398</td>
<td>79.40</td>
</tr>
<tr>
<td></td>
<td>LAB-LAB</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*M* Missing data.

TUC = wild flies from Tucumán obtained from guava fruits; non-irradiated controls.

LAB = laboratory strain reared at the Estación Experimental Agroindustrial Obispo Columbres.
themselves before having the opportunity to mate with wild counterparts (Moreno et al. 1991; Hernández et al. 2003). Liedo et al. (2002) observed that laboratory-reared females of *C. capitata* have greater mating propensity than wild females, and their age of maximum mating activity is earlier. Furthermore, Cayol (2000) reported that the high densities of flies in breeding cages may affect courtship, and matings tend to be faster.

During the strain colonization process for SIT application, the insects are faced with artificial conditions very different from nature and may experience genetic changes due to genetic drift and particular selective forces. These factors sometimes affect the efficiency of the SIT (Cayol 2000). The irradiation treatment to induce sterility was claimed to affect courtship behavior (Lux et al. 2002). Thus, the strain of *A. fraterculus* that is reared under semi-mass rearing conditions at the Obispo Colombres facility was evaluated under conditions that imitate those in nature as a prerequisite to being used in control programs with an SIT component. Outdoor field cages are an acceptable compromise between natural conditions and a controlled laboratory experimental system for monitoring strains (Robinson et al. 2002; FAO/IAEA/USDA 2003).

The present results show that the behavior of this laboratory strain is not substantially modified with respect to the natural population for *Horco Molle*. Average copulation start time was not statistically different between LAB and TUC. The preferred position in the tree for mating was conserved. Copulation duration was similar in non-irradiated LAB and TUC, but irradiation treatment significantly reduced this time. A similar trend was observed in *C. capitata* (Cayol et al. 1999), but the importance of this effect on the efficiency of the SIT is not clear. This is because there is not a direct relationship between copulation duration and the ability of males to transfer sperm. However, matings that are too short might increase the probability of female remating (FAO/IAEA/USDA 2003).

The estimated ISI (-0.03 to 0.07) and RSI (0.44 to 0.54) values suggest total compatibility between the laboratory strain and the natural population of *A. fraterculus* analyzed here. MRPI (-0.05 to 0.05) did not differ from the expected, indicating similar male mating competitiveness of LAB and TUC. An important result linked to the possibility of applying the SIT to control *A. fraterculus* is that compatibility and mating performance of male LAB flies are not affected by irradiation for all tested doses. This results contrasts with those of Cayol et al. (1999), who observed that under similar conditions LAB flies of *C. capitata* had reduced competitiveness (MRPI ≈ 0.09; RSI ≈ 0.33) and compatibility ISI ≈ 0.31).

According to our FRPI estimates, LAB females have higher mating propensity than TUC females. A similar result was observed in other tephritids (Cayol 2000; Liedo et al. 2002), which suggests that LAB females are sexually more active and less selective. However, this higher mating propensity of LAB females was reduced as the applied irradiation dose increased.

Some authors observed that irradiated females do not lay eggs depending on the radiation dose and the developmental stage at the time of the irradiation treatment (Burditt et al. 1975; Velasco & Enkerlin 1982; Calkins et al. 1988). According to the present analysis of egg laying and hatching, *A. fraterculus* females treated with 40 Gy oviposited a reduced number of eggs compared to control females. Higher doses prevented all egg laying. Moreover, the treatment with 70 Gy of gamma irradiation applied 48 h before adult emergence ensured 100% sterility both in males and females. Furthermore, during the evaluation period (12 d) there was no evidence of recovery of fertility in females or males.

Recent results by Vera et al. (2006) indicate that some *A. fraterculus* populations from different regions in South America might be sexually incompatible and reproductively isolated, while Petit-Marty et al. (2004) observed complete compatibility between TUC and several geographically isolated populations from within Argentina. Alberti et al. (2002) also concluded that TUC and other populations from Argentina and southern Brazil (Pelotas) are not differentiated genetically. Therefore, it is expected that the LAB population from Obispo Colombres facility will behave similarly when facing natural populations from Argentina and southern Brazil. The high compatibility of LAB and TUC flies and the good competitiveness of irradiated LAB males observed in the present work encourage the application of the SIT at least at a sub-regional level to control *A. fraterculus* populations from Argentina and southern Brazil.

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IMPROVING MATING PERFORMANCE OF MASS-REARED STERILE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) THROUGH CHANGES IN ADULT HOLDING CONDITIONS: DEMOGRAPHY AND MATING COMPETITIVENESS

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ABSTRACT

Mass rearing conditions affect the mating behavior of Mediterranean fruit flies (medflies) Ceratitis capitata (Wiedemann). We evaluated the effect of slight changes in the adult holding conditions of adult flies maintained for egg production on their mating performance. Colonization was initiated from wild flies collected as larvae from infested coffee berries (Coffea arabica L.). When pupae were close to adult emergence, they were randomly divided into 3 groups and the emerging adults were reared under the following conditions: (1) Metapa System (MS, control), consisting of 70 × 45 × 15 cm aluminum frame, mesh covered cages, with a density of 2,200 flies per cage and a 1:1 initial sex ratio; (2) Insert System (IS), with the same type of cage, and the same fly density and sex ratio as in the MS treatment, but containing twelve Plexiglas® pieces (23 × 8.5 cm) to provide additional horizontal surface areas inside the cage; and (3) Sex-ratio System (SS), same as IS, but in this case the initial male:female ratio was 4:1. Three d later, 1:3 newly emerged females were introduced, so the ratio became 3:1 and on the 6th d another group of newly emerged females was added to provide a 2:1 final sex ratio, at which the final density reached 1,675 flies per cage. The eggs collected from each of the 3 treatments were reared independently following standard procedures and the adults were held under the same experimental conditions. This process was repeated for over 10 to 13 generations (1 year). The experiment was repeated 3 times in 3 consecutive years, starting each replicate with a new collection of wild flies. Life tables were constructed for each treatment at the parental, 3rd, 6th, and 9th generations. Standard quality control parameters (pupation at 24 h, pupal weight, adult emergence, and flight ability), were estimated for each treatment every third generation in the third year. For the last generation each year, mating competitiveness was evaluated in field cage tests with wild flies. As colonization progressed, life expectancy and fecundity rates increased in the 3 rearing systems. There was no significant difference in standard quality control parameters among the 3 rearing systems. Wild males always achieved more matings than any of the mass reared males. Mating competitiveness of males from the IS, although surprisingly not from the SS, was significantly greater than that of males from the MS. Our results indicate that these slight changes in the adult holding conditions can significantly reduce the harmful effects of mass rearing on the mating performance of sterile flies.

Key Words: Ceratitis capitata, sterile insect technique, colonization, mating behavior, insect demography, mother colony

RESUMEN

Se ha demostrado que las condiciones de cría masiva afectan el comportamiento de apareamiento de la mosca del Mediterráneo Ceratitis capitata (Wiedemann). Nosotros evaluamos el efecto de ligeros cambios en las condiciones en las que los adultos son mantenidos para la producción de huevos, en el desempeño de apareamiento de las moscas estériles. La colonización se inició con moscas silvestres colectadas como larvas en cerezas de café (Coffea arabica L.) infestadas. Cuando las pupas estuvieron cerca de la emergencia de los adultos, se dividieron en tres grupos al azar y los adultos recién emergidos fueron criados en las siguientes condiciones: (1) Sistema Metapa (MS, testigo), consistente en jaulas con marco de aluminio de 70 × 45 × 15 cm, cubiertas con malla, con una densidad de 2,200 moscas por jaula y una relación de sexos inicial de 1:1; (2) Sistema Insertos (IS), con el mismo tipo de jaula, densidad de moscas, y relación de sexos que en el MS, pero conteniendo 12 piezas de plexiglas (23 × 8.5 cm) para proporcionar superficie horizontal al interior de la jaula; y (3) Sistema de Relación de Sexos (SS), igual que el IS, pero en este caso la relación inicial macho: hembra fue de 4:1, tres días después se introdujeron hembras recién emergidas para tener una relación de 3:1 y en el 6° día se añadió otro grupo de hembras para tener una relación final de sexos de 2:1, que equivale a una densidad final de 1,675 moscas por jaula. Los huevos colectados de cada tratamiento fueron criados independientemente siguiendo los procedimientos estándares y los adultos fueron mantenidos en las mismas condiciones experimentales. Esto
se repitió por 10 a 13 generaciones (un año). El experimento se repitió en tres ocasiones en años consecutivos, iniciando cada repetición con una nueva colecta de moscas silvestres. Se construyeron tablas de vida de cada tratamiento en las generaciones parental, 3°, 6° y 9°. Se estimaron los parámetros estándares de calidad (pupación a las 24 h, peso de pupa, emergencia de adultos y habilidad de vuelo) para cada tratamiento, cada tercera generación en el tercer año. En la última generación de cada año, se evaluó la competitividad sexual en pruebas en jaulas de campo con moscas silvestres. Conforme avanzó la colonización, se encontró que la esperanza de vida y las tasas de fecundidad se incrementaron en los tres sistemas de cría. No hubo diferencia significativa en los parámetros estándar de control de calidad entre los tres sistemas. Los machos silvestres siempre lograron más apareamientos que los machos procedentes de cada sistema de cría masiva. La competitividad de los machos del sistema IS fue significativamente mayor que la de los machos del sistema MS. Nuestros resultados indican que estas ligera modificaciones en las condiciones de la colonia de adultos reducen los efectos adversos de la cría masiva sobre el desempeño de apareamiento de los machos estériles.

Translation provided by the authors.

Since the early stages of the sterile insect technique (SIT), it was recognized that the mating competitiveness of the sterile insects was a critical factor for the successful application of the technique (Knipping 1955). Research results showed that the exposure to irradiation for sterilization affected the mating performance of the sterile fruit flies (Holbrook & Fujimoto 1970; Hooper 1971; Ohinata et al. 1977; Knipping 1979; Lux et al. 2002a). Later, it was found that both irradiation and the selection that occurs during colonization could adversely affect the mating performance of sterile flies (Rössler 1975; Wong & Nakahara 1978; Leppla et al. 1983; Wong et al. 1983; Harris et al. 1986).

In the case of the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann) however, it has been shown that, despite a long time under mass rearing conditions, sterile males are still capable of locating hosts, mating arenas or leks, and mix and interact with their wild counterparts under natural conditions (Zapien et al. 1983; Whittier et al. 1992; Shelly & Whittier 1996; Katsoyannis et al. 1999). Also, it has been documented that the courtship patterns of flies from different geographical areas are sexually compatible (Cayol et al. 2002; Lux et al. 2002b). However, it has been shown that slight quantitative changes in the courtship displays of males might result in female rejection and that these changes could be attributed to the selection that occurs under mass rearing conditions. Male courtship behavior of mass reared flies tends to be less elaborate, and the degree to which it is affected was found to be associated with the time under mass rearing conditions (McInnis et al. 1996; Eberhard & D. Briceño, personal communication).

Under natural conditions, this fast mating behavior results in less competitive sterile males in view of wild female mate choice, and therefore, less effective programs integrating the SIT. The goal of this study was to evaluate whether slight changes in the colony holding conditions, where adult flies are maintained for egg production, could reduce this selection for fast mating and thus produce more competitive flies. Two changes from the standard mass-rearing procedures (Schwarz et al. 1985) were tested: (1) horizontal clear inserts were introduced inside the rearing cages to increase the overall resting surface available and to imitate the undersurfaces of leaves where males usually perform their courtship under natural conditions (Prokopy & Hendrichs 1979), possibly reducing the frequency of courtship interruption; and (2) variation in the operational sex ratio by introducing the females into the cages at four different times, so the number of sexually mature males was always greater than the number of sexually mature females (Calkins 1989).

**Materials and Methods**

**Biological Material**

The study was initiated with wild flies collected from naturally infested coffee berries (Coffee arabica L.) in southwestern Guatemala. New collections were made in each of 3 consecutive years, each year being considered as a replicate of the whole experiment. The location, amount of coffee collected, and the approximate number of larvae and adults obtained for each collection are shown in Table 1.
Rearing Systems

Experimental work was carried out at the Moscamed mass rearing facility in Metapa, Chiapas, Mexico. Standard rearing procedures and environmental conditions were used (Schwarz et al. 1985). 3 adult rearing systems were evaluated: (1) Metapa System (MS, control), which consisted of an aluminum frame, mesh covered cage (70 × 45 × 15 cm) with an initial density of 1,100 males and 1,100 females per cage, and an average surface area of 3.91 cm$^2$ per fly; (2) Insert System (IS), as above but with the addition of 12 pieces of clear plexiglas (polycarbonate) (23 × 8.5 cm) inside the cage as horizontal surface areas, resulting in a surface area of 5.85 cm$^2$ per fly; and (3) Sex-ratio System (SS), same as IS, but with an initial density of 1,100 males and 275 females (4:1 male: female ratio). Three d later 92 recently emerged virgin females were introduced to make a 3:1 ratio, and at the 6th day 183 recently emerged virgin females were introduced to make a 2:1 ratio, and a total of 1,100 males and 550 females. The surface area was 6.94 cm$^2$ per fly.

Adults were fed ad libitum with a mixture of enzymatic yeast hydrolysate (ICN Biomedical, Costa Mesa, CA) and sucrose (1:3). Water was provided in test tubes covered with cotton plugs. On both sides at the bottom of the cages, water channels were placed for egg collection. These eggs were reared following the standard procedures at the Metapa facility (Schwarz et al. 1985).

Demographic Analysis

To compute life tables, the number of dead flies and the volume of eggs collected were recorded daily from the cages. In addition, a sample of 30 pairs from each treatment, every third generation, was taken and placed in plastic cages (8 cm diameter by 15 cm long, one male and one female per cage) with food, water, and a 2-cm diameter agar sphere (3 L of water + 80 g of agar dyed with green food coloring and wrapped in Parafilm®) as an oviposition device (Boller 1968, Freeman & Carey 1990). These spheres were replaced every 24 h and the number of eggs laid were recorded. This was done until the last female in the cohort died.

Male Sexual Competitiveness

Each year, after 10 to 13 generations, field cage mating tests with host trees were conducted (FAO/IAEA/USDA 2003). In each cage, 50 wild females, 50 wild males, and 50 males of each rearing system were released. Wild flies were 9-13 d old and mass-reared sterile flies were 7-11 d old. These ages were selected following the results of Liedo et al. (2002). The tests were conducted at coffee plantations in Guatemala during 5 consecutive d. Each d, 3 replicates (field cages) were set up. The males were color marked on the thorax for treatment identification.

In the third year, in addition to these mating tests, the “Fried” field cage test was used (Fried 1971). In each cage, 50 wild females, 50 wild males, and 150 sterile mass-reared males were released (one field cage for each treatment), and 25 agar oviposition devices (as described above) were placed inside each cage. After 24 h, the agar devices were removed, and egg hatch was determined from the eggs obtained from these devices. One hundred wild flies (1:1 male: female ratio) were placed in a field control cage to collect eggs and determine egg hatch without sterile fly competition. Sterility induced was estimated from the difference between egg hatch in the control and egg hatch in competition. There were 3 cages per treatment, and the test was run during 2 d, making 6 replicates per adult holding system.

Standard Quality Control Tests

In the third year, standard quality control parameters (FAO/IAEA/USDA 2003) were determined for each treatment at the parental, 3rd, 6th, and 9th generations. The parameters evaluated were pupal weight, adult emergence, and flight ability.

Statistical Analysis

Life table demographic parameters used in this study are defined by Carey (1993). Laboratory and field tests followed the methods described in the international quality control manual for tephritid flies (FAO/IAEA/USDA 2003). Data from observed proportions were transformed as $\sqrt{x + 0.5}$,
subjected to analysis of variance (ANOVA), followed by means separations by the Tukey test ($P \leq 0.05$) (SAS Institute 1992).

RESULTS

Demographic Analysis

Survival rapidly increased through colonization in the 3 treatments. Mean adult life expectancy increased significantly from the parental to the 3rd generation, then gradually increased or remained stable in the following generations, both in males and females. This trend was observed when the flies were evaluated individually (Fig. 1), although differences among generations were not significant ($F = 0.4355, P = 0.6556$ for males; $F = 2.9684, P = 0.0801$ for females). There were no significant differences among rearing systems ($F = 0.0790, P = 0.9244$ for males; $F = 0.1569, P = 0.8561$ for females) and there was no significant interaction between rearing systems and generations ($F = 0.2214, P = 0.9225$ for males; $F = 0.4244, P = 0.7888$ for females).

When survival data were taken directly from the rearing cages, there were highly significant differences among generations ($F = 7.7843, P = 0.0010$ for males; $F = 8.4050, P = 0.0006$). However, the differences among rearing systems were not significant ($F = 1.4461, P = 0.2570$ for males; $F = 0.5255, P = 0.5985$ for females) and there were also no interactions between generations and rearing systems ($F = 0.2589, P = 0.9502$ for males; $F = 0.1551, P = 0.9859$ for females) (Fig. 2).

Fecundity increased in a similar pattern. The number of eggs laid per female increased significantly from the parental generation to the 3rd generation in all treatments, then gradually increased every third generation. This was observed both in the data collected from single pairs (Fig. 3 top), as well as in those from the rearing cages (Fig. 3 bottom). There was wide variation in this parameter among treatments, particularly during the first 3 to 6 generations, but no statistical differences among treatments were found ($F = 11.0348, P = 0.0009$), and when data were collected from the rearing cages ($F = 35.8365, P = 1.208 \times 10^{-8}$). The interaction between rearing systems and generations was not significant ($F = 0.2406, P = 0.9111$ for single pairs; $F = 0.4048, P = 0.8678$ for rearing cages). It is important to note the demographic implications of the significant

Fig. 1. Life expectancy ($e_0$) (days ± SE) of male (top) and female (bottom) Mediterranean fruit flies from 3 different adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System), estimated from single pair cages.

Fig. 2. Life expectancy ($e_0$) (days ± SE) of male (top) and female (bottom) Mediterranean fruit flies from 3 adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System), estimated from rearing cages.
differences between the parental and the 9th generations in both, survival and fecundity, in the 3 rearing systems.

Male Sexual Competitiveness

Results from the field cage mating tests during the 3 years were rather consistent. Wild males were always the most successful in terms of the average percent of matings achieved and the differences were statistically significant ($F = 26.92; df = 3, 6; P < 0.001$) (Fig. 4). Among the 3 rearing systems, there was a significant difference between the IS and the MS (control). The differences between the SS and the other 2 rearing systems were not significant.

The average mating index ($\pm$ SE) estimated for each rearing system, according to the international quality control manual (FAO/IAEA/USDA 2003), also showed a significant difference between the IS and MS, and a non significant difference between the SS and the other 2 rearing systems ($F = 35.08; df = 2, 6; P = 0.042$) (Fig. 5).

Results from the Fried test showed that males from the IS were the ones that induced the greatest level of sterility (34%). Males from the SS and MS treatments only induced 18.3 and 16.3% sterility, respectively. However, differences among treatments were not statistically significant ($F = 32.87; df = 2, 15; P = 0.101$). Fig. 6 shows the average ($\pm$ SE) level of sterility induced by each treatment. Natural sterility was 13.3%.

Standard Quality Control Tests

The results of the standard quality control tests applied to the 3 rearing systems at the parental, 3rd, 6th, and 9th generations are shown in Fig. 7. All these values were within acceptable international ranges (FAO/IAEA/USDA 2003). There was a significant increase in pupal weight, from the parental flies to the mass reared flies. In the other 2 parameters, there were no significant differences among generations, although a similar pattern can be observed.

Pupal weight in the 3rd generation was greater in the SS compared with the other 2 treatments ($F = 0.84; df = 2, 9; P < 0.001$). There were no significant differences at the 6th generation ($F = 1.36; df = 2, 9; P = 0.052$). In the 9th generation, the IS produced the heaviest pupae ($F = 1.62; df = 2, 9; P = 0.011$).

Mean adult emergence was greater in the IS and SS than in the MS at the 3rd ($F = 4.66; df =$
2, 9; $P = 0.013$) and 6th generations ($F = 9.36; df = 2, 9; P = 0.021$). Differences in this parameter were not statistically significant at the 9th generation ($F = 9.08; df = 2, 9; P = 0.301$).

There were no significant differences among treatments in flight ability at the 3rd ($F = 2.43; df = 2, 9; P = 0.655$) and 9th ($F = 3.67; df = 2, 9; P = 0.231$) generations. At the 6th generation, flight ability was significantly greater in the IS than in the MS ($F = 5.64; df = 2, 9; P = 0.037$).

**DISCUSSION**

Demographic data confirm that mass-reared flies have greater reproductive rates than wild flies (Liedo & Carey 1996) and show that colonization for mass-rearing is a selection process in which insects adapt to the new rearing conditions (Leppla et al. 1983; Leppla 1989). For mass rearing purposes, this is desirable and necessary in order to produce large number of insects in an efficient manner. However, this same selection process can result in negative effects on other biological attributes, such as mating behavior.

The results from the single pair cages and the rearing cages showed that the conditions in which flies are held affect the demographic parameters obtained, with greater values at the single pair cages than at the more stressful adult holding cages. However, in both cases, the general trends were similar, with mean expectation of life and net reproductive rates increasing with generations, as the flies gradually adapted to the crowded mass-rearing conditions. At the same time only very small or no differences were found among rearing systems.

Our results from the field cage mating tests corroborate that mass-rearing adversely affects the mating competitiveness of the reared insects compared to wild flies (Wong & Nakahara 1978; Wong et al. 1983; McInnis et al. 1996). The introduction of horizontal inserts in the rearing cages contributed to a significantly better mating performance of the IS mass-reared insects when compared to the standard-produced MS males. Although there were no significant differences in the level of sterility induced (Fried test), the pattern was similar (IS > SS > MS). This suggests that the number of matings recorded during the observation period in the field cage mating test is correlated with the induction of sterility in the wild population and that males from the IS were more competitive than males from the other 2 rearing systems.

The manipulation of the sex ratio did not have a significant effect on the mating performance of mass-reared flies. This result was unexpected. We

![Fig. 6. Sterility levels (mean ± SE) induced by sterile Mediterranean fruit fly males reared under 3 different adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System) when competing with wild males in field cages (the natural sterility in the control was 13.3%).](image)

![Fig. 7. Standard quality control tests: (A) pupal weight (mg), (B) adult emergence (%), (C) flight ability (%) of the 3 adult colony holding systems (MS = conventional Metapa System, IS = Insert System, SS = Sex-ratio System).](image)
were expecting that the biased sex ratio in favor of males would allow females to be more selective and result in more competitive males. One explanation for this could be the reduced offspring produced by the smaller number of females and as a result of harassment of ovipositing females by the excess of males in the cage; however, there are other potential causes that need to be investigated. The small number of offspring was particularly critical in the second year. Manipulation of operational sex ratio in adult holding cages is now feasible due to the current availability of genetic sexing strains. We believe that this research line, and the interaction with increased surface area in cages, should be further explored.

Data from the standard quality control tests demonstrate that the 3 colonization methods have no detrimental effect on most of these parameters. Pupal weight was the only attribute that significantly changed (increased) through colonization. These findings suggest that while the demographic and mating attributes, as well as pupal weight, were under selection pressure during colonization, this was not the case for attributes such as adult emergence and flight ability. This raises the question of whether other biological attributes could be under selection pressure during colonization (Harris 1988; Calkins 1989; Miyatake & Haraguchi 1996). Rodrigueiro et al. (2002) reported differences between wild and mass-reared medflies in some morphological traits. Lux et al. (2002b) found quantitative differences in the courtship behavior of wild and mass-reared Mediterranean fruit fly males. The biological attributes that show significant differences between wild and mass-reared flies deserve further research.

In the current study, we started all 3 treatments from wild collected flies. It will be interesting to investigate whether the introduction of inserts might have a reverse effect. Will a long term mass-reared strain increase its competitiveness if horizontal inserts are introduced to the mass rearing process, without starting a new colony from wild flies? Based on our results, the introduction of inserts in the rearing cages is strongly recommended, because its represent a minor change in the production process, with negligible costs, and important benefits in the application of the SIT.

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PRODUCTION AND QUALITY ASSURANCE IN THE SIT AFRICA MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) REARING FACILITY IN SOUTH AFRICA

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ABSTRACT

A mass-rearing facility for Mediterranean fruit fly Ceratitis capitata (Wiedemann) was commissioned in Stellenbosch in 1999 to produce sterile male fruit flies for a sterile insect technique (SIT) project in commercial fruit orchards and vineyards in the Western Cape province of South Africa. The mass-rearing procedure was largely based on systems developed by the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria. A number of genetic sexing strains were used to produce only males for release. Initial cramped rearing and quality management conditions were alleviated in 2001 with the construction of a new adult rearing room and quality control laboratory. In 2002 a comprehensive Quality Management System was implemented, and in 2003 an improved genetic sexing strain, VIENNA 8, was supplied by the FAO/IAEA Laboratory in Seibersdorf. For most of the first 3 years the facility was unable to supply the required number of sterile male Mediterranean fruit flies for the SIT program without importing sterile male pupae from another facility. From mid-2002, after the quality management system was implemented, both production and quality improved but remained below optimum. After the introduction of the VIENNA 8 genetic sexing strain, and together with an improvement in the climate control equipment, production stability, and quality assurance parameters improved substantially. The critical factors influencing production and quality were an inadequate rearing infrastructure, problems with the quality of the larval diet, and the initial absence of a quality management system. The results highlight the importance of effective quality management, the value of a stable and productive genetic sexing strain, and the necessity for a sound funding base for the mass-rearing facility.

Key Words: genetic sexing strain, mass rearing, Mediterranean fruit fly, sterile insect technique, quality management

RESUMEN

La facilidad para criar en masa la mosca mediterránea de la fruta, Ceratitis capitata (Wiedemann) fue comisionada en Stellenbosch en 1999 para producir machos estériles de moscas para el proyecto de la técnica del insecto estéril (TIE) en huertos de frutos y viñas comerciales en la provincia del Cabo Occidental del Sudáfrica. El procedimiento de criar en masa fue en su mayor parte basado en los sistemas desarrollados por el Laboratorio de Agricultura y Biotecnología de la FAO/IAEA, Seibersdorf, Austria. Un número de razas que separaba los sexos genéticamente fueron utilizadas para producir solo machos para la liberación. La congestión de la condición inicial para criar las moscas y su manejo de calidad fueron aliviadas en 2001 con la construcción de un nuevo cuarto de cria para adultos y un laboratorio de control de calidad. En 2002, un Sistema de Manejo de Calidad comprensivo fue implementado, y en 2003 una raza mejorada que separa los sexos genéticamente, VIENNA 8, fue proveído por el Laboratorio de la FAO/IAEA en Seibersdorf. En la mayor parte de los primeros 3 años la facilidad no pudo suplir el número requerido de machos estériles de la mosca mediterránea de la fruta para el programa de TIE sin la necesidad para importar machos estériles de otra facilidad. Desde medio del año de 2002, después que el sistema de manejo de calidad fue implementado, producción y la calidad mejoraron pero aún quedaron por debajo del nivel óptimo. Después de la introducción de la raza VIENNA 8 que separa los sexos genéticamente, y junto con el equipo mejorado de control de clima, la estabilidad y los parámetros de seguridad de calidad mejoraron substancialmente. Los factores críticos que influyeron en la producción y la calidad fueron la infraestructura inadecuada para criar las moscas, problemas con la calidad del dieta para las larvas y la ausencia inicial de un sistema de manejo de calidad. Los resultados muestran claramente la importancia de un manejo eficaz de la calidad, el valor de una raza productiva que separa los sexos genéticamente y la necesidad de contar con una base sólida de financiamiento para la infraestructura de una cria en masa.
The export deciduous fruit industry is of great economic importance to South Africa. Nearly 90 million cartons are exported annually, with total earnings of approximately US$1 billion per annum. The Western Cape is the most important region for the production of deciduous fruit, with approximately 58,000 ha under cultivation (Optimal Agricultural Business Systems 2005).

The Western Cape is host to 2 species of tephritid fruit flies of economic importance, the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann), and the Natal fruit fly Ceratitis rosa (Karsch). Between them, they attack a wide variety of subtropical, tropical, and deciduous fruits (Annecke & Moran 1982). Both species are international quarantine pests with the potential to restrict international fruit trade with South Africa. Further details of their occurrence, behavior, and management in the Western Cape is given by Myburgh (1964) and Barnes (1994). It has been estimated that crop losses and control costs due to fruit flies in the Western Cape alone exceed US$3.2 million per annum (Mumford & Tween 1997). While the economic impact of tephritid fruit flies country-wide has not been determined, the impact on the South African export fruit industry of a quarantine embargo on South African fruit due to the presence of fruit flies would be devastating. For the South African export fruit industry to remain viable, the creation of fruit fly-free or low prevalence areas is therefore an urgent necessity. The sterile insect technique (SIT), integrated with other measures, is widely regarded as the most practical and cost-effective means of establishing such areas.

A pilot project to suppress C. capitata in an isolated export table grape production area in the Western Cape, the Hex River Valley, with an SIT component was initiated in 1997. Sterile C. capitata were produced in a mass-rearing facility located at the Infruitec-Nietvoorbij Fruit, Vine and Wine Research Institute of the Agricultural Research Council (ARC) in Stellenbosch, with a number of different temperature-sensitive lethal (tsl) genetic sexing strains (Franz 2005). Production at the facility started in Apr 1999, with aerial releases of sterile males over 10,000 ha starting in Oct that year. Further details of the pilot project are described by Barnes et al. (2004). Aerial releases over the entire 10,000 ha were later replaced by ground releases due to the high cost of aerial releases in view of the relative small release area.

The SIT operations were not supported financially by the national government, although the provincial government sporadically funded the mass-rearing facility. As a result, the facility was initially financed through a formal SIT Partnership between the ARC, which coordinated the SIT component of the program, and the Deciduous Fruit Producer’s Trust, a fruit-grower organization. Continued lack of national support, together with the inability of the SIT Partnership to continue funding the mass-rearing facility, led to the commercialization of the production and distribution of sterile medflies via SIT Africa (Pty) Ltd. in 2003. The SIT programme has since expanded to 2 other production areas, and at the time of writing a total of 6 million sterile male medflies per week were being ground-released, specifically targeting backyards and host plants, over a total fruit production area of 15,600 ha.

Production volumes and quality parameters of sterile medflies produced by the rearing facility were well below optimum and varied a great deal during the course of the program. This article describes the rearing process, the genetic sexing strains used, and the production and quality parameters achieved over a period of 1 to 5 years, and discusses causes of the poor rearing performance and the factors that led to improved production and quality in the facility.

**MATERIALS AND METHODS**

**Genetic Sexing Strains**

A number of genetic sexing strains obtained from the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria, were used to produce sterile male medflies. In such strains, the females carry a tsl mutation that results in their mortality as embryos by heat treatment so that females can be eliminated before mass rearing of the males destined for sterilization and release (Franz et al. 1994). This results in greater cost-effectiveness as only the males are the active agent in the SIT (Hendrichs et al. 1995). In addition, the females are homozygous for the mutation white pupae (wp). This allows the integrity of the sexing system and the accuracy of the temperature treatment to be monitored and is required for a Filter Rearing System to manage the mother colony (Fishé & Cáceres 2000).

The genetic sexing strain VIENNA 7-97 was initially used when mass-rearing started in Apr 1999. This strain was replaced in Aug/Sep 1999 with a refreshed genetic sexing strain, VIENNA 7/Mix-99 (Fisher 1999) that was initially used for the first sterile male releases that started in Oct 1999. Due to genetic instability of this genetic sexing strain under local rearing conditions, especially in the absence of a filter rearing system, the colony strain was replaced three times between May 2000 and Dec 2001, with strains VIENNA 7/Tol 2000 in May 2000, VIENNA 7/Mix 2000 in Nov 2000, and VIENNA 7-D53/Mix 2001 in Dec 2001 (Robinson et al. 1999).

A filter rearing system to control the accumulation of genetic recombinants (Franz 2002) in the genetic sexing strain was set up in mid-2000. In
Production and Release Procedure

Production. Initially, an old, disused building at the Pest Management Division of ARC Infruitec-Nietvoorbij was refurbished and used as a sterile fruit fly production facility. In Apr 2001, a new building was erected to house the adult colony and a quality control laboratory, alleviating cramped rearing conditions in the old building and providing for better management of quality control. This raised the maximum production potential to an estimated 10 million sterile males per week.

The mass-rearing procedure was largely developed by the FAO/IAEA Laboratory in Seibersdorf. Larvae were reared on the following artificial diet: digestive bran (28.75%), torula yeast (7.00%), white sugar (13.00%), sodium benzoate (0.25%), 30% hydrochloric acid (1.50%), formalin (0.08%) and water (49.43%). The pH of the diet was buffered to between 3.2 and 3.5. Five kg of diet were placed in each rearing tray, and 3.2 mL of eggs (for the colony stream) and 12.5 mL of eggs (male-only stream) were seeded per tray on an egg raft of toilet tissue.

After seeding, the trays were moved to Larvae Room #1 (25°C, 90-100% R.H.). After 3 d the trays were moved to Larvae Room #2 (22°C, 75-80% R.H.). After 3 d, trays were then moved to Larvae Room #3 (20°C, 65-70% R.H.), where mature larvae left the medium and were collected in water-filled gutters for either 5 d (colony production) or 2 d (male-only production). Each day’s larval collection was mixed with fine vermiculite and kept at 20°C and 80-85% RH for pupation.

In the adult room, egging cages measuring 0.74 m × 0.84 m in cross section and 2 m in height were surrounded on all 4 sides with fine mesh screen through which the females oviposited. The cages were mounted on wheels that ran on rails in a water bath. Each cage was loaded with 3.2 liters of pupae at a male:female ratio of 1:3, resulting in a total cage content of approximately 186,600 flies. Adult food consisted of a 1:3 mixture of enzymatic yeast hydrolysate (Separations, Johannesburg, South Africa) and sugar, and water was provided through soft cloths protruding through pipes filled with water. Eggs oviposited through the screen sides fell into the water bath that was drained once a day and the eggs were collected in a sieve. Each cage was kept in production for 12 d. Conditions in the adult room were maintained at 25°C and 60-65% R.H., with a photoperiod of 15.5:8.5 (L:D). All eggs were bubbled in water containing 0.1% sodium benzoate for 48 h under the same conditions. Eggs for the male-only stream were additionally heat-treated at 34°C for 16 h, which killed the female eggs.

Irradiation. Male pupae were sterilized 1 d before adult emergence (based on eye color; FAO/IAEA/USDA 2003) with 90 Gy from 60Co in the ARC Infruitec-Nietvoorbij walk-in irradiator. It was equipped with a sealed point-source of 60Co (Mayak Production Association, Ozorsk, Russia) stored in a below-floor, lead-filled drum that was raised hydraulically to the required level when needed. The dose-rate was 5.5 Gy/min, initially determined with Fricke dosimeters and verified by Gafchromic dosimetry (IAEA 2004). Pupae packed in plastic bags (sample size = 180 mm × 150 mm; volume = 5 L) were irradiated under hypoxia on a rotating table (diameter 1.0 m; 60 s for 1 rotation) fixed around the vertical axis of the 60Co source lifting rods. Eight rotating discs (diameter 0.2 m; 20 s for 1 rotation) were built into the table around its perimeter. The resulting dual rotation of the pupae facilitated optimum dose distribution throughout the sample. The dose was verified by Sterin® or RadTag® indicators in each container of pupae.

Irradiated pupae were dyed with Day-Glo® fluorescent dye (Radiant Color, Houthalen, Belgium) and were placed in paper bags (110 mL per bag) in Plastic Adult Release Containers (“PARC boxes”). During the period of aerial releases (VIENNA 7 genetic sexing strain) this yielded approximately 4,250 fliers/bag at 65% flight ability. During later ground releases (with VIENNA 8 genetic sexing strain) an improved flight ability of 75% yielded approximately 5,000 fliers/bag.

Food for the flies was provided by means of cakes of food grade agar plus sugar placed onto gauze vents on top of each PARC box for aerial releases, or for ground releases, by brown paper strips soaked in the agar and sugar mixture and placed into each bag. After poor performance of the food strips, food was later provided more effectively by means of small agar and sugar cakes in plastic containers (50 mm diameter, 20 mm deep) placed in the bottom of the bag.

Release. Details of aerial releases over the Hex River Valley are given by Barnes et al. (2004). These were replaced by ground releases in Jun 2003. For ground releases, bags of sterile flies...
were transported to the release areas from 3 to 5 d after emergence and released the following day. All flies were released in fruit fly host plants in gardens and backyards, and in any other neglected fruit trees, at a density of 2,000 flies per hectare (Ortíz-Moreno 2002). This release system has resulted in effective suppression of *C. capitata* in the Hex River Valley at a reduced cost to the growers (I. Sutherland, SIT Africa [Pty] Ltd., Stellenbosch, South Africa, personal communication).

The targeted nature of ground releases resulted in a decrease in demand for sterile medflies in the Hex River Valley to 1.4 million sterile flies per week. In Dec 2003 releases of sterile flies started in a second area (Elgin, Grabouw, Vye-boom and Villiersdorp) increasing the demand to 4.2 million sterile flies per week. In Aug 2004 a third area (Riebeek Valley) joined the SIT program, and the total requirement for releases was increased to 6 million sterile flies per week. The facility’s production output goal was not reduced following the decrease in demand for sterile flies—the facility management decided rather to have an output safety ‘cushion’ to accommodate any unexpected decrease in production.

Production Performance and Quality Assurance

All rearing procedures and quality control measurements were carried out according to the international fruit fly quality control manual (FAO/IAEA/USDA 2003). Up to Dec 2001 the facility had no formal quality management system. Following consistent problems encountered with production volumes and sterile fly quality, a comprehensive Quality Management System was introduced in Jan 2002.

The quality management system covered two main aspects: production and quality assurance. A weekly review meeting evaluated each of these aspects according to set targets as follows: (1) Production (all reproductive streams)—number of adult cages set up, egg production (mL per day), number of trays seeded, number of pupae irradiated, number of sterile flies delivered; (2) Quality control (all streams)—egg hatch (%; 0 h and 48 h), egg to pupa efficiency (%); flight ability (%), genetic recombination (males in white pupae; %), females in the male-only stream (%), sterility of males for release (% fertility). Definitions of these parameters and the methods of assessment are given in FAO/IAEA/USDA (2003). Other quality parameters, e.g., of raw diet ingredients and water, were not at that point incorporated into the quality management system.

For the purposes of this article only the following production parameters are discussed: production—daily egg production, and number of pupae irradiated per week; quality assurance—egg hatch (48 h), egg to pupa efficiency in the male-only stream, flight ability of sterile males, and percentage females in the male-only stream (as an indication of genetic recombination of the genetic sexing strain). Some records from the earlier part of the programme, before the new quality control laboratory was established, are incomplete and the data unreliable. Results are therefore given from as far back in each case as they were considered reliable. Data are presented as means ± SD.

**RESULTS**

**Egg Production**

Daily egg production with the genetic sexing strains VIENNA 7/Mix 2000 and VIENNA 7-D53/Mix 2001 from Jan 2001, and with genetic sexing strain VIENNA 8 from Aug 2003 to Sep 2004, is illustrated in Fig. 1a and b. A target of 495 mL of eggs per day was initially set in order to achieve production levels of five million sterile males per week for aerial releases over the Hex River Valley. As illustrated by Fig. 1a between Jan 2001 and Aug 2002, this target was seldom achieved. From Aug 2002 the target was exceeded virtually without exception. After the introduction of VIENNA 8, the daily egg production target was reduced to 360 mL per day due to the better egg to pupa efficiency of this strain. Fig. 1b shows that with VIENNA 8, this target also was exceeded on all but one occasion (Feb to Mar 2004). Relatively wide fluctuations in egg production from Sep to Dec 2003 narrowed noticeably thereafter, probably as a result of adaptation by the new strain to local conditions.

**Egg Hatch**

Egg hatch after 48 h in the male-only stream is illustrated in Fig. 2a (VIENNA 7-D53/Mix 2001) and Fig. 2b (VIENNA 8). Except for a sharp drop in Sep/Oct 2002, egg hatch in the VIENNA 7 strain fluctuated (mean = 55.3 ± 9.65%) for most of the reported period until Mar 2003, when it dropped an average of 5% below the target level. In the case of VIENNA 8, mean egg hatch was somewhat lower at 38.8 ± 11.01%, but very close to the target of 40% for this strain (C. Cáceres, FAO/IAEA Biotechnology Laboratory, Seibersdorf, Austria, personal communication).

**Egg to Pupa Efficiency**

Egg to pupa efficiency in the male-only stream is illustrated in Fig. 3a (VIENNA 7D53/Mix 2001) and Fig. 3b (VIENNA 8). The target egg to pupa efficiency for the VIENNA 7 strain was 12%. Although the FAO/IAEA (2002) target for the VIENNA 8 strain is 20%, the rearing facility set an interim target of 16%. Efficiency in the VIENNA 7 strain fluctuated between 5 and 15%, with a
mean of 11.2 ± 4.60% and was characterized by three periods of substantial decreases in efficiency. There were only 2 periods of relative stability in efficiency, between May and Sep 2002 and Mar and Jul 2003. After the introduction of the VIENNA 8 strain, egg to pupa efficiency immediately improved to a mean of 16.8 ± 4.18%. Two significant decreases in efficiency occurred from Dec 2003 to Jan 2004 and from Mar to May 2004, both as a result of equipment malfunction.

Number of Pupae Irradiated per Week

The number of pupae irradiated per week from Oct 1999 to Sep 2004, as an illustration of production of sterile flies by the facility, is presented in

Fig. 1. Daily egg production (14-day moving average) by two genetic sexing strains of C. capitata; (a) by two strains of VIENNA 7 from Jan 2001 to Jul 2003, and (b) by VIENNA 8 from Aug 2003 to Sep 2004.
Fig. 2. Egg hatch at 48 h (7-day moving average) of two genetic sexing strains of *C. capitata*; (a) VIENNA 7-D53/Mix 2001, from Apr 2002 to Jul 2003, and (b) by VIENNA 8 from Sep 2003 to Sep 2004.

**Vienna 7**
- Target = 55%
- Mean = 55.3%
- SD = 9.65

**Vienna 8**
- Target = 40%
- Mean = 38.8%
- SD = 11.01

Fig. 4. With few exceptions, production by the facility was very poor and variable during the first two and a half years until mid-2002. On only three occasions, Jun and Aug 2001 and Nov/Dec 2002, did production match demand. During this period, sterile *C. capitata* pupae were frequently imported from the El Pino facility in Guatemala to supplement aerial releases. From Aug 2002, production steadily increased to more than 8 million sterilized pupae per week for the next 10 months, albeit with 1 major slump in Jan and Feb 2003. In Jun 2003 production was deliberately decreased to approximately one million sterilized pupae per week when aerial releases were changed to ground releases. Production was then increased to 4 million and later 6 million sterilized pupae per week between Dec 2003 and Aug 2004 to accommodate additional fruit production areas implementing SIT.
Flight Ability of Sterile Males

Flight ability of sterile males is given in Fig. 5a (VIENNA 7-D53/Mix 2001) and Fig. 5b (VIENNA 8). The target flight ability for the VIENNA 7 and VIENNA 8 strains was 75% (C. Cáceres, Seibersdorf, Austria, personal communication). A target of 82% for the VIENNA 8 strain is specified by FAO/IAEA (2002), but this is for the smaller colony reared by the IAEA at Seibersdorf. Flight ability for the VIENNA 7 strain fluctuated between 60 and 85%, with a mean of 73.0 ± 10.92%. Flight ability improved with the VIENNA 8 strain, increasing to a mean of 78.3 ± 7.37%.
Females in Male-Only Stream

The occurrence of females in the male-only stream is summarized in Fig. 6a (VIENNA 7-D53/Mix 2001) and Fig. 6b (VIENNA 8). As sterile stings by females in commercial fruit can lead to infection by pathogens and secondary pests, a maximum level of 2% females in the male-only stream was set. From Dec 2002 to Apr 2003, the occurrence of females from the VIENNA 7 strain varied from about 1 to 3%, exceeding the maximum nearly 30% of the time. From Apr 2003, a steady increase in females of up to 11% was recorded until just before the introduction of the VIENNA 8 strain. The mean was 2.63 ± 2.46%.

Following the introduction of VIENNA 8 in Aug 2004, the occurrence of females in the male-only stream dropped dramatically to a maximum of less than 0.4%, with females being recorded on only three occasions in 13 months. The mean was 0.02 ± 0.09%.

DISCUSSION

During the period 1999 to mid-2002, production of sterile *C. capitata* by the facility was seldom sufficient to supply the requirements for aerial releases in the Hex River Valley, i.e., 5 million per week from Sep to May and 1 million per week from Jun to Aug. From mid-2002, after the positive effect of the implementation of the quality management system took effect, production was generally adequate with only one exception. Production and quality further improved and stabilized after the introduction of the VIENNA 8 strain in Aug 2003. Many factors contributed to the initial sub-standard production and quality parameters, the most important of which were the following:

1. **Lack of adequate funding.** In the absence of sustained government funding for operational expenses, and with an inadequate budget, the mass-rearing facility was constantly under financial duress. This negatively affected the integrity of the entire mass-rearing infrastructure, and consequently production and quality, and affected the overall success of the project.

2. **Short start-up time for rearing facility.** The rearing facility was required to supply 5 million sterile males per week to a fully operational SIT program within 7 months of starting up in a converted facility with new equipment and with new rearing staff with little experience. There was little opportunity for analyzing and solving initial problems common in a new mass-rearing operation. The rearing technicians had to gain most of their experience while the release program was in operation.

3. **Cramped rearing conditions.** Until mid-2001, all rearing took place in a small building that was converted into a rearing facility on a low budget. Inadequate space in both the adult...
and larval rooms led to poor egg production (e.g., Jan and Jun 2001) and poor larval production, which in turn resulted in failure to meet production targets. A new, spacious and well-equipped adult room was commissioned in Jun 2001, with a concomitant improvement in egg production thereafter.

(4) Problems with larval diet. During late 1999, very poor larval production was ascribed to bran contaminated at source with the insecticide chlorpyriphos. This resulted in reduced numbers of pupae being produced between Dec 1999 and Jan 2000. During Nov 2002 to Feb 2003, a build up of rust in the larval diet
mixture resulted in the diet containing toxic levels of rust (C. Cáceres, FAO/IAEA Biotechnology Laboratory, Seibersdorf, Austria, personal communication), that reduced the egg to pupa efficiency and thus the number of pupae irradiated from Dec 2002 to Jan/Feb 2003. On a number of occasions, bran of varying consistency was delivered, being sometimes too fine and sometimes of mixed size grading. This resulted in sub-standard larval diet and egg to pupa efficiency (Mar to May 2002; Apr/May 2004) and in the decrease in the number of pupae irradiated during Mar to May 2002.

Colony replacements. The lack of an effective filter rearing system until mid-2001 contributed to unacceptable levels of genetic recombination of the genetic sexing strain and to consequent poor production and quality. As a result, the rearing colony had to be replaced 4

Fig. 6. Percentage females in the male-only stream for (a) VIENNA 7-D53/Mix 2001 genetic sexing strain from Dec 2002 to Sep 2003, and (b) VIENNA 8 genetic sexing strain from Aug 2003 to Sep 2004.
times during which production was severely affected (May 2000, Nov 2000, Oct/Nov, and July 2003). Production quantity and quality, and strain stability, started improving once a filter rearing system was introduced.

(6) Equipment malfunction. Equipment installed in the new facility in 1999, in particular climate control equipment, broke down repeatedly. This was due mainly to budget restrictions precluding the purchase of high-performance equipment, but also because high ambient temperatures during the hot summer months (Dec to Feb) raised temperatures in the larval rooms and put stress on the climate control equipment. Power failures also occurred. Production was negatively affected on each occasion.

(7) Lack of a quality management system. Production and quality were generally poor in the absence of a quality management system. The benefit of the introduction of the quality management system in Jun 2002 can best be seen in daily egg production and number of pupae irradiated with the VIENNA 7 strains. Egg production increased from an average of 250 mL per day for 17 months pre-quality management system to 690 mL per day for 13 months post-quality management system. The average number of VIENNA 7 pupae irradiated increased from 1.4 million per week for 32 weeks pre-quality management system to 5.8 million per week for 13 months post-quality management system.

Performance of the VIENNA 8 Genetic Sexing Strain

The VIENNA 8 strain has been reported to show an approximate 20% improvement in performance relative to VIENNA 7-D53/Mix 2001 (Cáceres 2002). A comparison of data in Figs. 1a, 1b, 3a, 3b, 5a, 5b, 6a and 6b confirms the superiority of the VIENNA 8 strain. Egg production with VIENNA 8 was easily maintained at a high level and, once the strain had adapted to the new conditions, was relatively stable. Mean egg to pupa efficiency increased by 50% from 11.2% for VIENNA 7-D53/Mix 2001 to 16.8% for VIENNA 8. Mean flight ability increased by 7.3% from 73.0% with VIENNA 7-D53/Mix 2001 to 78.3% with VIENNA 8. The occurrence of females in the male-only stream decreased from a mean of 2.63% with VIENNA 7-D53/Mix 2001 to 0.02% with VIENNA 8, an improvement of 99.2%.

VIENNA 8 proved to be more genetically stable than the VIENNA 7 strains, exhibiting less genetic recombination (occurrence of ‘wrong sex’ pupae) following handling and environmental stress. After the introduction of VIENNA 8, equipment failure occurred on numerous occasions, yet, very low levels of recombination were recorded. Due to the better performance of VIENNA 8 it was possible to reduce the number of adult cages set up for the male-only stream from 12 per week to 8 per week without compromising egg production. This in turn led to an estimated savings in production costs of 30%.

In conclusion, the experiences in South Africa have highlighted the importance for effective fruit fly SIT operations of the following factors: (a) sound rearing infrastructure with high quality equipment; (b) an adequate period of staff training and equipment testing before delivering sterile flies to an operational program; (c) an effective quality management system during the production of sterile insects; (d) a stable and productive genetic sexing strain; and (e) a sound funding base for the mass-rearing facility.

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DEMOGRAPHIC AND QUALITY CONTROL PARAMETERS OF ANASTREPHA FRATERCULUS (DIPTERA: TEPHRITIDAE) MAINTAINED UNDER ARTIFICIAL REARING

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ABSTRACT

The integration of the sterile insect technique (SIT) in the management of the South American fruit fly Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) is a promising alternative to chemically-based control in those areas where it is sympatric with Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) or other tephritid species for which the SIT is being used. Implementation of the SIT requires the development of a cost effective mass-rearing protocol. In this work, we present demographic and quality control parameters for the A. fraterculus strain reared at the Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina. Considering the rearing cage as the reproduction unit, we observed that fecundity is optimal during the first 3 weeks after the onset of oviposition. Fertility was constant during this period. During 2003 and 2004, some improvements were made to the existing rearing protocol, which resulted in increased larval viability, pupal weight, and adult emergence. Current weekly egg production is 1 million per week. These eggs are used to maintain the colony and to assess quality parameters. Finally, research needs leading to improved yields and fly quality are discussed.

Key Words: Anastrepha fraterculus, sterile insect technique, mass-rearing, larval viability, fertility, fecundity

RESUMEN

La integración de la Técnica del Insecto Estéril (TIE) en el combate integrado de la mosca Sudamericana de la fruta, Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae), es una alternativa interesante para reemplazar al control químico en aquellas zonas donde esta especie es simpática con Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) u otros tefrítidos para los que ya se utiliza la TIE. La implementación de la TIE requiere del desarrollo de un protocolo de cría masiva que sea costo-efectivo. En este trabajo presentamos parámetros demográficos y de control de calidad de la cepa criada en la Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina. Considerando a la jaula de cría como unidad reproductiva, se observó que la fecundidad es óptima durante las tres primeras semanas de iniciada la oviposición y que la fertilidad se mantiene constante durante ese periodo. Durante 2003-2004 se implementaron mejoras en el protocolo de cría existente lo que resultó en un incremento de la viabilidad larvaria, del peso de pupas y del porcentaje de emergencia de adultos. La producción actual semanal es de un millón de huevos. Los mismos son utilizados para mantener la colonia y realizar distintos estudios de calidad de esta cepa. Por último, se sugieren necesidades de investigación para alcanzar mejores rendimientos.

Translation provided by the authors.

The South American fruit fly Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) is a serious pest that occurs from the southern United States (Texas) to Argentina (Salles 1995; Steck 1998). It attacks over 80 species of plants, including major fruit crops, and represents a serious threat in some fruit production areas. In addition, its presence results in quarantine restrictions for fresh fruit exports by importing countries (Steck 1998). At present the only control method available is the use of bait sprays. This presents a problem in areas where it coexists with other fruit fly pests against which the sterile insect technique (SIT) is being used. Such is the case for some regions in Argentina, where A. fraterculus is sympatric with the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann) (Diptera: Tephritidae). In such situations, the application of the SIT against A. fraterculus can be considered an attractive alternative (Ortíz 1999).

One paramount prerequisite for SIT implementation is the development of cost-effective
mass-rearing protocols. Large-scale mass rearing has not been achieved for A. fraterculus. Efforts to colonize this species and to develop mass rearing methods have been reported in many countries (Ortiz 1999). Major constraints were related to oviposition and egg fertility. A preliminary mass-rearing strategy was developed by Jaldo et al. (2001). This procedure followed a simple egg collection method with minimal handling and high egg fertility. However, egg to pupae recovery was not optimal and rearing parameters reported were obtained from small-scale rearing in Petri dishes with egg densities lower than those used in the routine maintenance of the colony. Additional demographic parameters were published by Salles (1992) and Jaldo (2001).

Although individual females are responsible for total fecundity and fertility of the colony, under mass rearing conditions, demographic parameters such as egg production are more informative when the rearing cage is considered as the production unit.

In this work, we evaluate the fecundity and fertility of the A. fraterculus colony maintained at the Estación Experimental Agroindustrial Obispo Colombres in Tucumán, Argentina and provide rearing and quality control parameters.

**Materials and Methods**

**Colony Maintenance**

The colony established at the Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina, was derived from infested guava collected in 1997 from the vicinity of Tafi Viejo, Tucumán province (northwestern Argentina). Since then, no wild material has been introduced into the colony. Rearing conditions were those proposed by Jaldo et al. (2001) with minor modifications, mainly related to humidity conditions in the rearing room and egg seeding density.

Adult colony cages were set up with 10,000 pupae and provided with water and adult food, which consisted of a mixture of hydrolyzed yeast, corn protein, and sugar (1:1:4) with a supply of vitamins and amino acids (Jaldo et al. 2001). Cages were held in a rearing room with 25 ± 1°C and a photoperiod of 12:12 (L:D). Humidity control was difficult during 2002-2003, and ranged from 50 to 90%, but normally it was at the lower values. From Jan 2004, with the addition of a new humidifier, the relative humidity in the rearing room was higher and more stable (80 ± 10% R.H.).

Egg collection began 1 week after adult emergence and eggs were collected 3 times per week. Females laid their eggs through one of the panels of the cage that was coated with a thin layer of silicone rubber (hereafter referred to as the oviposition panel). To avoid dehydration, moist foam rubber was applied to the outer side of the oviposition panel and held with clips to a transparent polycarbonate panel which was attached to the cage. After 24 h the foam rubber was removed and eggs were collected from the oviposition panel with the aid of a rubber sponge. The volume of eggs obtained for each cage was measured. Eggs were air bubbled in water within a plastic bottle (v/v ratio of 1:100) for 48 h and later seeded on larval diet (Salles 1992).

Seeding density decreased from 34 eggs/g of diet in 2003, to 22-15 eggs/g of diet during Jan-May 2004, to 11 eggs/g of diet from Jun 2004 onwards. Egg seeding density was reduced due to an increase in egg-pupae recovery and hence a need to avoid larval overcrowding. Approximately at d 7, larvae started leaving the diet and pupated in sand placed in a pan below the trays. Two d after pupation began; sand was sieved to remove pupae. The same pupal collection procedure was repeated twice. Pupae were placed in trays with a thin layer of sand and held 10-12 d for maturation. Two d before emergence, the total pupal production was weighted. Each week 2 batches of 120 g of pupae were prepared to set up 2 new adult holding cages. From Jun 2004, 2 batches of 45,000 eggs each were seeded per week to obtain enough pupae (around 60,000) to maintain the colony; any remaining eggs were discarded.

**Demographic Analysis**

Egg production was estimated by considering the total number of eggs collected during the lifespan of the production cages. Egg collection was carried out for the period of 4 weeks, for a total of 12 collections per cage. For each cage in each collection the volume of eggs collected was measured and the number of eggs obtained in each cage for each collection was determined by multiplying the volume of eggs (in mL) in each collection by 14,900, which was estimated as the number of eggs in 1 mL. In addition, from each cage in each egg collection, 3 samples of 100 eggs were placed in a Petri dish with moistened sponge to assess egg hatch over the oviposition period. This procedure was repeated in 7 cages from Oct to Nov 2003. Differences in mean values of egg hatch along the collections dates were tested by means of ANOVA with InfoStat (2004).

**Quality Control Parameters**

Several parameters related to the process of rearing and the quality of the pupae produced were estimated, including egg hatch, egg to pupae recovery, larval viability, number of eggs obtained per cage in each collection, number of eggs obtained per female in each collection, weekly egg and pupal production, pupal weight, adult emergence, and sex ratio. Egg hatch was determined 3 d after eggs were placed in Petri dishes containing a moistened cotton sponge. The number of unhatched eggs was counted.
and the percentage of egg hatch was estimated. Egg to pupae recovery was estimated as the number of pupae recovered in each batch/eggs seeded \times 100. For larval viability, the formula considered the number of viable eggs (obtained from the percentage of egg hatch). The number of eggs per female in each egg collection was determined by dividing the number of eggs obtained in each cage by the number of estimated females per cage. The number of females per cage was estimated from the amount of pupae used to set up the cage, the percentage of adult emergence, and the sex ratio. No mortality was assumed during this collection period.

Weekly egg production was determined by adding the number of eggs obtained for each cage in each of the 3 collections performed each week. Pupal weekly production was determined considering all the pupae obtained each week from the different batches. Pupal weight was assessed from 3 samples of 100 pupae weighed 2 d before adult emergence. These pupae were kept without food and water until all flies emerged and died. The number of emerged flies of each sex was recorded taking into account whether they were non-deformed, deformed, or partially emerged. Adult emergence and sex ratio were estimated from these figures as explained in the international fruit fly quality control manual (FAO/IAEA/USDA 2003). According to the changes in seeding density implemented from 2002 to the present, rearing and quality control parameters were estimated for 4 periods: 2002, 2003, Jan-May 2004 and Jun-Aug 2004. Egg hatch, egg to pupae recovery, and larval survival were determined for each pupal batch.

**RESULTS AND DISCUSSION**

Fecundity and fertility for each egg collection during the period Oct-Nov 2003 is presented in Table 1. Fecundity over the 4-week period totaled 413,179 ± 53,026 eggs per cage. Considering the period in which this value was obtained, as well as the percentage of emergence, pupal weight, and sex ratio, the number of eggs per female along the complete oviposition period (i.e., until the cage was discarded) was 102 eggs. In other studies values from 394 eggs per female (Salles 1992) to 625 eggs per female (Jaldo et al. 2001) have been recorded. Our value is underestimated since it assumes no mortality during the collection period; and as mentioned before, values reported previously come from studies in which eggs were collected during the complete reproductive period of females confined in small cages and under relaxed, non-crowded, conditions. As such, they neither provide an estimate of the number of eggs to be collected per female or cage, nor an informative figure for the rearing facilities regarding the optimal time to discard the production cage. The eggs from the first 3 weeks of collection represented approximately 90% of the total collected (Table 1). During the fourth week the production dropped and it is expected that collecting for longer periods, where flies are more than 35 d old, would not increase overall egg production. This, and the fact that old cages are sources of fungal and mite infections in the rearing rooms, prompts us to suggest that 3 weeks of egg collection is optimal for a rearing facility, hence production cages should be discarded on d 28.

For *Anastrepha obliqua* (Macquart), *Anastrepha ludens* (Loew), and *Anastrepha serpentina* (Wiedemann), 41, 64, and 36 d, respectively, have been proposed as the life of the production cage (Liedo & Carey 1994). These periods take into account the amount of pupae that will be harvested in the facility to be released in the field and have been estimated from wild flies, which probably explains the higher values obtained (Liedo & Carey 1994). Our suggestion is more in agreement with

<table>
<thead>
<tr>
<th>Week</th>
<th>Collection</th>
<th>Number of eggs/cage</th>
<th>Cumulative percentage</th>
<th>Egg hatch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>1</td>
<td>40,443 ± 9,437</td>
<td>9.8</td>
<td>76.0 ± 5.6</td>
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<tr>
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<td>2</td>
<td>46,214 ± 8,076</td>
<td>21.0</td>
<td>76.8 ± 6.7</td>
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<tr>
<td></td>
<td>3</td>
<td>45,551 ± 8,321</td>
<td>32.0</td>
<td>85.8 ± 3.6</td>
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<tr>
<td>Second</td>
<td>1</td>
<td>46,829 ± 8,003</td>
<td>43.3</td>
<td>86.3 ± 1.8</td>
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<tr>
<td></td>
<td>2</td>
<td>43,849 ± 5,067</td>
<td>53.9</td>
<td>87.9 ± 1.3</td>
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<tr>
<td></td>
<td>3</td>
<td>43,423 ± 7,786</td>
<td>64.5</td>
<td>85.2 ± 3.1</td>
</tr>
<tr>
<td>Third</td>
<td>1</td>
<td>40,656 ± 11,025</td>
<td>74.3</td>
<td>87.2 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>38,101 ± 8,963</td>
<td>83.5</td>
<td>87.5 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25,543 ± 5,775</td>
<td>89.7</td>
<td>88.4 ± 1.1</td>
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<tr>
<td>Fourth</td>
<td>1</td>
<td>17,241 ± 4,940</td>
<td>93.9</td>
<td>85.6 ± 3.2</td>
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<tr>
<td></td>
<td>2</td>
<td>10,856 ± 2,383</td>
<td>96.5</td>
<td>82.2 ± 2.8</td>
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<tr>
<td></td>
<td>3</td>
<td>14,474 ± 3,230</td>
<td>100.0</td>
<td>80.0 ± 4.3</td>
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<tr>
<td>Mean</td>
<td></td>
<td>34,432 ± 3,895</td>
<td></td>
<td>84.1 ± 1.2</td>
</tr>
</tbody>
</table>

1Adult colony cages were set up with approximately 10,000 pupae which produced approximately 4,060 females.
values proposed by Carey & Vargas (1985), obtained from other tephritids, which were already adapted to mass rearing conditions, as well as the 21 and 17 d used in the Moscafroot Metapa facility in Mexico to mass rear A. ludens and A. obliqua, respectively, (Artiaga-López & Hernández, pers. comm.). More recent values obtained from Jun-Aug 2004 revealed that females laid approximately 135 eggs during the first 3 weeks of oviposition before the cage was discarded (Table 2). This value increased, compared to the one obtained during the trial in 2003, as a result of an improvement of some rearing parameters such as pupal weight, which resulted in larger and probably more fecund females (see below).

During the 4 weeks of egg collection from Oct to Nov 2003, egg hatch averaged 84.1 ± 1.2% (Table 1). Although for the first collections mean values were lower, probably due to oviposition of virgins, there were no statistical differences (F = 1.39; df = 11.56; P > 0.05). Because the differences are not significant, and the production of eggs during the first week is important, it is not recommended to discard these eggs nor to start egg collection later.

Rearing and quality control data are presented in Table 2. The higher humidity in the rearing room from Jan 2004 may have been responsible for the higher egg to pupae recovery, because the larval diet did not dry out during the first days in which first instars require high humidity. This led to the need to reduce the seeding density from 34 eggs/g of diet to 22-15 eggs/g of diet. By Jun 2004, it was decided to seed eggs at an even lower density (11 eggs/g of diet). To prevent water loss from the diet, trays were covered with a polystyrene tray for the first 5 d of larval development. Covering rearing trays to prevent dehydration is a common practice in other insectaries and for other Anastrepha species (Pinsón et al. 1993). Subsequently, trays were uncovered and sugarcane bagasse was added to the diet to allow larvae to crawl outside the diet. Because the diet uses agar as a gelling agent (Salles 1992), we found that some larvae become stuck in the diet when trying to leave to pupate. The addition of sugarcane bagasse, which is produced locally as a by-product of the sugar industry, provides an adequate substrate for the larvae to crawl out of the diet and enhances larval recovery. In all, changes applied to the rearing conditions (higher and more stable relative humidity in the rearing room, lower seeding density and handling during the larval development), resulted in the increase of the viability, recovery, and quality control parameters (Table 2).

Egg to pupae recovery was higher than the 44% reported by Jaldo et al. (2001), even when those values were obtained from batches of 100 eggs seeded on Petri dishes under very relaxed conditions. Our values under such favorable conditions are approximately 83% recovery. Besides the increase in larval viability, the reduction in egg seeding density resulted in an increase in pupal weight and adult emergence. A gain in pupal weight may have resulted in larger and probably more fecund females. This may also explain the increase in the number of eggs collected per female as shown for other tephritids (Krainacker et al. 1989; Liedo et al. 1992).

Although larval viability improved, more improvement is still needed. The results obtained in the small-scale trials suggest that nutrients are not affecting the viability, but probably the structure of the diet. Finding the optimal moisture level and a good bulking agent to maximize intake of nutrients from the diet is an important goal to achieve. The present diet, which uses agar as a gelling agent, is too expensive for mass-rearing, and presents the added drawback of being difficult for larvae to leave and pupate. Sugarcane bagasse can be used to help the larvae leave the diet, it is locally available from the sugarcane ind-

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg hatch (%)</td>
<td>74.1 ± 4.2</td>
<td>84.9 ± 1.3</td>
<td>83.1 ± 1.1</td>
<td>84.4 ± 0.9</td>
</tr>
<tr>
<td>Egg-pupae recovery (%)</td>
<td>—</td>
<td>29.3 ± 1.7</td>
<td>37.9 ± 2.1</td>
<td>56.3 ± 2.1</td>
</tr>
<tr>
<td>Larval viability (%)</td>
<td>—</td>
<td>33.8 ± 1.2</td>
<td>49.3 ± 3.8</td>
<td>66.6 ± 2.3</td>
</tr>
<tr>
<td>Eggs/cage/collection</td>
<td>—</td>
<td>28,221 ± 3,181</td>
<td>43,822 ± 3,099</td>
<td>63,323 ± 3,183</td>
</tr>
<tr>
<td>Eggs/female/collection</td>
<td>—</td>
<td>6 ± 1</td>
<td>11 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Weekly egg production</td>
<td>—</td>
<td>83,531 ± 11,077</td>
<td>691,740 ± 41,580</td>
<td>1,074,425 ± 43,733</td>
</tr>
<tr>
<td>Weekly pupae production¹</td>
<td>—</td>
<td>—</td>
<td>124,963 ± 17,754</td>
<td>55,894 ± 4,559</td>
</tr>
<tr>
<td>Pupal weight (mg)</td>
<td>10.9 ± 0.4</td>
<td>11.3 ± 0.4</td>
<td>12.0 ± 0.3</td>
<td>13.1 ± 0.2</td>
</tr>
<tr>
<td>Non-deformed adult emergence (%)</td>
<td>73.0 ± 2.5</td>
<td>77.3 ± 1.8</td>
<td>75.8 ± 3.2</td>
<td>85.0 ± 2.5</td>
</tr>
<tr>
<td>Adult emergence (%)</td>
<td>77.1 ± 2.3</td>
<td>81.6 ± 1.7</td>
<td>80.0 ± 3.1</td>
<td>88.6 ± 2.6</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>0.85 ± 0.03</td>
<td>0.95 ± 0.03</td>
<td>0.97 ± 0.04</td>
<td>0.93 ± 0.05</td>
</tr>
</tbody>
</table>

¹From Jun 2004 onwards only 90,000 eggs per week were seeded; this explains the drop in the production of pupae.
dustry in the region, and it is free from insecticides. However, preliminary studies (unpublished data) have shown that yields with bagasse are not as good as those obtained with agar, indicating more improvement is still possible.

ACKNOWLEDGMENTS

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REFERENCES CITED


DEVELOPMENT OF QUALITY CONTROL PROCEDURES FOR
MASS PRODUCED AND RELEASED BACTROCERA PHILIPPINENSIS
(DIPTERA: TEPHRITIDAE) FOR STERILE INSECT TECHNIQUE PROGRAMS

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ABSTRACT

Quality control procedures for Bactrocera philippinensis Drew & Hancock 1994 (Diptera: Tephritidae) used in sterile insect technique (SIT) programs were established in the mass rearing facility at the Philippine Nuclear Research Institute. Basic studies on pupal irradiation, holding/packaging systems, shipping procedures, longevity, sterility studies, and pupal eye color determination in relation to physiological development at different temperature regimes were investigated. These studies will provide baseline data for the development of quality control protocols for an expansion of B. philippinensis field programs with an SIT component in the future.

Key Words: Bactrocera philippinensis, quality control, Oriental fruit flies, pupal eye color, longevity, sterility

RESUMEN

Los procedimientos de control de calidad para Bactrocera philippinensis Drew & Hancock 1994 (Diptera: Tephritidae) usados en programas de la técnica de insecto estéril (TIE) fueron establecidos en la facilidad de cria en masa del Instituto Filippo de Investigación Nuclear. Estudios básicos sobre la irradiación de las pupas, sistemas de almacenaje/empaque, procedimientos del envío, longevidad, estudios de esterilidad y la determinación del color de ojo de la pupa en relación con el desarrollo fisiológico en regímenes diferentes de temperatura fueron investigados. Estos estudios proveerán una línea de información básica para el desarrollo de protocolos de control de calidad para una expansión de los programas de campo para B. philippinensis con un componente de TIS en el futuro.

Quality control is important for monitoring the performance of mass reared insects for use in the sterile insect technique (SIT) (Boller et al. 1981). To meet this requirement, routine quality control tests on egg hatchability, pupal weight and size, percent adult emergence, longevity, flight dispersal, and mating ability are used. The effect of pupal holding conditions, irradiation, and packaging procedures must also be assessed and threshold values for each quality control parameter need to be established.

After eradication of Bactrocera philippinensis Drew & Hancock 1994 (Diptera: Tephritidae) with the SIT in Naoway Islet, Philippines (Manoto et al. 1996), a feasibility study based on an integrated control program was initiated in Guimaras Island (Covacha et al. 2000). The Philippine Nuclear Research Institute upgraded the fruit fly mass rearing facility in order to produce 25 million pupae per week. The B. philippinensis colony has been mass reared in the laboratory for more than 100 generations (Rejesus et al. 1975). The quality control procedures being developed for this species were based on those developed for the Mediterranean fruit fly Ceratitis capitata (Wiedemann).

MATERIALS AND METHODS

Standard Specifications

Routine quality control includes measurements of pupal size, percent adult emergence, flight ability, sex ratio, response to stress, and mating propensity. Preparation of samples, observation, and gathering of data were done by following or modifying the procedures in the manual for “Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies” (FAO/IAEA/USDA 2003). Minimum specifications required for weekly and monthly routine quality control tests were established on pre-irradiation, post-irradiation, and post shipment pupae to serve as guides in monitoring fly quality and for inclusion into the manual.
Monitoring Fruit Fly Quality

Release of sterile flies in Guimaras Island by ground commenced in Apr 2001 and for every batch of released sterile flies routine quality control checks in the pre-irradiation, post-irradiation, and post-shipment were carried out. Data from quality control tests were tabulated and analyzed to assess variation in the quality specifications. A database of results for routine quality control tests was constructed for reference purposes.

Pupal Irradiation

Samples of *B. philippinensis* pupae obtained from the stock colony were marked with 1.5 g fluorescent dye (Dayglo®) and held in glass vials 2 d before emergence. The glass vials containing 25 mL of pupae were irradiated with $^{60}$Co Gamma Cell 220 Irradiator facility with doses of 0, 25, 40, 50, 75, 100, 150, and 200 Gy. After irradiation, samples of pupae were prepared for the following tests.

Emergence and Flight Ability Tests

One hundred pupae, counted into a Petri dish, were placed at the base of a 10-cm black PVC pipe coated with talcum powder, inside a large cage. Percent adult emergence was based on the number of adults emerging from the pupal samples. Non-flying, fully emerged, partially-emerged, and deformed flies were counted and recorded. Flight ability (flies escaping from the black PVC pipe) was determined based on the number of unemerged pupae and residual flies remaining in the Petri dish.

Fecundity and Sterility Tests

Five replicates of 100 pupae of each dose were counted and placed for emergence in screened cages (30 × 30 × 40 cm) and provided with food and water. The flies were allowed to lay eggs for 10 d after emergence in a small egging device containing a wet sponge. Samples of eggs were counted, held in Petri dishes on damp cloth, and observed for egg hatch 3 d later.

Holding/Packaging of Pupae for Irradiation and Shipment to Guimaras Island

Trials on horizontal and vertical holding/packaging of pupae for irradiation and shipment were conducted and compared with the standard packaging system currently used. Each holding/packaging method was evaluated to determine the effect of length, size, and position/arrangement of polyethylene plastic bags. Three soft ice packs measuring 11 × 7.5 inches were placed inside the cardboard box as coolant. A laboratory thermometer was inserted inside the box to check the temperature at 4 h intervals for 48 h. At the same time, samples of pupae were taken at random from different sausage bags to determine the effect of each treatment on adult emergence and flight ability. The packages used with their corresponding specifications are shown in Fig. 1. Percent adult emergence and fliers were tabulated and checked to see if they passed the minimum specifications (Obra & Resilva 2003).

Fig. 1. Specifications and arrangement of 3 different packaging systems evaluated for irradiation and pupal shipment. a. Standard arrangement size of bags (cm) = 10.16 × 28, weight of pupae/sausage = 460 g, No. of sausage/box = 52, No. pupae/box = 1.9 million. b. Vertical arrangement size of bags (cm) = 10 × 40, weight. of pupae/sausage = 600 g, No. of sausage/box = 36, No. pupae/box = 1.7 million. c. Horizontal arrangement size of bags (cm) = 15 × 51, weight of pupae/sausage = 2000 g, No. of sausage/box = 12, No. pupae/box = 1.9 million.
Determination of Pupal Eye Color in Relation to Physiological Development

Development of pupae based on daily eye color changes (Ruhm & Calkins 1981) at different temperatures of 22-32°C (room temperature), 15, 19, and 28°C were determined. Pupal samples at 15, 19, and 28°C were placed in a controlled environment with an Echo Therm Chilling Incubator. Daily changes of eye color at each temperature were monitored by taking close up photographs with an Intel QX3 Computer Microscope at 60× magnification. Duration of pupal development and eye color changes of each pupal group were noted and matched with the color scale of the Soil Munsel Color Charts (Anonymous 2000).

Evaluation of Different Shipping Coolants for Sterile Pupae

Different shipping coolants such as soft ice, ice packs, and plastic ice trays were evaluated to determine suitable cooling materials for pupal shipment. Three boxes of pupal samples, arranged in the standard packaging system, were prepared and irradiated with the multi-purpose gamma irradiation facility. After irradiation, three sets of each coolant wrapped in newspaper were placed on the top of each cardboard box. The lids of the boxes were closed and secured with packaging tape. Data collection procedures were similar to those used for packing/holding.

Determination of Optimal Irradiation Doses Range

Samples of pupae were prepared and irradiated with doses inside of the irradiation chamber ranging from 52-56 Gy, 63-67 Gy, and 67-74 Gy. Sterility was checked by mating 50 irradiated males or females with 50 non-irradiated males or females in cages 30 × 30 × 40 cm. Samples of eggs were collected weekly in moistened plastic vials over a 3-week period to determine egg hatch for each treatment. As many as 500 eggs were collected and counted during each egging.

Statistical Analysis

All data obtained in all experiments were tested in a randomized complete block design with 5 replications, evaluated, and subjected to an analysis of variance (ANOVA), with the honestly significant difference value calculated as Tukey’s statistic at α = 0.05 (SAS Institute 1990).

RESULTS AND DISCUSSION

Standard Specifications for B. philippinensis

Table 1 shows the standard specifications for the essential weekly and monthly quality control tests of mass-reared B. philippinensis. These values were based on the minimum mean data obtained in a year’s production in the rearing facility. The minimum weight set for pupae was 11.13 mg with a diameter of 1.75 mm. Minimum emergence rate and fliers with a 10-cm flight tube in pre-, post-irradiation, and post-shipment were 90.3, 85.2, and 80.4% for emergence, respectively, and 77.3, 73.2, and 70.1% for fliers, respectively. Minimum values of 50.2, 45.3, and 40.3% survival after 28-32 h was acceptable when newly emerged flies were subjected to stress tests in pre- and post-irradiation and post shipment, respectively. Mating propensity indicates an acceptable mean mating index of 50.2% (pre-irradiation), 45.1% (post-irradiation), and 40.3% (post-shipment) for 10 day-old flies.

Routine quality control checks were done on sterile flies sent to Guimaras for release. Released sterile flies passed the minimum specifications set for pupal size, adult emergence, adult fliers, and other quality control parameters tested.

Irradiation Studies

Table 2 shows data on the effects of different doses of gamma radiation on adult emergence, flight ability, fertility, and longevity. Statistical analysis of the adult emergence data showed no significant difference for all doses tested, compared to the control group. Flight ability data indicate that a high proportion of flies, 93.3-97.7%, escaped from the flight tube after doses of 25-100 Gy. At higher doses, the number of fliers progressively decreased from 73.9 to 69.0%. Females irradiated as pupae with 25-40 Gy were sterile with egg hatch of 25.0 and 3.2%, respectively. When pupae were irradiated at 50 Gy and above, 100% sterility was achieved in all adult females. With regard to longevity tests, no significant difference was observed following irradiation with 25-75 Gy. However, increasing the dose beyond 100 Gy progressively affected the survival, resulting in a decrease in adult longevity after 5 weeks.

Holding/Packaging of Pupae for Irradiation and Shipment to Guimaras

Mean percent emergence and adult fliers in all packaging arrangements showed satisfactory results on pupae randomly-sampled every 4 h from 0-48 h. Similar results were observed in flight ability tests in which a high proportion of adults capable of flight (75-99%) escaped from a 10-cm flight tube. These findings indicate that horizontal and vertical arrangement of pupae were acceptable holding/packaging methods comparable to the standard packing system currently used for sterile fly shipment in Guimaras. Packing of pupae in cardboard boxes with 3 ice packs eliminates overheating of the pupae inside the box.
Temperature was maintained between 15 to 28°C for 48 h. In addition, lining with plastic bubble wrap between the layers of “sausage bags” protects the pupae from mechanical injury by serving as a cushion while in transit.

Determination of Pupal Eye Color in Relation to Physiological Development

Daily changes in eye color during pupal development at 22-32°C (room temperature), 15, 19, and 28°C are shown in Table 3. The method for estimation of the pupal age is based on color changes compared with the color scale in the Munsel Soil Color Charts (Anonymous 2000).

At 22-32°C (room temperature), pupal development is approximately 9 d. Dissection of the anterior part of the puparium is possible on the second day. On d 7 when pupae are irradiated, eye color is dark yellowish brown (HUE 10 YR 3/6). Adult flies start to emerge at 9 d and emergence is complete at 10 d.

### Table 1. Specifications for Required Weekly or Monthly Quality Control Parameters of Mass-Reared B. philippinensis for Use in SIT Programs in the Philippines.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Frequency</th>
<th>Pre-irradiation</th>
<th>Post-irradiation</th>
<th>Post-Shipment</th>
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<tr>
<td>A. Established minimum specifications¹</td>
<td>weekly</td>
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<td>nr</td>
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<tr>
<td>Pupal size</td>
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<tr>
<td>min. pupal weight (mg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>min. pupal diameter (mm)</td>
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<td></td>
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<tr>
<td>Sex ratio: min. % males</td>
<td>weekly</td>
<td>50.00</td>
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<tr>
<td>Emergence &amp; flight ability</td>
<td>weekly</td>
<td>90.00</td>
<td>85.0</td>
<td>80.0</td>
</tr>
<tr>
<td>min. % emergence</td>
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</tr>
<tr>
<td>min. % fliers (10-cm tube)</td>
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<td>73.0</td>
<td>70.0</td>
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<td>50.00</td>
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</tr>
<tr>
<td>Mating propensity</td>
<td>monthly</td>
<td>50.00</td>
<td>45.0</td>
<td>40.0</td>
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<td>Boller’s index (min), 10-d-old flies</td>
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<tr>
<td>B. Mean QC test results²</td>
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<td>Pupal size</td>
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<tr>
<td>min. pupal weight (mg)</td>
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<td>min. pupal diameter (mm)</td>
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<td>50.30</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>Emergence &amp; flight ability</td>
<td>weekly</td>
<td>94.50</td>
<td>92.7</td>
<td>91.6</td>
</tr>
<tr>
<td>min. % emergence</td>
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<tr>
<td>min. % fliers (10-cm tube)</td>
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<td>86.10</td>
<td>81.5</td>
<td>71.7</td>
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<td>56.3</td>
<td>53.4</td>
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<tr>
<td>Mating propensity</td>
<td>monthly</td>
<td>46.00</td>
<td>39.0</td>
<td>48.0</td>
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<tr>
<td>Boller’s index (min), 10-d-old flies</td>
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</table>

¹Minimum standard specification established for one year period.
²Mean quality control test results of flies used in SIT release program. All data were collected in replicates. nr = not required.

### Table 2. Effects of Different Doses of Gamma Radiation (X ± SEM) on the Oriental Fruit Fly Bactrocera philippinensis Irradiated 2 Days Before Emergence.* Within a row, means followed by the same letter are not significantly different (α = 0.05; ANOVA test).

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Emergence¹ (%)</th>
<th>Fliers¹ (%)</th>
<th>Mortality¹ (%)</th>
<th>Egg hatch¹ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98.7 ± 0.4 ab</td>
<td>97.9 ± 0.6 a</td>
<td>14.8 ± 3.8 a</td>
<td>83.8 ± 4.6 a</td>
</tr>
<tr>
<td>25</td>
<td>98.4 ± 1.0 ab</td>
<td>94.4 ± 1.7 ab</td>
<td>15.9 ± 2.9 ab</td>
<td>25.1 ± 5.2 b</td>
</tr>
<tr>
<td>40</td>
<td>97.4 ± 1.9 ab</td>
<td>94.9 ± 2.1 b</td>
<td>14.9 ± 3.8 a</td>
<td>3.2 ± 2.8 c</td>
</tr>
<tr>
<td>50</td>
<td>98.8 ± 0.8 a</td>
<td>94.2 ± 0.9 b</td>
<td>12.4 ± 2.4 a</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>98.1 ± 0.6 ab</td>
<td>93.2 ± 0.8 b</td>
<td>13.5 ± 3.3 a</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>97.9 ± 0.6 ab</td>
<td>93.3 ± 0.9 b</td>
<td>26.3 ± 5.45 c</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>97.5 ± 0.8 b</td>
<td>73.9 ± 4.6 c</td>
<td>19.4 ± 3.8 b</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>95.3 ± 2.7 c</td>
<td>69.0 ± 5.0 d</td>
<td>30.7 ± 5.2 d</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data are means of 5 replicates. Analysis of variance results for % emergence were \( F = 2.06; df = 7,16; P > 0.11030 \), % fliers \( F = 49.99; df = 7,16; P < 0.0001 \), % mortality \( F = 8.43; df = 7,16; P < 0.00002 \), % egg hatch \( F = 371.31; df = 7,16; P < 0.0001 \).
At 15°C, pupal development takes about 29 d. Dissection of the puparium is possible 4 d after pupation, and at d 22-23 the eyes are dark yellowish brown (HUE 10 YR 3/6 and 3/4, respectively) and the pupae can be irradiated. Adult flies begin emerging on d 30.

Pupal development is approximately 15 d at 19°C. Radiation can be applied at d 12 with eye color of dark yellowish brown (HUE 10 YR 3/8). Adult flies start to emerge after 16 d.

At 28°C, pupal development requires 9 d. Eye color is dark yellowish brown (HUE 10 YR 3/6). Adult flies start to emerge after 16 d.

---

**TABLE 3. CHANGES IN EYE-COLOR OF THE ORIENTAL FRUIT FLY, B. PHILIPPINENSIS AT DIFFERENT TEMPERATURE OF PUPAL DEVELOPMENT.**

| Colour identification | Temperature | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| Pale yellow           | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HUE 2.5 Y 8/2*        | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Yellow                | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HUE 2.5 Y 7/8*        | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Brownish yellow       | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HUE 10 YR 6/8*        | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Dark yellowish        | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HUE 10 YR 5/6*        | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Very dark grayish     | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HUE 10 YR 3/2*       | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Dark bluish gray      | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| GLEY 2 3/1*          | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Dark grayish green    | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| GLEY 1 2.5/2*        | 15          |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

*Color codes were compared to the Munsell® Soil Color Charts (Year 2000 Revised Washable Edition). E = Adult emergence

---

**TABLE 4. STERILITY OF ORIENTAL FRUIT FLY, B. PHILIPPINENSIS IRRADIATED WITH DIFFERENT RANGE DOSES OF GAMMA IRRADIATION.**

<table>
<thead>
<tr>
<th>Dose range (Gy)</th>
<th>Crosses1 (50 Males × 50 Females)</th>
<th>Number of eggs sampled</th>
<th>% Egg hatched</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 52-56 Gy</td>
<td>U × U</td>
<td>8,000</td>
<td>86.76</td>
</tr>
<tr>
<td></td>
<td>U × IR</td>
<td>1,055</td>
<td>8.93</td>
</tr>
<tr>
<td></td>
<td>IR × U</td>
<td>8,000</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>IR × IR</td>
<td>360</td>
<td>3.10</td>
</tr>
<tr>
<td>B. 63-67 Gy</td>
<td>U × U</td>
<td>8,000</td>
<td>90.40</td>
</tr>
<tr>
<td></td>
<td>U × IR</td>
<td>687</td>
<td>7.13</td>
</tr>
<tr>
<td></td>
<td>IR × U</td>
<td>7,900</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>IR × IR</td>
<td>176</td>
<td>2.50</td>
</tr>
<tr>
<td>C. 67-74 Gy</td>
<td>U × U</td>
<td>8,000</td>
<td>87.71</td>
</tr>
<tr>
<td></td>
<td>U × IR</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>IR × U</td>
<td>7,956</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>IR × IR</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1U = non-irradiated flies; IR = irradiated flies. Eggs were collected starting 10 d after emergence for 3 consecutive weeks.
and is noticeable on d 7 when the pupae can be irradiated. Adult flies started emerging after 9 d and emergence was complete in 10 d.

Evaluation of Different Shipping Coolants

Mean adult emergence and flight ability data were obtained from pupae packed in boxes with soft ice, ice packs, and plastic ice trays in a standard arrangement/packaging system. The use of ice packs and soft ice were equivalent and met the minimum specifications set for emergence and adult fliers. Similar results were also observed for plastic ice trays; however, a decrease in adult fliers was noted when pupae were stored more than 44 h. A possible explanation for low fliers in plastic ice trays appeared to be due to an increase in temperature up to 32°C that begins after 44 h.

Determination of Optimal Irradiation Doses Range

Table 4 shows the effects of irradiation on the sterility of irradiated with 4 different dose ranges. Pupal irradiation with doses lower than 67 Gy did not prevent egg hatch. When pupae were irradiated with doses ranging from 63-67 Gy, egg hatch was between 0.3-0.7% when irradiated males were paired with non-irradiated females, or from 7.1-8.9% when non-irradiated males were paired with irradiated females. However, when the dose range was increased to 67-74 Gy, egg hatch was completely suppressed. These results suggested that the best irradiation range to achieve complete sterility with a Gamma-cell 220 should be between 67 and 74 Gy.

ACKNOWLEDGMENTS

The authors thank the International Atomic Energy Agency (IAEA) for partial funding of this project under Research Contract 10841, and for procurement of the chilling incubator under the Human Resource Development Program No. PHI/0012. The support of Dr. Alumanda M. de la Rosa, PNRI Director is gratefully acknowledged. We also give our thanks and appreciation for technical assistance of Restituto B. Ilagan, Dolores Lazo, Ricky Garcia, and Flora Isip.

REFERENCES CITED


QUALITY CONTROL METHOD TO MEASURE PREDATOR EVASION IN WILD AND MASS-REAURED MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Sterile male insects, mass-reared and released as part of sterile insect technique (SIT) programs, must survive long enough in the field to mature sexually and compete effectively with wild males for wild females. An often reported problem in Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann) SIT programs is that numbers of released sterile males decrease rapidly in the field for various reasons, including losses to different types of predators. This is a serious issue in view that most operational programs release sterile flies at an age when they are still immature. Previous field and field-cage tests have confirmed that flies of laboratory strains are less able to evade predators than wild flies. Such tests involve, however, considerable manipulation and observation of predators and are therefore not suitable for routine measurements of predator evasion. Here we describe a simple quality control method with aspirators to measure agility in medflies and show that this parameter is related to the capacity of flies to evade predators. Although further standardization of the test is necessary to allow more accurate inter-strain comparisons, results confirm the relevance of measuring predator evasion in mass-reared medfly strains. Besides being a measure of this sterile male quality parameter, the described method could be used for the systematic selection of strains with a higher capacity for predator evasion.

Key Words: predator evasion, predation, survival, sterile males, Tephritidae, Ceratitis capitata

RESUMEN

Insectos machos estériles criados en forma masiva para ser liberados en programas que utilizan la técnica del insecto estéril (TIE), tienen que tener la capacidad de sobrevivir en el campo el tiempo necesario para poder madurar sexualmente y competir efectivamente con los machos silvestres por hembras silvestres. Un problema frecuentemente reportado por diversos programas de la mosca del Mediterráneo, Ceratitis capitata (Wiedemann), es que el número de machos estériles de laboratorio liberados en el campo, decrecen rápidamente por varias razones, incluyendo pérdidas debidas a diferentes tipos de depredadores. Estudios anteriores conducidos en el campo, y en jaulas de campo, han confirmado que las cepas de machos de laboratorio tienen menos capacidad de evadir depredadores que los machos silvestres. Estos estudios involucran, sin embargo, una considerable cantidad de manipulación y observación de depredadores, por lo que no son adecuados para ser usados como medidas rutinarias en los programas de cría masiva. Aquí describimos un método sencillo de control de calidad usando aspiradores para medir agilidad en la mosca del Mediterráneo y mostramos que este parámetro está relacionado a la capacidad de la mosca a evadir a depredadores. Aunque aún es necesario refinar la estandarización de éste método para permitir la comparación entre cepas, los resultados confirman la importancia de tener un método rutinario para medir la capacidad de evasión de depredadores en cepas de cría de laboratorio de la mosca del Mediterráneo. Además de medir este parámetro de control de calidad de los machos estériles, el método descrito podría también ser usado para la selección sistemática de cepas con una mayor capacidad de evasión de depredadores.

Translation provided by the authors.
An often-reported problem in Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann), area-wide control programs integrating the SIT, is the short period after sterile male release during which the males can be recaptured. To date, a number of very thorough field assessments of the dispersal and survival of sterile medflies have been carried out (Wong et al. 1986; Baker & Chan 1991; Plant & Cunningham 1991; Baker & van der Valk 1992; Bloem et al. 1994). Most of these studies report rapid declines in numbers of released sterile flies. On average, the last recaptures of released medflies in these studies have been on d 8. This rapid decrease of flies occurred even where the recapture covered a 1 km² area, and fly emigration was shown to be insignificant as evidenced by the few flies that reached the outer perimeter of traps (Plant & Cunningham 1991). As a result, medfly SIT programs often have to release twice a week to ensure a sufficient presence of sterile males in a given area (Bloem et al. 1994; Dowell et al. 2000).

The rapid decrease of released sterile flies is probably the result of many abiotic and biotic factors, including predation. Various studies under natural conditions confirm the importance of vertebrate and arthropod predators in medfly biology (Hendrichs & Hendrichs 1990; Hendrichs et al. 1991; Baker & van der Valk 1992). During field-testing of dispersal and survival of a temperature sensitive lethal (tsl) genetic sexing strain, VIENNA 42 (Franz et al. 1994) in a citrus orchard, the rate of decrease was again high, and recaptured males appeared to be locating and feeding at the same natural food sites as wild males (Hendrichs et al. 1993). At the same time, there was a ten-fold difference in survival in favor of VIENNA 42 males held in control field cages in the orchard compared with those released into the same orchard. However, during the same study it was shown that in control cages with orange trees, in which the entrance was left slightly open, sterile males suffered heavy losses due to predation by a yellowjacket wasp Vespula germanica L. On each of the 4 occasions that the study was replicated, wasps entered the open field cage in large numbers and within 5-7 h captured all of the flies that had been released onto the field-caged host tree (Hendrichs et al. 1993). In another related field study, it could be demonstrated that foraging V. germanica wasps followed the odor plume to pheromone-calling medfly males aggregated in leks within dense host foliage (Hendrichs et al. 1994). Other field and field-cage studies have confirmed the sexually-biased predation suffered by mature medflies in nature (Hendrichs & Hendrichs 1998).

In view of the above findings, the objective of the present study was to develop a simple quality control test to measure predator evasion in wild and mass-reared medfly males. The development of such a test is the first step in assessing the low escape ability of mass-reared flies, with the eventual goal of improving the effectiveness of application of SIT against medfly.

**Materials and Methods**

**Tests in Chios, Greece**

All tests were conducted on orange trees, Citrus sinensis Ob., (approximately 2.0 m tall with 2.2 m wide crowns) within field-cages used for behavioral studies (2.2 m height × 3 m diameter). The first 2 tests (Tests 1 and 2) took place in a citrus orchard in Chios, Greece, with wild medfly males, originating from sour oranges and figs, and laboratory males of a medfly genetic sexing strain VIENNA 42 (a temperature shock in the egg stage kills the female eggs, Franz et al. 1994), mass-reared and sterilized (90 Gy) at the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria. The males were shipped from Vienna to Chios, Greece (total transport time 12 h), for field-testing (Hendrichs et al. 1993). Wild and sterile laboratory reared males were marked with a different color on the thorax and released together into the field-cage the day before testing. The methodology followed was described in Hendrichs & Hendrichs (1998).

In Test 1, the field cage entrance zipper was left open (approximately 0.15 m wide) to allow access to foraging yellowjacket wasps, V. germanica L. Whenever a wasp entered the cage, the zipper was closed and all attacks of the wasp on wild and sterile medfly males were followed by two observers and recorded until the wasp captured a fly and was allowed to leave the cage.

In Test 2, no wasps were allowed into the field cage by keeping the cage zipper closed. Two observers simulated predation attacks on flies by attempting to capture flies by sucking them into aspirators normally used to handle flies in laboratories. The 2 observers alternated every 20 attempts each to capture wild (10 attempts) and sterile males (10 attempts) within the orange tree foliage. The aspirators were held at the lower end of a 20-cm glass tube inserted into a flexible plastic hose, the end of which was held in the mouth of the person attempting to capture flies. The opposite end of the glass tubing, through which flies were sucked, had a 5-mm diameter opening that was bent at an angle of about 70-75 degrees to facilitate reaching for flies present on or below surfaces such as host foliage. On average there were 2 capture attempts per min. The ability of flies, involved either in pheromone-calling or other activities such as resting or feeding, to escape from aspirating humans was quantified.

**Tests in Seibersdorf, Austria**

Tests 3 and 4, carried out at Seibersdorf, Austria, were based on the use of aspirators to cap-
ture flies on potted orange trees in field cages (same dimensions as above). Unmarked flies of different treatments were placed into separate but adjacent field cages. In Test 3, with laboratory flies of the pupal color dimorphism medfly genetic sexing strain SEIB 30-C (Robinson et al. 1999), we compared non-irradiated and irradiated flies, which were exposed to gamma radiation (90 Gy) as mature pupae two days before adult emergence. Flies were compared, while involved in different activities on the host tree, in their capacity to evade capture with aspirators. This comparison was carried at 2-d intervals, starting with adult emergence, to assess changes in evasive ability during the fly maturation period.

In Test 4, we carried out inter-strain comparisons to measure the capacity to evade capture in 3 different laboratory strains: genetic sexing strain VIENNA 42, pupal color genetic sexing strain SEIB 1-61, and a strain originating from coffee in Guatemala and held in the laboratory for 22 generations. Flies of all strains were mature (6-8 d old) when tested. One-half of all capture attempts in Test 4 were made by an observer with no previous experience with aspirators, the other by an experienced user of aspirators. In addition, both observers repeated the comparison of the 3 strains under 2 environmental regimes: sunny with approximately 23-25°C, or overcast and only 20-21°C.

In all 4 tests, conducted in Chios as well as Seibersdorf, care was taken that flies captured did not exceed 40% of flies released or originally emerged in a cage, in order to prevent the fly population in the cage from being constituted predominantly of individuals who had succeeded in escaping capture. Furthermore, although not recorded as part of the tests, every effort was made to remove flies that had escaped in the first capture attempt to eliminate them from the population to be sampled. However, this was not possible in the first test where wasps were often seen attacking the same fly several times in succession before capture or definite escape of the fly. Data of all tests were analyzed by Kruskal-Wallis non-parametric one-way analysis of variance. In Tests 1 and 2 each replicate consisted of the percent capture in 10 capture attempts; in Tests 3 and 4 each replicate consisted of 5 capture attempts.

**RESULTS**

Tests in Chios, Greece

Results of Tests 1 and 2 conducted in Chios, comparing wasp attacks and simulated attacks with aspirators, are shown in Table 1. Three to 4 times more sterile VIENNA 42 males than wild males were captured by wasps. This highly significant difference was found for both pheromone-calling males and for males engaged in other activities (Table 1A).

In Test 2 (Table 1B), the simulated predator attacks with aspirators resulted also in significantly higher captures of sterile VIENNA 42 males, which were 2 to 3 times more likely to be captured than wild males. Overall, the percent capture of medfly males, irrespective of fly type or fly activity, was 18.2 ± 13.6% (SD) for wasps and 55.7 ± 25.6% (SD) for aspirators. In spite of this difference between wasps (Test 1) and observers with aspirators (Test 2) in capturing flies (P<0.0001), the capture ratios between fly types and fly activities in general were similar for both tests. In addition (Test 2), there were no differences in percentage captures between the two observers experienced with the aspirators used to handle flies (P = 0.4576).

Tests in Seibersdorf, Austria

Results of Test 3, again measuring evasion of capture with aspirators, are shown in Tables 2 and 3. Overall, capacity to avoid capture increased directly with days since adult emergence in the field.

### Table 1. Success of Capture Attempts (Percentage ± SD) of Wild or Laboratory-Rearing Medfly Males by Yellowjacket Wasps (A) or by Experimenter with Aspirators (B) on a Field-Caged Orange Tree in Chios, Greece. Numbers in parenthesis represent fly capture attempts.

| Fly activity | Successful capture attempts (%) | Fertile wild males | Sterile lab. males | Significance
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Wasps</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexual activities</td>
<td>8.2 ± 8.7 (100)</td>
<td>36.7 ± 13.4 (90)</td>
<td>P = 0.0003**</td>
<td></td>
</tr>
<tr>
<td>Other activities</td>
<td>13.7 ± 6.8 (540)</td>
<td>40.6 ± 12.1 (80)</td>
<td>P = 0.0001**</td>
<td></td>
</tr>
<tr>
<td>B. Aspirators</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexual activities</td>
<td>27.6 ± 6.9 (29)</td>
<td>80.7 ± 8.0 (150)</td>
<td>P = 0.0058**</td>
<td></td>
</tr>
<tr>
<td>Other activities</td>
<td>28.4 ± 11.7 (101)</td>
<td>62.3 ± 19.9 (220)</td>
<td>P = 0.0001**</td>
<td></td>
</tr>
</tbody>
</table>

1Kruskal-Wallis One-Way Nonparametric ANOVA.
2Includes feeding and resting males, both on the cage wall and foliage.
cage (Table 2). Although this trend was observable in non-irradiated flies, it was not statistically significant. For irradiated flies the difference was highly significant, with recently emerged flies having the lowest capacity for evading predators. The comparison of non-irradiated and irradiated flies involved in different activities on an orange tree is shown in Table 3. Overall, irradiated flies were significantly more susceptible to being captured than non-irradiated flies ($P = 0.0019$). This was particularly so for activities or locations for which alertness or wariness of flies is normally higher, such as male presence on leaf tops or female approaching male leks (females on the same or neighboring leaves within a radius of 10-15 cm of pheromone calling males). On the other hand, there was no significant difference for activities in which the attention of males is diverted in sexual activities, for example pheromone-calling, male-aggressive encounters, courting or mating.

Results of Test 4 are presented in Tables 4 and 5. As shown in Table 4, no differences were found in capture avoidance between the 3 laboratory strains under comparison ($P = 0.2544$) and capture rates were only slightly, but not significantly, higher at the cooler temperatures (Table 4). While differences in capture rates between the respective fly activities (Table 5) were similar to the preceding aspirator tests, observer experience in with aspirators played a role for those fly activities in which alertness is normally higher, such as male presence on leaf tops or females on fruit. For these activities significant differences in fly capture were obtained between the experienced and the inexperienced observer, whereas for those activities in which flies are less wary because of involvement in sexual activities or feeding no such differences were found (Table 5).

**DISCUSSION**

Our results, comparing wasp attacks and simulated attacks with aspirators under semi-natural conditions, show that sterile laboratory medflies are less likely to evade capture than wild flies. This confirms the relevance of measuring predator evasion in strains mass-produced for many generations under standard medfly mass-rearing conditions.

Considering the significantly higher susceptibility of laboratory reared flies to capture by foraging predators, resulting in significant losses for fruit fly control programs that integrate the SIT, it appears important to develop and apply a simple quality control test for tephritid fruit flies that addresses sterile fly capacity to avoid capture. Presently, the FAO/IAEA/USDA (2003) manual, which is used as a standard for quality control procedures in fruit fly programs incorporating the SIT, does not make reference to this aspect of sterile fly behavior, or fly agility or irritability, in general. Even the “startle” test, recommended as part of the RAPID quality control system (Boller et al. 1981), and listed in a quality control manual used in Latin America (Orozco et al. 1983), is not included in the above international fruit fly quality control manual.

Open field mating tests consistently require much higher sterile to wild male overflooding ratios than are needed within relatively protected field cages or large field enclosures to achieve the same levels of egg sterility (Rendón et al. 2004; Shelly et al. 2005). As the successful food foraging ability of sterile males has been repeatedly confirmed (Maor et al. 2004), this discrepancy between open field and field enclosure results must derive from the very high mortality that sterile males incur in the field before maturing sexually and encountering potential mates. Thus, ability to avoid capture is a fundamental aspect of sterile male quality that needs to be considered in operational programs.

The startle test measures a complex of reactions involved in fly response to light. It was originally developed by Schroeder et al. (1973), later modified by Boller et al. (1981), and further improved by C. O. Calkins (personal communication), including a compact mobile startle test.

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### Table 2. Success of Capture Attempts (Percentage ± SD) of Irradiated and Non-Irradiated Laboratory Reared Male and Female Medflies by Experimenters with Aspirators on a Field-Caged Orange Tree Over Successive Periods After Fly Emergence. Numbers in parenthesis represent fly capture attempts.

<table>
<thead>
<tr>
<th>Fly age (days after emergence)</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>Successful capture attempts (%)</th>
<th>Significance¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-irradiated flies²</td>
<td>85.0 ± 15.5 (80)</td>
<td>78.0 ± 17.4 (200)</td>
<td>71.3 ± 25.3 (160)</td>
<td>$P = 0.2113^{**}$</td>
<td></td>
</tr>
<tr>
<td>Irradiated flies²</td>
<td>93.8 ± 12.0 (80)</td>
<td>83.5 ± 14.2 (200)</td>
<td>73.9 ± 23.0 (160)</td>
<td>$P = 0.0022^{**}$</td>
<td></td>
</tr>
</tbody>
</table>

¹Kruskal-Wallis One-Way Nonparametric ANOVA.
²Mass reared flies of pupal color sexing strain SEIB 30-C.
machine with 10 test units. It is the only test currently available that, indirectly, may give an indication of fly agility or predator evasion capacity in mass-reared flies. However, the startle test is actually a type of flight propensity test that distinguishes between flies that will fly when stimulated from those that do so at a much slower rate. This flight is not in response to a sudden approach by a dark object (e.g., a predator) or a sudden falling object (Schroeder & Chambers 1977), but to a sudden onset of light experienced by flies held previously in total darkness for 30 min.

In terms of measuring capacity to avoid capture, there are additional disadvantages with the startle test. Not only is the test carried out under unnatural conditions, but previous placement of flies into the test units requires considerable fly manipulation, often involving CO2. There is also the possibility of pheromone contamination of the test units. However, it is not possible to distinguish between fly activities nor to detect some of these problems because flies are concealed within black holding containers. Finally, the startle test lasts for a period of 3 min, allowing flies during this rather long period to fly in the direction of the light source, whereas responses to a predator attack occur within fractions of a second to, at most, several seconds.

Fly response to real predation attacks is complex and its measurement even more so. It requires extensive observation not readily amenable to standardization, as well as the availability of predators (Hendrichs & Hendrichs 1998). Predators are not available at all locations and during all seasons. To overcome these complications, we have described here a simple quality control method for measuring male medfly capacity to avoid capture. Unlike the startle test, the test requires no special equipment and does not measure flight response to onset of light and other stimuli but rather reflects more directly fly escape ability under more realistic conditions. Although we found absolute losses to simulated predation with aspirators higher than to real predation by foraging yellowjacket wasps, the relative ability to avoid capture is similar in mass-reared and wild males.

Our quality control method for measuring capacity to avoid capture in fruit fly males would not require routine testing as part of the RAPID laboratory quality control tests (Boller et al. 1981). Rather, it could be conducted at longer intervals as part of the more valuable confirmation quality control tests carried out under semi-natural conditions (Chambers et al. 1983). Only such tests, which must be carried out in greenhouses or on

### Table 3. Success of Capture Attempts (Percentage ± SD) of Irradiated and Non-Irradiated Laboratory Rearing Male and Female Medflies in Relation to Fly Activities by Experimenters with Aspirators on a Field-Caged Orange Tree. Numbers in parenthesis represent fly capture attempts.

<table>
<thead>
<tr>
<th>Fly activity</th>
<th>Successful capture attempts (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-irradiated flies¹</td>
<td>Irradiated flies¹</td>
</tr>
<tr>
<td>Females approaching leks</td>
<td>62.5 ± 21.8 (80)</td>
<td>81.3 ± 20.0 (80)</td>
</tr>
<tr>
<td>Males present on leaf tops</td>
<td>65.8 ± 16.1 (120)</td>
<td>80.0 ± 19.6 (120)</td>
</tr>
<tr>
<td>Males resting on leaf bottoms</td>
<td>75.8 ± 22.1 (120)</td>
<td>82.5 ± 18.9 (120)</td>
</tr>
<tr>
<td>Males involved in sexual activities</td>
<td>81.3 ± 18.6 (80)</td>
<td>80.0 ± 14.6 (80)</td>
</tr>
<tr>
<td>Mating pairs</td>
<td>86.7 ± 17.3 (40)</td>
<td>95.0 ± 8.9 (40)</td>
</tr>
</tbody>
</table>

¹Mass reared flies of pupal color sexing strain SEIB 30-C.
²Kruskal-Wallis One-Way Nonparametric ANOVA.

### Table 4. Success of Capture Attempts (Percentage ± SD) of Laboratory Rearing Medflies with Aspirators on a Field-Caged Orange Tree under Different Environmental Conditions. Numbers in parenthesis represent fly capture attempts.

<table>
<thead>
<tr>
<th>Laboratory strain</th>
<th>Sunny (23-25°C)</th>
<th>Overcast (20-21°C)</th>
<th>Significance²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupal color sexing strain SEIB 1-61</td>
<td>88.9 ± 14.1 (90)</td>
<td>96.0 ± 8.2 (100)</td>
<td><em>P = 0.0845</em></td>
</tr>
<tr>
<td>tsl sexing strain VIENNA 42</td>
<td>92.0 ± 10.1 (200)</td>
<td>95.0 ± 8.9 (200)</td>
<td><em>P = 0.3173</em></td>
</tr>
<tr>
<td>Bisexual strain from Guatemala</td>
<td>95.0 ± 8.9 (100)</td>
<td>97.9 ± 6.3 (95)</td>
<td><em>P = 0.2452</em></td>
</tr>
</tbody>
</table>

¹Only those fly activities are included in which there were no differences in capture rates between experimenters (see Table 5). No difference between the 3 strains in percent capture (*P = 0.2544*).
²Kruskal-Wallis One-Way Nonparametric ANOVA.
field-caged host trees, and preferably including wild females as a quality baseline, will provide a more definite verification of sterile fly quality.

Results, both with wasps and aspirators, confirm that mass-rearing conditions favor the production of flies with a decreased irritability or “nervousness”. Highly irritable flies appear to die sooner in crowded adult colony cages of mass-rearing facilities than do more sedentary flies (Calkins, personal communication), and less irritable flies appear to have a higher mating success under these conditions (Briceño & Eberhard 2002).

To counter this inadvertent selection, the described method, besides measuring capacity to avoid capture, could be useful for the systematic selection of strains with a lower threshold for startle activity (Ewing 1963; Schroeder & Chambers 1977). The “filter rearing system” (Fisher & Cáceres 2000; Cáceres et al. 2004), involves managing a mother colony that is filtered to maintain the purity of genetic sexing strains, and from which large colonies are regularly derived for sterile male mass-production. This same approach could be extended to establish small “pre-filter” mother colonies under relaxed low density conditions, preferably in greenhouses or field-enclosures with trees and include the selection for males that achieve matings with wild females, and that maintain a certain irritability to reduce easy capture.

As in the case of the startle test, a number of variables have to be controlled to standardize the aspirator method to make it more reproducible. These include temperature and light conditions, fly location, fly sex, fly age, fly numbers (in relation to foliage surface), and nutritional history of the flies. For example, temperatures during testing should not fall outside the 24-28°C range, and flies located on the cage wall should not be included in the measurements, in view that flies away from the host tree are less able to evade capture attempt than those present within the host foliage.

A major disadvantage, as shown by our tests, is individual variation in skill in the use of aspirators. However, preliminary tests have shown that better uniformity can be achieved by using battery-powered mechanical aspirators or by not applying suction until just before the tip of the aspirator reaches the fly. In addition, as shown in Table 5, the observer effect can largely be reduced by restricting measurements to males involved in sexual activities, as they are the main targets of foraging predators (Hendrichs et al. 1994; Hendrichs & Hendrichs 1998), presumably because sexually active males are, in general, less wary of predator attacks (Nagamine & Itô 1980; Burk 1982; M.A.H., unpublished data).

In summary, we present a method that allows measuring the capacity of medflies to evade capture by predators, essential for sterile males to reach sexual maturation under open field conditions. It is recognized, that further standardization of the test is required to allow more accurate inter-strain comparisons to be made. However, it can be concluded that standard medfly mass-rearing conditions result in the production of sterile males that are much less able to evade predation than are wild males. Recognition of this problem and development of a corresponding quality control test could significantly improve the reliability and economics of sterile insect technology for fruit flies.

ACKNOWLEDGMENTS

This work was supported in part by the EU Agroindustry Grant AIR3-CT92-0300 to B. I. K. We thank C. O. Calkins, W. Klassen, P. Rendón, L. LaChance, and A. S. Robinson for critical reviews of an earlier version of this manuscript, C. O. Calkins for useful information and K. Gagg and Harry Baumgartner (FAO/IAEA, Seibersdorf, Austria) for technical assistance. This work is part of a dissertation by M. A. H. at the University of Vienna, Austria.

<table>
<thead>
<tr>
<th>Fly activity¹</th>
<th>Inexperienced² person</th>
<th>Experienced person</th>
<th>Significance³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females present on fruit</td>
<td>72.0 ± 11.0 (25)</td>
<td>96.0 ± 8.9 (25)</td>
<td>P = 0.0139*</td>
</tr>
<tr>
<td>Males present on leaf tops</td>
<td>72.0 ± 29.1 (75)</td>
<td>94.7 ± 9.2 (75)</td>
<td>P = 0.0057**</td>
</tr>
<tr>
<td>Males resting on leaf bottoms</td>
<td>88.0 ± 14.7 (75)</td>
<td>93.3 ± 9.8 (75)</td>
<td>P = 0.3375NS</td>
</tr>
<tr>
<td>Males feeding</td>
<td>96.0 ± 8.3 (75)</td>
<td>98.7 ± 5.2 (75)</td>
<td>P = 0.2909NS</td>
</tr>
<tr>
<td>Males pheromone-calling</td>
<td>90.7 ± 10.3 (75)</td>
<td>92.0 ± 10.1 (75)</td>
<td>P = 0.7172NS</td>
</tr>
<tr>
<td>Mating pairs</td>
<td>98.5 ± 5.5 (65)</td>
<td>97.8 ± 7.3 (70)</td>
<td>P = 0.5930NS</td>
</tr>
</tbody>
</table>

¹Combined data of comparison of 3 mass reared laboratory strains.
²No previous experience.
³Kruskal-Wallis One-Way Nonparametric ANOVA.
GAS-EXCHANGE PATTERNS OF MEDITERRANEAN FRUIT FLY PUPAE (DIPTERA: TEPHRITIDAE): A TOOL TO FORECAST DEVELOPMENTAL STAGE

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ABSTRACT
The pattern of gas-exchange (CO$_2$ emission) was investigated for developing Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann) pupae incubated at different temperatures. This study was undertaken to explore the usefulness of gas-exchange systems in the determination of physiological age in developing pupae that are mass produced for sterile insect technique projects. The rate of CO$_2$ emission was measured in a closed flow-through system connected to commercial infrared gas analysis equipment. Metabolic activity (rate of CO$_2$ emission) was related to pupal eye-color, which is the current technique used to determine physiological age. Eye-color was characterized digitally with 3 variables (Hue, Saturation and Intensity), and color separated by discriminant analysis. The rate of CO$_2$ emission throughout pupal development followed a U-shape, with high levels of emission during pupariation, pupal transformation and final pharate adult stages. Temperature affected the development time of pupae, but not the basic CO$_2$ emission patterns during development. In all temperatures, rates of CO$_2$ emission 1 and 2 d before adult emergence were very similar. After mid larval-adult transition (e.g., phanerocephalic pupa), digital eye-color was significantly correlated with CO$_2$ emission. Results support the suggestion that gas-exchange should be explored further as a system to determine pupal physiological age in mass production of fruit flies.

Key Words: carbon dioxide emission, sterile insect technique, metabolic rate, pupal respiration, physiological age, irradiation time, digital eye-color

RESUMEN
En el presente estudio se investigaron los patrones de intercambio gaseoso (emisión de CO$_2$) en pupas de la mosca de las frutas del Mediterráneo (Ceratitis capitata Wiedemann) incubadas a diferentes temperaturas. El estudio fue realizado con la finalidad de explorar la utilización de sistemas de intercambio gaseoso en la determinación de la edad fisiológica de pupas durante su producción masiva en proyectos de mosca estéril. La proporción de emisión de CO$_2$ fue medida en un sistema cerrado de “flujo a través del sistema” conectado a un detector infrarrojo de gases. La actividad metabólica de la pupa (emisión de CO$_2$) fue contrastada al color del ojo de la pupa en desarrollo, que constituye la actual técnica de determinación de la edad fisiológica. El color de ojos en pupa fue determinado digitalmente, usando tres variables (Tendencia, Saturación e Intensidad). Los colores fueron separados utilizando el análisis discriminatorio. Los patrones de emisión de CO$_2$ durante el desarrollo de la pupa sugieren una tendencia de U: una alta actividad metabólica durante la fase inicial de pupación y transformación y durante la fase final del adulto. La temperatura de incubación afectó el tiempo de desarrollo pero no el patrón básico de actividad metabólica. La proporción de emisión de CO$_2$ uno y dos días antes de la emergencia del adulto fue muy similar para pupas mantenidas en las diversas temperaturas. El color digital del ojo de la pupa se correlacionó significativamente con los patrones de emisión de CO$_2$, detectados a partir de la fase media de la transformación de larva a adulto. Los resultados soportan la utilización de sistemas de intercambio gaseoso como un sistema auxiliar para la determinación de la edad fisiológica en cría masiva de moscas de la fruta.

Translation provided by the authors.

The sterile insect production capacity has greatly expanded during the last decades and evolved into an industrial process. For fruit flies, many mass-produces sterile insects for field release to control the damage exerted by these pest insects; released sterile males copulate with wild fertile females curtailing their ability to produce a new generation of wild flies. The success and increased interest in the SIT, is based on scientific achievements and technological innovations de-
developed during the last decades, and on the ability to produce and deliver sterile fruit flies of good quality and sexual competitiveness (Fisher 1997; Hendrichs et al. 2002; Tween 2002).

This technological advance has opened the doors for the participation of the private sector in this enterprise, which was solely financed in the past by the public sector. New facilities, some of them based on private capital (such as “Bio-Fly” in Israel and “Insecta” in Europe) are being opened, where labor is expensive and production is capital intensive. These new tendencies in the industrial production process of sterile insects are driving research and development in the direction of process automation, labor saving activities, and the reduction in production uncertainties. Our aim in this study was to support this tendency by exploring the ability of available gas-exchange measuring technologies to forecast important biological events of the developing flies. Specifically, we characterized the gas-exchange patterns of developing pupae and explored analytical methods to forecast and monitor key events in the production process.

Key biological events during mass production of Mediterranean fruit flies (medflies) *Ceratitis capitata* (Wiedemann) for SIT purposes include, among others, egg hatching, larval jumping, pupation (Quesada-Allue et al. 1996), stage of pupal development, and adult emergence. Knowledge on the precise pupal physiological age is of importance for the management of mass-production and for sterilization purposes (Ruhm & Calkins 1981). Medflies are sterilized by exposing pupae to gamma-radiation. The outcome of such exposure on induced sterility and fly quality depends on the physiological age and dose at which pupae are irradiated (Ruhm & Calkins 1981). Pupae irradiated earlier than the optimal time could be damaged, affecting their adult performance and ability to effectively mate (Ohinata et al. 1971). On the other hand, late irradiation could compromise sterility (FAO/IAEA/USDA 2003), leading to the release of fertile flies. These fertile flies may copulate with wild ones, affecting the sterile/wild relationship and effectiveness of the program. In addition, the ability to monitor and forecast the physiological stage of the developing pupae could be of use during the daily activity and working program of mass-rearing facilities. As an example, mass-rearing facilities manipulate the environmental conditions (such as temperature) of developing pupae to synchronize, and adjust, production timing to field logistics and release schedules (Ruhm & Calkins 1981). In this sense, a system able to automatically determine the physiological stage of the developing pupae could serve to manage and set incubation temperatures (i.e., automatically) as required by field timetables.

The current system to determine pupal physiological age consists of removing the pupal case and visually inspecting the color of the eye (FAO/IAEA/USDA 2003). This subjective system is based on the fact that eye-color is known to change with pupal physiological age (Ruhm & Calkins 1981; Resilva et al. 2007). Eye color in developing pupae becomes apparent during mid-pupation with the eversion of the head and the initiation of the “phanerocephalic” pupal stage (Quesada-Allue et al. 1996). At this stage, pupal eyes are whitish. Eye coloration starts to change at the end of the pupal stage and beginning of the pharate-adult stage, with the increase in metabolic activity: at this stage, eyes become yellowish (Quesada-Allue 1994). Subsequently, eye color rapidly changes from yellow to orange, to red, to brown orange and, before adult emergence, to iridescent (Ruhm & Calkins 1981; Quesada-Allue 1994; Quesada-Allue et al. 1996). At 23°C, the whole larval-adult transition may be accomplished in 13 d (Quesada-Allue et al. 1996). At this temperature, the onset of the phanerocephalic stage, where eyes have a whitish coloration, occurs 48 h after larval immobilization, while the finalization of the pupal stage and initiation of the pharate adult stage, with the subsequent acceleration of metabolism and change of eye color to yellow, is observed 120 h (5 d) after larval immobilization (Quesada-Allue et al. 1996).

Recently, Donoso et al. (2003) suggested monitoring temperature in pupal incubation rooms to determine physiological age and time of irradiation. These authors proposed that since insect development is dependent upon temperature (Ratte 1984), degree-day models could be used to determine emergence time and the precise time for irradiation. While this method could provide us with a good approximation of physiological age, it is an indirect estimation of age which is based on parameters estimated under constant conditions. As a result, it could be insensitive to temperature oscillations and other environmental factors inside incubation rooms that could affect development.

Direct measurements of metabolic activity, on the other hand, could provide a more reliable system for the estimation of physiological age. Oxygen consumption and carbon dioxide (CO₂) production in living organisms, as an example, are the direct outcome of metabolic activity. The rates at which these gases are consumed or produced are directly related to the rate at which metabolism proceeds in the organism (Keister & Buck 1973; Lighton & Wehner 1993). Thus, the measurement of gas exchange is expected to be a good indication of metabolic activity. The rate of gas exchange during metamorphosis of some Diptera species (e.g., *Calliphora erythrocephala* Macquart) has already been described and was shown to follow a U-shaped curve (Agrell & Lundquist 1973), with a high metabolic activity during early and late developmental stages and a slow-down during mid-pupal stage. This same U-shaped
curve was described for the oxygen consumption of developing medfly pupae (Langley 1970).

The present study is based on this previously generated knowledge. We suggest that the measurement of metabolic activity in medfly pupae through gas exchange systems could provide us with a reliable tool to forecast physiological age and adult emergence time in mass-rearing facilities. In order to investigate this idea, we first digitally characterized the eye-color of developing medfly pupae as a reference, and to have an objective method of comparison. We then characterized the daily patterns of CO₂ emission on pupae developing at different constant and variable incubation temperatures (ranging from 15 to 30°C). Finally, the ability of the gas-exchange system to determine pupal physiological age was inferred from correlating and contrasting digital eye color with pupal respiration patterns.

Materials and Methods

Study Insects

Larvae were obtained from the colony of the medfly strain ‘Sade’ (more than 20 years old) of the Board of Fruit and Vegetable Growers, Israel. This is a bisexual strain, reared on artificial diet, which is regularly refreshed with material from the wild (2-3 times a year). Larvae were collected as they were leaving the diet (“crawling phase”). Collections were conducted during a short period of time to synchronize immobilization and pupation.

Digital Determination of Medfly Pupal Eye-color

Approximately 330 pupae from different ages (and developing under different temperatures) were sampled, dissected, and their eyes exposed. Dissected pupae were positioned, always following the same orientation on a mini-stage (5 cm in diameter), where the background was always the same, illumination was provided from the same sources and from the same directions, and shade was reduced by a series of mini-reflectors surrounding the stage. Pupae were photographed at a magnification of 20× with a stereoscopic microscope equipped with a 3-CCD color digital camera (Sony DXC 990P). A small area of the pupal eye was focused and a picture taken. The digital image consisted of 3 components: Red, Green, and Blue (RGB space). The digital image was analyzed with Image-Pro PLUS version 4.5 software (Media Cybernetics, Inc.). The 3 basic color components, RGB, were transformed to an alternative color space, defined by Hue, Saturation, and Intensity (HSI). In the HSI color space, the information on the object’s color is expressed mainly through Hue, because this variable is not affected by the illumination intensity. The Saturation expresses the vividness of the color, while the Intensity is affected mainly by the illumination intensity. Eye colors included: white, yellow, orange, red-orange, brown, dark-brown, and iridescent. For each color category we analyzed at least 20 specimens.

Eye color data derived from all the samples were analyzed by “Discriminant Analysis” (Statgraphics 5 Plus 2000, Manugistics, Inc.). This analysis produced 3 canonical variables (F1, F2, and F3) that are derived from the original variables (Hue, Intensity and Saturation). These canonical variables are used to classify the data into groups (“standardized digital eye-color”). Resulting groups were correlated with their respiration patterns.

Respiratory Patterns of Medfly Pupae as Affected by Incubation Temperature

CO₂ emission was measured with a closed flow-through system connected to commercial infrared gas analysis equipment (Model No. S-151, Qubit Systems, Inc., Kingston, Ontario, Canada). Pupae were placed inside a glass Erlenmeyer flask (250 mL), which was hermetically sealed with a rubber stopper with lure connectors. Air was pumped (0.4 L/min) through the flask to collect the pupal-emitted CO₂, which was directed to the infrared carbon dioxide analyzer (Qubit Systems, Model No. S-151, with a resolution of ±1 ppm CO₂). Air emerging from the CO₂ analyzer was pumped back into the Erlenmeyer flask. The whole system was kept closed with vinyl tubing. Closed circulation of air provided the cumulative amount of CO₂ emitted by the pupae in a period of time. In order to measure the rate of CO₂ emission, we recorded its accumulation in a period of 10 min and obtained the rate from the slope. The data-logger connected to the CO₂ analyzer (Vernier Software and Technology, Beaverton, Oregon, USA) generated 1 measurement per min.

We measured the rate of CO₂ production in pupae incubated at several constant temperatures (15, 20, 25, and 30°C), and at variable room temperature (R.T.), with temperatures oscillating between 18-25°C. This design was expected to create different pupal physiological ages independent of chronological age. Each experiment (e.g., replicate) consisted of simultaneously incubating 5 g of recently immobilized larvae at the different temperatures. Incubation in the different environments was kept constant from the moment of larval immobilization until adult emergence.

Gas-exchange measurements took place around mid-day. During the last days of pupal development, there are differences in metabolic rate throughout the day (e.g., circadian rhythms), which tend to increase after mid-day (Nestel, unpublished data). Due to this we kept constant the time of the day at which gas-exchange was measured. Batches of pupae were taken from the incubator, weighed (pupae lose water throughout development, reduc-
ing their initial weight at larval immobilization by approximately 20%, Nestel et al. 2003), placed inside the Erlenmeyer flask, and connected to the flow-through Erlenmeyer flask system to measure CO₂ production during a period of 10 min. At the end of the measurement, pupae were returned to their respective incubator until the following measurement next day. This experiment was repeated 2 to 3 times on different dates. For room temperature (R.T.), we only conducted 1 replicate.

CO₂ emission patterns during pupal development under the different temperatures were obtained by fitting the data to quadratic functions (Statgraphics 5 Plus 2000, Manugistics, Inc.). Average rate of CO₂ production 1 and 2 d before adult emergence were extrapolated from the calculated functions. During all the experiments, we sampled more than 10 pupae per temperature and day. These were dissected and their eye-color determined with the digital ImagePro System. Canonical variables were derived from the original variables (Hue, Intensity and Saturation) as explained earlier, and the derived standardized digital eye-color was later correlated with rate of CO₂ emission during the specific day and temperature regime (see the following section).

Relationship between Standardized Digital Eye-color and Respiration Patterns

Pupae collected in the above experiment were immediately dissected and their eye-color digitally characterized by the 3 variables with ImagePro. Based on the derived canonical variables, a “standardized digital eye-color” was determined for each pupa. The average “standardized digital eye-color” for a specific temperature treatment during a specific date was related to the rate of CO₂ production measured during that day based upon linear regression (Statgraphics 5 Plus 2000, Manugistics, Inc.). Due to the typical U-shape pattern of gas exchange, we decided to investigate this relationship with data on CO₂ emission obtained in all temperatures and replicates from mid-pupal development period until adult emergence. Thus, digital eye-color was correlated with CO₂ emission from d 10 in pupae developing at 15°C, from d 6 in pupae developing at 20°C, from d 4 in pupae developing at 25°C, from d 2 in pupae developing at 30°C, and from d 6 in pupae developing at room temperature (R.T.). These dates corresponded in all of the treatments with pupae having white-eyes.

RESULTS

Digital Characterization of Pupal Eye-color and Standardization

After pupariation and the eversion of the head during mid-pupal stage, pupal eyes have a whitish coloration. As pupal development proceeds, eye-color changes from white to yellow, then orange, red-orange, brown, dark-brown, and finally iridescent (slightly before adult emergence). Fig. 1a shows the scatter graph derived from the digital characterization of pupal eye-color using the Hue, Saturation and Intensity system. As can be seen, the colors are not well separated by these 3 variables. As a result we applied a multivariate analysis to separate between groups. The canonical discriminant analysis resulted in 3 significant “canonical variables” able to discriminate between the 7 levels of pupal eye-color (Variable F1, χ² = 1377, df = 18, P < 0.01; Variable F2, χ² = 651, df = 10, P < 0.01; Variable F3, χ² = 101, df = 4, P < 0.01). Fig. 1b shows the clusters of pupal eye colors formed by using the 2 first canonical variables F1 and F2. The ability of canonical variable F1 to predict pupal eye-color from digital data of Hue, Saturation and Intensity used for the calibration is provided by Table 1. The overall classification accuracy was 74.01%. The classification accuracy decreases (to 53% of cases accurately predicted) when the pupal eye-color obtains an iridescent coloration.

Effect of Incubation Temperature upon Pupal Development Time and CO₂ Emission

Fig. 2 shows the daily patterns of CO₂ emission at mid-day as affected by incubation temperature and chronological pupal age. The figure shows actual measurements and the fitted quadratic functions. In all the cases, the fitted quadratic function resulted in a high coefficient of determination (above 0.85). Rate of gas exchange patterns followed a typical U-shape, with a high metabolic activity during the first hours and days of metamorphosis, and a lower level by mid-pupal period. After this drop in metabolic activity, CO₂ emission steadily increases up to adult emergence. Using the resultant quadratic functions obtained for each incubation temperature, we derived the level of CO₂ emission 1 and 2 d before adult emergence by extrapolation (Table 2). Rate of CO₂ emission 2 d before adult emergence ranged from 22.5 to 30.8 nmol CO₂/g of pupae/minute while 1 d before adult emergence it ranged from 30.3 to 39.4 nmol. In elevated incubation temperatures, the rate of CO₂ emission was higher than at lower temperatures. In contrast, at lower temperatures, rate of emission was more reduced (Table 2). At 25 and 30°C, rate of CO₂ emission 1 and 2 d before adult emergence was comparable. At 20°C rate of CO₂ emission was slightly lower than at these 2 temperatures. Incubation at room temperature produced the lowest rates of CO₂ emission for 1 and 2 d before adult emergence.

Relationship between Standardized Pupal Eye-color and Patterns of CO₂ Emission

Fig. 3 shows the relationship between standardize pupal eye-color and rate of CO₂ emission.
Eye color was standardized based upon canonical variable F1 (see previous section). As explained in the methodology section ("Relationship between Standardized Digital Eye-color and Respiration Patterns"), the relationship between the 2 variables was performed from mid-pupal stage until
adult emergence (eyes in the pupae are only distinguished after the “phanerocephalic” stage). As can be seen from the figure, there is a good linear relationship between these 2 variables. The coefficient of determination ($R^2$) was 0.78, suggesting that at least 78% of the variability can be explained by the linear relationship between these 2 variables.

**DISCUSSION**

The typical U-shape metabolic (and CO$_2$ emission) pattern during metamorphosis is basically the manifestation of the early histolysis and later histogenesis and differentiation processes of the larval-adult transition (Agrell & Lunquist 1973). During the pre-pupal and early pupal stage, all the machinery involved in tissue histolysis (e.g., proteolytic enzymes) is highly active (Agrell & Lunquist 1973; Rabossi et al. 2000; Tolmasky et al. 2001). This was manifested in our study by the high levels of CO$_2$ production during the first hours and days of metamorphosis. Following the evagination of the head and thoracic appendages, and formation of definite body proportions (i.e., end of the puation process), metabolic activity and CO$_2$ emission drops (Agrell & Lunquist 1973; Rabossi et al. 2000; Tolmasky et al. 2001). At this stage, pupal eyes are whitish. Metabolic activity rises again during the pharate adult stage, when tissues are increasing and further differentiating (Agrell & Lundquist 1973). During this period eyes start to change in coloration (Quesada-Allue 1994). As expected and as previously reported for the medfly (Langley et al. 1972), reduced incubation temperatures delayed the metamorphosis process and the time needed for the completion of development. However, the basic U-shape metabolic patterns did not differ for any of the tested temperatures, and metabolic activity as measured from CO$_2$ production followed the expected trend.

The ability of the digital system and discriminant function to predict eye-color was within acceptable ranges (overall, more than 70% of the cases were correctly classified). The success of this system to correctly classify pupal eye-color decreased when the eye color became fully iridescent. The main source of misclassifications was with iridescent eyes, when 43% of the cases where classified as dark-brown when our subjective classification was iridescent. The lack of accuracy in this case could be related to our inability of subjectively classifying eye-color correctly, or to the inability of the discriminant function to completely separate between these 2 similar classes of eye-color. In any case, this misclassification at a sensitive moment of production highlights the problem of the system to rely on a subjective measurement to make decisions, which is mainly based on the perspective of the human eye.

The significant linear relationships after mid-pupal stage between standardized pupal eye-color and average pupal emission of CO$_2$ strengthened the working hypothesis of this study that the rate of gas exchange, and therefore of metabolic activity, is an indication of mid-pupal and pharate adult physiological age. The variability of the estimated regression (mainly at advanced pupal ages and larger rates of CO$_2$ emission) can probably be attributed to 2 aspects: (1) the reduced synchronicity of pupal age resulting from incubations at low temperatures (e.g., 15 and 20°C), and (2) the mentioned misclassification of eye-colors close to adult emergence.

At low temperatures, adult emergence usually extends over a longer period of time (Nestel, unpublished data). In contrast, pupae incubated simultaneously at higher temperatures result in adult emergence occurring more synchronously within a shorter period of time (Nestel, unpublished data). This lack of synchronicity at lower incubation temperatures, and the resulting mixture of physiological ages within the samples may explain the slightly lower rate of CO$_2$ emission observed 1 and 2 d before adult emergence in pupae incubated at 15°C, 20°C, and room temperature.

**Table 1. Classification accuracy of eye color (visually determined) by discriminant analysis based on canonical variable F1 \(F_1 = (-0.220486 \times \text{HUE}) + (0.470136 \times \text{SATURATION}) + (-1.2807 \times \text{INTENSITY})\). The diagonal elements in the table represent the percentage of cases accurately classified.**

<table>
<thead>
<tr>
<th>Actual eye color visually determined</th>
<th>White</th>
<th>Yellow</th>
<th>Orange</th>
<th>Red-orange</th>
<th>Brown</th>
<th>Dark-brown</th>
<th>Iridescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>White ((n = 150))</td>
<td>73.3</td>
<td>24.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Yellow ((n = 33))</td>
<td>15.2</td>
<td>78.8</td>
<td>6.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Orange ((n = 55))</td>
<td>0.0</td>
<td>3.6</td>
<td>76.4</td>
<td>20.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Red-Orange ((n = 25))</td>
<td>0.0</td>
<td>0.0</td>
<td>1.20</td>
<td>76.0</td>
<td>12.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Brown ((n = 30))</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>16.7</td>
<td>70.0</td>
<td>13.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Dark-Brown ((n = 17))</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>17.7</td>
<td>70.6</td>
<td>11.7</td>
</tr>
<tr>
<td>Iridescent ((n = 17))</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>41.7</td>
<td>52.9</td>
</tr>
</tbody>
</table>
This aspect requires further studies, corrections and fine-tunings before the gas-exchange system can be suggested for mass-rearing facilities.

The present study was conducted under laboratory conditions and a colony of a bisexual strain was used. Production levels in this colony are
small, and sample size was tailored to the availability of pupae for experimentation. If the gas exchange system is going to be adopted by the SIT industry however, modifications and adaptation would be required. One possibility includes the sampling of developing pupae at critical stages and measurement of CO₂ emission to determine physiological age in a similar way to the one presented in this study. A completely different approach may include the establishment of incubation rooms in the mass-rearing facilities for advanced pupal ages, and the automatic monitoring of CO₂ accumulation in the room air. These options, and other possibilities, however, would need to be further explored.

**ACKNOWLEDGMENTS**

The present study was partially financed by the IAEA Research Contract 11474. We appreciate the inspiring discussions with Hernán F. Donoso Rifo (Centro de Producción de Insectos Estériles, Arica, Chile), who seeded the idea of this project on the authors. We thank Yoav Gazit and Ruth Akiva (Board of Fruit and Vegetable Growers, Israel) for providing medfly larvae and pupae for this project. We appreciate the suggestions brought by several anonymous reviewers to previous drafts; their contribution enormously helped improve the contents and understanding of the manuscript.

**REFERENCES CITED**


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**TABLE 2. AVERAGE CO₂ EMISSION 1 AND 2 D BEFORE ADULT EMERGENCE FROM PUPAE INCUBATED AT SEVERAL CONSTANT TEMPERATURES, AND AT VARIABLE ROOM TEMPERATURE. THE LEVEL OF CO₂ WAS DERIVED BY EXTRAPOLATION FROM THE QUADRATIC FUNCTIONS FITTED TO THE DATA (SEE FIG. 2).**

<table>
<thead>
<tr>
<th>Conditions of pupal incubation</th>
<th>2 d before adult emergence</th>
<th>1 d before adult emergence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°C (constant temperature)</td>
<td>27.9</td>
<td>32.0</td>
</tr>
<tr>
<td>20°C (constant temperature)</td>
<td>27.1</td>
<td>33.6</td>
</tr>
<tr>
<td>25°C (constant temperature)</td>
<td>30.8</td>
<td>39.4</td>
</tr>
<tr>
<td>30°C (constant temperature)</td>
<td>27.1</td>
<td>38.1</td>
</tr>
<tr>
<td>Room Temperature (variable)</td>
<td>22.6</td>
<td>30.3</td>
</tr>
</tbody>
</table>

---

**Fig. 3. Relationship between the average “standardized digital pupal eye-color” (derived from canonical variable 1 based on the measured Hue, Saturation, and Intensity) and the rate of emitted CO₂ by pupae. The linear relation was calculated for pupae that have already completed the first half of their development, and that are starting to increase their metabolic rate (i.e., the ascending portion of the polynomial functions in Fig. 2). Data represent measurements of CO₂ and eye-color from pupae developing at several constant temperatures, and at room temperature. R² stands for the coefficient of determination for the linear regression.**
Mediterranean fruit fly (*Ceratitis capitata*): pupal metabolism in relation to mass-rearing techniques. Entomol. Exp. & Appl. 15: 23-34.


EFFECTS OF PRE-IRRADIATION CONDITIONING OF MEDFLY PUPAE (DIPTERA: TEPHRITIDAE): HYPOXIA AND QUALITY OF STERILE MALES

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ABSTRACT

Irradiation of pupae in sterile insect technique (SIT) projects is usually undertaken in hypoxic atmospheres, which have been shown to lessen the deleterious effects of irradiation on the quality of adult sterile flies. Although this is the accepted technology in most mass-rearing and sterilization facilities, to date no information has been generated on the actual levels of oxygen (O2) in pupae-packing containers during irradiation. The present study utilized recently-developed technology to investigate the O2 level inside bags in which pupae of Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann) are packed prior to irradiation, the ability of pupae to create hypoxic environments in these bags, and the effect of O2 atmospheres on the quality of irradiated males. Pupae, 1 d before adult emergence, were shown to deplete the O2 level in sealed bags in approximately 1 h. The rate of O2 consumption was dependent upon pupal age and incubation temperature. Incubation temperature did not significantly affect the quality of pupae or mating capacity of resultant adult males if pupae were irradiated under maximal hypoxic conditions inside packing bags. In contrast, mating competitiveness drastically decreased when pupae were irradiated under ambient O2 conditions, with the packing bag open. There was no difference in the mating capacity of males when pupae were irradiated in sealed bags under either 10% or 2% O2 levels, or under maximal hypoxia. Normal doses of fluorescent dye, applied to pupae to mark sterile flies, did not affect the ability of pupae to create hypoxic conditions inside packing bags, nor the quality control parameters of either pupae or adults. Current practices in mass-rearing facilities are discussed in the light of these results.

Key Words: Ceratitis capitata, oxygen levels, pupal respiration, mating competitiveness, irradiation, sterile insect technique

RESUMEN

La irradiación de pupas en proyectos de mosca estéril usualmente se hace bajo condiciones de hipoxia. Esta condición ha demostrado ser menos perjudicial a la calidad de las moscas que la irradiación en atmósferas con proporción normal de oxígeno. Aunque esta ha sido por mucho tiempo parte del protocolo de irradiación en plantas de producción de mosca estéril, hasta ahora no se ha medido el contenido de oxígeno dentro de los recipientes de empaque de pupa durante la irradiación. El presente estudio investigó los contenidos de O2 en los contenedores de pupas de la mosca de las frutas del Mediterráneo (Ceratitis capitata Wiedemann), la habilidad de pupas de crear hipoxia dentro de los contenedores, y los efectos del contenido de O2 durante la irradiación del contenedor en la calidad y capacidad de apareamiento de moscas estériles. Pupas de un día antes de emerger como adultos crearon atmósferas de máxima hipoxia dentro del empaque en aproximadamente una hora. La proporción de consumo de O2 en contenedores sellados es dependiente de la edad de la pupa, y de la temperatura de incubación. La temperatura de incubación no afecta significativamente la calidad ni la capacidad de apareamiento de machos derivados de pupas irradiadas bajo condiciones de hipoxia. Sin embargo, la capacidad de apareamiento de machos irradiados como pupas en contenedores abiertos y en condiciones oxigenadas fue drásticamente afectada. En comparación a los resultados anteriores, atmósferas de 2% y 10% O2, durante la irradiación no afectaron la capacidad de apareamiento de moscas estériles. Polvo fluorescente, aplicado a pupas para marcar las moscas estériles, no tuvo efectos sobre la capacidad de las pupas de crear hipoxia. Los resultados de este estudio se discuten en base a las prácticas actuales de producción e irradiación.

Translation provided by the authors.

Routine irradiation of fruit flies in control programs integrating the sterile insect technique (SIT) is usually undertaken when pupae are packed in containers that have been closed or her-
metically sealed for some period prior to their exposure to the radiation source (Schwarz et al. 1985). One variation of this method consists of the constant flushing of the pupae with nitrogen gas during irradiation (Fisher 1997). These techniques have been adopted to attain “reduced-oxygen atmospheres” during irradiation. These procedures have been shown to lessen the “oxygen effects” of “air irradiation” on mating performance and competitiveness of sterile males (Hooper 1971; Ohinata et al. 1977). While these practices are common in many fruit fly mass-rearing and irradiation facilities, and are recommended in the international fruit fly quality control manual (FAO/IAEA/USDA 2003), the actual levels of oxygen (O$_2$) inside sealed bags during irradiation have not, to our knowledge, been previously determined. The present study took advantage of recently developed technology for O$_2$ measurements in air environments to characterize O$_2$ atmospheres during packing and irradiation of pupae of the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann).

Initially, we investigated how pupae of different ages, packed in sealed polyethylene bags commonly used in many mass-rearing facilities, modify the O$_2$ environment inside the bag. A second objective investigated the effect on fruit fly quality of several “pupae packing pre-irradiation protocols” commonly performed in different mass-rearing facilities, on the O$_2$ environments in packing bags, and on the effect of irradiation upon resultant adult males. Specifically, we investigated the effect of incubating pupae for a certain period of time (usually 1 h) at low temperatures before sending the pupae to the irradiator, as reported by Schwarz et al. (1985). This type of manipulation, and the transfer of pupae from the “cold environment” into the room where the irradiation chamber is located, may expose the pupae to several changing temperature regimes (in tropical facilities temperatures in irradiation rooms may be above 25°C). We also investigated the effect of irradiating pupae at different periods after the bags were sealed, and determined O$_2$ level in the sealed bag environment at the start of irradiation. Finally, we investigated the effect of dusting pupae with fluorescent dye during packing and before irradiation upon the ability of pupae to modify the O$_2$ environments inside the sealed bags. Treating pupae with fluorescent dye prior to irradiation is a routine method commonly used to identify released sterile flies in traps in the field.

**Materials and Methods**

**Source of Insects**

Male medfly pupae of the temperature sensitive lethal genetic sexing strain VIENNA 8 (Franz 2005) were obtained from the colony maintained in the research facility of the Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, at Seibersdorf, Austria. Pupae were collected at the appropriate age, as specified in each of the experiments.

**Measurement of Oxygen Levels**

Between 400 and 500 mL of pupae were placed inside 4.5-L polyethylene bags (15 cm width × 45 cm height, 1.5 mm thick), commonly used to pack, irradiate, and ship medfly pupae in some mass-rearing facilities (e.g., El Pino, Guatemala). The bags were perforated in two places by screwing male and female luer connectors, which opened to the inner and outer sides of the bag (a hole in the plastic was punctured through the connectors). The connectors facing the outside were then attached to vinyl tubing. One of the connectors directed the air emerging from the sealed bag into an O$_2$ sensor (a lead-O$_2$ battery, Model No. S-102, Qubit Systems Inc., Kingston, Ontario, Canada). The bags were sealed, leaving an empty space of 3–5 cm above the pupae. An air pump (0.4 liters/min) pumped air through the O$_2$ sensor after which it was directed into the sealed polyethylene bag. Thus, air was circulated throughout the entire experimental period in a closed circuit. The depletion of O$_2$ over time, as affected by respiration of the pupae in the sealed system, was registered by a data logger (Vernier Software and Technology, Beaverton, Oregon, U. S. A.) that generated 1 measurement per min. Each experiment was discontinued when measurements showed a stable low O$_2$ level (close to 0%) for a period of 10 min (“maximal hypoxia”). Experiments were conducted at 24°C except when otherwise specified.

**Irradiation and Quality Control Procedures**

In experiments where pupae were irradiated, a $^{60}$Co source in a Nordion Gamacell-220 (Nordion, Canada®) was used. Dose was calculated at 150 Gy. A Gafchromic dosimeter placed in the center of the bag, however, showed an average dose during the experiments of 157 Gy. The approximate time spent in the irradiation chamber for this dose was 8.5 min. After irradiation, samples of pupae were used to investigate the following quality control parameters: pupal weight, number of pupae in 5 mL, % adult emergence, and flight ability index (FAO/IAEA/USDA 2003). These quality control parameters were also determined for non-irradiated pupae of the same batch of the experiment (control).

An additional test investigated the mating competitiveness of flies in the different treatments in each of the experiments (including a non-irradiated control). For each treatment 25 sexually mature males (each marked with a dot of differently-colored paint on the prothorax to dif-
ferent treatments) were released into a field cage inside a greenhouse together with 25 non-irradiated and sexually mature virgin females (with an Egyptian genetic background that had been maintained in the laboratory since 1968). The greenhouse was kept at 25°C. A potted citrus tree, pruned to facilitate observations of mating pairs, was placed in the center of each cage. Matting pairs were counted once an hour for 12 continuous hours, giving 12 observations during the entire experiment. Each observation was considered a replicate. Differences in mating competitiveness between treatments was calculated from the average and variance of the number of mating pairs found during each 12-h observation period based on General Linear Models (Statgraphics 5 Plus 2000, Manugistics, Inc.).

Experiment 1: Effect of Pupal Age on \textit{O}_2\textit{Depletion in Sealed Bags}

Non-irradiated pupae 3, 2, and 1 d before adult emergence were used; 500 mL of pupae of each age were placed in separate polyethylene bags equipped with \textit{O}_2\textit{sensors as described above. For each pupal age, we used 2-3 replicates. A hermetically sealed bag with no pupae inside was used as a control. The real pupal ages were confirmed by using “pupal emergence grids”, which included a random sample of 100 pupae (one pupa per grid space), and by following the emergence from pupae over time. Oxygen levels were measured as described above at a constant temperature of 24°C. Airflow in the closed system was started when the bags were sealed (= time 0). The rate of \textit{O}_2\textit{ depletion was determined by measuring the \textit{O}_2\textit{ levels every min. Differences in \textit{O}_2\textit{ depletion rates (at 10 min after sealing), and time until the attainment of maximal hypoxia inside the bags, were investigated with a one-way ANOVA; means were separated by LSD (Statgraphics 5 Plus 2000, Manugistics, Inc.).

Experiment 2: Influence of Incubation Temperature on Attainment of Maximal Hypoxia and Quality of Irradiated Males

Polyethylene bags equipped with \textit{O}_2\textit{sensors as described above were filled with 450 mL of non-irradiated pupae at an estimated age of 1 d before adult emergence. The bags were laid flat to reduce the accumulation of metabolic heat and the pupae were then incubated for 1 h with the bags open (“preconditioning incubation”). Two preconditioning incubation temperatures were used, 16°C and 24°C; 16°C was selected because preconditioning incubations at 16°C are performed in some facilities to halt pupal development before irradiation (FAO/IAEA/USDA 2003). An incubator was used to attain 16°C while 24°C was the temperature in the experimental room. After incubation, bags were hermetically sealed and immediately connected to the closed airflow system to measure \textit{O}_2\textit{ consumption as specified above. Oxygen consumption was investigated under two constant temperature regimes (“post-sealing incubation temperatures”): 16°C and 24°C.

Measurements at 16°C were performed with the bag and the air line inside the incubator. For this, the door of the incubator was closed with the vinyl tubes emerging through the rubber door seal to conduct the air of the closed system to the \textit{O}_2\textit{ sensor. We ensured a free flow of air through the system without affecting the temperature inside the incubator. In the case of incubation at 24°C, the entire system was in a room maintained at this constant temperature. Thus, we had four treatments which combined the two preconditioning incubation temperatures (PC) and the two post-sealing-incubation temperature (PS): (1) 24°C PC and 24°C PS; (2) 24°C PC and 16°C PS; (3) 16°C PC and 24°C PS; and (4) 16°C PC and 16°C PS.

After reaching a constant level of maximal hypoxia in the sealed bag system, \textit{O}_2\textit{ measurements were discontinued, pupae were irradiated inside the sealed bags, bags were opened, and samples of pupae were subjected to quality control tests as specified above. We repeated the experiment twice with different batches of pupae.

Experiment 3: Oxygen levels in Packing Bags during Irradiation and Effect upon Male Fly Quality

As in Experiment 2 we used pupae at an estimated age of 1 d before adult emergence, placing 450 mL of non-irradiated pupae in four polyethylene bags. Three out of the 4 bags were sealed simultaneously but only 1 of the bags was used to monitor the \textit{O}_2\textit{ level. Since bags were sealed simultaneously with the same type and quantity of pupae, we made the assumption that the reading in the monitored bag would be representative of those in the other bags. The 4th bag was left unsealed. The sealed bags were then individually irradiated 15, 30, and 60 min after sealing, this being when the \textit{O}_2\textit{ level inside the monitored bag reached approximately 10%, 2%, and maximal hypoxia, respectively. In addition, we irradiated the unsealed bag that was presumed to have an ambient \textit{O}_2\textit{ environment during irradiation. Total irradiation time lasted approximately 8.5 min, during which interval the \textit{O}_2\textit{ level inside the sealed bags are presumed to have decreased further. Thus, the \textit{O}_2\textit{ levels mentioned above are those expected at the onset of irradiation. The experiment was replicated twice with different batches of pupae of the same age. After irradiation the bags were opened and samples of pupae from each bag were separately subjected to the quality control tests. Non-irradiated pupae which were not exposed to hypoxia were used as a control.
Experiment 4: Effect of Fluorescent Dye on $O_2$ Consumption Patterns and Irradiation Effects

Two polyethylene bags were each loaded with 500 mL of pupae at an expected age of 1 d before adult emergence. The pupae in 1 bag were thoroughly mixed with 0.75 g of Day-Glo® fluorescent powder. The second bag was untreated and used as a control. The bags were hermetically sealed and the consumption of $O_2$ over time measured as described above. After reaching maximal hypoxia the bags were irradiated, opened, and separate samples of pupae subjected to quality control tests. In this experiment we did not investigate mating competitiveness.

RESULTS

All samples of pupae placed in adult emergence grids confirmed that the expected ages of pupae used for these experiments were true ages (results not shown).

Experiment 1: Effect of Pupal Age on $O_2$ Depletion in Sealed Bags

Fig. 1 shows the effect of pupal age upon the consumption of $O_2$ by pupae in the closed air system. These data show that older pupae consume $O_2$ more rapidly than younger pupae. The rate of $O_2$ consumption 10 min after the bag was sealed was significantly faster in pupae 1 d before adult emergence than in pupae 2 or 3 d before emergence (Fig. 2; $F = 20.9; df = 2.6; P < 0.01$). Similarly, the consumption rate was significantly faster in pupae 2 d before adult emergence than 3 d before emergence. The time needed by pupae to reach maximal hypoxia in the bag was significantly longer in pupae 3 d before adult emergence than in pupae 2 or 1 d before emergence (Fig. 2; $F = 9.3; df = 2.6; P < 0.05$).

Experiment 2: Influence of Incubation Temperature on Attainment of Maximal Hypoxia and Quality of Irradiated Males

The rate at which $O_2$ was consumed by pupae in hermetically sealed bags when incubated at different preconditioning (PC) and post-sealing-bag (PS) temperatures is shown in Fig. 3. Pupae incubated at 24°C during both PC and PS consumed $O_2$ faster than pupae incubated first at 24°C during PC and then transferred after 1 h to 16°C for PS incubation (Fig. 3A). Similarly, pupae incubated during PC at 16°C and then transferred to 24°C consumed $O_2$ faster than pupae incubated at 16°C for both PC and PS (Fig. 3B). The average durations (2 replicates) needed for pupae to reach maximal hypoxic conditions during these different schedules were 57.5 min for 24°C PC + 24°C PS; 87.0 min for 24°C PC + 16°C PS; 98.0 min for 16°C PC + 24°C PS; and 148.5 min for 16°C PC + 16°C PS.

Table 1 shows results of the quality control tests after pupal irradiation under the 4 temperature combination protocols and in the control. Average and standard deviation are for 2 replicate experiments. Mating competitiveness test was performed only with 1 of the replicates. Pupal weight, number of pupae in 5 mL, % adult emer-
gence and flight ability did not differ significantly between treatments and control. Similarly, incubation temperature protocols did not have any significant effect upon the mating competitiveness of irradiated males (Fig. 4). However, as expected the mating competitiveness of non-irradiated males (control) was significantly greater than that of irradiated males (Fig. 4).

Experiment 3: Oxygen levels in Packing Bags during Irradiation and Effect upon Male Fly Quality

Fig. 5 shows the approximate O₂ levels: I, in an open bag (ambient O₂ environment); II, 15 min after sealing (± 10% O₂ level); III, 30 min after sealing (± 2% O₂ level); IV, maximal hypoxia (close to 0% O₂ level). The rate of O₂ consumption and the time needed to reach maximal hypoxia were very similar in the 2 replicates: at 10 min after bag-sealing O₂ consumption was 9.6 and 9.2 µL O₂/mL air/min, and maximal hypoxia was reached after 52 and 58 min, in replicates 1 and 2, respectively.

Table 2 shows the effects of irradiation under several O₂ environments in packing bags upon pupal weight, number of pupae in 5 mL, % adult emergence, and flight ability. Average and variance are for 2 replicate experiments. The mating competitiveness test was performed only with one of the replicates. None of the treatments with reduced O₂ levels had a significant effect upon the
quality control parameters (Table 2). In contrast, irradiation under ambient O$_2$ level, although not statistically significant, reduced adult emergence and flight ability, and significantly affected the mating competitiveness of irradiated males (Table 2, Fig. 6). As expected, non-irradiated males (control) performed significantly better than sterile males in the mating competitiveness test (Fig. 6).

**DISCUSSION**

With no renewal of O$_2$, pupae sealed in bags were expected to totally deplete the O$_2$ levels of the air through their metabolic activity (FAO/IAEA/USDA 2003). This study clearly showed that the O$_2$ level inside sealed bags loaded with pupae steadily declines over time. Time needed for the attainment of maximal hypoxia was dependent not only on the age of the pupae, but also similar: 8.7 and 8.6 µL O$_2$/mL air/min, respectively. Time to reach maximal hypoxia was 58 min for dyed pupae and 60 min for undyed pupae. Likewise, pupal weight, number of pupae in 5 mL, % emergence and flight ability was very similar between the 2 treatments, and comparable to the unirradiated control (data not shown).

**TABLE 1. Effect of different pre-irradiation temperature regimes on quality control parameters of irradiated medflies.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg. pupal weight (mg/pupae) ± SD</th>
<th>Avg. no. pupae in 5 mL ± SD</th>
<th>Avg. adult emergence (%) ± SD</th>
<th>Flight ability index (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>24°C→24°C</td>
<td>8.65 ± 0.07</td>
<td>285 ± 1</td>
<td>87.7 ± 9.1</td>
<td>84.8 ± 8.6</td>
</tr>
<tr>
<td>24°C→16°C</td>
<td>8.50 ± 0.28</td>
<td>270 ± 25</td>
<td>83.1 ± 16.5</td>
<td>85.7 ± 10.4</td>
</tr>
<tr>
<td>16°C→24°C</td>
<td>8.70 ± 0.14</td>
<td>285 ± 3</td>
<td>86.7 ± 12.3</td>
<td>86.2 ± 9.7</td>
</tr>
<tr>
<td>16°C→16°C</td>
<td>8.55 ± 0.07</td>
<td>291 ± 13</td>
<td>84.2 ± 13.3</td>
<td>86.0 ± 7.5</td>
</tr>
<tr>
<td>Control**</td>
<td>8.75 ± 0.07</td>
<td>298 ± 12</td>
<td>92.7 ± 2.6</td>
<td>91.1 ± 7.5</td>
</tr>
</tbody>
</table>

$\text{H}^{***}\quad 4.1 \quad 3.7\quad 1.0\quad 1.5$

$P >0.05\quad >0.05\quad >0.05\quad >0.05$

*Preconditioning Temperature → Post-Sealing Temperature.

**Control consisted of non-irradiated pupae, and pupae that did not undergo hypoxia treatment and preconditioning and post-sealing-bag temperature incubations. Control pupae were maintained at 24°C until processing.

***$H$—Kruskal-Wallis Non-Parametric one way ANOVA.

Experiment 4: Effect of Fluorescent Dye on O$_2$ Consumption Patterns and Irradiation Effects

Oxygen consumption curves of dyed pupae and undyed pupae 10 min after bag sealing were very similar: 8.7 and 8.6 µL O$_2$/mL air/min, respectively. Time to reach maximal hypoxia was 58 min for dyed pupae and 60 min for undyed pupae. Likewise, pupal weight, number of pupae in 5 mL, % emergence and flight ability was very similar between the 2 treatments, and comparable to the unirradiated control (data not shown).

**Fig. 4. Mating competitiveness (ability to form copulating pairs) of irradiated and non-irradiated (control) male medflies (VIENNA 8). Irradiated males were subjected to different incubation protocols (preconditioning, PC, and post-sealing-bag, PS, incubation temperatures protocols) before irradiation. Irradiation was performed after reaching maximal hypoxia in sealed bags during PS incubation. The figure includes the resultant $F$ and $P$ (General Linear Models) and step-wise separation of means (lower case letters).**

**Fig. 5. Oxygen consumption curve and level of hypoxia attained for medfly pupae (VIENNA 8) 1 d day before emergence packed in sealed bags: I. ambient O$_2$ level during irradiation; II. 10% O$_2$ level at the onset of irradiation; III. 2% O$_2$ level at the onset of irradiation; IV. Maximal hypoxia at the onset of irradiation.**
on the temperature at which pupae are maintained. These results support the findings of Langley (1970) and Seo et al. (1990), that the rate of O₂ consumption is strongly dependent upon the rate of metabolic activity. Metabolic activity and respiration rate increased significantly in pupae close to adult emergence, and in pupae kept at high temperatures (Keister & Buck 1973; Seo et al. 1990).

Pre-conditioning (PC) and post-sealing (PS) incubation temperatures had a marked effect upon the consumption of O₂ by pupae. As expected for a poikilotherm organism, incubation at 24°C accelerated the consumption of O₂ and the metabolism of pupae, while 16°C had a depressing effect upon both metabolism and O₂ consumption. The transfer of pupae from a PC temperature of 24°C to a PS temperature of 16°C reduced O₂ consumption to a lesser extent than the opposite situation, suggesting that it takes more time to warm up pupae and accelerate metabolic rate than to slow down metabolism with cooler temperatures. While temperature manipulation prior to attainment of hypoxia and irradiation affected the rate of O₂ consumption, these incubation protocols did not have any noticeable effect upon pupal quality and mating performance. In contrast, ambient O₂ levels inside bags during irradiation had, as previously demonstrated (Ohinata et al. 1977), an important negative effect upon mating activity and pupal quality.

The mechanism by which different O₂ environments during irradiation affect the quality of the fly has still not been fully investigated. However, the most acceptable hypotheses suggest that low O₂ tension in pupal tissue reduces the formation of toxic free radicals and peroxides during irradiation (Ohinata et al. 1977). Regardless of the mechanism by which the O₂ atmosphere influences the quality of the irradiated fly, the present study confirmed the fact that a hypoxic environment during irradiation enhances the mating performance and quality of sterile pupae (Bakri et al. 2005).

It is interesting to note that irradiation under 10% and 2% O₂ environments resulted in flies with similar mating competitiveness and quality to those irradiated under maximal hypoxia. This phenomenon could be the result of ongoing pupal metabolic activity, and O₂ consumption, during the time spent inside the irradiation chamber (8.5 min), which may have reduced further the O₂ levels inside the sealed bags. A further possibility is that below certain O₂ level inside the bags, the “Oxygen effect” (Hooper 1971; Ohinata et al. 1977) is not manifested. Our study was not able to detect this “threshold” O₂ level, which may be of theoretical, but not practical, interest.

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**Table 2. Effect of different pre-irradiation oxygen levels on quality control parameters of the irradiated medfly.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg. pupal weight (mg/pupae) ± SD</th>
<th>Avg. no. pupae in 5 mL ± SD</th>
<th>Avg. adult emergence (%) ± SD</th>
<th>Flight ability index (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum hypoxia</td>
<td>8.40 ± 0.30</td>
<td>298 ± 8</td>
<td>89.2 ± 0.9</td>
<td>82.6 ± 1.8</td>
</tr>
<tr>
<td>2% O₂</td>
<td>8.50 ± 0.30</td>
<td>303 ± 1</td>
<td>90.9 ± 2.9</td>
<td>84.2 ± 1.9</td>
</tr>
<tr>
<td>10% O₂</td>
<td>8.45 ± 0.20</td>
<td>302 ± 4</td>
<td>89.6 ± 1.8</td>
<td>81.6 ± 4.7</td>
</tr>
<tr>
<td>Ambient O₂</td>
<td>8.55 ± 0.20</td>
<td>303 ± 11</td>
<td>85.3 ± 6.8</td>
<td>67.4 ± 13.4</td>
</tr>
<tr>
<td>Control*</td>
<td>8.45 ± 0.40</td>
<td>301 ± 8</td>
<td>89.4 ± 2.3</td>
<td>86.3 ± 0.7</td>
</tr>
</tbody>
</table>

*Control consisted on non-irradiated pupae that did not undergo hypoxia treatment. Control pupae were maintained at 24°C until processing.

**H—Kruskal-Wallis Non-Parametric One Way ANOVA.

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**Fig 6. Mating competitiveness (ability to form copulating pairs) of irradiated and non-irradiated (control) male medflies (VIENNA 8) as affected by irradiation under different O₂ environments. Hypoxia levels as shown in Fig. 5: 60 min = maximal hypoxia inside sealed packing bags; 30 min = 2% O₂ level at the onset of irradiation; 15 min = 10% O₂ level at the onset of irradiation; 0 min = open bags with ambient O₂ levels. Control males were neither irradiated nor underwent hypoxia. The figure includes the resultant F and P (General Linear Models) and step-wise separation of means (lower case letters).**
In practical terms, this study demonstrated that hermetically sealing bags containing pupae for irradiation, and keeping these pupae for approximately 1 h at temperatures of 24°C, is sufficient to create an optimal hypoxic environment inside these bags. It also demonstrated that routine treatment of pupae with fluorescent dye (1.5 g/kg of pupae) did not appear to affect the ability of pupae to consume O₂ inside the bags. The study also provided data on the effect of different incubation temperatures upon the ability of pupae to create hypoxic atmospheres inside packing bags, and upon the effects of some pre-irradiation incubation protocols currently carried out in several medfly rearing facilities. Specifically, our data suggests that keeping pupae in open bags at 16°C until irradiation may affect their later ability to create an optimal hypoxic environment inside sealed bags before and during irradiation. These last results, and the cost/benefit evaluations of incubating procedures, need to be re-assessed before final recommendations are made.

ACKNOWLEDGMENTS

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REFERENCES CITED


STERILE INSECT TECHNIQUE: A MODEL FOR DOSE OPTIMIZATION FOR IMPROVED STERILE INSECT QUALITY

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ABSTRACT

The sterile insect technique (SIT) is an environment-friendly pest control technique with application in the area-wide integrated control of key pests, including the suppression or elimination of introduced populations and the exclusion of new introductions. Reproductive sterility is normally induced by ionizing radiation, a convenient and consistent method that maintains a reasonable degree of competitiveness in the released insects. The cost and effectiveness of a control program integrating the SIT depend on the balance between sterility and competitiveness, but it appears that current operational programs with an SIT component are not achieving an appropriate balance. In this paper we discuss optimization of the sterilization process and present a simple model and procedure for determining the optimum dose.

Key Words: SIT, model, competitiveness, sterility, radiation dose

RESUMEN

La técnica de insecto estéril (TIE) es una tecnología de control de plagas favorable para el medio ambiente con una aplicación de un control integrado de plagas claves para toda la área, incluyendo la supresión o eliminación de poblaciones introducidas y la exclusión de nuevas introducciones. La esterilidad reproductiva es normalmente inducida por radiación ionizada, un método conveniente y consistente que mantiene un grado razonable para la capacidad de competencia en insectos liberados. El costo y la eficacia de un programa de control que incluye TIE dependen en tener un balance entre la esterilidad y la capacidad para competir, pero parece que los programas operacionales corrientes con TIS como un componente no están logrando el tener un balance apropiado. En esta publicación, nosotros discutimos la optimización del proceso de esterilización y presentamos un modelo y procedimiento sencillos para determinar la dosis óptima.

The sterile insect technique (SIT) was conceived in the 1930s (Knipling 1955), and first applied on a significant scale in the 1950s against the New World screwworm Cochliomyia hominivorax (Coquerel) (Baumhover et al. 1955; Knipling 1960) and subsequently to a number of other pest species (Dyck et al. 2005). The principle of the technique is to introduce sterility by rearing large numbers of the target pest, reproductively sterilize them, and release them into the wild. When the sterile males mate with wild females, the females produce no viable offspring. With a constant rate of release of sterile insects this results in an increasingly rapid decline in the overall population over several generations. This technique has been used successfully against a number of pest species such as Mediterranean fruit fly Ceratitis capitata (Wiedemann), melon fly Bactrocera cucurbitae (Coquillet), pink bollworm Pectinophora gossypiella (Saunders), codling moth Cydia pomonella (L.) and tsetse fly Glossina austeni Newstead (Tan 2000; Wyss 2000; Hendrichs et al. 2005; Klassen & Curtis 2005).

The attractive features of the SIT are that it is absolutely specific to the targeted pest, integrates well with other controls, reduces the use of toxic insecticides, and its action is inverse-density dependent. This latter characteristic implies that as the field population declines, the pressure increases on the population from a constant rate of sterile insect release; this characteristic makes it desirable for eradication, suppression, containment, or the exclusion of sporadic introductions in a preventive release program (Hendrichs et al. 2005). The inverse-density dependence of the technique makes it possible, as part of a systems approach, to eliminate or reduce pests to such low levels as to allow export of important commodity crops to areas with quarantine restrictions against the pest.

Sterility can be induced by chemicals or ionizing radiation. Chemical sterilization was used in early work (Borkovec 1966; LaChance 1967; Labrecque & Smith 1968), but because of the hazard of handling these substances, problems with controlling the dose, and the risks of environmental
contamination, chemical sterilization has been replaced by irradiation (Hayes 1968; Bakri et al. 2005a; Bakri et al. 2005b).

When biological material is irradiated, free radicals are formed, and breaks are created in the chromosomes. If breakage occurs in chromosomes of the germ line, this leads to the formation of dominant lethal mutations in eggs and sperm (LaChance 1967; Curtis 1971). Radiation sterilization is a simple process with easy and reliable quality control procedures. The action of the radiation is immediate so there is no requirement to hold the sterile insects after treatment, and radiation can pass through packaging material allowing the insects to be treated after sealing in secure packaging enhancing biosecurity and reducing handling.

**DOSE OPTIMIZATION**

The radiation absorbed dose (referred to hereafter as dose) that is used to induce sterility is of prime importance to programs that include the release of sterile insects. Insects that receive too low a dose are not sufficiently sterile and those that receive too high a dose may be uncompetitive, reducing the effectiveness of the program by requiring that a greater number of sterile insects must be released (Robinson 2002).

While competitiveness has often been investigated (Hooper 1970; Hooper & Katiyar 1971; Hooper 1972; Katiyar 1973a, b; Hooper 1975; Zumreoglu et al. 1979; Winstead et al. 1990; Haynes & Smith 1992; Boshra 1994; Saour & Makkee 1997; Bloem et al. 1998; Bloem et al. 1999; Bloem et al. 2004; Toledo et al. 2004), the critical balance between sterility and competitiveness has rarely been investigated or discussed in sufficient detail, and few data have been presented in the literature in a form that permits a proper analysis of this balance (Bakri et al. 2005a). In order to perform the analysis, data are required simultaneously for the variation of both sterility and competitiveness with dose. Where competitiveness has been studied, frequently only one or two doses have been investigated. Further, for the competitiveness data to be realistic, the tests should be performed in field cages or open plots.

The relationship between residual fertility and log(dose) is well known and is sigmoid in form. Not enough data are available to be certain of the relationship between competitiveness and log(dose), but for simplicity we assume it to be similar to most response-to-dose relationships, which are sigmoid (Finney 1971); however any monotonic decreasing function will lead to similar conclusions to those presented below. Fig. 1 illustrates the relationships of fertility and competitiveness to log(dose) following this assumption, where the scale on the x-axis is such that one unit represents the change in log(dose) needed to produce one $\sigma$ change in the response (competitiveness or fertility). The displacement, $\delta x$ (in units of $\sigma$) of the competitiveness curve to the right (or left) of the fertility curve is generally unknown, but must vary with species and other factors such as the oxygen content of the atmosphere and temperature during irradiation, free radical scavengers provided in the diet, quality of rearing, and possibly other factors. Considerable research related to the SIT is to improve the competitiveness of the insects for a given sterility level, that is to move the competitiveness line as far to the right as possible, and thus to increase the value of $\delta x$.

Knipling (1955) presented a simple relationship for the effect of released sterile insects on a wild population. This may be written as:

$$F_1 = P \times (1 - S) \times R$$

where $F_1$ is the population size in the filial generation, $P$ is the parental generation size, $R$ is the net population growth rate per generation, and $S$ is the sterility induced by the released sterilized insects (IAEA 1992, pp. 108-109). In practice $R$ is likely to be density dependent, but for this simple model it is assumed to be density independent, and $S$ is dependent on the number of sterile insects released ($N$) if it is assumed that the released insects are both fully sterile and fully competitive:

$$S = \frac{N}{(N + P)}$$

This, however, does not take into account either of incomplete sterility induced by the irradiation ($S_l$), or of the reduced competitiveness ($Q$). To sim-
plify the equations, the reduced competitiveness of the 𝑁 released insects can be represented as 𝑁𝑄 fully competitive insects (and 𝑁(1-𝑄) non-competitive insects that have no effect on the target population), and the reduced sterility as 𝑁𝑄𝑺 steriles and 𝑁𝑄(1-𝑺) fertile insects. This simplification does not affect the final form of the relationship. These 𝑁𝑄(1-𝑺) fertile individuals add to the pool of breeding individuals, so that:

\[ P' = P + NQ(1 - S_I) \]

and equation [2] becomes:

\[ S' = \frac{NQS_I}{NQS_I + P + NQ(1 - S_I)} = \frac{NQS_I}{NQ + P} \]

The original equation [1] now becomes:

\[ F_I = P' \times (1 - S') \times R \]

or:

\[ \frac{F_I}{R} = (P + NQ(1 - S_I)) \times \left(1 - \frac{NQS_I}{NQ + P}\right) \]

Regression analysis of both Probit transformed competitiveness (𝑄) and residual fertility (1-𝑺) against log radiation dose will yield a relationship that may be used to predict both parameters for any radiation dose. Equation [6] can then be solved numerically by iteration to find the minimum value of \(F_1/R\) for given values of \(\delta x\) and 𝑁/𝑃. Using values of \(R = 1\), 𝑃 = 1 and 𝑁 = 9, Fig. 2 shows the effect on the subsequent generation \(F_I\) of log(dose) at 3 values of \(\delta x\) for a fixed release rate. This clearly shows that as the value of \(\delta x\) increases the value of \(F_{I_{\text{Minimum}}}^1\) decreases and this minimum point occurs at a higher sterility. At the same time the slope of the \(F_I\) curve each side of the optimum point gets shallower, implying that a larger dose variation may be tolerated. This has the potential to increase the throughput of the irradiation process as less strict limits need to be applied.

This indicates that research is essential to establish the relationship of dose to the level of sterility and competitiveness in the treated insects, and that a standardized dosimetry system and recognized dosimetry procedures are used (ISO/ASTM 2005a). The dose to be used for any given SIT program is then based on the results of such studies. The program manager should specify the optimum dose to achieve the best combination of competitiveness and sterility (Table 1), and this dose should be reviewed when changes in any procedure alter the value of \(\delta x\).

Ideally, all the insects should be irradiated to receive this optimum dose, but as the dose rate varies spatially within a container, it is inevitable that insects within will receive a range of doses. Because of this dose variability the program manager should also specify the minimum and maximum acceptable dose that insects may receive. If the dose variability within the container is too high, it may be necessary to modify the radiation field (e.g., with a field flattener, a shaped lead shield that improves the dose uniformity ratio) or limit the volume used for irradiation by blocking off areas with unacceptably high and/or low dose rates. The range of acceptable doses should be approximately symmetric about the optimum dose (in log(dose)), as shown by the symmetry of the \(F_I\) curves (Fig. 2). We suggest that the maximum and minimum dose should be set to yield \(F_I\) values not more than 110% of \(F_{I_{\text{Minimum}}}^1\).

For many insects, the dose required in the late pupal stage to stop egg production or egg hatch in females is lower than the dose required to induce sterility in males (Bakri et al. 2005a). For most purposes, therefore, the minimum dose will be set higher than the dose at which egg production or hatch stops. For legal or other justifiable program requirements a higher minimum dose may be specified, but it must be recognized that this may affect the program efficiency (Toledo et al. 2004).

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Fig. 2. Size of next generation \(F_I\) as a function of log(dose) for \(\delta x = 1\sigma, 3\sigma\) and \(5\sigma\).
The actual dose applied in different programs and research projects has varied widely, by a factor of almost 3 for some species (i.e., *Sitophilus granarius* L. which varies between 50 and 135 Gy) as shown by the International Database on Insect Disinfestation and Sterilization website (IAEA 2003; Bakri et al. 2005a). From Table 1 it can be seen that the optimum dose only yields 95% sterility (5% residual fertility) when \( \delta x \) is about 1.8 and 99.9% when \( \delta x \) is greater than 5. It is unlikely that \( \delta x \) will ever be as large as 5, but because of the lack of appreciation for the insect competitiveness issues involved, many programs continue to use 99.9% sterility when lower doses would yield better control.

The optimum dose depends furthermore on the ratio \( N/P \) (Table 2). In the early stages of a suppression or eradication campaign, while the wild population is still relatively large and the ratio \( N/P \) is small, the optimum dose is lower than later in the program when the value of \( N/P \) is larger. It would thus appear that current operational programs releasing sterile insects are not achieving the appropriate balance between sterility and competitiveness at each stage in the program. Table 2 may be used to estimate the optimum dose in Gy for any given value of \( \delta x \) at various ratios of \( N/P \). If the regression equation for the dose-fertility relationship, with dose in Gy transformed to log(dose) and fertility to normal equivalent deviates (NED) is:

\[
NED(fertility) = a + b \times \log(dose)
\]

then the actual dose in Gy can be calculated from the values of D in Table 2 as:

\[
dose(Gy) = \frac{-D + a}{b}
\]

The value of \( \delta x \) can be estimated from a simple field cage experiment, but an adequate set of field cage data to determine the dose-response relationship has not been published. In order to illustrate the concept, the extensive set of laboratory data for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) presented by Hooper (1972) for fertility and competitiveness (Haisch 1970; Fried 1971) at various treatment doses is used. The main purpose of this illustration is to demonstrate the procedure for determining the optimum dose from relevant data. Using Hooper’s data, we show the relationships of fertility and competitiveness to the radiation dose in Fig. 3, with the linear regression lines and 95% confidence intervals for the regressions. The regression fit for the fertility is very good, but there is a larger scatter in the competitiveness values. This is inherent in the method of measuring and calculating competitiveness (Haisch 1970; Fried 1971; Hooper & Horton 1981; Iwahashi et al. 1983). The regression coefficients for competitiveness and fertility do not differ significantly from each other (competitiveness: regression coefficient = -3.4032, SE = 0.3326; fertility: regression coefficient = -3.8866, SE = 0.5403) (Sokal & Rohlf 1981). The value of \( \delta x \) from these data is 1.44, and from the fertility relationship the increase in log(dose) that results in a 1σ change in fertility is 0.26, the reciprocal of the slope of the linear regression line.

From these values the optimum dose can be estimated from Table 2 and equation [8]. In the present example, where \( \delta x = 1.44 \), for \( N/P = 8 \), from the table the value of D is 1.45. Based on equation [8]:

\[
dose(Gy) = \frac{-1.45 + 5.4955}{-3.8866} \approx 61
\]
**Table 3. Optimum radiation dose (D, in units of \( \sigma \)) above the log(dose) that yields 0.5 residual fertility and corresponding sterility level (in italics) for selected values of \( \delta x \) and \( N/P \) (the ratio of sterile to wild males).**

<table>
<thead>
<tr>
<th>( \delta x )</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.22</td>
<td>0.34</td>
<td>0.50</td>
<td>0.67</td>
<td>0.86</td>
<td>1.04</td>
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<td>1.40</td>
</tr>
<tr>
<td></td>
<td>58.7%</td>
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<td>80.5%</td>
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<td>88.9%</td>
<td>91.9%</td>
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<td>1.32</td>
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<td>4.5</td>
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<td>2.49</td>
<td>2.60</td>
<td>2.72</td>
<td>2.86</td>
<td>3.00</td>
<td>3.14</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>99.2%</td>
<td>99.4%</td>
<td>99.5%</td>
<td>99.7%</td>
<td>99.8%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>99.9%</td>
</tr>
<tr>
<td>5</td>
<td>2.64</td>
<td>2.72</td>
<td>2.82</td>
<td>2.93</td>
<td>3.06</td>
<td>3.19</td>
<td>3.32</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td>99.6%</td>
<td>99.7%</td>
<td>99.8%</td>
<td>99.8%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

The value of \( \delta x \) is overestimated by the data in Hooper (1972), as the competition was between irradiated and unirradiated colony flies under laboratory conditions, not between irradiated colony flies and wild flies in the field. This colony has been maintained under artificial conditions for many generations and can be expected to have competitiveness less than 1 before irradiation. Irradiated colony flies could therefore be expected to perform worse against wild flies than against colony flies. Wong et al. (1983) compared mating success between irradiated and wild males of *Ceratitis capitata*, and found no difference over a range

![Fig. 3. Relationships of fertility (squares) and competitiveness (diamonds) with dose for the Mediterranean fruit fly (data from Hooper 1972). NED = normal equivalent deviates.](image-url)
of doses, but there is an urgent need for additional field cage or field data on competitiveness over a range of radiation doses to determine the dose relationship and thereby the magnitude of $\delta x$.

**DOSIMETRY AND THE IRRADIATION PROCESS**

Dosimetry plays a crucial role throughout the radiation sterilization process of insects. At the research phase, where the effect of radiation on sterility as well as on competitiveness of the insects is investigated, radiation dose is the key quantity. At the production facility, dosimetry also has several essential roles. First, it assists in the characterization of the irradiator, and in the regular monitoring of its consistent operation. It also helps in determining the correct size and shape of the canister and other key process parameters for irradiation of the insects. And later during the sterilization process, it provides an important element of process control.

Considering the importance of dosimetry in programs applying the SIT, the specific requirements of SIT programs, especially the useful dose range of 50-600 Gy and a low cost (IAEA 2004). This reference also describes relevant dosimetry procedures as well as various components of this dosimetry system.

Accidental release of insects that are significantly under-dosed will require rapid correction by release of additional sterile insects and other measures, especially for programs like those in California and Florida, USA., where SIT is used for eradication of extremely small introductions and/or as a prophylactic measure to prevent establishment of newly introduced flies (Dowell et al. 2000). Besides administrative control, there are 3 main process control elements that are in place that would minimize the chances of such accidents (FAO/IAEA/USDA 2003). These different elements control various steps in the process and thus complement each other as follows: (1) Sterility Testing—In any SIT program, sterility testing through bioassays should be carried out on a regular basis to confirm that all the procedures are being followed correctly, including the rearing, the pre-irradiation preparation (such as age-based selection of insects, packaging for hypoxia or nitrogen, if used), temperature control, irradiation dose control, and post irradiation handling, (2) Routine Dosimetry—The purpose of dosimetry in process control is to monitor that all the canisters (and hence all the insects) are receiving the dose within the specified range, and (3) Radiation-Sensitive Indicators—This control element provides an immediate visual check at irradiation facilities and at pupal reception/fly emergence.

### Table 3. Optimum Dose, Minimum and Maximum Doses, and the Corresponding Sterility, Competitiveness and $F_1$ for Mediterranean Fruit Fly, for $\delta x = 1.44$ and Various Values of $N/P$ (Derived from Data in Hooper 1972).

<table>
<thead>
<tr>
<th>Dose</th>
<th>Gy</th>
<th>Sterility</th>
<th>Competitiveness</th>
<th>$F_1$</th>
<th>$F_1/F_1$ Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta x = 1.44$</td>
<td>$N/P = 3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>39</td>
<td>74.8%</td>
<td>78.0%</td>
<td>0.476</td>
<td>1.10</td>
</tr>
<tr>
<td>Optimum</td>
<td>52</td>
<td>88.0%</td>
<td>60.4%</td>
<td>0.433</td>
<td>1.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>70</td>
<td>95.3%</td>
<td>40.7%</td>
<td>0.476</td>
<td>1.10</td>
</tr>
<tr>
<td>103 Gy</td>
<td>103</td>
<td>99.9%</td>
<td>18.8%</td>
<td>0.643</td>
<td>1.49</td>
</tr>
<tr>
<td>162 Gy</td>
<td>103</td>
<td>99.9%</td>
<td>4.9%</td>
<td>0.871</td>
<td>2.01</td>
</tr>
<tr>
<td>$\delta x = 1.44$</td>
<td>$N/P = 9$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>49</td>
<td>85.9%</td>
<td>64.1%</td>
<td>0.267</td>
<td>1.10</td>
</tr>
<tr>
<td>Optimum</td>
<td>61</td>
<td>92.7%</td>
<td>49.3%</td>
<td>0.243</td>
<td>1.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>77</td>
<td>96.6%</td>
<td>34.8%</td>
<td>0.267</td>
<td>1.10</td>
</tr>
<tr>
<td>103 Gy</td>
<td>103</td>
<td>99.0%</td>
<td>18.8%</td>
<td>0.378</td>
<td>1.56</td>
</tr>
<tr>
<td>162 Gy</td>
<td>103</td>
<td>99.9%</td>
<td>4.9%</td>
<td>0.692</td>
<td>2.85</td>
</tr>
<tr>
<td>$\delta x = 1.44$</td>
<td>$N/P = 100$</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>77</td>
<td>96.7%</td>
<td>34.7%</td>
<td>0.060</td>
<td>1.10</td>
</tr>
<tr>
<td>Optimum</td>
<td>89</td>
<td>98.1%</td>
<td>26.0%</td>
<td>0.055</td>
<td>1.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>103</td>
<td>99.0%</td>
<td>18.5%</td>
<td>0.060</td>
<td>1.10</td>
</tr>
<tr>
<td>103 Gy</td>
<td>103</td>
<td>99.0%</td>
<td>18.8%</td>
<td>0.060</td>
<td>1.09</td>
</tr>
<tr>
<td>162 Gy</td>
<td>103</td>
<td>99.9%</td>
<td>4.9%</td>
<td>0.169</td>
<td>3.08</td>
</tr>
</tbody>
</table>
centers that a given container has gone through the irradiation process.

CONCLUSIONS

For SIT, ionizing radiation is the method of choice for inducing reproductive sterility. The sterilization process is important in determining the quality of the released insects and their ability to compete with the wild population. Thus, optimization of the sterilization process is critical for the efficacy of SIT programs and should be given due consideration. We believe that doses lower than currently applied will result in a more effective SIT program, with any increase in residual fertility more than compensated for by the increased competitiveness of the released insects.

We have developed a quantitative procedure for determining the optimum dose based on fertility and competitiveness data. In order to estimate the optimum dose, it will be necessary to calculate correlations between dose and both fertility and competitiveness. The fertility relationship is already known for many insects, so attention should be concentrated on collecting data on competitiveness over a suitable range of doses. As the optimum also depends on the ratio of sterile to fertile males, the treatment dose should be reviewed constantly during the progress of a program. Optimization can lead to significant reduction in program cost and increase in programme efficiency.

The dose of radiation can be readily measured with a standardized dosimetry system, such as the Gafchromic® system (IAEA 2004; ISO/ASTM 2005c). A dosimetry system that is traceable to national or international standards can be reliably used both for setting the dose for the radiation sterilization process and for routine process control.

ACKNOWLEDGMENTS

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REFERENCES CITED


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INDUCTION OF STERILITY IN ANASTREPHA FRATERCULUS (DIPTERA: TEPHRITIDAE) BY GAMMA RADIATION

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1Comisión Nacional de Energía Atómica, CNEA
2Estación Experimental Agro-industrial Obispo Columbres, Tucumán, Argentina

ABSTRACT

In relation to the application of the sterile insect technique (SIT) for the South American fruit fly Anastrepha fraterculus (Wiedemann), we analyzed the effect on adult fertility of different doses of gamma irradiation and the age of pupae at the time of irradiation. In a first experiment, we applied doses of 50, 70, and 90 Gy to pupae at 24, 48, 72, and 96 h before adult emergence. In a second experiment we irradiated pupae 48 h before emergence with 20, 40, and 60 Gy and estimated male and female fertility and sperm transfer by irradiated males. The results indicated pupal age at irradiation does not significantly affect male fertility. If males irradiated with 60 Gy are crossed to non-irradiated females the fertility is about 1%. Females irradiated with 40 Gy did not lay eggs independently of the male to which they mated. No significant effects of radiation were observed with respect to the ability of males to transfer sperm. A dose of 70 Gy applied 48 h before adult emergence induces 100% sterility in both males and females.

Key Words: SIT, South American fruit fly, fertility, sperm transfer, sterility, pupal age

RESUMEN

Para la aplicación de la técnica del insecto estéril (TIE) en Anastrepha fraterculus (Wiedemann), en este trabajo analizamos el efecto de diferentes dosis de irradiación gamma y la edad óptima de la pupa al momento de la irradiación. En el primer experimento se evaluaron las dosis de 50, 70, y 90 G y en pupas de 24, 48, 72, y 96 h antes de la emergencia del adulto. En el segundo experimento se irradiaron pupas 48 h antes de la emergencia con dosis de 20, 40, 60 G y se estimó la fertilidad de los machos y las hembras, y la transferencia de espermas por los machos irradiados. Los resultados indicaron que la irradiación no modificó significativamente la fertilidad de los machos. En las cruzas de machos irradiados a 60 G con hembras no irradiadas se observó 1% de eclosión larvaria, mientras que las hembras irradiadas a 40 G no pusieron huevos. La irradiación no afectó significativamente la transferencia de espermas de los machos tratados. Por lo tanto, una dosis de 70 G aplicada 48 h antes de la emergencia del adulto induce 100% de esterilidad tanto en machos como en hembras.

Translation provided by the authors.

The South American fruit fly Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) is an important pest for fruit production in Argentina (Stone 1942). This species is native to the Americas, most probably South America, and is widely distributed throughout the tropical and subtropical regions (between the latitudes 27°N and 35°S). Its range includes southern USA (South Florida and Rio Grande Valley, Texas), Central America, Caribbean Islands, and South America, from Trinidad and Guyana to Central Argentina (Steck 1999; Aluja 1994; Hernández-Ortiz 1992).

There are at least 80 host species of A. fraterculus, including many economically important fruit species (Norrbom & Kim 1988). Tropical fruit flies not only cause great losses in fruit and vegetable production, but they also seriously impede international trade because of quarantine regulations (Klassen & Curtis 2005). In particular, the presence of A. fraterculus in the orchards reduces the possibility of exporting fruits and other horticultural products to the northern hemisphere (SENASA 1997). The export of fruits and vegetables to pest free areas or those that have implemented control programs against this pest requires the application of a quarantine treatment. Another common problem is that the intensive use of chemical insecticides is associated with environmental contamination. Furthermore, insects have been found to develop resistance to almost every chemical class of insecti-
cide (Brown & Payne 1988). This includes some tephritids, such as Bactrocera oleae (Gmelin) (Vontas et al. 2002) and Bactrocera dorsalis Hendel (Hsu et al. 2004).

Recent studies indicate that populations of A. fraterculus from Argentina and Southern Brazil are not differentiated genetically (Alberti et al. 2002) and that 4 populations from different regions of Argentina do not show reproductive isolation (Petit-Marty et al. 2004). These findings suggest that the sterile insect technique (SIT) might be applied successfully against A. fraterculus at least at a regional scale.

In other tephritids, such as Ceratitis capitata (Wiedemann), the irradiation process may reduce the mating performance of the sterilized males (Calcagno et al. 2002; Lux et al. 2002). An essential requirement for a successful SIT is the application of a sterilization protocol to mass reared insects that ensures sterility with a minimal detriment of the mating competitiveness and viability of the released insect. Germ cells (oocytes and spermatids) are highly radiosensitive and when exposed to ionizing radiation, dominant lethal mutations are induced (Muller 1927). The dominant lethal mutations produced by radiation in insects depend mainly on the dose, insect type, size, and sex (Hooper 1989). Radiosensitivity also depends on other factors such as irradiation temperature, humidity, ploidy level, mitotic cycle phase, and metabolic condition (Enkerlin et al. 1997).

In species of the genus Anastrepha, studies on the effect of pupal age and radiation dose on the induced sterility are not completely consistent. Rhode et al. (1961) reported that Anastrepha ludens (Loew) pupae irradiated 96 h before emergence with 40 Gy showed 100% male sterility. By contrast, according to Velasco & Enkerlin (1982), the dose needed to induce sterility in the same species should be much higher. They reported that 40 Gy and 100 Gy induced 90% and 99% sterility, respectively, when pupae were irradiated 72 h before emergence. In the case of Anastrepha suspensa (Loew), Burditt et al. (1975) irradiated pupae with 40 Gy at 48 h before emergence and observed complete adult sterility whereas Calkins et al. (1988) reported that lower irradiation doses (30 Gy) applied 24-48 h before emergence induced high levels of sterility.

The efficiency of sterilized insect release programs depends to a great extent on the ability of laboratory reared sterile males to mate with, and transfer sperm to, wild females in the field (McInnis 1993). Usually, immediately after copulation 90% of sperm is found in the spermathecae of the female (Yuval et al. 1996). Mossinson & Yuval (2003) have shown that females with fewer sperm in their spermathecae show a higher tendency to remate. Remating may reduce the efficiency of the SIT if the second mating occurs with a wild male. However, this is very unlikely as sterile males far outnumber wild males in an SIT programme.

We analyzed under laboratory conditions the effect of different doses of gamma irradiation and the age of pupae at the time of irradiation on the induced sterility and the ability to transfer sperm in A. fraterculus.

MATERIALS AND METHODS

The A. fraterculus individuals studied were from a strain reared since 1997 at Estación Experimental Provincial Obispo Colombes, Tucumán, Argentina. Pupae were sent to Buenos Aires (Centro Atómico Ezeiza, Grupo Agronómico, CNEA) by surface and there were kept under controlled conditions (25 ± 1°C, 75 ± 5% RH, and a photoperiod of 12:12 (L:D)). Adult diet was composed of white sugar: yeast (Calsia, S.A.; Tucuman, Argentina) (3:1). Water was provided as 1% agar in 12-mL vials. Food and water were changed each once a week.

Pupae were irradiated at the Centro Atómico Ezeiza facility (Comisión Nacional de Energía Atómica, Argentina) in a Gammacell 220 (MDS Nordion, Canada) irradiator, with 60Co source (dose rate for the first and second experiment: 1.67 Gy min⁻¹ and 1.60 Gy min⁻¹).

Experiment 1: Optimal Pupal Age for the Irradiation Treatment

Pupae were irradiated 24, 48, 72, or 96 h before adult emergence. In each case four different radiation doses were applied: 0 (control), 50, 70, and 90 Gy. Upon emergence adults were separated by sex and kept for 15 d under controlled conditions at 25°C, 80% RH, and a photoperiod of 13:11 (L:D), and light intensity of 3500 lux. At this age all individuals are sexually mature (De Lima et al. 1994). For each treatment, male fertility was evaluated by exposing ten fertile (non-irradiated) females to a sample of ten treated males for 20 d in a 3000-cm³ flask.

After exposure to males, females were transferred to egg collecting flasks that were similar to those used for the crossing, but they had an artificial egg laying substrate hanging from the top. It consisted of 3-cm diameter sphere made of 3.5 g agar and 0.05 g red dye (color index 14700) dissolved in 300 mL water. The sphere was wrapped in Paraflim® (Boiler 1968; Manso 1998). The artificial substrates were removed after an exposure period of 48 h to the inseminated females, The Paraflim® was removed, and the eggs were manually extracted and transferred to a wet Petri dish. Eggs were kept for 72 h at 25°C. After this period, the numbers of hatched and non-hatched eggs were recorded. The experiment was replicated 3 times for each treatment.

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Experiment 2: Optimal Dose for the Sterilization of Pupae 48 h before Adult Emergence

The effect of irradiation on male or female fertility was analyzed by mating flies irradiated with different doses of gamma rays with non-irradiated flies of the opposite sex. Pupae were treated 48 h before emergence with 0 (control), 20, 40, and 60 Gy. Emerged adults were kept under the same conditions as those of Experiment 1. Male fertility was evaluated in a similar way as that described for Experiment 1, but in this case 15 males and females were used. Six replicates were obtained for the 20, 40, and 60 Gy treatments. Female fertility was tested by exposing 15 irradiated females to 15 mature fertile males. In this case 2 replicates were obtained for each radiation dose. Four replicates of the crossing of fertile males and females were used as the control treatment for both male and female fertility tests.

The method of egg collecting was similar to that described for Experiment 1. In this case females were allowed to lay eggs for 1 month. During this period 7 egg collections (one every 3-5 days) were made for each treatment. A total of 28 samples were obtained for the control (7 collections × 4 replicates) and 14 for each group of irradiated females (7 collections × 2 replicates).

Sperm Transfer

In order to determine if sterile males are able to transfer sperm, spermathecae of fertile females mated to irradiated and non-irradiated males in Experiment 2 were observed. Females were sacrificed and fixed in 70% ethanol. Spermathecae were dissected on a paraffin wax layer with the help of entomological needles. Spermathecae were transferred onto a slide with a drop of acetic orcein (Guillén-Aguilar 1983), covered with a coverslip, and pressure applied with the thumb to break them and release sperm into the dyeing solution. The presence or absence of sperm was scored with a stereoscopic microscope at 100× magnification.

Statistical Methods

To determine the best time to irradiate pupae, the percent of egg hatch was compared among eggs laid by females inseminated by fertile males and males irradiated with 50, 70, and 90 Gy at 24, 48, 72, and 96 h before adult emergence by means of a homogeneity chi square test. To evaluate the effect of different radiation doses 48 h before adult emergence on male fertility, the percent of egg hatch was compared among eggs laid by fertile females inseminated by males treated with different radiation doses. The method used was non-parametric Kruskall-Wallis analysis of variance. Pair wise comparisons were performed by Mann-Whitney U test. The percentage of egg hatch was compared for each treatment among the 7 consecutive egg collections by means of Kruskall-Wallis analysis of variance.

Irradiated females tended to lay lower numbers of eggs and their eggs showed a reduced egg hatch. Because only 2 classes were able to lay eggs, i.e., control and females irradiated with 20 Gy, they were compared by means of Mann-Whitney U test. The proportion of spermathecae with or without sperm was compared by means of Fisher's exact test. All statistical tests were performed with the program STATISTICA, ver. 5.1 (StatSoft 2000).

RESULTS

Optimal Pupal Age for Irradiation

The percent egg hatch of the control group (females inseminated with non-irradiated males) was about 87%. This value drops dramatically (P = 0) when the males were irradiated even with the lowest dose (50 Gy). The doses of 70 and 90 Gy induced total sterility independent of pupal age at the time of irradiation (Table 1). The comparison of percent egg hatch among ages for the treatment with 50 Gy indicated that the differences are not significant (χ² = 2.49, P = 0.93). These results indicate that within the interval considered, pupal

<table>
<thead>
<tr>
<th>Pupal age</th>
<th>0 (Control)</th>
<th>50</th>
<th>70</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.56 (533)</td>
<td>0 (646)</td>
<td>0 (387)</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>0.32 (930)</td>
<td>0 (382)</td>
<td>0 (845)</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>0.18 (550)</td>
<td>0 (625)</td>
<td>0 (886)</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>0.51 (787)</td>
<td>0 (614)</td>
<td>0 (777)</td>
<td></td>
</tr>
</tbody>
</table>

--- 86.98 (976)

1 Hours before emergence.

**TABLE 1.** PERCENT OF EGG HATCH AND TOTAL NUMBER OF SCORED EGGS (IN PARENTHESES) OVIPOSITED BY FERTILE FEMALES INSEMINATED BY NON-IRRADIATED MALES (CONTROL) AND MALES IRRADIATED WITH DIFFERENT DOSES OF GAMMA RAYS AND AT DIFFERENT STAGES OF PUPAL DEVELOPMENT.
age at the time of irradiation does not affect the sterility induced by gamma radiation in males.

Evaluation of Optimal Dose for Pupal Irradiation

Radiation reduced male fertility as determined by egg hatch, from about 80% in the control group (0 Gy) to about 1% in the group treated with 60 Gy (Table 2). According to Kruskall-Wallis analysis of variance, the differences among treatments were highly significant ($H = 123.08, P \leq 0$). Pairwise comparisons by Mann-Whitney tests indicated that all pairs differ significantly ($U = 0-362, Z = 4.7-7.6, P < 10^{-5}$). The percentage of egg hatch did not differ significantly within treatments among the collections throughout the one-month period the females were allowed to oviposit. The percent of egg hatch was slightly higher in females mated to fertile males (34/45) than in females mated to irradiated males (46/70) but these differences were not significant according to Fisher’s exact test ($P = 0.24-0.09$).

In the case of females, the radiation treatment affected both the number of eggs laid and the percent hatch (Table 3). Females irradiated with 40-60 Gy were not able to lay eggs at all. Females irradiated with 20 Gy laid only a third of the eggs as compared with non-irradiated females. The differences between the control (0 Gy) and the treatment with 20 Gy were highly significant for both percent of egg hatch ($U = 16, Z = -4.8, P = 1.6 \times 10^{-4}$) and number of eggs that were laid ($U = 1.5, Z = -5.2, P = 2 \times 10^{-2}$).

Evaluation of Effects of Radiation on Sperm Transfer

The proportion of spermathecae containing sperm was slightly higher in females mated to fertile males (34/45) than in females mated to irradiated males (46/70) but these differences were not significant according to Fisher’s exact test ($P = 0.37$).

DISCUSSION

The efficient application of the SIT requires the precise determination of the optimal conditions for pupal irradiation. This question is very important to avoid undesirable side effects of the radiation treatment such as physiological or behavioral alterations that might reduce the competitiveness of irradiated males. Irradiation of larvae and young pupae may produce adult sterility but also extensive somatic damage with unwanted effects as aspermia or reduced adult survival. The irradiation of mature pupae has been shown to improve the field performance of mass reared and sterilized males (Hooper 1989). In most insects meiosis during spermatogenesis occurs before the last molt (Chapman 1998) and mature spermatozoa have already left the testis at the time of adult emergence from the pupa (Wigglesworth 1965). Radiation may induce high levels of dominant lethal mutations in spermatids and spermatozoids as well as the death of premeiotic cell stages and atrophy of germinal tissues. Usually, sterility is permanent, although in exceptional cases non-damaged spermatozoids may be regenerated from spermatogonia.

With respect to the best time to apply radiation treatment, studies on other tephritid species such as B. dorsalis, Bactrocera cucurbitae (Coquillet), Bactrocera oleae (Gmelin), C. capitata, A. ludens, Anastrepha obliqua (Macquart), and A. suspensa demonstrate that pupae irradiated 24-48 h before emergence exhibit high levels of sterility (Velasco & Enkerlin 1982; Hooper 1989; Walder & Calkins 1993; Toledo 1993).

Our results in A. fraterculus indicate no differences in the percentage of egg hatch among eggs produced by individuals irradiated at different ages within the interval 24-96 h before emergence. Therefore, the question remains as to what is optimal age within this period to produce sterile males with the best competitiveness. Although this aspect remains to be studied, we adopted the generalized criterion applied in SIT operations against other tephritid flies of irradiating 48 h before emergence (Velasco & Enkerlin 1982; Hooper 1989; Walder & Calkins 1993; Toledo 1993). As stated by Hooper (1989) irradiation at early developmental stages is highly detrimental due to the high metabolic activity and morphological changes during the metamorphosis process. If radiation is applied to mature pupae 24-48 h before

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Hatching (%) ± SE</th>
<th>Mean number of eggs collected ± SE</th>
<th>Total number of eggs collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80.60 ± 8.06 a</td>
<td>120.90 ± 39.50</td>
<td>3384</td>
</tr>
<tr>
<td>20</td>
<td>39.30 ± 22.18</td>
<td>37.90 ± 13.36</td>
<td>530</td>
</tr>
<tr>
<td>40</td>
<td>—</td>
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<td>0</td>
</tr>
<tr>
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<td>—</td>
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<table>
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<tr>
<th>Dose (Gy)</th>
<th>Egg hatch (%) ± SD</th>
<th>n</th>
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</thead>
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<td>0</td>
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<td>3384</td>
</tr>
<tr>
<td>20</td>
<td>9.8 ± 4.53 b</td>
<td>4760</td>
</tr>
<tr>
<td>40</td>
<td>3.0 ± 2.00 c</td>
<td>5042</td>
</tr>
<tr>
<td>60</td>
<td>1.3 ± 1.70 d</td>
<td>5175</td>
</tr>
</tbody>
</table>

Different letters mean groups that differ statistically.

TABLE 2. Effect of Dose of Gamma Irradiation on the Percent of Hatch of Eggs Laid by Fertile Females Mated to Males Irradiated 48 H Before Emergence. n = Number of Eggs Scored.

TABLE 3. Number of Eggs Collected from Females Treated with Different Doses of Gamma Rays and their Corresponding Percent of Hatch.
emergence, the metamorphosis is almost complete and the detrimental effects of irradiation on organs with low metabolic rate is minimized. However, the spermatogenesis is still ongoing, spermatogonia and spermatozoids are still differentiating and they constitute the main target for dominant lethal mutation induction.

Competitiveness of irradiated males is negatively correlated with the absorbed radiation dose (Calcagno et al. 2001; Calcagno et al. 2002; Lux et al. 2002). Therefore, in order to optimize the efficiency of the SIT it is necessary to reach the best compromise between sterility and competitiveness (Parker & Mehta 2007). Taking into account the results obtained in Experiment 1 and the arguments discussed above, Experiment 2 was based on pupae irradiated 48 h before emergence.

The relationship between dose and percent egg hatch observed in the present research was similar to that observed in other tephritid species. The results are consistent with the “one-hit” hypothesis (La Chance & Graham 1984) which predicts a linear response at low doses, but as the dose increases an increasing proportion of sperm carry multiple dominant lethal mutations. One dominant lethal mutation, however, is sufficient to cause lethality. Furthermore, high irradiation doses produce unwanted side effects reducing the relative efficiency of the radiation (Hooper 1989).

According to the results presented here, relatively low doses of irradiation, e.g., 20-40 Gy, cause 90-97% sterility. This agrees with other authors who observed that 40 Gy can sterilize A. fraterculus (González et al. 1971), 50 Gy (48 h before emergence) produces 100% male sterility in A. suspensa (Walder & Calkins 1993), 60 Gy (24-48 h before emergence) produces high levels of sterility in A. obliqua (Toledo 1993), and 40 Gy at 96 h before emergence causes 100% male sterility in A. ludens (Rhode et al. 1961).

We observed that the induced sterility remained across the 7 egg collections made over a month for all treatments. This consistency among egg collections indicates that males do not recover their fertility during this period for any of the doses considered. These results are consistent with those of González et al. (1971) in the same species, and those of Velasco & Enkerlin (1982) in A. ludens.

In tephritid females the oviposition is reduced as the irradiation dose increases. Moreover, the eggs laid show evidence of dominant lethal mutations. The doses that cause males sterility also completely inhibit oviposition in females (Burditt et al. 1977; Calkins et al. 1988; Hooper 1989). According to Velasco & Enkerlin (1982) females of A. ludens are more susceptible to radiation than males are, showing effects with extremely low doses (5-20 Gy). The reason is that 48 h before emergence the ovaries are in an early developmental stage and the radiation, depending on the dose, may cause complete ovarian atrophy (Walder & Calkins 1992).

According to our results, the irradiation of females of A. fraterculus results in a reduction in the number of eggs laid, compared with non-irradiated females after mating to fertile males. Doses of 20 Gy induced a 40% reduction in egg hatch and a 67% reduction in egg laying. The dose of 40 Gy was sufficient to induce complete sterility by preventing egg laying. The fact that irradiated females are not able to lay eggs is favorable to SIT implementation based on bisexual laboratory strains because the potential damage to some fruits (stings) by released females would be eliminated.

The analysis of sperm transfer indicated that empty spermatothecae can be found in females exposed to both non-irradiated and irradiated males. The difference in the proportions of empty spermatothecae between control and males irradiated with 60 Gy was not significant. Although we did not quantify the number of sperm transferred, our results indicate that the sterility of irradiated males should be attributed to the induction of dominant lethal mutations and not to atrophy of testes, seminal ducts, or aspermia.

Taken as a whole the results obtained in the present work support the use of a dose of 70 Gy applied 48 h before adult emergence to induce 100% sterility in males and females of A. fraterculus.

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The operational use of Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann), genetic sexing strains in Sterile Insect Technique applications can be maximized by developing methods for effective shipment of eggs. This would enable a central production facility to maintain the relevant mother stocks and large colonies to supply eggs to satellite centers that would mass produce only males for irradiation and release. In order to achieve this, the survival of medfly embryos of different ages was assessed after storage at 5, 10, 15, 20, and 25°C in water for different periods of time. Survival was affected by all 3 variables, i.e., embryo age, water temperature, and length of storage. Storage of embryos at any temperature for 120 h resulted in almost no survival. Controlling the age of the embryo at the time of the temperature treatment is crucial for the success of this procedure. Embryos collected between 0 to 12 h after oviposition and pre-incubated at 25°C for 12 h provide a suitable 72 h window for shipment when maintained between 10 to 15°C. Under these conditions, no significant reductions in survival during all the developmental stages were observed.

**Key Words:** Mediterranean fruit fly, *Ceratitis capitata*, egg shipment, SIT, genetic sexing

Currently, a majority of area-wide programs that integrate the Sterile Insect Technique (SIT) against the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), are using genetic sexing strains based on male-linked translocations that generate temperature sensitive lethality in female embryos (Robinson et al. 1999). In order to supply field programs with sterile flies for release, sterilized male pupae are shipped to emergence and release centers (FAO/IAEA/USDA. 2003; Enkerlin et al. 2003) with the shipment being carried out 1 or 2 d prior to adult emergence and immediately after irradiation (Schwarz et al. 1981; Vargas 1989; Cáceres 2002). This approach can reduce insect quality through prolonged exposure of pupae to reduced-oxygen atmospheres during transport.

Males of the genetic sexing strains carry a translocation that reduces fertility and the females have reduced viability due to being homozygous for the *temperature sensitive lethal* (*tsl*) mutation. These 2 factors impact design and
implementation of production systems in mass rearing facilities (Cáceres et al. 2004). Consequently it is obligatory to have 2 specific systems, one for the production of only male pupae for sterilization and release and the second for maintenance of the production colony (Cáceres et al. 2000). In addition, a Filter Rearing System (FRS) is needed in order to maintain the genetic integrity of the genetic sexing mother stock (Fisher and Cáceres 2000). In rearing systems for colony production, where females are required, the larval rearing conditions have to be very carefully controlled so as to keep temperatures below those that would impact viability of females. For male only production no such precautions are needed and larval rearing is straightforward. The relatively complex, but manageable, rearing systems associated with the use of tsl based genetic sexing strain has led to the idea of separating the colony production process, including the FRS, from that of the production of male pupae for irradiation and release. In this concept, a large central facility would be responsible for the more complex task of maintaining the mother stock and large production colony, and this central facility would supply eggs to satellite facilities where male only production would be carried out.

In order to determine the feasibility of this concept, a first step is to assess if protocols can be developed which enable routine long-distance shipments of eggs to be carried out. The objective of this study was to analyze the effects of embryo age, storage periods, and storage temperatures on egg viability and egg to adult survival.

MATERIALS AND METHODS

Egg Storage Experiment

Eggs of the medfly genetic sexing strain VIENNA-8/D53 (Franz 2005) were collected 1 h after oviposition and 150 aliquots were transferred to moist black filter paper for incubation at 25°C and 90% RH for different incubation periods. To obtain embryos of different ages, 25 aliquots were incubated for each the following periods: 1, 6, 12, 18, 24, and 36 h. After incubation the aliquots were transferred to flasks with water and for each incubation period 5 flasks were placed at each of the following storage temperatures: 5, 10, 15, 20, and 25°C, with 1 of the 5 flasks being held for the following storage periods: 12, 24, 48, 72, or 120 h. Immediately after this treatment 5 samples of 200 eggs were collected from each flask and placed on moist black filter paper located over standard carrot powder larval diet (Heather & Corcoran 1985) in a Petri dish held at 25°C. Each Petri dish was kept individually in a small plastic box with sand as pupation medium and covered with a mesh lid that allowed ventilation. The percent egg hatch for each sample of 200 eggs was determined. The larvae completed development in the Petri dish, left the diet, and pupated in the sand. 2 days before adult eclosion, pupae were collected and placed in a separate Petri dish to determine adult emergence. The effect of storage time on egg hatch and egg to adult survival was analyzed by 1-way analyses of variance (ANOVA) for each temperature and embryo incubation period and means compared by the Tukey multiple range test.

Male Mating Competitiveness

In a separate experiment, following incubation for 24 h at 25°C, eggs of strain VIENNA 8/D53 were stored in water for 120 h at 5, 10, 15, and 20°C. Mating competitiveness of the males emerging from these different treatments was measured in field cage tests by comparing 50 individually marked, protein-fed, virgin 5-d-old males from each temperature treatment with 50 untreated VIENNA 8/D53 control males, introduced into a field cage that was 2 m high and 3 m diameter (Calkins & Webb 1983) and containing orange trees (FAO/IAEA/USDA 2003). The field cage was set up in an environmentally controlled greenhouse at 25°C and 60% RH. Males were released early in the morning of the day of the test and 50 protein fed laboratory wild type females of strain Egypt-II) were released 1 h after the males. Propensity of Mating (PM) (Cayol 2000) and the proportion of males that participated in mating were calculated based on the number and type of mating recorded for the control and treatment flies. The experiment lasted for 3 h and was replicated 12 times for each temperature treatment. The effect of the storage time and temperature treatment on the percent of participation of mating was analyzed by one-way analyses of variance (ANOVA) and means compared by the Tukey multiple range test. Results from all experiments were analyzed with statistical software MINITAB for windows.

RESULTS

Egg Hatchability

The data for egg hatchability following the different treatments are shown in Fig. 1a-e. Fig. 1a shows the data for storage at 5°C and it is clear that the shorter the incubation period (i.e., the younger the embryos), the more susceptible they are to long-term storage. In general longer storage times for any age of embryos result in lower egg hatch (F = 2, P < 0.01). Egg hatch for embryos incubated for 1 h and stored for 12 h was 48.9 ± 0.5%, however, this value declined to 0.1% following storage for 120 h (Fig. 1a). In general, egg hatch improved at 10°C, with only the 2 shortest incubation periods showing a decline following the longer storage periods (Fig. 1b). Egg hatch for embryos stored at 15°C showed a similar pattern.
for all incubation periods and storage times, with only long storage times significantly reducing survival \((F = 10, P < 0.01)\) (Fig. 1c). Egg hatchability values at 15°C ranged from 77.2 ± 9.4% to 60.8 ± 3.1%. There was a general decline in egg hatch when storage temperature was increased to 20°C with again shorter incubation periods leading to higher lethality (Fig. 1d). Egg hatch for embryos incubated for 1, 6, and 12 h was reduced significantly after 24 h of storage \((F = 25, P < 0.05)\), while embryos incubated for 18, 24 and 36 h showed significant reductions in egg hatch only after 72 h of storage \((F = 34, P < 0.05)\). Egg hatchability values ranged from 69.0 ± 1.0% to 36.0 ± 0.1%. Egg hatch of embryos stored at 25°C showed significant reductions in survival after 24 h of storage \((F = 16, P < 0.05)\) and egg hatchability ranged from 79.2 ± 0.4% to 16.2 ± 0.9% (Fig. 1e).

### Egg to Adult Survival

The effect of storage for different periods of time at different temperatures following egg incubation for different times on the overall egg to adult survival is shown in Fig. 2a-e and Table 1. The egg to adult survival is calculated based on the number of adults produced from a certain number of eggs; it therefore includes the egg hatch data shown in Fig. 1. Egg to adult survival declined with time of storage for all embryo ages and temperature treatments. Egg to adult survival was lowest for embryos stored at 5°C reaching nearly zero after 72 h of storage \((R^2 = 0.95, P < 0.05)\). The same tendency was observed for embryos incubated for 6, 12, and 18 h, where the values ranged from 45 to 0%, 48 to 0%, and 49 to 4%, respectively, \((R^2 = 0.95, 0.94, \text{and } 0.97; P < \text{...})\).
Egg to adult survival from embryos incubated for 24 and 36 h fell significantly after 48 h of storage ($F = 189$ and $170$ respectively, $P < 0.05$), and values ranged from 49 to 19% and 48 to 1%, respectively.

For storage at 10°C the trend of egg to adult survival was similar to that for 5°C, but values were in general higher. Embryos incubated for shorter periods had the lowest values. Egg to adult survival for embryos incubated for 18, 24, and 36 h was significantly reduced following 48 h of storage, but the effect was not as great as for the 5°C treatment ($P < 0.05$).

Following storage at 15°C, egg to adult survival decreased significantly only after 72 h of storage for embryos incubated for 1 and 6 h ($F = 12$, $P < 0.05$). Values for these incubation periods ranged from 52 ± 1% to 31 ± 1.8%. For embryos incubated for 18 and 24 h, a significant reduction was only observed after 24 h of storage ($F = 2.72$ and $0.10$ respectively, $P < 0.05$). Values ranged from 52 ± 5.2% to 32 ± 1.6% and 53 ± 0.8% to 38 ± 3.3%, respectively. For embryos incubated for 36 h significant differences were only observed after 48 h of storage ($F = 44$, $P < 0.05$).

For embryos stored at 20°C the adult emergence pattern for all incubation periods was similar. Egg to adult survival of embryos incubated for 1 h was the lowest for most of the storage periods.
periods with decreasing values as storage times increased. A similar trend was shown for embryos incubated for 1, 6, 12, and 36 h (R² = 0.92, 0.97, 0.95 and 0.89, respectively). The egg to adult survival of embryos incubated for 18 and 24 h was not correlated with storage time (R² = 0.65 and 0.79, respectively).

The egg to adult survival for embryos stored at 25°C showed a reduction as a function of storage time (R² = 0.94, 0.96, 0.97, 0.93, 0.99, and 0.99; P < 0.05, respectively) for 1, 6, 12, 18, 24, and 36 h of incubation periods. Values ranged from 55 to 4%, 57 to 1% 51 to 3%, 51 to 2%, 58 to 10%, and 59 to 10%, respectively, for these incubation periods.

Male Mating Competitiveness

In all field cage tests the Proportion in Mating (PM) index was high (91.3 ± 5%) indicating that there was a high degree of sexual activity during the test period. The proportion of copulations by control males was 19.3 ± 3.8% while that for males that emerged from the different temperature treatments oscillated between 16.5 ± 5.3% to 19.3 ± 4.4%. Results on the basis of the proportion of male participation in mating have shown no significant reduction in the sexual performance of males emerging from embryos stored for 120 h at any of the temperatures tested (F = 0.71, P < 0.5) (Fig. 3).

**DISCUSSION**

The most appropriate procedure for egg storage or for egg shipment allowing the subsequent male only production in satellite facilities is to select eggs 0 to 12 h post oviposition and incubate them for at least 12 h at 25°C. The embryos can then be stored or shipped for up to 72 h when maintained at 10 to 15°C without any significant reduction in egg to adult survival or adult male quality.

Overall, the optimal conditions for storage or shipping were to maintain the embryos in water up to 72 h at a temperature of 15°C. Eggs can be placed into storage at any development stage 1 h

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**Table 1. ADULT EMERGENCE (%) FROM 1, 6, 12, 18, 24, AND 36 H OLD EMBRYOS HELD AT DIFFERENT TEMPERATURE (5, 10, 15, 20, AND 25°C) FOR PERIODS BETWEEN 12 AND 120 H. MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (P < 0.05). *CONTROL, EGGS WERE COLLECTED 1 H AFTER OVIPOSITION THEN INCUBATED AT 25°C FOR 48 H THEN TRANSFERRED TO THE LARVAL MEDIUM.**
Hatching and egg to adult survival was relatively unaffected after storage at this temperature for up to 72 h. After 72 h storage, hatching of the 1 h embryos was still 94% of the control, but egg to adult survival fell to 85% of the control. Hatching of older embryos (18, 24, and 36 h old) was unaffected; however, adult emergence was significantly reduced after 72 h of exposure (70% and 71% of the control, respectively).

Storage at 10°C affected the rate of adult survival when the embryos were younger than 12 h and a significant reduction in egg to adult survival was observed when embryos of 1 or 6 h of age were stored for more than 48 h. Consequently, for storing eggs in water at 10°C, the embryos should be at least 12 h old. Following 120 h storage, hatching was still 66% of the control, but the rate of egg to adult survival fell to 50% of the control. A similar pattern was observed for any of the storage times tested for embryos 18, 24, and 36 h old.

Storage at temperatures higher than 15°C had a negative effect on embryo survival probably due to the fact that at these temperatures normal embryo development continues. For embryos stored at 20°C and incubated for 1, 6, and 12 h, this effect was more obvious after 24 h of storage. Results showed that short storage of embryos at 20°C is possible but the embryos should be at least 18 h old. After 72 or 120 h storage, hatching of the embryos incubated for 18 h was still 86 and 59% of the control, respectively, but rate of egg to adult emergence fell to 63 and 10% of the control, respectively.

In general, the most sensitive development stages are the 1s during early embryogenesis, i.e., 1-6 h old eggs. Similar effects were observed by Leopold (2000) in housefly Musca domestica (L.), but Arakaki et al. (1984) reported no effect in melon fly Bactrocera cucurbitae Coquillett on egg hatch, larval growth, pupal and adult development when 20-h-old embryos were stored in water at 5 and 10°C after 168 h of storage. There is good evidence that chilling intolerance of the very young embryos is related to the formation of the blastoderm (Strong-Gunderson & Leopold 1989; Callaini & Marchini 1989). In recent studies, Stefani et al. (2004) determined that in medfly, 1-2 h after oviposition, the eggs are at stage embryonic stage 2 with 2 nuclei. At 2.5 h the number of nuclei had multiplied to 64 nuclei, and at about 3.5 h embryos reach stage ten and the beginning of the formation of the syncytial blastoderm. At about 8.5 h, more than 256 nuclei can be observed, and cellularization of the blastoderm occurs.

In conclusion, the optimal protocol for storage or long distance shipment of medfly eggs is to collect eggs between 0 to 12 h post oviposition and incubate them for 12 h at 25°C, when these embryos reach the optimal storage age of 12 to 24 h old and can then be transported during a 72 h window when maintained at 10 to 15°C. N1 of the treatments affected male mating competitiveness. Similar results were obtained by Rajamohan et al. (2003) during quality assurance tests carried out to compare cryopreserved medfly eggs. Thus, these results support the viability of routine long distance egg shipment from central egg production centers to male-only satellite production facilities.
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A PROTOCOL FOR STORAGE AND LONG-DISTANCE SHIPMENT OF MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) EGGS.
II. ASSESSMENT OF THE OPTIMAL TEMPERATURE AND SUBSTRATE FOR MALE-ONLY PRODUCTION

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ABSTRACT

The present study has been conducted to assess the effect and interaction of various storage substrates and conditions on eggs of the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann). Tests were carried out with the genetic sexing strain VIENNA 8/D53, a strain that carries a temperature sensitive lethal (tsl) mutation that allows the selective killing of female zygotes. This study identifies strategies to enhance the storage and transport conditions through assessment of effect on egg, pupal and adult survival in order to facilitate the establishment of satellite mass rearing facilities for the production of male medflies. Eggs were immersed in two different substrates and stored at different temperatures and for different time periods. Findings from this study suggest that egg storage periods, and to some extent, the storage substrates have significant effects on pupal and adult survival. For 72-h storage periods, the eggs preserved in agar solution at 10°C produced the most pupae. There was an inverse relationship between the concentration of dissolved oxygen in the substrate during storage and the quality and survival of the stored/transported eggs. Apparently low levels of dissolved oxygen reduce metabolic rates, allowing the storage period to be prolonged.

Key Words: SIT, Ceratitis capitata, Mediterranean fruit fly, egg storage, genetic sexing, egg shipment

RESUMEN

El presente estudio fue conducido para evaluar el efecto e interacción de varios substratos y condiciones de almacenamiento en huevos de la mosca mediterránea de la fruta, Ceratitis capitata (Wiedemann). Las pruebas se realizaron con la cepa en la cual es posible separar los sexos genéticamente VIENNA 8/D53, la cual contiene una mutación letal sensible a la temperatura que permite la eliminación selectiva de los zigotos femeninos. Este estudio identifica estrategias para mejorar las condiciones de almacenamiento y transporte por medio de la evaluación de su efecto en la supervivencia de huevos, pupas y adultos, esto para facilitar el establecimiento de laboratorios satélites de cría masiva para la producción de machos de la mosca mediterránea de la fruta. Los huevos fueron sumergidos en dos substratos diferentes y almacenados a diferente temperatura a diferentes periodos tiempos. Los resultados de este estudio sugieren que el periodo y hasta cierto punto el estrato de almacenamiento tienen un efecto significativo en la supervivencia de las pupas y los adultos. Para un periodo de almacenamiento de 72-h los huevos almacenados en solución de agar a 10°C producen un número mayor de pupas. Hubo una relación inversa entre la concentración de oxígeno disuelto en el substrato durante el almacenamiento y la calidad y supervivencia de los huevos almacenados/transportados. Aparentemente los niveles bajos de oxígeno disuelto reducen el metabolismo y permiten que el periodo de almacenamiento pueda ser prolongado.

Previous studies (Cáceres et al. 2007) have identified various protocols that can be used in order to store and transport eggs of the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann) from a central facility to satellite facilities where the eggs would be used to produce only sterile males. These protocols considered 3 variables, the age of the eggs, the temperature of storage in water, and the length of the storage period.

Currently, all programs applying the sterile insect technique (SIT) against the medfly utilize genetic sexing strains based on a temperature sensitive lethal mutation and a male linked translocation (Cáceres et al. 2004; Dyck et al. 2005). These strains produce only males following elimination of females as a result of a high temperature treatment during embryo development (Fisher 1998). It is therefore important to assess the effect of different storage conditions on the high temperature treatment that would be given to the eggs following transport to a satellite mass rearing facility.

For the present study, eggs of the Mediterranean fruit fly genetic sexing strain VIENNA 8/D53 (Franz 2005) were routinely collected and
stored in either water or a dilute agar solution at different temperatures and for different time periods. The purpose of using the agar solution was to provide a medium with a higher density than distilled water in order to prevent egg sedimentation, and thus avoid damage. Following storage, the eggs were incubated according to the standard protocol for elimination of females (Fisher 1998; Fisher & Cáceres 2000; Cáceres 2002). Following the temperature treatment, the embryos were placed on standard larval diet and various quality control parameters were assessed during the larval, pupal, and adult stages. In addition, the dissolved oxygen was measured during storage to determine any possible relation between the dissolved oxygen concentration and the quality and survival of the stored eggs.

The results are discussed in the framework of improving the efficiency of programs integrating the SIT by providing a strategy for long distance egg shipment to supply satellite medfly mass-rearing facilities with fertile eggs for male-only pupal production.

MATERIALS AND METHODS

Strain

The genetic sexing strain VIENNA 8/D53 was selected for all experiments. This strain carries a male-linked translocation, a chromosome inversion, and the females are homozygous for two selectable markers, white pupae (wp) and temperature sensitive lethal (tsl) (Robinson et al. 1999; Franz 2005). Females are eliminated by exposing the eggs to 34°C for 12 to 24 h during the last half of embryo development (Franz et al. 1996; Cáceres et al. 2004).

Egg Treatment

Eggs from strain VIENNA 8/D53 that were 24 h old were immersed in different storage substrates, and subjected to different storage temperatures and time periods. The storage conditions consisted of 2 substrates, distilled water or agar (0.1%) solution, 2 temperatures 10 or 25°C, and 3 storage periods, 0, 24, and 72 h. The egg collections were performed following standard guidelines and protocols for medfly genetic sexing strains (Cáceres 2002). Following collection, the eggs were incubated inside plastic bottles containing distilled water (v/v ratio of 1:20, respectively), into which compressed air was provided via an aquarium stone, for 24 h at 24°C. For this study, nine 8-mL aliquots of eggs were removed. One served as control and was incubated for an additional 12 h at 34°C to eliminate the female zygotes, and then maintained for an additional 12 h at 24°C. The other 8 aliquots were stored under different test conditions, which consisted of the combination of the 2 temperatures, the 3 storage periods and the 2 substrates. Each aliquot was stored in 70 mL of substrate.

Following storage, the eggs were transferred back into the plastic bottles to complete the 48-h incubation period as follows, 12 h at 34°C to eliminate the female embryos and then for another 12 h at 24°C.

Larval Rearing

Following the incubation period, the treated and the control eggs were washed with distilled water to remove traces of the storage substrate, and transferred into trays containing 5 kg of larval diet (Tanaka et al. 1969). An aliquot of 8 mL of eggs was put into each tray. The 3rd instars were collected in a tray containing sawdust, which served as the pupation medium. Larvae were collected for 3 consecutive days and the volume measured.

Quality Control

Egg Hatch

Samples of 1000 eggs were collected for each treatment and the control group, before transfer to the larval diet. Five days later, the number of unhatched eggs was counted and the percentage egg hatch calculated. During the 5-day incubation period, the egg samples were placed under the same conditions as the larval trays.

Egg to Pupal Survival

Egg to pupal survival was calculated based on the estimated original number of eggs (8 mL × 25000 egg/mL) transferred to the larval diet and the number of pupae produced for each treatment. The number of pupae recovered for every treatment was estimated volumetrically.

Egg to Adult Survival

Egg to adult survival was calculated based on the estimated number of eggs transferred to the larval diet for each treatment, the number of pupae recovered, as estimated for the egg to pupal survival, and the percentage of adult emergence from 5 mL of 8-d-old pupae. The total number of adults was determined 5 d after the first fly emerged.

Dissolved Oxygen

Dissolved oxygen and temperature were measured with a portable dissolved oxygen meter
microprocessor-based instrument, consisting of a cell-containing electrolyte enclosed by a selective membrane and two metallic electrodes (PAKTON® 35640-series). The electric current produced by the consumption of oxygen by the cathode is proportional to the partial pressure of the oxygen in the sample. The dissolved oxygen and temperatures were measured for all treatments, before and after the storage periods.

Data Analysis

Numerical data were analyzed by Analysis of Variance (ANOVA). The effect of the storage times, substrates, and temperatures on the egg hatch and egg to adult survival was analyzed by 3-way ANOVA and the means compared by the Tukey multiple range test. Interaction between the variables, storage temperature and time was determined by means of the generalized linear model of variance to perform univariate ANOVA. Results were analyzed with the statistical software MINITAB® for windows.

RESULTS

Egg Hatch

The length of the storage period had a significant effect on egg hatch ($F = 11.31, P = 0.002$). Eggs stored for 72 h (as well as control eggs) displayed higher hatch than those stored for 24 h (Table 1). Moreover, within a given storage period, egg hatch was higher for eggs stored at 10°C. Overall egg hatch did not differ between storage in water and in an agar solution, although storage in water tended to result in slightly higher egg hatch than storage in agar. The highest egg hatch was recorded for the eggs stored in water and in the agar solution at 10°C for 72 h (59.7 ± 7.2% and 59.4 ± 9.9%, respectively).

Egg to Pupal Survival

Significant variations were observed when assessing the egg to pupal survival among the different treatments (Table 1). In contrast to egg hatch, 0 and 24 h storage periods produced higher values for egg to pupal survival ($F = 12.39, P = 0.029$) than 72 h storage. For eggs stored for 72 h, the highest egg to pupal survival was recorded for eggs stored at 10°C. The lowest egg to pupal survival values were observed among the eggs stored for 72 h at 25°C, regardless of the storage substrate. Nevertheless, statistically significant differences were only found between those eggs stored in agar solution at 25°C for 24 h (27.4 ± 3.0%), and at 10°C for 24 h (26.4 ± 2.2%), when compared with those stored at 25°C for 72 h either in agar solution (19.9 ± 1.4%), or water (8.4 ± 3.4%) ($F = 6.72, P < 0.5$). Eggs stored for 24 h produced higher numbers of pupae than those stored for 72 h, regardless of the storage substrates or temperatures. Overall, the storage of medfly eggs in agar solution at 25°C for 24 h appeared to be the most suitable condition for pupal production. However, when considering 72 h storage periods, the eggs preserved in agar solution at 10°C produced the most pupae (22.9 ± 5.0%), but was not significantly different from the treatment in agar solution at 25°C for 24 h that produced the highest number of pupae (27.4 ± 3.0%) or the control (27.1 ± 4.4%).

Egg to Adult Emergence

There was a significant correlation in terms of the storage conditions between egg to pupal survival and egg to adult survival ($R^2 = 0.984, P =$

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<th>Treatments</th>
<th>Parameters</th>
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**Table 1. Egg Hatch, Egg to Pupal Survival, and Egg to Adult Survival (Mean % ± SE) from 24-H-Old Eggs of Genetic Sexing Strain VIENNA 8/D53 Held Under Different Storage Conditions. Control Eggs were Incubated in Water for 12 H at 34°C to Eliminate the Female Zygotes, and Then Maintained for an Additional 12 H at 24°C. Means Within Columns Followed by the Same Letter Are Not Significantly Different, P < 0.05.**
However, since this was contrary to the effect seen with egg hatch, this indicates that storage directly affects larval and pupal survival. As for the egg to pupal survival, egg to adult survival was greater for those eggs stored for 24 h than those stored for 72 h (Table 1), although the survival was only significantly lower for the eggs stored in water at 25°C or 72 h (6.5 ± 2.8%). For eggs stored for 72 h in agar solution at 10°C, the egg to adult survival was 18.4 ± 3.8%, which was not statistically different from the control (21.5 ± 3.4%).

**Dissolved Oxygen**

Concentrations of dissolved oxygen were 5 times higher in water than in the agar solution prior to and following storage (Fig. 1). A relationship between the concentration of oxygen available for the duration of the different treatments and the storage substrates, temperatures and periods was observed. The water substrate, as well as low temperatures (10°C), were associated with higher oxygen concentrations. Moreover, at any given temperature higher concentrations of oxygen occurred during the shorter storage periods. The water substrate at 10°C for 24 h displayed the highest concentrations of dissolved oxygen available at the end of the storage period (Fig. 1). Nevertheless, in spite of these findings, there was no direct effect of the concentration of dissolved oxygen on adult fly emergence. There was no control for this test as bubbling and aerated was continuous and there was no variation in the concentration of dissolved oxygen at the beginning and the end of storage period.

**DISCUSSION**

The optimal storage conditions for 72 h storage are at 10°C in an agar solution. In general increasing storage reduces egg hatch, pupal production and adult emergence. Storage periods of 24 h appeared to have no damaging effects on adult emergence and pupal production, although there was a direct effect on egg hatch. Storage periods of 72 h on the contrary, appeared to have no effect on egg hatch, whereas there was a negative effect on the pupal and adult production, but only when stored at 25°C. Overall, storage in agar solution appeared to be better than storage in water, although the effect was only significant for 72 h storage at 25°C. The detrimental effect of storage in water at 25°C for 72 h on the rate of adult emergence and pupal production observed in this study are in line with data reported by Cáceres et al. (2007) and with Arakaki et al. (1984), who showed that with melon fly Bactrocera cucurbitae Coquillett at temperatures of 5 or 10°C, eggs preserved in water maintained high hatch rates, even after 7 d of storage.
The results gathered from this study on the number of pupae produced and the rate of adult emergence suggest that storage in agar at 25°C for 24 h constitutes the most suitable condition for eggs of the VIENNA 8/D53 genetic sexing strain. This is acceptable for situations where the satellite mass rearing facility is within a 24-h shipment period from the central facility mass-producing the eggs. However, for storage up to 72 h, storage in agar solution at 10°C provides the best conditions in terms of pupal production and adult emergence. Overall, the effect of the storage times was not significant for egg hatch, but for the larval development stage this effect was reflected directly on the significantly diminished number of pupae collected particularly on the treatments where eggs were stored up to 72 h. The concentration of dissolved oxygen available in the storage substrate affected the emergence of adult flies. As initially suggested, we can assume that the agar solution provides better physical conditions for egg storage, as it prevents egg sedimentation. In addition, the low levels of dissolved oxygen may reduce metabolic rates, allowing the storage period to be prolonged. Experiments with housefly Musca domestica (L.) have demonstrated that the gaseous composition of the atmosphere during incubation has an effect on the survival of the embryos and oxygen-enhanced environments during cold storage appear to have a detrimental effect on survival (Bucher et al. 1947). However, recent experiments have demonstrated that although exposure of housefly embryos to hypoxic conditions was not detrimental, it did not increase chilling tolerance (Leopold 2000). In the present study it was established that egg storage in agar solution is better than in water, but the specific characteristics that enhance this storage effectiveness, were not determined.

REFERENCES CITED


BREAKFAST OF CHAMPIONS OR KISS OF DEATH?
SURVIVAL AND SEXUAL PERFORMANCE OF PROTEIN-FED,
STERILE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

BOAZ YUVAL, MEYRAV MAOR, KARMIT LEVY, ROY KASPI, PHILLIP TAYLOR AND TODD SHELLY

ABSTRACT
The sterile insect technique (SIT) is increasingly being used around the world to control Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), the Mediterranean fruit fly as part of an area-wide integrated approach. One option that may improve the effectiveness of the SIT, by increasing the sexual competitiveness of released sterile males, consists of feeding males protein during the post-teneral stage, a diet that increases sexual performance of wild males. We examine the effects of diet on the successive hurdles males must overcome in order to inseminate females, i.e., joining leks, copulating females, having their sperm stored and inhibition of female remating. In addition, we address the effects of diet on post-release foraging success, longevity, and the ability to withstand starvation. While protein feeding universally increases the sexual success of wild males, its effect on sterile males varies with strain, experimental settings, and environmental conditions. In some cases, treatments that resulted in the best sexual performance were significantly associated with increased vulnerability to starvation. However, no particular diet affected the ability of sterile males to find nutrients in the field when these were available. We suggest it may be better to release relatively short-lived flies that are highly competitive, rather than long-lived, sexually ineffective ones.

Key Words: Tephritidae, Ceratitis capitata, Sterile Insect Technique, nutrition, sexual behavior, longevity, starvation

RESUMEN
El uso de la técnica de insecto estéril (TIE) está aumentando alrededor del mundo para el control de Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), la mosca mediterránea de la fruta como parte de un enfoque integrado por toda el área. Una opción que puede mejorar la eficiencia de TIE, por medio del aumento de la capacidad de los machos estériles liberados para competir, consiste en la alimentación de los machos con proteína durante la etapa de post-teneral, una dieta que aumenta el desempeño sexual de los machos naturales. Nosotros examinamos los efectos de la dieta sobre los obstáculos sucesivos que los machos tienen que superar para inseminar las hembras, i.e., unir con otros machos en áreas para el apareamiento, copula de las hembras, almacenar esperma e inhibir el re-apareamiento de hembras. Además discutimos los efectos de la dieta sobre el éxito de su actividad forrajera después de ser liberados, la longevidad y la habilidad para aguantar la inanición. Mientras que la alimentación con proteína universalmente aumentan el éxito sexual de los machos naturales, su efecto sobre los machos estériles varía según la raza, el lugar de los experimentos y las condiciones ambientales. En algunos casos, los tratamientos que resultaron con mejor desempeño sexual fueron asociados significativamente con el aumento de la vulnerabilidad a la inanición. Sin embargo, ninguna dieta en particular afectó la habilidad de los machos estériles para encontrar nutrientes en el campo cuando fueron disponibles. Nosotros sugerimos que puede ser mejor el liberar moscas que tienen una vida relativamente corta y que son mas competidoras, en vez de moscas que tienen una vida larga y sexualmente inefectiva.

Most of the tephritid fruit flies are anautogamous, i.e., females need to feed on a source of protein to mature eggs, while males need protein for pheromone production, the renewal of sperm supplies, and production of male accessory gland secretions (Drew & Yuval 2000). Male Mediterranean fruit flies (medflies) Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), are no exception. We have documented, for both wild and laboratory reared males, how post-teneral pro-
tein feeding affects their ability to join leks (Yuval et al. 1998), copulate in leks (Kaspi et al. 2000; Shelly et al. 2002), transfer a substantial ejaculate (Taylor & Yuval 1997), and inhibit female remating (Blay & Yuval 1997). When synthesizing and reviewing these findings (Yuval & Hendrichs 2000; Yuval et al. 2002), the importance of post-teneral nutrition for the effective performance of mass-reared, sterile males in the field was suggested.

For the sterile insect technique (SIT) to succeed, it is imperative that released sterile males compete successfully against males from the wild population. This entails joining or establishing leks in the field, attracting and courting wild females, copulating them, and, finally, inhibiting their receptivity to further copulations, all the while competing with wild males. Accordingly, a significant research effort has focused on establishing ways to understand the basis of male competitiveness and enhance that of sterile males (Shelly 1999; Kaspi & Yuval 2000; Shelly & McInnis 2001; Briceño et al. 2002; Robinson et al. 2002). Furthermore, although some released males may be quite long-lived in the field (Plant & Cunningham 1991), their effectiveness in copulating and inseminating females as they age was unknown. If their potency declines significantly, their survivability becomes less crucial for the success of the SIT.

In this review, we examine a number of recent studies that look at the effect of protein diet on sexual performance and survival of sterile, mass reared male medflies in various experimental settings. We examine the effects of diet on the successive hurdles males must overcome in order to inseminate females, namely joining leks, copulating females, transferring sperm, and inhibition of female remating. In addition, we address the effects of diet on male post-release foraging success, longevity, and the ability to withstand starvation. Because the effects of protein diet on sterile male sexual performance and survival are equivocal, we dwell on the experimental conditions of the various studies, the strains used, and environmental effects that may have affected the results.

SEXUAL PERFORMANCE OF STERILE MALES

Participation in Leks

Kaspi & Yuval (2000), working in field cages, found that protein-fed sterile males of the VIENNA 4/Tol-94 sexing strain (Robinson et al. 1999), (flown from Guatemala to Israel), were significantly more likely to join leks and emit pheromone (call) than sterile males fed only sugar. This effect was significant for the 2 age groups tested, 4-d-old and 6–8-d-old males. These experiments were performed without females and without competition between males of the 2 diet treatments.

In another experiment with the VIENNA 7/Tol-2000 sexing strain (Franz 2005), participation of protein-fed or protein-deprived (sugar-fed) sterile males in leks on the days following their release into a large field enclosure (8 × 3 square meters, 2.5 meters high) was monitored (Maor et al. 2004). Males emitting pheromone in leks were sampled for 30 min each day after release. Numbers of calling males from both diets declined significantly from day to day, but the post-teneral diet had no significant effect on calling in leks.

Maor (2004) attempted to find an alternative protein-rich diet to the hydrolyzed yeast commonly used, one that would have a significant effect on lek joining in simulated field conditions. Sterile males of the VIENNA 7/Tol-2000 strain were offered one of the following diets after they emerged: (i) sugar, (ii) sugar and protein (hydrolyzed yeast presented in a separate dish to allow optimal self selection (e.g., Cangussu & Zucoloto 1995), (iii) sugar and a protein pulse on d 2, or (iv) a slice of dry, but rehydrated, apricot to mimic a protein rich natural diet. Four d after emergence, flies were released in a field enclosure, and lek participation was monitored for 6 d. A significant temporal decline in lek participation was observed for all diets. Furthermore, the post-teneral diet significantly affected lek participation. The highest level of participation was exhibited by apricot fed males (iv), followed by the sugar (i) and sugar + protein-fed (ii) flies. The flies fed a protein pulse on d 2 (iii) contributed the fewest numbers to the lekking population (Fig. 1).

Fig. 1. Effect of pre-release diet on subsequent participation in leks by sterile male Mediterranean fruit flies. Diets were: 1. “apricot”—dry, sliced apricot; 2. “sug”—20% sucrose; 3. “sug + pro”—sucrose plus unlimited hydrolyzed protein; 3. “pro day 2”—unlimited sucrose, protein only on d 2. Bars represent 2 standard errors. Different letters in columns denote a significant difference ($P < 0.05$) in male abundance.
The total amount of protein and lipid in each male was assayed. Diet significantly affected protein levels ($H = 128$, $df = 4$, $P < 0.0001$). On emergence, the males contained on average $28.47 \pm 1.23$ μg of protein. This increase significantly in all groups at the time of release, with the greatest increase found in males who enjoyed diets containing hydrolyzed yeast (Fig. 2a). Lipid levels were significantly different between diets ($F = 6.51$; $df = 4.223$; $P < 0.0001$), but show a different pattern (Fig. 2b). The highest levels of lipid, at 4 d of age (day of release), were found in the flies that were fed sugar only or sugar with a pulse of protein.

This experiment demonstrated that the apricot diet, a natural source of protein and carbohydrate, enhanced the sexual performance of the sterile males. This enhancement was not reflected in the total amounts of nutrients present on the day of release (Fig. 2), suggesting either a synergistic effect or the action of qualitative agents present in the dried fruit. We repeated this experiment in field cages on the Hawaiian island of Oahu, using sterile males of the VIENNA 4/Tol-94 strain obtained as pupae from the California Department of Food and Agriculture rearing facility in Hawaii (Maor 2004). In these experiments, despite similar temporal trends in lekking behavior, the apricot diet did not confer a significant advantage to the males who consumed it.

Copulatory Success

Kaspi & Yuval (2000) allowed protein and sugar fed sterile males of the VIENNA 4/Tol-94 strain to compete against wild males fed only sugar for copulations with wild females (providing a 2:1 sex ratio). Significantly, protein-fed males were more likely to copulate. This effect was significant for the 2 age groups tested—4-d-old males and 6-8-d-old males. Similarly, Shelly & Kennelly (2002) investigated the effects of dietary protein on the mating competition of wild and mass-reared (Maui-Med bisexual strain from Hawaii) males for copulations with wild females. Importantly, they found that although protein in the adult diet improved the copulatory success of wild males, it did not improve the ability of sterile, mass reared males to compete against wild males for copulations with wild females.

Shelly & McInnis (2003) reconfirmed this finding in a follow up study conducted in field cages. Protein-fed, sterile males of 2 mass reared strains—VIENNA 7/Tol-2000 and Maui-Med bisexual strain—were unable to compete any better against wild males for copulations with wild females than only sugar-fed males.

In another experiment, artificial diets were removed from 4-d-old sterile males, and a natural diet (apple slice) was offered. After 24 h, there was no difference between sugar-fed and protein-fed males in copulatory success with wild females in a field cage (Kaspi & Yuval 2000). Furthermore, when males were starved instead of offered a natural diet, virtually none copulated. A similar result was obtained by Shelly & Kennelly (2003) when starved wild males were assayed for copulatory success. They found that wild males unable to locate food for 1 d have reduced copulatory success on the following day compared to successful foragers. Males starved for 24 h obtained only half as many matings as fed males; this outcome was independent of the pre-starvation diet. When the starvation period was only 18 h, diet did affect mating performance—protein fed males performed poorly, while sugar fed males mated as often as fed males.

The combined effects of diet and exposure to α-copaene, a plant derived volatile that enhances male sexual success (Shelly 2001), on copulatory success of sterile males of the VIENNA 7/Tol-2000 strain was examined in field enclosures in Guatemala (Shelly et al. 2003). This study pitted sterile males against wild males and was replicated at 2 elevations, 700 and 1,200 m above sea level. While exposure to ginger root oil (containing α-copaene) enhanced male performance irrespective of diet or elevation, protein-fed males performed significantly better than sugar-fed males only at the high elevation site. Intriguingly, overall performance of sterile males at the higher elevation was significantly worse than at the lower elevation. Accordingly, this study, while confirming the utility of exposure to α-copaene, could not provide unambiguous support for the inclusion of protein in the pre-release diet, and indicated that the conditions favoring such a diet need to be established with greater specificity.

Post-Copulatory Effects

Sperm Storage. Taylor et al. (2001), working with males of the VIENNA 4/Tol-94 strain mating with wild females in field cages, found that the probability of sperm storage decreased significantly with male age but was not significantly affected by male diet. As males aged, there was a significant decline in the number of sperm stored by their mates (median: 1263 sperm; range: 20-5684 sperm), yet number of sperm stored did not vary with male diet, male size, or female size.

Copula Duration. Taylor et al. (2000, 2001) showed that copulations of sterile males with wild females culminating in sperm storage lasted a median of 186.5 min, whereas failed copulations (no sperm storage) lasted a median of 157.5 min. Despite the considerable overlap in duration of these copulations, the relationship between copula duration and probability of sperm storage was significant. However, duration of inseminating copulations did not vary with any of the investigated fly qualities (male age, diet, or size of males and females). Similarly, Shelly & Kennelly (2002) found...
Fig. 2. Effect of pre-release diet on nutritional status of sterile male Mediterranean fruit flies. a. Protein. b. Lipid. Diets as in Fig. 1. Emergence refers to flies sampled within hours of leaving the puparium; all other flies were assayed at 4 d of age before release. Bars represent 2 standard errors. Different letters in columns denote a significant difference ($P < 0.05$) in nutrient content.
that copula durations of sterile males (of the Maui-Med bisexual strain), mating with wild females in the laboratory, varied independently of male diet.

In the field enclosure experiment in Guatemala (see above), Shelly et al. (2003) found that copula duration varied with diet but not with exposure to ginger root oil. Significantly, protein-deprived (sugar-fed) males at the high elevation site copulated longer (average 141.4 min; range 11-255 min) than protein-fed males (120 min on average, range 15-225 min). Furthermore, copulations starting later in the day were significantly shorter than copulations starting early in the day (see also Vera et al. 2003). Assuming that males control copula duration, these findings suggest that males adaptively prolong copulation when the probability of their partner copulating again is highest and their own chances of being accepted as a mate, lowest.

Female Remating. A number of studies have shown that females first mated to a sterile male are more likely to remate than are females first mated to a wild male (e.g., Mossinson & Yuval 2003; Vera et al. 2003). Furthermore, Blay & Yuval (1997) found that females whose first mate was protein-deprived (sugar-fed) were more likely to remate than females first mated to a protein-fed wild male. However, in the only laboratory study combining the effects of diet and irradiation, Shelly & Kennelly (2002) found that the frequency of female remating varied independently of male diet regardless of whether her first mate was a wild or mass reared male. One difference between this study and those of Blay & Yuval (1997) and Mossinson & Yuval (2003) is that the first remating opportunity in the latter 2 experiments was offered on the day following mating, as opposed to 2 d following the initial mating in this study. It has been shown that male accessory gland secretions affect female receptivity (Jang 2002) and that their effect intensifies several days after mating (Miyatake et al. 1999; Mossinson & Yuval 2003). Although the effect of irradiation and post-teneral diet on these secretions has not been studied, the results of Shelly & Kennelly (2002) are consistent with a mechanism in which accessory gland secretions affect remating 2 d after the initial copulation.

The amount of sperm stored by female medflies determines, to a large extent, their short-term receptivity to further copulations (Miyatake et al. 1999; Mossinson & Yuval 2003). In comparison to the average ejaculate of wild males, which contains approximately 3,000 sperm (Yuval et al. 1996; Taylor et al. 2000), the ejaculate of sterile males contains fewer sperm, and this alone may prejudice the effectiveness of SIT. Furthermore, the amount of sperm stored by females significantly declines as the sterile male ages. Thus, sterile males are effective inseminators only on the 2-3 d following release. Wild females may copulate with older sterile males (but see Liedo et al. 2002), but they will probably rapidly copulate again, either on the same day or on the following one (Mossinson & Yuval 2003; Vera et al. 2003). Accordingly, the first few days following release are the critical period for sterile male effectiveness. If protein fed males can survive during this period, and bring competitive advantages to bear, the SIT operation will benefit.

**Survival and Longevity**

In the laboratory, no difference was documented in survival of sugar-fed or sugar and protein-fed sterile males when they had access to a natural source of food (apple slice) from the fifth d of life onwards. However, when these males were starved, a dramatic effect was observed. Diet significantly affected the probability of surviving starvation, with males who had previously also fed on protein dying significantly faster than sugar-fed males (Kaspi & Yuval 2000).

Maor et al. (2004) replicated these findings in a field enclosure experiment. A sample of males from each diet treatment was held in a cage within the field enclosure, either with or without food. After 2 d without food, most (>95%) of the flies (protein-fed and protein-deprived males) died. Conversely, most of the flies that had continuous access to a dry fig survived for as long as 18 d. Furthermore, post-teneral diet had a significant effect on the survival of the flies. Protein-deprived (sugar-fed) males survived longer without food, but protein-fed males survived longer when food was provided (Maor et al., 2004). Apparently, the ingestion of protein, while increasing male sexual activity, also affects male metabolism in such a manner that interruption of protein feeding has greater negative consequences than does interruption of a diet lacking protein. The protein rich diet provided to males may commit them metabolically to reproduction (Carey et al. 2002) by diverting resources to pheromone and accessory glands and energy to sexual advertisement. This commitment carries higher sexual rewards in some environments, but also the penalty of inability to weather periods of nutritional stress.

Recognizing the importance of olfactory cues containing α-copaene in enhancing sexual performance of male medflies (Shelly 2001), the synergistic effect of olfactory cues and dietary regimes on survival was recently investigated (Levy et al. 2005). Working with wild flies from Israel and the VIENNA 7/Tol-2000 and (in Hawaii) VIENNA 4/Tol-94 strains, a series of experiments were conducted to determine how various diets, combined with exposure to volatiles containing α-copaene, affect the ability of male medflies to withstand starvation.

Flies were presented with diets and olfactory stimuli from the moment of emergence for 4 d, simulating the pre-release period. From the
fourth day onwards, simulating release, diets and other stimuli were removed, and flies monitored until all had died. A comparison of the pooled survival rates of the 3 strains revealed that sterile males have a significantly lower ability to resist starvation compared to wild males. Furthermore, males from the 2 mass reared strains also differed significantly in their ability to endure starvation. Males from the strain shipped during approximately 72 h from Guatemala to Israel (VIENNA 7/Tol-2000) died significantly faster than males from the strain (VIENNA 4/Tol-94) tested in Hawaii without long-distance shipping. The sugar diet, alone or in combination with α-copaene, was associated with the highest ability to resist starvation, followed closely by the sugar + protein diet (again, alone or in combination with α-copaene). Paradoxically, the apricot diet, which in some trials was associated with the best sexual performance (see above), contributed most significantly to the rapid death of the flies ingesting it. Furthermore, when the apricot diet was coupled with α-copaene, rates of demise were faster yet (Levy et al. 2005).

**PRE-RELEASE DIET AND FORAGING SUCCESS IN THE FIELD**

The findings covered above prompt a key question—does pre-release diet affect the ability of sterile males to find food in the field? Results of a number of studies in field cages and enclosures shed some light on this matter. Shelly & McInnis (2003) released protein + sugar-fed and protein-deprived (sugar-fed) sterile males from 2 different mass rearing strains into field cages containing a single guava tree. In 1 experiment, no food was provided, and all surviving males were collected after 2 d. Sixty one percent of males of the VIENNA 7/Tol-2000 strain from both diet treatments survived. In the other strain tested, Maui-Med bisexual strain, survival was lower overall, but similarly did not differ between diet treatments. In another experiment, food (papaya and orange slices) and water were placed in the field cages and surviving males collected 4 d later. Again, pre-release diet did not affect survival. Close to 40% of the males from both strains survived, irrespective of diet. The survival of males in the cages without food was probably due to the presence of bacteria or residual honeydew. The low survival after 4 d with food may be due to predation by ants, spiders or other invertebrate predators within the field cage.

To seek conclusive evidence for the ability of protein-fed sterile males to forage for food, Maor et al. (2004) determined the nutritional status of males sampled in leks within a field enclosure for 6 d following release. Post-teneral diet had no significant effect on the level of sugar in the flies, as most had high levels of carbohydrates that must have been recently acquired. Diet did have a significant effect on the level of protein in the flies. Protein-fed males contained significantly more protein than protein-deprived (sugar-fed) males on the day of release and on the day following release. Finally, post-teneral diet had a significant effect on the level of lipids in the flies. Protein-fed males contained significantly less lipids than protein-deprived (sugar-fed) males on the day of release and on the first day following release. The level of lipids decreased significantly from d 0 to d 6 in both protein-fed and protein-deprived males.

These results demonstrate conclusively that when food is available at the release site, most sterile males are able to find and ingest it, irrespective of their pre-release diet.

**PROSPECTS FOR THE FUTURE—MANIPULATION OF THE POST-TENERAL ENVIRONMENT**

While some might hurry to conclude that sugar alone is the best diet for sterile males, we interpret the emerging picture in a different light. First, post-teneral diet does not affect foraging ability of sterile males (Shelly & McInnis 2003; Maor et al. 2004; Barry et al. 2003). Thus, if nutrition is available in the field, most sterile males will find it. Furthermore, due to their reduced ability to inseminate as they age, the sexual effectiveness of sterile males is diminished following the first 24-48 h after release (Taylor et al. 2001; Liedo et al. 2002). Such males may serve as distractions for wild females but will fail to inhibit their receptivity (Mossinson & Yuval 2003). Thus, it may be better to release relatively short-lived flies that are highly competitive, rather than long lived, sexually ineffective ones. Better still would be long-lived sterile males with prolonged competitive ability (e.g., McInnis et al. 2002). The enhancing effect of protein feeding, while found in all studies of wild flies, is not evident in all studies of sterile flies. This may be due to a strain-environment interaction, the ability of sugar fed flies to acquire protein by feeding on feces, pupal frass or the carcasses of dead conspecifics, or variations in the bacterial communities present in the guts of these insects.

Accordingly, more research is needed on the critical post-teneral period, a time when the sterile males and their environment are completely under the control of the SIT program personnel. An optimal protocol would incorporate diet, olfactory cues, and possibly hormonal and social stimuli as well. Its validation must be based on sexual performance of the sterile males in a competitive setting that emulates field conditions.

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DISPERSAL AND LONGEVITY OF WILD AND MASS-REARED ANASTREPHA LUDENS AND ANASTREPHA OBLIQUA (DIPTERA: TEPHRITIDAE)

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ABSTRACT
The rates of dispersal and survival of sterile mass-reared laboratory flies and sterile wild flies of Anastrepha ludens (Loew) and Anastrepha obliqua (Macquart) were estimated and compared with a regular rectangular array of 64 food-baited traps spaced 60 m between traps around the release point in Tapachula Chiapas, Mexico. The traps were scored every day during the first week, and then every 3 d over a 30-d period. For A. obliqua, the number of males recaptured was higher than that of females, while for A. ludens, females were re-captured more frequently than males. The recapture rate for the wild strains ranged from 0.6-24.8% for A. ludens and 1.3-16.2% for A. obliqua and the corresponding ranges for the mass-reared strains were 0.5-7.1% and 0.5-3.0% respectively. The life expectancy was 4.7 d for wild and 4.3 d for mass-reared A. obliqua males but 3 and 2 d, respectively, for wild and mass-reared A. ludens males. The net displacement of A. ludens and A. obliqua ranged approximately from 100-250 m and took place mostly on the first day. Wild A. ludens moved to the northwest from the release point while the mass-reared strain moved to the west. The A. obliqua wild flies moved to the west, while the mass-reared strain shifted to the southwest. We discuss the implications of our findings as to the spacing and frequency of sterile fly releases for the suppression of wild populations.

Key Words: fruit fly, Anastrepha ludens, Anastrepha obliqua, trapping, life expectancy, sterile insect technique, dispersal, longevity

RESUMEN
La dispersión y longevidad de las moscas estériles silvestres y de cría masiva de Anastrepha ludens (Loew) y A. obliqua (Macquart) fueron determinadas y comparadas utilizando un arreglo rectangular de 64 trampas espaciadas a 60 metros entre trampas alrededor del punto de liberación en Tapachula Chiapas, México. Las trampas fueron revisadas y evaluadas diariamente durante la primera semana y después cada tres días hasta completar 30 días. Para A. obliqua la cantidad de machos capturados fue mayor que la cantidad de hembras; mientras que para A. ludens las hembras fueron capturadas con mayor frecuencia que los machos. La recaptura de moscas silvestres de A. ludens fue de 0.6 a 24.8%, para A. obliqua fue del 1.3 al 16.2%, para moscas de laboratorio fue de 0.5 a 7.1 y 0.5 a 3%, respectivamente. La esperanza de vida correspondió a 4.7 y 4.3 días para machos silvestres y de laboratorio de A. obliqua respectivamente; mientras que 3 y 2 días fueron para los machos silvestres y de laboratorio de A. ludens. La dispersión para A. ludens y A. obliqua fue de 100 a 250 m tanto para individuos silvestres como de laboratorio. Los adultos de A. ludens silvestre se desplazaron del punto central de liberación al noroeste, los individuos de laboratorio se movieron hacia el oeste del plano Cartesiano. A su vez los adultos de A. obliqua silvestre se movieron hacia el oeste y las de laboratorio hacia el sureste. Discutimos las implicaciones de nuestros resultados con relación al espaciamiento y frecuencia de las liberaciones de moscas estériles para la supresión de poblaciones silvestres.

Translation provided by the authors.

The Anastrepha (Schiner) genus (Diptera: Tephritidae) is deemed to be one of the most significant pests of commercially grown fruit from the southern United States to northern Argentina (Aluja 1994; Aluja et al. 1996). Anastrepha ludens (Loew) is the major pest of citrus fruits in Mexico, Belize, Guatemala, and the lower Rio Grande valley of Texas (Aluja et al. 1996; Thomas & Loera-Gallardo 1998). Anastrepha obliqua (Macquart) is the main fruit fly species to attack mango in commercial orchards situated at lower altitudes, while A. ludens infests orchards located at higher altitudes (Aluja et al. 1996). The sterile insect technique (SIT), as part of an area-wide integrated management approach, has been continuously deployed in northern Mexico since 1994 against A. ludens, and since 2001 against A. obliqua.

Maximum effectiveness in the application of the SIT requires a detailed knowledge of the various ecological parameters that can predict the
population dynamics of the target populations. It is intuitively obvious that the dispersal of an insect species at its reproductive stage has a considerable effect on the dynamics of the local population (Royama 1979). This highlights the importance of measuring the fitness of the released insects in relation to the wild population, and the need to determine the ways in which possible differences could affect the success of the control program (Fletcher & Economopoulos 1976). The international fruit fly quality control manual (FAO/IAEA/USDA 2003) defines the estimation of the ability of released sterile insects to survive in the field and to move from the point of release to feed and mate as the main objective of release-recapture tests of dispersal and survival.

The field quality of sterile flies has 2 main components, mating competitiveness and survival rate. Sterile insects must survive under natural conditions at the target site until they are sexually mature and achieve copulation with fertile wild females in order to be effective as agents of control. The basic questions are how long do the flies live after they are released, and how far do they disperse? Such information is necessary for determining the frequency with which releases should be made and the distance between release points (Thomas & Loera-Gallardo 1998).

Recapture studies have been the traditional method by which the dispersal and longevity information is obtained. Shaw et al. (1967) used a design to measure long-distance dispersal and reported some recaptures at 5-8 km from the release point. Baker et al. (1986) and Baker & Chan (1991) calculated orientation ellipses to visualize the dispersal of mass-reared populations of Ceratitis capitata (Wiedemann) and A. ludens. They concluded that in all cases the mean fly position moved significantly away from the release point indicating a drifting of the released population. Most movements were towards the north and east, the direction of prevailing daily winds. Thomas & Loera-Gallardo (1998) used a regression model and estimated the average dispersal distance to be 240 m for sterile mass-reared A. ludens and found that life expectancy after release ranged from 5 to 10 d.

The purpose of this work is to compare the dispersal rates and longevity of sterile mass-reared flies and wild flies of 2 species, A. ludens and A. obliqua. We present data from fly releases performed over 4 years of research in commercial mango orchards and discuss the implications of our findings as to the spacing and frequency of sterile fly releases for the suppression of wild populations.

**MATERIALS AND METHODS**

**Release and Recapture of Wild and Mass-reared Irradiated Flies**

The flies were released in commercial mango orchards (Mangifera indica L. v. Ataulfo) in Tapa-chula, Chiapas, Mexico. The mango trees were 20 years old and 15 m high with spacing of 15 m between them. The sterile flies used in this study were obtained from the Moscafrut mass-rearing facility in Metapa, Mexico, which is the same source of flies used for the suppression programs in Mexico. Mass-reared colonies of A. ludens and A. obliqua were maintained on corn cob larval diet (Artiaga-López et al. 2004; Stevens 1991). Wild A. obliqua flies were obtained from an intensive collection in Comitán, Chiapas (16°15'13"N, 92°07'35"W) of infested fruits of Spondias mombin L., and wild A. ludens flies from infested fruits of Citrus aurantium L. collected in Tapachula, Chiapas (14°54'10"N, 92°15'32"W). Fruits were placed in plastic containers and stored for 4 d to allow most of the larvae to reach physiological maturity. Later, fruits were dissected to obtain larvae, which were placed in a container along with moist vermiculite in order for them to pupate under indoor conditions of 26 ± 1°C and 75 ± 5% R.H.

The mass-reared flies were sterilized by exposing the late pupal stage, 48 h before adult emergence, to 80 Gy from a 60Co source. The irradiated pupae were held in 4-L paper bags until complete adult emergence. Adults were provided sugar and water and held in the bags for 5 d at which time they were released. In all replicates, the number of flies per release was about 6,000 (3,000 per sex). The pupae were marked with fluorescent dyes of different colors to discriminate between the wild flies from the laboratory flies, and also to distinguish among different release dates. Both A. ludens and A. obliqua were released shortly after sunrise at the center of the orchard. For each species, 9 releases were performed during the years 1999 to 2004. The “Jasso” mango orchard, 30 m above sea level and located within 20 km from Tapachula at km 4 of the Jaritas-Cd. Hidalgo road, was chosen as the release site.

For fly capture, 64 McPhail traps were baited with a mixture of 10 mL of protein hydrolysate (Captor 300), 5 g of borax, and 240 mL of water and deployed in a regular rectangular 8 × 8 array with 60 m spacing between the traps. The traps were placed 4 m above ground in a mango tree, and were installed the day after fly release. To estimate longevity, the traps were scored every day. The fluorescent color dye in marked flies was distinguished with a Leica MMZ FLIII fluorescence microscope. The recaptured flies were separated by origin (mass-reared or wild), sex, trap, and release date for each species.

The analysis of recapture rates was done after arc-sine transformation of the square-root of the proportions recaptured (Underwood 2005). The transformed data were given by the formula

$$Y = \sin^{-1}\sqrt{Y/100}$$

where Y values are the percentage of captured flies.
Longevity of Wild and Mass-reared Irradiated Flies

The longevity was estimated with fly captures as a function of time after the release (Carey 1989). To form the first (age) column of a life table, the release day was considered as age zero, while the subsequent d were considered as specific age \( (x) \). The number of flies captured on a given day \( (Y) \) over successive d formed the next column and the following items formed subsequent ones and were calculated as follows. The accumulated number of flies captured from a given day was found by

\[
n_{x+1} = n_x - Y_x
\]

Survival rate up to a given day was found by

\[
l_x = n_x / n_0
\]

so that

\[
\sum_{x=0}^{n} l_x = 1
\]

The age specific death rate (proportion of flies that died on a given day) was found by

\[
q_x = l_x - l_{x+1}
\]

and the survival rate from release to halfway through a given day was estimated as

\[
L_x = \left[ l_x - (q_x / 2) \right]
\]

Finally, whereas the mean life expectation at the day of release could be estimated as

\[
e_0 = \sum_{x=0}^{n} L_x
\]

the expectations at other ages were calculated in two stages (columns) as shown in Tables 3 and 4. Mean values were compared by one-way analysis of variance, while the daily survival population curves and life expectancy curves were compared by a survival analysis and Log-Rank test between groups by JMP Statistical Discovery Software (SAS Institute 2003). The survival curves were fitted to the natural logarithm model with the number of individuals trapped expressed in logarithmic scale and time in arithmetical scale.

Dispersal of Wild and Mass-reared Irradiated Flies

Mean dispersal distance \( d \), was estimated by relating the number of flies captured in each trap, the distances between traps, and the release point according to the following formula:

\[
\tilde{d} = \frac{\sum_{i=1}^{n} x_i C_i}{\sum_{i=1}^{n} C_i}
\]

where \( C_i \) is the number of flies captured in trap i, and \( x_i \) is the distance between trap i and the release point (FAO/IAEA/USDA 2003). The dispersal area was modeled by contour plot analysis with JMP Statistical Discovery Software (SAS Institute 2003), in which the distance (m) was the average displacement from the release site towards any point, without considering the direction. The magnitude (m) was the average displacement in the Cartesian plane, considering the direction. The direction angle (°) was the direction of the displacement in the Cartesian plane. The r vector consisted in the adjustment of the displacements to a “swarm” type movement. The occupied area (%) was determined from the area occupied by the traps that captured flies.

RESULTS

Recapture of Wild and Mass-reared Irradiated A. ludens

The number of flies captured on each date according to origin and sex are presented in Table 1. The mean capture percentage (± SE) was 7.62 ± 2.81% for wild flies and 2.88 ± 0.88% for laboratory flies, but there was a high variation among replicates and the means were not significantly different \( (F = 2.80; df = 1,16; P = 0.1134) \). The first (May 17, 1999) and fifth (July 1, 2001) releases resulted in the highest recapture rates for both wild and mass-reared flies (Table 1). The mean percentage of recaptured males was 50.90 ± 1.50% and 45.13 ± 3.65% laboratory and wild flies, respectively, was non-significant \( (F = 2.14; df = 1,16; P = 0.1627) \). Fig. 1 shows the number of flies recaptured per release cohort (identified by release date). The points were fitted to exponential regression and we observed that for A. ludens the laboratory line was above the wild line.

Recapture of Wild and Mass-reared Irradiated A. obliqua

The percentage of recaptured males was 55.26 ± 2.10% and 52.75 ± 2.72% for wild and laboratory flies, respectively (but the difference between the two types of male was not significant; \( F = 0.53; df = 1,16; P = 0.475 \)). For the releases performed on June 06, 1999, July 12, 2001, August 17, 2001, and September 06, 2001, more than 2.0% of the released flies that were recaptured were of laboratory origin; while on June 06, 1999, July 12, 2000, and September 06, 2001, the percentage was over 8% for wild flies (Table 1). The mean re-
capture rates of laboratory and wild flies were significantly different ($F = 16.23; df = 1, 16; P = 0.001$). Fig. 1 shows the number of flies captured per release date and the fit for exponential regression; the lines for wild flies are consistently lighter than those for laboratory flies.

### Longevity of Wild and Mass-reared *Anastrepha ludens*

Longevity, expressed as the life expectancy value within the trap array (Table 2) indicates that the life expectancy ($e_0$) after release for *A. ludens* was 3.1 d for the wild strain and 2.2 d for the laboratory strain. Both wild and laboratory flies showed similar values ($F = 1.25; df = 1, 16; P = 0.2783$). Moreover, there were no significant differences between wild and laboratory females ($F = 0.65, df = 1, 16; P = 0.4291$) or between wild and laboratory males ($F = 0.91, df = 1, 16; P = 0.3538$). Table 3 shows the calculation of the life expectancy of the laboratory males of *A. ludens* within the trap array; the values are based on the sum of males captured during the first 15 d of trapping following each of the 9 release dates. The life expectancy of each release cohort is given in Fig. 4; wild *A. ludens* had high values in 2000 and 2004, whereas the laboratory flies tended to be more constant.

### Longevity of Wild and Mass-reared *A. obliqua*

The life expectancy ($e_0$) after release for *A. obliqua* was estimated at 4.3 d for the wild strain and 4.2 d for the laboratory strain, and there was no significant difference between them ($F = 0.16; df = 1, 16; P = 0.6932$). The mean life expectancy was not significantly different for wild and laboratory females ($F = 0.017; df = 1, 16; P = 0.6793$), or males ($F = 0.11; df = 1, 16; P = 0.7376$) (Table 2). Table 4 shows the life expectancy values based on the males captured during the first 15 d following each of the 9 release dates. The life expectancy pertinent to each release cohort appears in

### Table 1. Total recapture of *Anastrepha ludens* and *A. obliqua* in mango orchard “Jasso” after releases in the center of the sampling area.

<table>
<thead>
<tr>
<th>Release date</th>
<th>Total recapture</th>
<th>Percent male recapture</th>
<th>Percent recapture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W</td>
<td>L</td>
<td>W</td>
</tr>
<tr>
<td><em>Anastrepha ludens</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17/05/99</td>
<td>814</td>
<td>560</td>
<td>42.63</td>
</tr>
<tr>
<td>27/05/00</td>
<td>90</td>
<td>76</td>
<td>31.11</td>
</tr>
<tr>
<td>30/05/01</td>
<td>112</td>
<td>258</td>
<td>31.25</td>
</tr>
<tr>
<td>22/06/01</td>
<td>53</td>
<td>41</td>
<td>39.62</td>
</tr>
<tr>
<td>01/07/01</td>
<td>167</td>
<td>830</td>
<td>63.47</td>
</tr>
<tr>
<td>05/06/04</td>
<td>72</td>
<td>298</td>
<td>50.00</td>
</tr>
<tr>
<td>13/06/04</td>
<td>45</td>
<td>625</td>
<td>44.45</td>
</tr>
<tr>
<td>13/06/04</td>
<td>115</td>
<td>1006</td>
<td>58.26</td>
</tr>
<tr>
<td>08/06/04</td>
<td>141</td>
<td>153</td>
<td>45.39</td>
</tr>
<tr>
<td>Average ± SE</td>
<td></td>
<td></td>
<td>45.13 ± 3.65 a</td>
</tr>
<tr>
<td><em>Anastrepha obliqua</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06/06/99</td>
<td>675</td>
<td>386</td>
<td>43.11</td>
</tr>
<tr>
<td>12/07/99</td>
<td>423</td>
<td>321</td>
<td>56.50</td>
</tr>
<tr>
<td>17/08/99</td>
<td>51</td>
<td>41</td>
<td>62.75</td>
</tr>
<tr>
<td>12/07/00</td>
<td>207</td>
<td>94</td>
<td>58.45</td>
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<tr>
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<td>154</td>
<td>60.27</td>
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<tr>
<td>10/08/01</td>
<td>116</td>
<td>68</td>
<td>58.62</td>
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<td>17/08/01</td>
<td>114</td>
<td>108</td>
<td>52.63</td>
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<tr>
<td>06/09/01</td>
<td>196</td>
<td>126</td>
<td>57.14</td>
</tr>
<tr>
<td>08/06/04</td>
<td>186</td>
<td>245</td>
<td>47.85</td>
</tr>
<tr>
<td>Average ± SE</td>
<td></td>
<td></td>
<td>55.26 ± 2.10 a</td>
</tr>
</tbody>
</table>

W = Wild strain, L = Laboratory strain. Values for the same species in a row with the same letter do not differ significantly (ANOVA on arcsine transformed percentages, $\alpha = 0.05$). The values are expressed by average ± SE.
Hernández et al.: Dispersal and Longevity of Sterile Flies

Fig. 4; life expectancy at release fell to less than 2 d during the months of Jul and Aug 1999, but much higher values were achieved by later release cohorts.

Wild and laboratory flies were captured over 20 d (Fig. 1), but less than 50% of the flies were alive after about 4-5 d (Fig. 3). Equations corresponding to the curves shown in Fig. 3 were ln(nx) wild female = 4.9870 – 0.1733(age) ($F = 399.12; df = 1,21; P < 0.0001$); ln(nx) wild males = 4.9425 – 0.1710(age) ($F = 477.32; df = 1,21; P < 0.0001$); ln(nx) laboratory female = 4.5568 – 0.1852(age) ($F = 1720.81; df = 1,21; P < 0.0001$); ln(nx) laboratory males = 4.6642 – 0.2004(age) ($F = 561.69; df = 1,21; P < 0.0001$).

Dispersal of Wild and Mass-reared *Anastrepha ludens*

Dispersal parameters are presented in Table 5. In all cases, wild flies did not differ from laboratory flies (ANOVA, $P < 0.05$). *Anastrepha ludens* males and females could have dispersed over
150 m during the first 15 d after release (Fig. 5). Fig. 6 shows how the movements are mostly close to the release point. There was no statistical difference between laboratory and wild strains.

Dispersal of Wild and Mass-reared A. obliqua

Dispersal parameters are presented in Table 5. In all cases, wild flies did not differ from laboratory flies (ANOVA, \( P < 0.05 \)). The mean distance moved away from the release point indicates that the total displacement was almost 139 m for both wild and laboratory strains. A. obliqua males and females dispersed over 200 m during the first 13 d after release (Fig. 4). The A. obliqua laboratory strain has a displacement similar to that of A. ludens (Fig. 6). However, for the wild strain, the displacement was very irregular (i.e., displacement not concentric) with more area occupied by the flies as a whole.

DISCUSSION

In all cases, the life expectancy values obtained for the flies released during this investigation were lower than the 9.85 d obtained by Thomas & Loera-Gallardo (1998) in the arid subtropics for mass-reared and sterilized A. ludens and the 17.3 d mentioned by Celedonio-Hurtado et al. (1988) within the laboratory. The shorter life span obtained in this study may have been due to different biotic and also abiotic conditions in the tropics, in particular the rainy weather and higher relative humidity. Also, there may have been spray drift from the chemical control in neighboring commercial mango orchards at times

TABLE 2. LIFE EXPECTANCY (\( e_0 \)) FOR ANASTREPHA LUDENS AND A. OBLIQUA IN MANGO ORCHARD “JASSO” AFTER THE RELEASES.

<table>
<thead>
<tr>
<th></th>
<th>Anastrepha ludens</th>
<th>Anastrepha obliqua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild (d)</td>
<td>Laboratory (d)</td>
</tr>
<tr>
<td></td>
<td>3.34 ± 0.56 a</td>
<td>2.39 ± 0.21 a</td>
</tr>
<tr>
<td>Female</td>
<td>4.45 ± 0.76 a</td>
<td>4.17 ± 0.71 a</td>
</tr>
<tr>
<td>Male</td>
<td>2.99 ± 0.43 a</td>
<td>2.03 ± 0.26 a</td>
</tr>
<tr>
<td></td>
<td>4.65 ± 0.88 a</td>
<td>4.25 ± 0.69 a</td>
</tr>
<tr>
<td>Total</td>
<td>3.12 ± 0.47 a</td>
<td>2.20 ± 0.21 a</td>
</tr>
<tr>
<td></td>
<td>4.35 ± 0.82 a</td>
<td>4.17 ± 0.63 a</td>
</tr>
</tbody>
</table>

The values are expressed by average ± SE. The mean life expectancy was estimated on flies captured during 20 (A. ludens) and 23 (A. obliqua) d of trapping following each of the 9 release dates. Values for the same species and sex in a row with the same letter do not differ significantly (ANOVA, \( \alpha = 0.05 \)).

TABLE 3. LIFE EXPECTANCY CALCULATIONS FOR LABORATORY MALES OF ANASTREPHA LUDENS.

<table>
<thead>
<tr>
<th>x Capture day</th>
<th>Captured flies</th>
<th>( Y_x )</th>
<th>( n_x )</th>
<th>( l_x = n_x/n_0 )</th>
<th>( q_x = l_x - l_{x+1} )</th>
<th>( L_x = l_x - (q_x/2) )</th>
<th>( T_x = \Sigma L_x + L_{x+1} + \ldots L_n )</th>
<th>( e_x = T_x/l_x )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1577</td>
<td>3542</td>
<td>1.00</td>
<td>0.45</td>
<td>0.78</td>
<td>1.98</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>601</td>
<td>1965</td>
<td>0.55</td>
<td>0.17</td>
<td>0.47</td>
<td>1.20</td>
<td>2.17</td>
<td></td>
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<tr>
<td>2</td>
<td>617</td>
<td>1364</td>
<td>0.39</td>
<td>0.17</td>
<td>0.30</td>
<td>0.73</td>
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<tr>
<td>3</td>
<td>250</td>
<td>747</td>
<td>0.21</td>
<td>0.07</td>
<td>0.18</td>
<td>0.44</td>
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<td></td>
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<tr>
<td>4</td>
<td>211</td>
<td>497</td>
<td>0.14</td>
<td>0.06</td>
<td>0.11</td>
<td>0.26</td>
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<tr>
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<td>114</td>
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<tr>
<td>7</td>
<td>28</td>
<td>93</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>1.84</td>
<td></td>
</tr>
<tr>
<td>8</td>
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<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>1.42</td>
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<tr>
<td>9</td>
<td>16</td>
<td>27</td>
<td>0.01</td>
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<td>0.01</td>
<td>0.01</td>
<td>1.72</td>
<td></td>
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<td>7</td>
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<td>0.01</td>
<td>2.64</td>
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<td>12</td>
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<td>6</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>2.00</td>
<td></td>
</tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

\( n_0 = 3542 \)

Life expectancy = \( \Sigma L_x = 1.98 \) d

The averages are based on the sum of males captured during the first 15 d of trapping following each of the 9 release dates.
Fig. 2. *Anastrepha ludens*. Survival proportion (lx)(Female, Log-Rank, $\chi^2 = 0.5478$, df = 1, $P = 0.4578$; Male, Log-Rank, $\chi^2 = 0.2727$, df = 1, $P = 0.6015$). Lives flies (ln nx) (Female, Log-Rank, $\chi^2 = 1.5345$, df = 1, $P = 0.2154$; Male, Log-Rank, $\chi^2 = 0.0061$, df = 1, $P = 0.9379$). Life expectancy (ex))(Female, Log-Rank, $\chi^2 = 13.9547$, df = 1, $P = 0.0002$; Male, Log-Rank, $\chi^2 = 53.7651$, df = 1, $P = 0.0 < 0.0001$). The values were estimated from recapture rates with McPhail glass traps.
of peak fruit production. Celedonio-Hurtado et al. (1995) and Aluja et al. (1996) were unable to find a correlation between weather and trap capture in the same release site (Tapachula, Chiapas, México), but they were recording natural populations and did not obtain data on fly survival.

The survival curves for *A. ludens* and *A. obliqua* correspond in both cases to the “diagonal type” in the classification proposed by Hutchinson (1981), where the $n_x$ curve descends in a straight line when it is plotted on a logarithmic scale, and time on an arithmetical scale. This type of curve indicates that throughout a fly’s life, the probability of death is fairly constant.

Anastrepha obliqua had a higher average displacement than *A. ludens*. In both species, during the first day, displacement distance was about 100 m while, for the rest of the time, further displacement was low and variable. *Anastrepha ludens* and *A. obliqua* showed the same tendency observed by Thomas & Loera-Gallardo (1998) for *A. ludens* releases along the Santa Rosa Canyon in the State of Nuevo León, Mexico, where recap- 

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>A. ludens</em></th>
<th><em>A. obliqua</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>111.58 ± 4.26 a</td>
<td>110.85 ± 0.98 a</td>
</tr>
<tr>
<td>L</td>
<td>151.73 ± 6.17 a</td>
<td>139.18 ± 9.26 a</td>
</tr>
</tbody>
</table>

*W* = Wild strain, *L* = Laboratory strain. The values are expressed by average ± S.E. Values in the columns for the same specie with the same letter are not different (ANOVA, $\alpha = 0.05$).

Distance.—Average displacement from the release site towards any point, without considering the direction.

Magnitude.—Average displacement in the Cartesian plane, considering the direction.

Direction angle.—Direction of the displacement in the Cartesian plane.

R vector.—Adjustment of the displacements to a “swarm” type movement.

Occupied area.—Area occupied determined from the traps that captured flies.

### Table 5. Dispersal and Orientation of *Anastrepha ludens* and *A. obliqua* in a Mango Orchard.

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>Anastrepha ludens</em></th>
<th><em>Anastrepha obliqua</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (m)</td>
<td>111.58 ± 4.26 a</td>
<td>110.85 ± 0.98 a</td>
</tr>
<tr>
<td>Magnitude (m)</td>
<td>47.12 ± 3.52 a</td>
<td>46.88 ± 2.70 a</td>
</tr>
<tr>
<td>Direction angle (°)</td>
<td>164.33 ± 15.01 a</td>
<td>184.17 ± 15.24 a</td>
</tr>
<tr>
<td>R vector</td>
<td>0.40 ± 0.03 a</td>
<td>0.22 ± 0.06 a</td>
</tr>
<tr>
<td>Occupied area (%)</td>
<td>48.61 ± 2.51 a</td>
<td>69.53 ± 12.44 a</td>
</tr>
</tbody>
</table>

$\alpha = 0.05$.
and the dispersion was not a process of simple diffusion. Displacement in the Cartesian plane for both species did not include the northern and southwestern quadrants. Thomas & Loera-Gallardo (1998) observed dispersal distances of 9 km from the release point, and also reported persis-

Fig. 3. *Anastrepha obliqua*. Proportion surviving on trap array by date (lx); number of trappable live flies on array (ln nₐ); life expectancy on trap array (eₓ). The values were estimated from recapture rates with McPhail glass traps.
tence of the sterile flies up to 78 d. The reason for such a difference in the present results remains to be established.

The life expectancy values obtained for mass-reared, male *Anastrepha ludens* and *A. obliqua* indicate that suppression or eradication pro-

---

**Fig. 4.** Life expectancy on trapping array ($e_0$) by release date; *Anastrepha ludens* (upper) and *A. obliqua* (lower).
grams should consider 2 releases per week for *A. ludens* and once per week for *A. obliqua*. The mean distance of displacement was about 100 m, indicating that in order to optimize the dispersal of sterile flies, release lines should be spaced not more than 200 m. In view of the low life expectancy of mass-reared *Anastrepha* spp. flies after release (3 to 4 d), and since they reach sexual maturity only around 10 d of age (Orozco et al. 2001), operational programs could consider holding sterile flies longer before release, or treating them with juvenile hormones to accelerate maturation (Teal et al. 2007), to avoid that a majority of released sterile males do not survive before being able to inseminate wild females.

**ACKNOWLEDGMENTS**

The authors are grateful to the authorities of the Mexican Fruit Fly National Campaign. We thank IAEA...
for supporting this project. We sincerely appreciate the technical support by Bigail Bravo and Mr. Orlando Rivera. We thank Teresa Vera and anonymous reviewers for constructive criticism of an earlier version of this manuscript.

REFERENCES CITED


Fig. 6. Dispersion of laboratory and wild Anastrepha ludens and A. obliqua determined by contour plot analysis from JMP. Number in each line indicates the number of flies captured inside the area.


DISPERSION OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) AT HIGH AND LOW DENSITIES AND CONSEQUENCES OF MISMATCHING DISPERSIONS OF WILD AND STERILE FLIES

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ABSTRACT
Both wild and released (sterile) Bactrocera tryoni (Froggatt) (Diptera: Tephritidae) and wild Bactrocera papayae (Drew and Hancock) in Australia had patchy distributions and comparisons with predictions of the negative binomial model indicated that the degree of clumping was sometimes very high, particularly at low densities during eradication. An increase of mean recapture rate of sterile B. tryoni on either of 2 trap arrays was not accompanied by a reduction in its coefficient of variation and when recapture rates were high, the percentage of traps catching zero decreased only slightly with increase in recapture rate, indicating that it is not practicable to decrease the heterogeneity of dispersion of sterile flies by increasing the number released. There was often a mismatch between the dispersion patterns of the wild and sterile flies, and the implications of this for the efficiency of the sterile insect technique (SIT) were investigated with a simulation study with the observed degrees of mismatch obtained from the monitoring data and assuming the overall ratio of sterile to wild flies to be 100:1. The simulation indicated that mismatches could result in the imposed rate of increase of wild flies being up to 3.5 times higher than that intended (i.e., 0.35 instead of 0.1). The effect of a mismatch always reduces the efficiency of SIT. The reason for this asymmetry is discussed and a comparison made with host-parasitoid and other systems. A release strategy to counter this effect is suggested.

Key Words: Bactrocera tryoni, Bactrocera papayae, patchiness, extinction, dispersion

RESUMEN
Las moscas naturales y liberadas (estériles) de Bactrocera tryoni (Froggatt) (Diptera: Tephritidae) y Bactrocera papayae (Drew and Hancock) en Australia tuvieron distribuciones en parches y sus comparaciones con las predicciones de un modelo binomial negativo indicaron un nivel de agregación a veces fue muy alto, particularmente en las densidades bajas durante la eradicación. Un aumento en el promedio de la tasa de B. tryoni estériles recapturadas en las dos formas de trampas no fue acompañado por una reducción en su coeficiente de variación y cuando las tasas de moscas recapturadas fue alto, el porcentaje de las trampas que capturaron ninguna mosca bajó solo un poco con un aumento en la tasa de las moscas recapturadas, esto indicó que no es practicable bajar la heterogenicidad de dispersión de las moscas estériles por medio de un aumento el número de moscas liberadas. Muy a menudo se encontró un desajuste entre los patrones de dispersión de las moscas naturales y estériles, y las implicaciones de esto para la eficiencia de la técnica del insecto estéril (TIE) fueron investigadas en un estudio de simulación con los grados de desajustes observados obtenidos de los datos del monitoreo y se consideró que la razón general del número de moscas estériles a moscas naturales fueron 100:1. La simulación indicó que los desajustes en los patrones de dispersión pueden resultar en una tasa impuesta sobre el aumento de las moscas naturales de hasta 3.5 veces más alta que la tasa intentada (i.e., 0.35 en vez de 0.1). El efecto de un desajuste siempre reduce la eficiencia de TIS. Se discute la razón para esta asimetría y una comparición hecha con el sistema de hospedero-parasitoid y otros sistemas. Se sugiere una estrategia de liberación para contrarrestar este efecto.

Patchy dispersion patterns are widespread in ecological systems and may have fundamental significance to their stability on a number of scales (Huffaker et al. 1963; Hassell & Waage 1984; May 1978; Harrison 1991; Hassell et al. 1991; Pacala & Hassell 1991; Taylor 1991; Murdoch & Briggs 1996). Clumped dispersion patterns of pests have been related to patchiness of the natural or managed habitat (Zalucki et al. 1984; Vargas et al. 1989; Clarke et al. 1997; Papadopoulos et al. 2003) and knowledge of the spatial heterogeneity in the density of a pest and its temporal variation can be utilized in strategies for its management (Clarke et al. 1997; Papadopoulos et al. 2003). To do this, however, degrees of dispersion must be quantified in suitable terms such as the coefficient of variation in density (Pacala & Hassell 1991) or its spatial autocorrelation (Buntin 1988; Clarke et al. 1997; Papadopoulos et al. 2003), the exponent of the negative binomial
Clumped dispersion has been reported for trap catches of adults of many fruit fly species (Diptera: Tephritidae) with examples in natural populations (Zalucki et al. 1984) and in an invading population subject to an eradication campaign (Clift & Meats 1998; Meats 1998) and in distributions of cohorts of released sterile flies (Teruya 1986; Plant & Cunningham 1991). During the outbreak stage of an exotic incursion of Bactrocera papayae Drew & Hancock (Diptera: Tephritidae) in northern Queensland (Australia), there was a gross pattern of localized distribution corresponding to discrete propagules of various sizes, and there was a further pattern of heterogeneous dispersion within them; a similar pattern was seen during the final stages of eradication of the incursion when the remnant populations were reduced to small isolated foci (Clift & Meats 1998; Meats 1998).

Heterogeneous dispersion of fruit flies must be taken into account when control measures are deployed and their effectiveness assessed. When the sterile insect technique (SIT) is used against fruit flies, the wild population is reduced to foci (as above), whereas the distribution of sterile flies should be more widespread (Meats 1983, 1996; Meats et al. 1988). If mating competitiveness is measured directly in open field conditions, the apparent decline in its value (Iwahashi et al. 1983; Iwahashi 1996) may be an artifact of the use of the wrong value for the ratio of sterile to wild flies in the calculations. This can be due to the inclusion of areas without wild flies in censuses to establish the ratio with the result that a much higher value is used that is really the case in the areas where sterile and wild flies are actually present together; this in turn will lead to an underestimate of the value for mating competitiveness (Meats 1983, 1996; Meats et al. 1988).

There are, however, less striking mismatches in the distribution of sterile and wild flies and these have consequences for the efficiency of SIT and may apply at many stages of eradication (Shiga 1986). As with control with cover-sprays of pesticide, an essential aim is to treat all the individuals of the target pest within the target area. Thus, whereas it may not be necessary for the treatment to reach all parts of the target area (for instance, if the target pest does not inhabit rocky outcrops or certain patches of vegetation), the distribution of the treatment should coincide with the distribution of the pest. If it does not, the pest may persist in localized patches or even increase to make control measures ineffective.

It is the purpose of this paper to examine the dispersion of wild and sterile fruit flies and in particular the simultaneous dispersion patterns of wild and sterile Bactrocera tryoni (Froggatt) (Diptera: Tephritidae) that were observed during trials of release techniques (Meats et al. 2003a) and to calculate the consequences that would apply if those patterns were present during an SIT campaign when the overall ratio sterile to wild flies was 100:1.

**Materials and Methods**

**General Analysis and Modelling**

Because all of the investigations reported here involved real or simulated results from trapping arrays, there were many analytical and modeling methods in common as follows. Means (m) of catch per trap per week (or per 2 weeks for B. papayae data) on a monitoring array, their standard deviations and errors (SD and SEM), coefficients of variation (CV), correlations, linear regressions, and the statistical significance and any differences between them were calculated with standard formulas (Snedecor & Cochran 1989). Data were tested for conformity to Taylor’s Power Law and negative binomial models for percentage of positive traps (traps with flies). Taylor’s power law (Kuno 1991; Nyrop & Binns 1991; Southwood & Henderson 2000) relates variance (s^2) to mean (m) as s^2 = am^b. The constants of this relationship were calculated as the intercept (log_{10} a) and slope (b), respectively, of the linear regression of log_{10} s^2 on log_{10} m, which has the form (log_{10} s^2 = log_{10} a + b log_{10} m). The equivalent regressions with SD substituted for variance have values of log_{10} a and b at half the corresponding amounts.

The negative binomial expectations for the percentage of positive traps in an array (% trapping > 0 in a given week) were found by

\[
\% > 0 = 100(1 - p_e)
\]

where p_e is the zero term of the negative binomial distribution found by

\[
p_e = \left[1 + \left(\frac{m}{k}\right)\right]^k
\]

and k is a constant related to the amount to which the distribution is more clumped than random. A k value of infinity gives the same result as the zero term for the Poisson distribution, and values down to about five have a very similar effect. However, a k value of one or less is considered to indicate a significantly clumped distribution from an ecological perspective. If predators or parasitoids distribute their attacks on their prey or hosts in such a manner, a sufficiently large proportion of the latter will escape giving potential stability to the ecological relationship (May 1978).

Model predictions for percentage of positive traps over a range of values of mean catch per trap were generated, with k values of 2.0, 1.0, 0.5, 0.3, 0.1, 0.05, and 0.02 plotted on the relevant Figs so that the range within which real or simulated values fell could be seen.
Data from Sterile Releases at Gilgandra and Narromine

Trials of release techniques for sterile B. tryoni were carried out in a number of small towns in New South Wales, Australia, from Feb 1996 to Apr 1998 (Meats et al. 2003a). ‘Conventional’ releases of newly emerged sterile flies were made in Gilgandra and Narromine, which are about 75 km apart and about 350 km northwest of Sydney. Both towns are of similar size (5 km²), have river frontage, a similar altitude (230–250 m), and are in a region that has an average annual rainfall of 500-600 mm. Both towns received marked sterile flies at an identical rate in any 1 week at weekly intervals during the study period. In a given week, equal numbers of flies were released at sites midway between the traps. It was estimated that the number of adults flown from the release sites varied from 48,000 to 115,000 males per km² per week but no flies were released in late autumn and winter period from mid Apr to mid Aug (Meats et al. 2003a).

Monitoring traps in each town were spaced at about 0.4 km from each other and cleared each week, and both the wild and sterile flies that were trapped by them were counted. The type of trap used was the Lynfield (pot) trap (Cowley et al. 1990). The wick of each trap was initially supplied with 4 mL of cue lure and 1 mL of malathion solution (50% in emulsifiable concentrate).

The sterile flies were produced at the facilities of NSW Agriculture (Meats et al. 2003a). The pupae were mixed with fluorescent marking powder from the ‘FEX’ series from Swada (London) Ltd. at a rate of 50 g per 100,000. The pupae (having completed about 75% of their development) were gamma-irradiated at the Australian Nuclear, Scientific and Technical Organization (ANSTO) from a 60Co source with 71-73 Gy at a rate of 7.6-10.2 Gy per min (depending on the age of the source). They were transported in thermally insulated boxes by air to Dubbo and then by road to an air conditioned insectary at the Trangie Agricultural Research Centre. During the first 2 seasons, the sterile pupae were kept in modified plastic garbage bins (45 liter capacity, 30,000 pupae per bin) and these were transported to the release sites (on a trailer covered with a tarpaulin) after the adults had emerged (Dominiaik et al. 1998). From Sep 1997, either small or large cages (0.5 × 0.5 × 0.5 m or 2.5 × 1.7 × 0.42 m) with shade cloth mesh were used. The smaller cages could receive up to 16,000 pupae and the larger ones up to approximately 225,000. Adult flies were supplied with sucrose and water up to the time of release at which time they were 2-3 d old.

Limited Data Set for Towns under Sterile Releases

A restricted set of data from Gilgandra and Narromine was selected in order to avoid the statistical problem of non-independence between weekly catches when calculating the slopes and standard errors (SEM) of the regressions of the log₁₀ values of s², SD or CV on log₁₀ means of trap catches. The reason for the non-independence problem is that despite the fact that new flies were released each week, many flies from previous releases would be trapped as well. Unless analysis is restricted to catches that are months apart, this problem cannot be overcome completely for a fly that can survive as long as B. tryoni. However it is worth investigating how the problem can be reduced to reasonable proportions (say, with trap catches at different times having only around 5-10% or less of flies in common from the same set of releases).

Fletcher (1973) gives the results of 9 releases of B. tryoni made between Feb and Apr 1969, and from his data one can calculate that 50% of mature adults within about a 200 m radius leave the area in any week. Recent results have yielded estimates of between 47% and 85% per week (unpublished data). With the figure of 50%, one can estimate the percentage R of the set of release cohorts trapped on 1 occasion that would be trapped on a subsequent occasion n weeks later. When there are weekly releases of fresh flies, R would be found by

\[ R = 100 \left(0.5^n / (0.5^n + 0.5^{n-1} + 0.5^{n-2})\right) \]

Thus, the mean expectation would be that a given trap clearance would have only 6.7%, 3.2%, and 1.6% of flies with release dates in common with clearances that were, respectively, 3, 4, and 5 weeks later. It was arbitrarily decided that sufficient independence of census counts was obtained if they were 3 weeks or more apart.

Because there were breaks in the release program, the complete data set comprised weekly censuses with means that ranged from less than 1 to over 200. The restricted data set was limited to censuses that had mean rates of catch per trap of 50 or more in order for the results to be relevant to a real SIT program when large numbers of sterile flies would be recaptured.

Extinction of B. papayae at Cairns

An exotic incursion of B. papayae in and around Cairns (north Queensland, Australia) was eradicated by a campaign starting in Oct 1995 that used male annihilation with caneite blocks impregnated with methyl eugenol and malathion bait sprays comprising protein hydrolysate and malathion (Hancock et al. 2000). With 1 exception, the traps that caught the highest number of flies were on the original monitoring array in Cairns (spaced about 1 km apart). Thus, the use of the data from traps on that array yields information on dispersion pertinent to a wide range of wild fly densities during a trend to extinction.
F fortnightly totals were used because data in the form of weekly totals were not available.

Dispersion after Sterile Releases Stopped

Data on sterile fly recaptures from the full data set for Gilgandra were arbitrarily selected by choosing the first 4 censuses (in chronological order) that occurred with mean catch per trap in each of the ranges 80-61, 60-36, 35-21, 20-11, 10-1, and <1 (a total 24 censuses). For censuses with higher means, the set of 8 that was used for simulating the results of sterile release at Gilgandra (see below) was employed.

Simulated Extinction Trend

The set of 8 censuses that were used for simulating the results of sterile releases at Gilgandra (see below) was employed and 3 sets of simulated data were generated by dividing the catch data from that original set by 10, 100, and 500, respectively. There was a problem in simulating values for the percentage of positive traps by dividing the original catches as above. This was due to the fact that the dividing procedure could generate trap catches for low-scoring traps that were less than 1 and, moreover, if all these were deemed positive then the number of positive traps would never reduce (as it would with real data) when the mean number of flies trapped declined. Thus, for the purposes of the simulation, a trap was no longer deemed positive if the simulation resulted in there being 0.5 or less flies in it.

Similarity of Sterile and Wild Fly Dispersions

An index of dispersion similarity was found by calculating, for each census, the percentage of variation in wild flies among traps that was explained by the linear regression of wild flies in each trap with the number of sterile flies recaptured in the same trap (or alternatively, the value of 100\(r^2\) where \(r\) is the correlation coefficient). The index was calculated for the dates of the trap clearances used in the simulated SIT exercise (see below) and also for each of the 4 weekly clearances that preceded them.

Simulated SIT with Mismatched Dispersions

The efficiency of SIT with various dispersions of sterile and wild flies can be assessed by comparing the aggregate result of the locally imposed generational rates of increase (\(\lambda_{\text{GIL}}\)) of wild flies with that expected if the both sterile and wild flies were evenly spread (i.e., if the same imposed ratio of sterile to wild flies prevailed in all parts of the release area). In the case of the simulation used here, it is assumed that the mating competitiveness of the sterile flies is 0.5 and the overall ratio of sterile to wild flies is 100:1 and the natural generational rate of increase (\(\lambda_{\text{GIL}}\)) of the wild flies is 5.0. Thus, if both kinds of fly were distributed evenly or their dispersions were identical (hence the sterile to wild ratio was the same in all parts) the imposed generational rate of increase both locally (\(\lambda_{\text{GIL}}\)) and overall (\(\lambda_{\text{GIA}}\)) would be 0.098. In this situation the imposed rate of increase is, as with all successful SIT, less than 1 indicating a decrease that in this case is just more than a ten-fold reduction per generation. If dispersion patterns of the 2 fly types were different, then \(\lambda_{\text{GIA}}\) would vary from place to place with further consequences for the overall result (\(\lambda_{\text{GIA}}\)).

The simulated SIT calculations were based on real weekly censuses (trap clearances) selected from the data from Gilgandra and Narromine. Censuses with large numbers of sterile recaptures (mean recaptures per trap exceeding 100) were chosen in order to conform to a realistic semblance of a successful SIT operation. However, there were only 4 such censuses at Gilgandra (range of means, 103-218) and 3 from Narromine (range of means, 102-185) that satisfied this criterion and that were also spaced more than 2 weeks apart. Thus, to augment the data set, censuses based on fortnightly totals were selected; this procedure made 4 more censuses available for Gilgandra and 1 more for Narromine (range of means, 87-185). The actual census dates used for Gilgandra were Mar 18 and 25 (1996), Dec 30 (1996), Mar 17 (1997), Apr 21 and 27 (1997), Sep 29 and Oct 6 (1997), Nov 10 (1997), Dec 29 (1997) and Jan 5 (1998), and Mar 16 (1998). For Narromine they were Mar 17 and 24 (1997), Mar 2 (1998), Mar 23 (1998), and Apr 20 (1998). It can be concluded, therefore, that less than 5% of flies in successive censuses came from the same release (see above) and virtually none did so in most cases.

For the SIT simulation, the dispersion of wild flies between traps was retained but the numbers for each trap were reduced by the same factor for any 1 census (trap clearance) date so that the overall sterile to wild ratio (i.e. the ratio pertaining to the recaptures of the whole trap set on that date) was 100:1. This was done by applying the following equation to the data for any given census date:

\[
W_i = w_i \cdot \left(0.01 \cdot S_i / w_i\right)
\]

where \(w_i\) is the unadjusted number of wild flies in a trap at the census date, \(W_i\) is the adjusted number, \(S_i\) is the total of sterile flies in all traps and \(w_i\) is the unadjusted total of wild flies in all traps.

For simulation of the effects of SIT with the given dispersions of sterile and wild flies on any one date, it was assumed that the natural rate of increase per generation (\(\lambda_{\text{GIL}}\)) of the wild flies would have been 5 and the locally imposed rate of increase (\(\lambda_{\text{GIA}}\)) pertaining to the data from any 1 trap was calculated as follows:
\[
\lambda_{\text{GIL}} = \lambda_{\text{GN}} / ((CS_L/W_L) + 1)
\]
and the imposed rate of increase over the whole trap array (\(\lambda_{\text{GIA}}\)) was found by
\[
\lambda_{\text{GIA}} = (\Sigma (\lambda_{\text{GIL}}W_L))/ (\Sigma W_L)
\]
where \(C\) is the mating competitiveness of the sterile flies, \(S_L\) is the number of sterile flies in the trap.

The relationship of various factors to the imposed rate of increase over the whole trap array (\(\lambda_{\text{GIA}}\)) was examined by means of correlation and regression analyses. The factors were as follows: the mean catch per trap of sterile flies and its SD, the percentages of positive traps with more than 0, 10, or 50 flies and the ‘index of similarity’ of the sterile and wild dispersion (see above).

**RESULTS**

**Complete Data for Gilgandra and Narromine**

The relation between log_{10} standard deviation (SD) and log_{10} mean catch per trap (m) of *B. tryoni* appears to be linear. Both wild and sterile flies fit a common slope \((b = 0.91)\) that explains 96.4% of the total variance, 91.3% of the variance of wild flies and 96.2% of that of sterile flies (Fig. 1). The slope for the analogous relation used by Taylor's Power Law (where variance replaces SD) is double of that in Fig. 1.

**Limited Data Set for Towns under Sterile Releases**

When restricted data sets were used for Gilgandra and Narromine in order to avoid non-independence between censuses, no significant differences between sterile and wild *B. tryoni* were found \((P > 0.05, n = 12)\) for the slope of the regression of log_{10} SD (and therefore variance) on log_{10} mean catch per trap (Fig. 2a, Table 1). The regression slopes for log_{10} SD on log_{10} mean catch per trap did not differ significantly from unity \((P > 0.05, n = 12)\) and this can be related to the finding that there were no significant relationships of...
coefficient of variation (SD/mean catch per trap) and mean catch per trap for either wild or sterile flies (Fig. 2b). The 3 regression lines shown in Fig. 2a pertain to the combined Gilgandra and Narromine data for sterile flies only (upper dashed line), wild flies only (lower dashed line), and wild and sterile flies combined (solid line). These regressions explain 71%, 93%, and 98% of the variance of the corresponding values of log10 SD. The common regression (solid line) explains 61% of the variance of sterile flies only and 90% of that of wild flies only.

The relationship between the percentage of positive traps and mean catch per trap (Fig. 2c) was variable but the values fell within the range predicted by negative binomial models for very clumped distributions having exponent (k) values in the range 0.1-1.0.

Extinction of B. papayae at Cairns

Data from the original monitoring grid at Cairns during the extinction of B. papayae are plotted on Fig. 3a-c in an analogous form to Fig. 2a-c. The slope of Fig. 3a appears to be similar to that of the slope for the combined data of Fig. 2a, but it is significantly lower than unity (< 0.01, n = 12) with the consequence that the slope of log10 CV on log10 mean catch per trap (Fig. 3b) declines significantly with increasing mean (P < 0.01, n = 12). However, it can be argued that because these data come from successive trapping intervals, they are not strictly independent and conclusions as to statistical significance should be treated with caution. The relation between the percentage positive traps and mean catch per trap (Fig. 3c) fell within a similar range to that seen in Fig. 2c.

### Table 1. Constants of Taylor’s Power Law determined from regressions pertaining to Bactrocera tryoni and B. papayae (Diptera: Tephritidae) in Australia.

<table>
<thead>
<tr>
<th>Regression of form: y = log10 a + bx^2</th>
<th>% var expl</th>
<th>Intercept, a (± SEM)</th>
<th>Slope, b (± SEM)</th>
<th>Traps</th>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Restricted range of trapping data at Gilgandra (wild B. tryoni)</td>
<td>94.0</td>
<td>0.26 (0.10)</td>
<td>1.92 (0.13)</td>
<td>31</td>
<td>16</td>
</tr>
<tr>
<td>(b) Restricted range of trapping data at Narromine (wild B. tryoni)</td>
<td>97.6</td>
<td>0.60 (0.06)</td>
<td>1.62 (0.10)</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>(c) Restricted range of trapping data at Gilgandra (sterile B. tryoni)</td>
<td>73.5</td>
<td>1.19 (0.51)</td>
<td>1.62 (0.26)</td>
<td>31</td>
<td>16</td>
</tr>
<tr>
<td>(d) Restricted range of trapping data at Narromine (sterile B. tryoni)</td>
<td>84.1</td>
<td>1.43 (0.56)</td>
<td>1.62 (0.29)</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>(e) Combined wild and sterile data (a-d)</td>
<td>97.7</td>
<td>0.26 (0.07)</td>
<td>2.11 (0.05)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>(f) Cairns original grid (monitoring eradication of B. papayae)^4</td>
<td>98.0</td>
<td>0.78 (0.08)</td>
<td>1.73 (0.08)</td>
<td>12</td>
<td>24^4</td>
</tr>
</tbody>
</table>

1Mean (m) relates to variance (s^2 as s^2 = am^2
2y = log10 (spatial variance); x = (log10, spatial mean).
3Percentage of variance of log10 (spatial variance) explained by regression.
4Trap data based on fortnightly counts; all other rows based on weekly counts. n.a. = not applicable.

Dispersion after Sterile Releases Stopped

Fig. 4c enables the comparison to be made of the natural decline of sterile B. tryoni at Gilgandra and Narromine after releases stopped with the forced decline of B. papayae at Cairns (above) that was due to male annihilation. Fig. 4c is very similar to Fig. 3c, with the percentage of positive traps falling within the predictions given by negative binomial models for very clumped distributions with k values in the range <0.05-1.0.

Simulated Extinction Trend

The results of the simulation of trends to extinction that were produced by manipulating data of sterile fly trapping at Gilgandra are shown in Figs. 4a-b. The original Gilgandra data are plotted on the right hand side of each graph. Three other sets of points are plotted which are the results of dividing the catch data from the original set by 10, 100, and 500, respectively.

The dashed lines in Fig. 4a illustrate how (because of the nature of the formula for SD) this process preserves, for each set of points, the original slope (b) of the regression log10 SD = log10 a + (b log10 m). The value of the original b is 0.79 (± SEM, 0.20) for each set of points, not significantly different from unity (P < 0.01). The intercepts (log10 a) are all different being (from right to left) 0.61, 0.40, 0.19, and 0.13. The common slope (solid line) has a slope (b = 0.996 ± SEM, 0.013) not significantly different from unity (P << 0.01).

The simulated values for percentage of positive traps (a trap ceasing to be deemed positive if the simulation results in there being 0.5 or less flies in it) show much less variation with decrease in mean...
catch per trap than is seen with real data from declining populations (compare Fig. 4b with Figs. 3c and 4c). With the simulated reductions, the original variation in percentage of positive traps appears to be almost exactly maintained. This is not consistent with Fig. 3c and 4c where the variation is encompassed by a wider range of negative binomial predictions when pertinent to lower mean catch rates.

**Similarity of Sterile and Wild Fly Dispersions**

Among the set of 8 sterile fly censuses used for the Gilgandra SIT simulation, there were only 3 instances of a significant correlation \( (P < 0.05) \) between immediately successive censuses (respectively 5, 6 and 10 weeks apart). For the 4 Narromine simulation SIT censuses, there were no significant correlations between successive censuses \( (P > 0.05) \). A similar lack of serial correlation applied to the wild flies trapped at each of the above dates. This is perhaps to be expected with such widely spaced dates.
When runs of 5 consecutive weekly censuses were compared (12 runs, each including weeks used for the SIT simulations) the correlation between dates was much stronger. The first census of any run was compared to the following 4 censuses with respect to both wild and sterile flies. For Gilgandra, the mean number of ‘following censuses’ (out of a possible maximum of 4) that was significantly correlated with the first \((P < 0.05)\) was 3.1 for sterile flies and 3.4 for wild flies. The Narromine data had less sequential correlation, the equivalent Figures being 2 and 1, respectively. Indices of dispersion similarity of wild and sterile flies in any one week showed analogous results. Such an index is expressed as a percentage and is found by \(100r^2\), where \(r\) is the correlation coefficient pertinent to numbers of wild and sterile flies in each trap in a given week. There was considerable consistency in the index at Gilgandra where, out of the 8 runs of 5 weeks, it stayed within a 10% band for 3 or more weeks for 6 of those runs. At Narromine, the similarity index was within a 10% band for never more than 2 weeks.

Simulated SIT with Mismatched Dispensions

The degree of mismatch between the distribution patterns of the wild and sterile flies varied over time. The imposed rate of increase \((\lambda_{GIA})\) varied from 0.13-0.35 despite the overall ratio of sterile to wild flies being one that should impose a value of 0.098 if the 2 types of fly were identical dispersed (see above). The mean catch per trap of sterile flies ranged from 87-218 and the associated SD values from 116-283. The percentages of the 3 categories of positive traps (those with >0, >10 and >100 sterile flies in) ranged from 52-90%, 23-53%, and 38-68%, respectively, whereas the index of dispersion similarity ranged from 23-73%.

Of the relationships between the imposed rate of increase and the various factors, only the 1 with the index of dispersion similarity (Fig. 5) was significant. The latter regression explained 60% of the variance of \(\lambda_{GIA}\). Despite the rather low value of percentage variance explained, the regression line in Fig. 5 predicts a value of \(\lambda_{GIA}\) for 100% similarity in dispersion that is very close to the theoretical value of 0.098.

**DISCUSSION**

Taylor’s Power Law, SD and CV

The exponent \((b)\) of Taylor’s Power Law (slope of \(\log_{10}\) variance of catch per trap on \(\log_{10}\) of its mean) was found to be, in all cases, not significantly different from a value of 2. Zalucki et al. (1984) found that its value for *B. tryoni* in a natural area of coastal rainforest and open *Eucalyptus* woodland in south east Queensland (Australia) was significantly higher at 2.27 (SEM ± 0.07). The range of mean values was similar to those used here, but the area was over 10 times larger than the trap arrays at Gilgandra and Narromine and the traps were spaced by 1 km or more. Thus, the difference may be a matter of scale in sampling frequency, or size of sampled area (Southwood & Henderson 2000), but it is also possible that the town landscapes relevant to the present paper may be associated with a slightly less clumped distribution than a heterogeneous natural one. If the dispersal pattern of wild flies were the product of the distribution of favorable and unfavorable microhabitats and recent demographic history, then there is no reason for the Taylor exponent to be the same in all places. Sterile flies are initially distributed as evenly as possible and have no local demographic history thus their dispersion when trapped need not necessarily be the same as that of wild flies in the same area. Nevertheless, no significant differences were found between the Taylor exponent \((b)\) of wild and sterile flies at Gilgandra and Narromine. However, the interpretation of regression slopes can depend on the range of values that are being used and this point will be developed in discussing the slopes of SD on mean.
The slopes for \( \log_{10} SD \) on \( \log_{10} \text{mean} \) are (inevitably) half the value of the Taylor exponent, and were not significantly different from unity in the case of \( B. \) tryoni and hence the CV of the same data did not decline with the mean. The equivalent slope for \( B. \) papayae was, in contrast, significantly lower than unity and hence the CV did decline with mean. The fact that CV did not decline with mean catch per trap in the case of sterile \( B. \) tryoni implies that a more even coverage in SIT will not necessarily be achieved by increasing the release rate. This is also supported by the relation of the percentage of positive traps to the mean (see later).

One of the problems of comparing regression slopes is that the greater the numerical range of the data series on abscissa, the steeper the slope of a significant relationship is likely to be. This is the result of the method of calculating the line with the method of ‘least squares’, which minimizes the squared deviations of the dependent variable from the line (Maelzer 1970; St. Amant 1970). Also, there is a similar effect on the measure of ‘goodness of fit’ as indicated by the percentage of variation of the dependent variable explained by the regression line. This is clearly illustrated by Fig. 2a where the common slope for sterile and wild flies (where the range of mean values is large) is compared with the slopes that pertain only to either the sterile or the wild flies (where the range of means is more restricted). The percentage of variance explained by the common slope is large (where the range of means is more restricted), the percentage explained by the ‘wild only’ slope is less (range of means of intermediate length) whereas the percentage explained by the ‘sterile only’ slope is least (range of means of smallest length). The case of the common slope versus either of the other 2 slopes, it is apparent that the fact that the variance explained by the former is greatest is an artifact of the process, whereby the mean of the dependent variables is roughly intermediate between the means of the wild and sterile variables taken separately. As a result, deviations of both kinds from the common means are much larger, whereas the deviations of either set from the common slope (the ‘unexplained’ deviations) are not much bigger than they are from their own specific slopes. A similar illustration can be seen in Fig. 4a for simulated extinction data, where the slopes of individual clusters of points can be compared with the common slope.

Relation to Negative Binomial Model

The relationship between the percentage of positive traps and mean catch per trap was variable in all cases, the values falling within the range predicted by negative binomial models for very clumped distributions having exponent \( k \) values in the range <0.05-1.0. The values of percentage of positive traps that are predicted by negative binomial models rise with decreasing slope as the mean increases. With such low values of \( k \), they indicate a very poor return in terms of increasing the percentage of positive traps by increasing the number of flies released. For example, if \( k \) were 0.2, the model would predict a percentage of traps with positive values of 71% when catch per trap was 100 and this would rise only to 82% when the catch per trap was 1000.

Extinction Trends

Real extinction trends (of wild \( B. \) papayae at Cairns and of sterile \( B. \) tryoni when releases stopped) were very similar to the trend simulated by dividing the catch data from an original set by 10, 100, and 500, respectively. However, the scatter of points in the slopes of graphs of percentage of positive traps on mean catch per trap is smaller in the simulated data than it is in the real data. No attempt has been made to quantify this, but the data suggest that clumping can be more pronounced at lower densities in real situations that it is at higher ones. This is consistent with the model of patterns of outbreak and extinction (see Introduction) where colonization starts with discrete propagules and only remnant foci are left as extinction is approached.

SIT with Mismatched Dispersions

The imposed rate of increase \( (\lambda_{\text{cum}}) \) varied from 0.13-0.35 despite the overall ratio of sterile to wild flies being of a value that should impose a rate of 0.098 if the 2 types of fly were identically dispersed. It is of interest that in no case did a mismatch result in an imposed rate of increase that was less than that expected from evenly matched dispersions. This was a consequence of the asymmetry of the effect of increasing the local density of sterile flies in one patch by decreasing it in another (as would happen with uneven dispersion with a given overall ratio of sterile to wild flies). This can be illustrated as follows by with the equation and competitiveness value (0.5) that were used in the simulations. Consider that 2 local patches have the same ratio of sterile to wild flies and that this ratio (100:1) imposed the same local rate of increase of 0.098. If the sterile flies were dispersed differently so that there were 170 sterile flies to every wild one in one patch and only 30 in the other then the rates of increase imposed on the wild flies would be 0.058 and 0.313, respectively, with the mean of the 2 patches taken together being 0.185 or about 1.9 times greater than would have been expected had the sterile flies been evenly dispersed. The effect is greater with greater mismatch and analogous results are obtained if the wild flies are varied instead of the sterile ones.
Such a phenomenon has analogies in the ecological model whereby sufficiently uneven distribution of attack rates by natural enemies (predators or parasitoids) on population patches of an organism can result in effective partial refuges for that organism that can facilitate the persistence of the relationship (Murdoch & Briggs 1996).

Implications for Release Strategy

It appears that the patchy distribution of released fruit flies is inevitable and that it is not practicable to decrease it by increasing the number released because the increase of mean recapture rate by an array of traps is not accompanied by a reduction in its coefficient of variation and with high recapture rates, the percentage of traps catching zero does not decrease appreciably with increase in recapture rate. It is probable that this would also apply to releases of other flying insects, whether for SIT or for inductive releases of natural enemies for augmentative biological control.

However, it appears from the simulation study that a clumped distribution would not be a problem if the target organism has a similar one (as could happen if patchiness of suitable microhabitats was the sole cause). The problem arises when the dispersal patterns are mismatched. In that case, the reason is not likely to be environmental unsuitability because the target flies are present where the mismatch is adverse. Shiga (1986) described such a situation. He found that in an SIT program involving releases of Bactrocera cucurbitae (Coquillett), the spatial correlation between wild and sterile trappings on a monitoring array was variable and often poor over a period of three months, the index of similarity ranging from 1-41%. This compares with the range 23-73% reported in the present paper. The more extreme mismatches reported by Shiga (1986) were associated with resurgent foci or ‘hot spots’ of wild flies and these were treated with supplementary releases of sterile flies. Of course, in the case of Shiga’s data one cannot tell whether the mismatches were the cause of the hotspots or vice versa. It would be highly likely that the more extreme mismatches were at least part of the cause of the hotspots if the mismatches preceded the eruptions and were also associated with locally low ratios of sterile to wild flies in the adjacent traps. In such circumstances, the population in the hot spots may have a rate of increase close to the natural one whereas the overall rate of increase pertaining to the whole trapping grid may still be below replacement rate ($\lambda < 1$). However, hotspots must be dealt with if eradication is to proceed to completion. It may be prudent, therefore, to avoid the occurrence of hot spots by identifying those traps where the mismatch produces an ineffective ratio of released to wild insects and augment areas surrounding the former with supplementary releases. This may be feasible because the degree of mismatch can be consistent for several weeks (as at Gilgandra).

When the numbers of wild insects become too low for reliable detection by traps, the problem of patchy coverage by wild flies is probably not serious, even in the vicinity of traps failing to recapture released sterile insects. This is because when population density is very low, the probability of persistence is also low (Meats et al. 2003b). However, continued monitoring would always be required for a given period after no target insects are trapped as a precaution against resurgence of the infestation (Shiga 1986; Clift & Meats 2004).

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COMPARISON OF LONGEVITY BETWEEN A LABORATORY STRAIN AND A NATURAL POPULATION OF \textit{ANASTREPHA FRATERCULUS} (DIPTERA: TEPHRITIDAE) UNDER FIELD CAGE CONDITIONS

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ABSTRACT

The South American fruit fly \textit{Anastrepha fraterculus} (Wiedemann) is one of the most destructive fruit pests in this region, infesting major fruit crops. Implementation of the sterile insect technique (SIT) as part of an area-wide integrated approach against this species requires information on the survival of mass-reared and sterilized insects in the field and their ability to mate with wild females. The survival rates in field cages of both non-irradiated and irradiated laboratory flies were compared with that of wild flies. Both types of laboratory flies survived longer than their wild counterparts over the 8 days under the experimental conditions. The irradiation dose (70 Gy) did not affect survival of the laboratory reared flies. Our results improve the prospect of integrating the SIT into the control of \textit{A. fraterculus} populations in Argentina.

Key Words: \textit{Anastrepha fraterculus}, SIT, genetic control, longevity, survival

RESUMEN

\textit{Anastrepha fraterculus} (Wiedemann), la mosca sudamericana de la fruta, es una de las plagas más destructivas en la región que infesta a los principales cultivos de frutas. La implementación de la Técnica del Insecto Estéril (TIE) como parte de un manejo integrado en áreas extensivas contra esta especie requiere ensayos que demuestren que los insectos producidos en forma masiva y esterilizados son capaces de sobrevivir en el campo y aparearse con las hembras silvestres. Se comparó la supervivencia de individuos de una línea de laboratorio, tanto irradiados como no irradiados con la de individuos de una población natural. Los dos tratamientos de moscas de laboratorio sobrevivieron más tiempo que las salvajes durante los 8 días y en las condiciones ensayadas. La dosis de radiación (70 Gy) no afectó la supervivencia de las moscas criadas en laboratorio. Nuestros resultados mejoran las perspectivas de integrar la TIE en el control de las poblaciones argentinas de \textit{A. fraterculus}.

Translation provided by the authors.
ulation, increasing the probability for a wild female to copulate with a sterile male. This method has been very successful in eradication of the New World screwworm, Cochliomyia hominivorax (Coquerel) (Diptera: Calliphoridae) from southern USA to Central America (Wyss 2000) and North Africa (Lindquist et al. 1992), the control of the Mediterranean fruit fly Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) in many Latin America regions (Hendrichs et al. 1995), and the suppression of the codling moth Cydia pomonella (L.) (Lepidoptera: Tortricidae) in Canada (IAEA 2001). The main advantages of SIT are that it is environmentally friendly, species-specific, compatible with other control methods, and its effectiveness increases as the size of the target population declines (Benedict & Robinson 2003).

In Argentina, the SIT has been successfully implemented against the most common tephritid pest, C. capitata (Aruni et al. 1996; De Longo et al. 2000). The use of this technique to control A. fraterculus requires political support and grower organization to allow an area-wide approach, and a more detailed knowledge of its biology. One requirement for SIT to be successful is that released insects must survive in the field and mate with wild insects. Mating success is probably related to interactions with other males and to female choice (Partridge & Halliday 1984). Also, at least in related tephritids, such as C. capitata, morphometric traits appear relevant in mate choice (Norry et al. 1999; Kotiaho et al. 2001; Rodriguero et al. 2002a, b).

Previous studies by our group verified that A. fraterculus populations of different regions in Argentina are fully compatible among themselves (Petit-Martí et al. 2004). Nevertheless, as the mass-rearing process and sterilization may cause a loss of fitness (Shelly et al. 1994; Lance et al. 2000; Alphey 2002; Benedict & Robinson 2003), survival of laboratory reared sterile insects should be evaluated under field conditions in order to predict their performance in control programs that integrate the SIT. Field cages with host trees provide a suitable model to simulate some aspects of field conditions.

The main objective of this study was to compare the survival of a laboratory-reared strain of A. fraterculus (irradiated and non-irradiated) with that of a wild population in order to develop a non-expensive, easy to follow method that can be applied as a routine quality control test for sterilized, mass-reared insects in SIT based control programs.

**MATERIALS AND METHODS**

Insects and Methods of Handling

Two populations were tested. The first one was collected from the wild and the second was a laboratory strain, reared since 1997 under semi mass-rearing conditions (Jaldo et al. 2001) at the Estación Experimental Agroindustrial Obispo Colombres, Tucumán Province, in the northwest of Argentina. Cages were held in a rearing room with 25 ± 1°C, 80 ± 10% R.H. and a photoperiod of 12:12 (L:D) (Vera et al. 2007). Wild flies were collected at Horco Molle, (26º48’S, 65º20’W), in the same province, from wild guava fruits, Psidium guajava L. (Myrtaceae).

Infested guava fruits were put on a sand layer to allow pupation. Emerging pupae were sieved and transferred to glass flasks. Both wild and laboratory reared pupae were sent to the Instituto Nacional de Tecnología Agropecuaria (Castelar, Buenos Aires, 58º40’W, 34º40’S), where they were kept in glass flasks (3 liters) and maintained under controlled conditions (23 ± 2°C, 70 ± 5% R.H. and a photoperiod of 12:12 (L:D) until adult emergence. Previously, half of the laboratory reared pupae were irradiated at the Centro Atómico Ezeiza (Comisión Nacional de Energía Atómica, Argentina) with a sterilizing median dose of 70 Gy in a GammaCell 220 60Co irradiator. Irradiation was performed 2 d before adult emergence, at room temperature, air atmosphere and 1 atmosphere of pressure with a dose rate between 1.0904 and 1.0785 Gy/min.

Sex Separation

Every day, emerging adults from all treatments were removed from the flasks and transferred to new 3-L glass containers. The following day they were sorted by sex and supplied with water and adult food composed of brown sugar and hydrolyzed corn protein. This diet promoted normal sexual development in laboratory strains (Manso 1998). Adults were kept under laboratory conditions (20-27°C, 60 ± 20% R.H. and a photoperiod of 12:12 (L:D) until the moment of release into the field cages.

Fly Marking

Two d after emergence flies were marked to identify their origin. They were placed in a netting bag (1 mm mesh), carefully immobilized and labeled with a dot of water-based paint (Témpera Alba, Alba, Inc., Argentina) on the mesonotum. Five colors (green, red, white, blue, and yellow) were interchanged sequentially each labeling day. After painting, groups of 25 flies were placed in 1-L containers, covered with a mesh, and provided with food and water and held under laboratory conditions. They were released into the field cages 2 d after marking.

Field Test

The experiments took place at Instituto Nacional de Tecnología Agropecuaria, between 25 Mar
and 8 Apr 2004. Eight outdoor nylon screened cages (2.5 m high × 3 m diameter) were placed over rooted young (about 1.5 m high) tangerine trees (one per cage). Two flasks (50 cm³) filled with water were placed in each cage. The flasks had a perforated lid with a gauze wick (partially outside the flask). As a result, flies could take water from the gauze without polluting the water inside the flask. Two pieces of dry peach suspended from a wire fixed to the roof of the cage were used as food source; this was shown to be a suitable food source in preliminary tests (data not shown).

The first day (Day 0), at noon, 18 males and 25 females of wild origin were released into each of the 8 cages. Additionally, the same number of male and female irradiated laboratory flies were release into 4 cages (#1, #2, #3, and #4) and non-irradiated laboratory flies into the remaining 4 (#1', #2', #3', and #4'). All the cages had the same number of flies, half from wild and half from laboratory origin. Two days later (Day 2) all surviving flies were recovered from 2 cages (#1 and #1'). In each case the number of flies of each strain (wild or laboratory) was recorded. Both cages were refilled with flies of the same age as that of Day 0. The same procedure was followed on Day 4 with cages #2 and #2', on Day 6 with cages #3 and #3', and on Day 8 with cages #4 and #4'. All flies recovered by aspiration from Day 2 to Day 8 had been released on Day 0 and represent, respectively, the survivors after 2, 4, 6, and 8 d. On 10 d all survivors were aspirated from all cages. At this moment the flies in cages #1, #2, #3, and #4 had survived respectively 8, 6, 4, and 2 d (they correspond to the second release into each cage).

With this protocol we obtained for each set of laboratory flies (non-irradiated and irradiated) 2 replicates of relative survival at 2, 4, 6, and 8 d with respect to wild flies. An additional release was performed on 11 d in two cages, one with non-irradiated laboratory flies and the other with irradiated laboratory flies, in both cases co-released with the wild flies (in proportion 1:1). On 13 d both cages were emptied, providing a third replicate of the survival after 2 d. These extra replicates allowed us to compensate for the fact that (due to lack of material), one of the earlier replicates for 2 d had wild insects 3-4 d older than laboratory insects.

In 1 replicate (corresponding to survivors at 4 d) 22 (instead of 25) females from the non-irradiated laboratory strain and 16 (instead of 18) wild males were released. These numbers were taken into account to estimate expected values when performing the statistical analysis. It was not possible to have more replicates because of the increasingly cold weather. Meteorological data (temperature, relative humidity, wind speed, and sunshine) were recorded every h at the meteorological station of the Instituto de Clima y Agua, located 2 km away from the experimental site.

Data Analysis

Homogeneity among replicates was analyzed by means of homogeneity \( \chi^2 \) tests. The statistical significance of any departure from equal performance of populations was tested by means of a \( \chi^2 \) test of goodness of fit comparing the number of wild and laboratory reared flies recovered alive with the expected 1:1 ratio (i.e., the null hypothesis is that the probability of survival is the same for both groups.) An alternative method of analyzing relative survival through time was based on evaluating the regression of the proportion of laboratory flies (number of laboratory flies/total number of flies) recovered on the number of days spent into the field cages.

In order to compare performance between populations, a new variable was defined as the proportion between laboratory-reared and total recovered flies. Arithmetic means over replicates were calculated for each period (2, 4, 6 and 8 d) and used to perform the regression analysis. The average proportion of laboratory flies recovered at each age (2, 4, 6, and 8 d) was compared between the cages with irradiated and non-irradiated laboratory flies by means of a Wilcoxon pair wise test. All statistical analyses were performed with Statistica (5.1) for Windows (Stat Soft, Inc. 2000).

Results

Temperature and humidity during the field tests (Table 1) varied within ranges that are considered favorable for A. fraterculus. Sunshine ranged between 0.3 and 10 h of direct light (effective heliophany, 3 to 88% of relative heliophany). The weather was benign, except for a single rainy day with strong winds.

Wild flies were compared with both irradiated and non-irradiated laboratory flies, and the total number of wild flies released in all was twice each of the 2 other groups. Total numbers of male and female flies recovered alive at each time period are presented in Tables 2 and 3, respectively. Results for males (Table 2) indicate that the numbers of flies recovered for both laboratory classes (irradiated and non-irradiated) were similar to wild flies for the shorter periods, but significantly higher for the longest period of 8 d. Results for females (Table 3) are similar for non-irradiated females, however wild females caged with irradiated females had an unusually high survival rate at 8 d (14% vs. 5.6-6.0% in equivalent cells for wild males and females of Tables 2 and 3), meaning that in this case no significant differences were found between wild females and irradiated laboratory females.

The regression analyses showed the same trend in all cases (Fig. 1). The association between the ratio of laboratory:wild in the recovered flies and the number of days in the cage was
positive and highly significant \((P < 0.01)\), indicating that on average laboratory flies survive longer under protected field cage conditions than wild flies. Wilcoxon matched pairs tests indicated that the differences were not significant \((P = 0.29\) and \(P = 0.72\) for male and female regressions, respectively). In order to avoid any bias due to the different age in 1 of the replicates (2 d), the complete analysis was repeated after removing this case (i.e., 2 replicates for 2 d in cage, as well as for the other periods). Significance levels remained unchanged.

**DISCUSSION**

Even though weather conditions affect absolute survival, the present analysis was based on relative viability of laboratory flies with respect to wild flies. As the weather was similar most of the days we consider that it was not a factor that could have modified the results in different cages.

Field cages, as a semi-natural environment, represent a compromise between the laboratory and the open field. Within closed field cages flies are much more protected from abiotic and biotic factors such as predators than under open field conditions (Hendrichs et al. 1993; Hernández et al. 2007). Nevertheless, the uncontrolled conditions in an outdoor field cage test significantly reduced survival of flies with respect to laboratory tests. Only 6-35% of flies could be recovered after 8 d inside the cages, whereas flies survived longer periods of time under laboratory conditions (data not shown). This result suggests that closed field cages still represent a challenging environment, where weather variation could impose extra mortality and where it is not possible to avoid completely the presence of predators such as spiders.

**Table 1. Weather conditions during the testing period. Wind speed was measured 2 m above soil surface. (All data came from the Instituto De Clima y Agua, Inta-Castelar, Meteorological Station.)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Max temp (°C)</th>
<th>Mean temp (°C)</th>
<th>Min temp (°C)</th>
<th>Wind speed (km/h)</th>
<th>Precipitation (mm)</th>
<th>Humidity (%)</th>
</tr>
</thead>
<tbody>
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<td>25 Mar</td>
<td>33.0</td>
<td>25.2</td>
<td>17.4</td>
<td>4.0</td>
<td>0.0</td>
<td>63</td>
</tr>
<tr>
<td>26 Mar</td>
<td>32.6</td>
<td>24.5</td>
<td>16.4</td>
<td>4.1</td>
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<td>61</td>
</tr>
<tr>
<td>27 Mar</td>
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<td>23.0</td>
<td>15.0</td>
<td>4.3</td>
<td>0.0</td>
<td>59</td>
</tr>
<tr>
<td>28 Mar</td>
<td>30.6</td>
<td>23.8</td>
<td>17.0</td>
<td>7.3</td>
<td>0.0</td>
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</tr>
<tr>
<td>29 Mar</td>
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<td>26.0</td>
<td>20.0</td>
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<td>56</td>
</tr>
<tr>
<td>30 Mar</td>
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<td>26.0</td>
<td>21.5</td>
<td>11.9</td>
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</tr>
<tr>
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<td>21.0</td>
<td>6.2</td>
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<td>79</td>
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</tr>
<tr>
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<td>19.6</td>
<td>3.3</td>
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</tr>
<tr>
<td>03 Apr</td>
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<td>22.0</td>
<td>3.7</td>
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</tr>
<tr>
<td>04 Apr</td>
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<td>28.8</td>
<td>22.8</td>
<td>10.9</td>
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<tr>
<td>05 Apr</td>
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<tr>
<td>06 Apr</td>
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<tr>
<td>07 Apr</td>
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<td>17.0</td>
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<td>74</td>
</tr>
<tr>
<td>08 Apr</td>
<td>28.0</td>
<td>22.0</td>
<td>16.0</td>
<td>9.2</td>
<td>5.0</td>
<td>87</td>
</tr>
</tbody>
</table>

**Table 2. Anastrepha fraterculus males recovered for each period (days in the field cage) and Chi square tests for Goodness of Fit to compare relative survival of laboratory flies with respect to wild flies. Data are presented as an absolute number \((n)\) of insects recovered in all the replicates for the same period and percent survival \((\%)\) \((100 \times recovered/released)\).**

<table>
<thead>
<tr>
<th>Period (days)</th>
<th>W</th>
<th></th>
<th></th>
<th>W*</th>
<th></th>
<th></th>
<th>Irr</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>(\chi^2)</td>
<td>P</td>
<td>n (%)</td>
<td>n (%)</td>
<td>(\chi^2)</td>
<td>P</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>2</td>
<td>27 50.00</td>
<td>31 57.41</td>
<td>0.28</td>
<td>0.60</td>
<td>31 57.41</td>
<td>44 81.48</td>
<td>2.25</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15 41.67</td>
<td>12 33.33</td>
<td>0.33</td>
<td>0.56</td>
<td>9 26.47</td>
<td>9 25.00</td>
<td>0.01</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6 16.67</td>
<td>14 38.89</td>
<td>3.20</td>
<td>0.07</td>
<td>2 5.56</td>
<td>5 13.89</td>
<td>1.29</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2 5.56</td>
<td>9 25.00</td>
<td>4.45</td>
<td>0.03</td>
<td>2 5.56</td>
<td>10 27.78</td>
<td>5.33</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50 30.86</td>
<td>66 40.74</td>
<td></td>
<td></td>
<td>44 27.5</td>
<td>68 41.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)W stands for wild flies released into the same cages that the laboratory reared stain (Lab), and W* for wild flies released together with laboratory reared and gamma irradiated flies (Irr).
In addition to that, food and water were supplied in a way that flies had to actively forage for them. The ability of a laboratory reared and sterilized fly to survive to sexual maturity under open field conditions is very important for the application of the SIT. Usually, in fruit fly suppression or eradication programs, releases of sterilized flies occur once or twice a week (Dyck et al. 2005). It would be desirable that a significant proportion of the released flies survive this period. Thus, if flies are released in an immature state, as in our experiments, and as is common in most opera-

**Table 3. Anastrepha fraterculus females recovered for each period (days in the field cage) and Chi square tests for goodness of fit to compare relative survival of laboratory flies with respect to wild flies. Data are presented as an absolute number (n) of insects recovered in all the replicates for the same period and percent survival (%) \((100 \times \text{recovered/released})\).1**

<table>
<thead>
<tr>
<th>Period (days)</th>
<th>W Lab</th>
<th>(\chi^2)</th>
<th>P</th>
<th>W* Irr</th>
<th>(\chi^2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>49</td>
<td>65.33</td>
<td>0.17</td>
<td>0.68</td>
<td>54</td>
<td>72.00</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>32.00</td>
<td>2.27</td>
<td>0.60</td>
<td>9</td>
<td>18.00</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>18.00</td>
<td>3.00</td>
<td>0.08</td>
<td>10</td>
<td>20.00</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>6.00</td>
<td>9.80</td>
<td>&lt;0.01</td>
<td>7</td>
<td>14.00</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>34.22</td>
<td>80</td>
<td>41.56</td>
<td>94</td>
<td>41.78</td>
</tr>
</tbody>
</table>

1W stands for wild flies released into the same cages that the laboratory reared stain (Lab), and W* for wild flies released together with laboratory reared and gamma irradiated flies (Irr).

**Fig. 1. Regression (95% confidence) of relative advantage of laboratory non-irradiated or irradiated flies with respect to wild flies on the number of days survived after release into field cages. Relative advantage was calculated as the proportion of non-irradiated or irradiated laboratory flies in the total number of recovered flies at each period: (a) non-irradiated males \((r = 0.569, P = 0.0004)\); (b) irradiated males \((r = 0.593, P = 0.0006)\); (c) non-irradiated females \((r = 0.602, P = 0.0006)\); and (d): irradiated females \((r = 0.316, P = 0.0000)\).**
tional programs, only a proportion of males reaches sexual maturity, because on average they require at least one week to achieve maturation (Segura et al. 2005). Results of the current test suggest that roughly only 25% of sterilized flies are able to survive 8 d in field cages.

The relation between the number of released flies and the survivors after a week should be considered when estimating the number of flies to be released and the frequency of releases. Most flies survived the first 2 d in field cages. Therefore, if mature sterilized males were released, significantly more males could participate in sexual activities, and a considerable proportion of males would have the ability to continue mating during the following days. This assumption is strongly supported by the fact that survival of the laboratory-reared flies was higher than that of wild flies even after irradiation.

Mass rearing can affect fly fitness (Cayol 2000). Additionally, irradiation can damage some physiological processes, leading to reduction of survival (Spates & Hightower 1970; Crystal & Whitten 1976) and/or mating competitiveness (Calcagno 2001; Allinghi et al. 2002; Calcagno et al. 2002; Lux et al. 2002). It is not unreasonable to suppose that the advantage in survival that laboratory-reared flies showed in our experiments is due to good rearing conditions in the laboratory facility. It is impossible to predict exactly how this fitness would change if a major *A. fraterculus* mass rearing system was used, but our results based on a small-scale rearing suggest that manipulation and irradiation did not reduce survival, which would be good for the application of the SIT to control this insect. Nevertheless, there are a number of factors that could modify these results as laboratory and wild flies have undergone entirely different selection pressures. Characteristics that are relevant for flies to survive and mate in the laboratory may not be the same as those needed to survive and mate in field (Mayer et al. 1998).

Obviously, all these considerations are to be added to the effect of handling and transportation of the flies to be released in field that could cause damage and possibly stress the insects. It is possible that released flies could have reduced dispersal compared to wild flies (Mayer et al. 1998). As in every laboratory strain, lack of diversity could affect the ability to survive, reach sexual maturity, find a potential mate, and copulate in the field. Implementation of SIT requires continuous surveillance to evaluate survival of sterile flies, probably through the implementation of regular field cage and open field tests.

ACKNOWLEDGMENTS

The authors thank Dr. M. M. Viscarret, Mr. F. H. Milla, and Misses B. Comas, V. López, and R. Sellaro for collaboration during the field cage tests. J. C. V. is a member of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina). This work was supported by the following grants: IAEA Research Contract 9897 to B.O. Saidman, IAEA Research Contract 1083, CONICET PIP 0722/98, UBACYT 2004 X241, and ANPCYT PICT 6628 to J.C.V., and IAEA Research Contract 8308 to J. L. C.

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LEKKING BEHAVIOR OF ANASTREPHA FRATERCULUS
(Diptera: Tephritidae)

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ABSTRACT
Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) displays a lek mating system. Males form groups in which they simultaneously display signals (acoustical, visual, or chemical) to attract females with the purpose of mating. Females visit the lek and choose among signaling and courting males to mate. Scarce information is available in A. fraterculus about the main factors involved in female choice and the behavior of displaying males. This information could be important within the context of pest control programs with a sterile insect technique (SIT) component, because departures from normal sexual behavior caused by artificial rearing could affect males’ performance in the field. In this study we assessed A. fraterculus male behavior within the leks and analyzed the importance of behavioral and morphological traits on their copulatory success. The existence of preferred places for lek formation was evaluated in field cages with trees inside and analyzed by dividing the trees in sectors according to a 3-dimensional system. Males were individually weighed, marked, and observed every 15 min. Morphometric and behavioral characteristics of successful and unsuccessful males were compared. Most successful males grouped in a region of the tree characterized by the highest light intensity in the first 2 h of the morning. Results showed that pheromone calling activity is positively associated with copulatory success. Copulations were more frequent for males calling inside the lek, indicating that pheromone calling activity and presence in the lek are key factors for copulatory success. A positive association between copulatory success and eye length was found; some characteristics of the face were also associated with copula duration and latency.

Key Words: Anastrepha fraterculus, field cages, lek, mating behavior, morphometric traits, sexual selection, South American fruit fly

RESUMEN
Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) presenta un sistema de apareamiento tipo lek. Los machos forman grupos y, en forma conjunta, emiten señales (acústicas, visuales, o químicas) para atraer a las hembras con el propósito de aparearse. Las hembras visitan el lek y eligen entre los machos para copular. La información acerca de los principales factores involucrados en la elección de la hembra y de la influencia del comportamiento de los machos en los leks en esta elección es escasa para A. fraterculus. Esta información es importante en el contexto de programas de control que incluyen la Técnica del Insecto Estéril. En el presente estudio se evaluó el comportamiento sexual de machos de A. fraterculus dentro de los leks, y la asociación de su comportamiento y de rasgos morfométricos con el éxito copulatorio. El lugar preferido de agrupamiento de los machos fue evaluado en jaulas de campo con árboles en su interior y dividiendo el árbol en sectores de acuerdo a un sistema de tres dimensiones. Los machos fueron individualmente pesados, marcados y observados cada quince minutos. Luego de finalizado el ensayo se midieron los rasgos morfométricos. El mayor éxito correspondió a machos agrupados en una región del árbol caracterizada por tener la mayor intensidad de luz en las dos primeras horas de la mañana. Los resultados mostraron que la actividad de llamado con feromonas está asociada con el éxito copulatorio. Las cópulas fueron más frecuentes para machos que llamaron dentro del lek, indicando que la actividad de llamado con feromonas y la presencia dentro del lek son factores importantes en la obtención de la copula. Los análisis morfométricos revelaron una asociación positiva entre el éxito copulatorio y el largo del ojo, y que algunas características de la cara están asociadas además con la duración de la cópula y la latencia.

Translation provided by the authors.
Anastrepha fraterculus (Wiedemann) is a quarantine fruit fly present in South America. It is a polyphagous species that attacks about 80 fruit species from more than 15 different families, many of them of economic importance (Malavasi et al. 2000; Norrbom 2004). Efforts to develop the sterile insect technique (SIT) to integrate it in the control for A. fraterculus are in progress and include the development of mass-rearing (Vera et al. 2006), irradiation protocols (Allinghi et al. 2007b), and studies of the sexual compatibility among different populations (Petit-Marty et al. 2004a; Petit-Marty et al. 2004b; Vera et al. 2006).

Sexual behavior of A. fraterculus has been analyzed for some Brazilian and Argentinean populations (Malavasi et al. 1983; Petit-Marty et al. 2004a). Mating occurs early in the morning, with most copulations taking place during the first 2 or 3 h after dawn, when males aggregate in groups (known as “leks”) to which females are attracted (Malavasi et al. 1983). Copulation takes place preferably on the undersides of leaves, half-way up in the tree (Malavasi et al. 1983). Environmental conditions such as temperature, sunshine, and wind speed affect mating behavior (Malavasi et al. 1983; de Lima et al. 1994; Petit-Marty et al. 2004a).

Lek formation is common in the genus Anastrepha (Aluja et al. 2000). Aluja & Birke (1993) defined the Anastrepha lek as “an aggregation of at least 3 males (pheromone) calling simultaneously in a clearly defined area, usually from adjacent leaves of a single branch”. The largest lek was observed in A. suspensa, containing 9 pheromone calling males (Sivinsky 1989). For A. fraterculus, Malavasi et al. (1983) recorded leks of 5 males separated by less than 80 cm from one another, grouped in the area of the tree with the highest incidence of sunlight and generally establishing territories on the underside of leaves of host and non-host trees. The stimuli eliciting lek formation in A. fraterculus are unknown but may involve light characteristics as well as male signaling behavior, mainly through pheromone release (Malavasi et al. 1983).

Sexual selection plays an important role in the evolution of species with lek mating systems. Males often defend a small territory and exhibit a wide repertory of signals (acoustic, visual, and/or chemical) to attract females. In this mating system, females visit the sites where males aggregate and display their calling behavior, and choose a mating partner (Burk 1983). This, in turn, could lead to sexual selection, through female choice, on male morphological or behavioral traits.

In the Mediterranean fruit fly Ceratitis capitata (Wiedemann), which is also a lekking species, male mating success may be associated, at least partially, to morphometric traits (Norry et al. 1999; Rodriguero et al. 2002a; Rodriguero et al. 2002b). In A. fraterculus, the relative importance of these traits has recently been studied (Sciurano et al. 2007), but the importance of attendance at leks for the male copulatory success remains unknown. Considering that a high proportion of the matings in this genus apparently occurs at the lek (Burk 1983; Hendrichs 1986; Aluja et al. 2000), strong selection in males for their ability to acquire a position within the lek (and perhaps even an intense competition for the female preferred positions) could be occurring. Descriptions of male-male agonistic interactions had been made for Anastrepha species (Hendrichs 1986; Sivinski & Burk 1989). In fact, Hendrichs (1986) found that A. suspensa males compete for leaves in the center of leks and that matings usually occur there. However, evidence that females choose males based on the location of the male may be difficult to reconcile with studies that suggest that individual qualities are important in mate choice (Kotiaho et al. 2001; Sciurano et al. 2007). Studies directed to assess the traits involved in female preference and male mating success should take into account the particular mating system of each species. Thus, any behavioral test must allow the males to form a lek, but also the females to choose among males inside and outside leks (Zapien et al. 1983).

The aims of our study were (1) to analyze the distribution of males inside the tree and determine regions of lek occurrence, (2) to evaluate the effect of morphological traits and pheromone calling behavior of the males on their lekking behavior and their copulatory success, and (3) to reveal potential advantages and requirements to join a lek.

**Materials and Methods**

**Biological Material**

A. fraterculus wild adults were obtained from infested guavas (Psidium guajava) collected at Horco Molle (Tucumán, Argentina). This population has been evaluated in terms of its mating compatibility with other populations (Petit-Marty et al. 2004a; Vera et al. 2006) and its sexual behavior (Petit-Marty et al. 2004a; Allinghi et al. 2007a). Upon emergence, flies were sorted by sex and fed with brown sugar and hydrolyzed corn protein (2:1), the regular diet used at Instituto de Genética, INTA Castelar, Argentina (Manso 1998). When tested, males were 20 d old, and females were 17 d old. All males were weighed and marked 1 day before testing. The mark consisted of a small piece of colored paper glued with a dot of acrylic paint on their notothorax. A letter (Microsoft Word Arial size 4) printed on the paper was used to individually recognize all the males. This technique has been used in sexual behavior studies in the medfly (McInnis et al. 2002; Vera et al. 2002; Vera et al. 2003).
Test Procedure

Lekking behavior and male copulatory success was assessed in standard outdoor field cages (2 m tall, 3 m diameter) containing a rooted tangerine tree (nearly 1.7 m tall), at the experimental field in INTA Castelar during Apr 2002 (time of sunrise 7:20 AM approximately). Sixteen marked males were released into each cage. Observations took place from 7:30 AM to 12:00 PM, covering the period of sexual activity for Argentinean populations of this species (Petit-Marty et al. 2004a; Vera et al. 2006). One hour after the males were released, 8 virgin females were released into each field cage.

Two observers were assigned to each cage. Fifteen min after male release, the position and activity of each male inside the cage was recorded. This recording was repeated every 15 min. To determine the position of the males inside the tree, all trees were divided in 24 sectors in a 3 dimensional arrangement: (1) according to cardinal axes we defined four quadrants (NE, NW, SW, and SE representing the northeastern, northwestern, southwestern, and southeastern quadrants, respectively); (2) regarding tree height we defined 3 evenly-spaced sections (approximately 40 cm high) of the canopy (1 for the lowest third, 2 for the middle third, and 3 for the highest third); and (3) according to the depth of the leaves in the canopy we defined 2 sectors (P), for leaves situated approximately 5 cm from the periphery or edge of the canopy, and (C), for central or core leaves. Both height and depth were relative to each tree and defined previously by all observers to avoid bias. The activities of the males were classified as pheromone calling (hereafter referred to as calling, the presence of an everted anal pouch at the tip of the anus), mating, walking, interacting with other males (face to face encounters between males), and resting (motionless). Mating start time and copula duration were recorded for each couple observed. The test was performed simultaneously in 4 field cages and repeated on 2 different days.

At the end of the observation period, all males were recovered and stored in the freezer. A sample of 25 successful males (70% of all mated males) and 68 unsuccessful males (74% of all unmated males) were selected at random and measured for 8 morphometric traits, as follows: head width (HW), face width (FW), eye length (EL), thorax length (THL), wing length (WL), wing width (WW), femur length (FL), and tibia length (TIL) (Fig. 1). All measurements were made with a stereoscopic microscope, Ernst Leitz Wetzler, with a 12.5× ocular containing a micrometric scale. WL and WW were measured with a 1× objective; HW, EL, THL, FL and TIL with 4× and FW with an 8× objective (for a more detailed description see Sciurano et al. 2007). The data were standardized to have mean zero and unit variance in each replicate (Norry et al. 1999) in order to overcome any possible effect of the cage or the day.

Data Analysis

Location of calling males was used to determine if leks were actually forming, and if so, to describe the number of males inside each lek, the number of leks per tree, and whether leks were randomly distributed or followed some sort of spatial organization inside the tree. For those males that moved and called in different parts of the tree during the observation period, we considered their most frequent position in the analysis. In order to see whether it was possible to pool the data from the 8 replicates, differences among replicates for number of males in each position (height, depth, or cardinal position inside the tree) were analyzed with an ANOVA test. Non-significant differences were found (P > 0.05) and as a consequence, the analysis was performed by grouping data from all replicates. The observed frequencies of calling males found in each sector (NE, NW, SW, or SE; 1, 2, or 3; and C or P) were compared with the expected frequencies under random distribution by a Chi square test.

Sexual behavior of males was described considering (1) the time elapsed between the release of the males and the beginning of calling behavior (time to call), (2) the time spent calling, and (3) the time doing other activities. Time spent calling was calculated as the number of observations in which an individual male was observed pheromone calling divided by the number of observations on this male before attaining copulation (in
the case of successful males) or by the total number of observations performed until the end of the observation period. Thus, if a male was pheromone calling all the time before mating, the time spent in calling behavior was one. The other activities registered such as walking, interacting with other males, or resting were grouped in a variable called ‘other activities’. The sum of time spent calling and the time doing other activities equals one. For those males that mated, the time since female release until the achievement of copulation (referred to as ‘latency’) and copula duration were computed.

The time to call, the time spent calling (calling activity), and the body weight were compared between successful and unsuccessful males by means of a one-way analysis of variance (ANOVA). The association of time to call, calling activity, and weight with latency and copula duration of successful males were analyzed by means of Pearson product-moment correlation analysis. Statistical relationship between 8 morphological traits and copulatory success, copula duration, and latency was studied by step-wise multiple regression analysis.

Those males that exhibited calling behavior were classified according to whether they called inside a lek, always, sometimes, or never. The percentage of males that successfully mated was compared among the 3 categories by means of a Chi square test of heterogeneity. In addition, differences in time to call, time spent calling, body weight, and the 8 morphometric traits were evaluated among the same 3 categories (males that always, sometimes, or never called inside a lek). For the first 3 variables (time to call, time spent calling, and body weight), a one-way ANOVA was performed and for morphometric traits a MANOVA was used. In those cases in which significant differences were found, a Tukey test was performed. All statistical analyses were performed with Statistica for Windows (StatSoft, Inc. 2000) and STATISTIX 7 (Analytical Software 2000).

RESULTS

Lekking Behavior and Mating Location

The distribution of the number of calling males on the different sections of the tree is shown in Fig. 2. Calling males tended to be grouped in the NE quadrant ($\chi^2 = 58.16$, $df = 3, P < 0.001$), in the peripheral leaves ($\chi^2 = 21.16$, $df = 1, P < 0.001$), and at a middle height of the tree canopy ($\chi^2 = 18.81$, $df = 2, P < 0.001$). Given that the preferred area to call was so small (one out of the 24 available, considering cardinal orientation, height of the tree, and depth in the canopy), only one group of calling males (i.e., lek) was evident in each tree. The largest lek in location NE2P contained 4 males. The position of the first male that called in the tree determined the location of the rest of the pheromone calling males. In 58.88% of the cases, the first calling male was detected in the central third of the canopy (with the rest evenly distributed between the lowest and the highest thirds); in 70.6% of the cases the first calling male was located in the NE quadrant, with the remaining cases found in the SE quadrant. And finally, in all cases the first calling male was located in the P leaves.

Mating started at 9:30 AM and continued until 11:30 AM, reaching a maximum around 10:15 AM. Matings lasted from 15 min to 105 min; with a mean ($\pm$ SE) of 67.30 $\pm$ 4.30 min. Mating couples had a spatial distribution similar to that of calling males (Fig. 2). They were found in the following areas: 90% in the P leaves, 72% in the NE quadrant, and 48% at height 2. In all, 35% of the couples were collected in the preferred area (periphery at an intermediate height of the NE quadrant). The percentage of males that mated was 28%. Considering that only half as many females as males were released per cage, this figure repre-
sented 56% of the possible copulations. The mean number of copulations per cage was 3.9. Most of the copulations (90%) were observed on the underside of the leaves. Mean latency was 30 min and the last copulation was registered 150 min after releasing the females.

Lekking Behavior and Copulatory Success

The comparison of behavioral and morphological traits between successful and unsuccessful males indicated that successful males spent significantly more time calling than unsuccessful ones \((F = 3.97, P = 0.048)\). The time to call was not different between the 2 classes of males \((F = 2.099, P = 0.151)\) and 20% of unsuccessful males did not call at all. The mean body weight was \(14.55 \pm 0.17 \text{ mg}\). This variable did not differ statistically between successful and unsuccessful males \((14.99 \pm 0.35 \text{ mg} \text{ and } 14.30 \pm 0.26 \text{ mg},\) respectively). Multiple stepwise regression analysis indicated that eye length (EL) was associated with copulatory success (Table 1).

Latency and copula duration were not associated with time to call, time spent calling, or body weight (Table 2). By contrast, the analysis of morphometric traits showed that latency was associated with face width (FW) and that copula duration was associated with FW and EL (Table 1).

We found that only 10% of the males called exclusively within the limits of the lek, while most of them (64%) never integrated to leks, and the rest (26%) entered the lek occasionally. Although males that called always inside the lek were less abundant, most of them (83.3%) copulated, while only 20.6% of the males that sometimes called inside the lek and 23.7% of the males that called always outside the lek mated. The differences among these classes were significant \((\chi^2 = 8.03, P = 0.018)\). No differences in body weight or any of the morphometric traits were found among males that called only, sometimes, or never inside the leks (ANOVA for body weight: \(F = 0.82, P = 0.441\); MANOVA for morphometric traits: \(0.853 \geq P \geq 0.155\)). However, significant differences were found in calling activity, both for the time spent calling (ANOVA: \(F = 5.47, P = 0.009\), Table 3) and for the time required to start calling (ANOVA: \(F = 6.35, P = 0.003\), Table 3). Males that never joined the lek spent less time calling than males that called alternatively outside or inside the lek (Tukey test: \(P < 0.001\), while those that always called inside the lek started their calling activity later than males that called alternatively inside or outside the lek \((P = 0.002)\). The males that always called inside the leks showed intermediate values \((P > 0.05)\). Interactions between males were not very frequent (only 8 observations) and therefore they were not analyzed.

**DISCUSSION**

In the present study we analyze *A. fraterculus* male behavior and location during the hours of sexual activity and its relevance in their copulatory success. Males were found pheromone calling in a restricted part of the trees (on the northeastern quadrant, at an intermediate height, and in the peripheral leaves). This area represents one out of the 24 parts into which the tree canopy was divided, indicating that the grouping was very restricted and only a small portion of the tree is suitable or selected by the flies for calling and mating activity. This was considered as the area of lek formation. Given that most matings started before 11.00 AM, the preferred place to call corresponded to the one that received sunlight during the first hours of the day (the experiment was conducted in middle of autumn and the sunlight around 10.00 AM is concentrated in the NE and SE quadrants). This seems to imply that light determines, at least to some extent, the area of lek.

**Table 1. Association between copulatory success, copula duration, latency, and the 8 morphometric variables.**

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Independent variables</th>
<th>Order</th>
<th>(\beta^1)</th>
<th>SD ((\beta^1))</th>
<th>t (df)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copulatory success</td>
<td>EL</td>
<td>1</td>
<td>0.43</td>
<td>0.18</td>
<td>2.36 (89)</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>2</td>
<td>-0.22</td>
<td>0.13</td>
<td>-1.68 (89)</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>TIL</td>
<td>3</td>
<td>-0.19</td>
<td>0.16</td>
<td>-1.17 (89)</td>
<td>0.244</td>
</tr>
<tr>
<td>Copula duration</td>
<td>EL</td>
<td>1</td>
<td>0.50</td>
<td>0.18</td>
<td>2.79 (22)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>2</td>
<td>-0.29</td>
<td>0.13</td>
<td>-2.24 (22)</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>TIL</td>
<td>3</td>
<td>-0.19</td>
<td>0.16</td>
<td>-1.20 (22)</td>
<td>0.234</td>
</tr>
<tr>
<td>Latency</td>
<td>FW</td>
<td>1</td>
<td>0.59</td>
<td>0.21</td>
<td>2.78 (22)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>2</td>
<td>-0.35</td>
<td>0.21</td>
<td>-1.65 (22)</td>
<td>0.113</td>
</tr>
</tbody>
</table>

\(^1\beta\), partial regression coefficient of the corresponding dependent variable on the independent variable; SD (\(\beta\)), standard deviation of \(\beta\); t (df), Student’s t-test (degrees of freedom). FW = face width; EL = eye length; WL = wing length; and TIL = tibia length.
TABLE 2. RESULTS OF THE PEARSON PRODUCT-MOMENT CORRELATION ANALYSIS. CORRELATION COEFFICIENT \( r \), AND THE ASSOCIATED \( P \)-VALUE IS PRESENTED FOR EACH PAIR OF VARIABLES.

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th>Calling activity</th>
<th>Time to call</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency</td>
<td>( r = 0.06 ), ( P = 0.49 )</td>
<td>( r = 0.04 ), ( P = 0.60 )</td>
<td>( r = 0.20 ), ( P = 0.35 )</td>
</tr>
<tr>
<td>Copula duration</td>
<td>( r = 0.14 ), ( P = 0.11 )</td>
<td>( r = 0.15 ), ( P = 0.09 )</td>
<td>( r = 0.22 ), ( P = 0.29 )</td>
</tr>
</tbody>
</table>

Formation. Probably flies look for the best place to display during the time of highest sexual activity (Petit-Marty et al. 2004a; Vera et al. 2006).

Factors such as light intensity, foliage density, and wind speed have been postulated to affect lek location in the medfly (Arita & Kaneshiro 1989; Hendrichs & Hendrichs 1990; Whittier et al. 1994). Hendrichs & Hendrichs (1990; Whittier et al. 1994) found that leks occurred on leaves receiving more sunlight in the morning. Given that tephritid males are usually attracted by pheromone emitting males (Kaspi & Yuval 1999), it could be proposed that in A. fraterculus the first active calling male positions himself in the best place of the tree (probably the one which receives more sunlight, NE2P in our case), and then new males arrive to join the calling male until the stimulus becomes sufficiently intense to attract females.

Fiske et al. (1998) found that male copulatory success in lekking species is associated mainly with male displaying activity, aggression rate, and lek attendance. Our results suggest that calling activity and the way in which the males participate in the lek are key factors determining copulatory success (successful males called more than unsuccessful ones, and those who called exclusively inside the lek attained a higher proportion of the copulas). Although agonistic interaction between males of other Anastrepha species has been proposed as an important factor to maintain the position in the lek (Aluja et al. 1983; Hendrichs 1986; Sivinski & Burk 1989), aggression did not seem to be important in A. fraterculus, given that direct interactions between males were infrequent in our experiment. According to Shelly (2000), several lines of evidence suggest that male-male conflict seems to have low influence on copulatory success compared with the effect of intersexual selection in C. capitata. Whittier et al. (1994) found that male-male contests in C. capitata are infrequent and that male copulatory frequency is unrelated to fighting ability. This low frequency of fights between males was also found in our study, probably indicating the intrasexual selection mediated by agonistic interaction is unimportant for the copulatory success of A. fraterculus males, at least at the density we set inside our field cages. Nonetheless, the method employed in our study to characterize male behavior could have underestimated male-male interactions, because these phenomena usually last only a few s. A more focused observation of male-male interactions will surely help to understand the importance of male aggressive behavior on copulatory success.

There is some evidence suggesting that larger males have higher copulatory success in A. fraterculus (Sciuiano et al. 2007), Anastrepha suspensa (Loew) (Burk & Webb 1983), and C. capitata (Churchill-Stanland et al. 1986; Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi et al. 2000). Conversely, other studies showed that size has no effect on male copulatory success, at least for C. capitata (Whittier et al. 1994; Vera 1996; Norry et al. 1999; Shelly 2000). In the present study, body weight, thorax length, and wing width and length were used as indicators of males’ size, and no relationship was found between these variables and copulatory success. Our males were collected from guavas, a highly nutritious host, and after emergence were fed ad libitum until the field cage trials. Thus, it is likely that the males used in our study cover only a small range of possible sizes, a range that perhaps was not large enough to include those sizes that diminish the copulatory success.

Although overall size was not associated with copulatory success, probably other morphological characteristics could be the target of female selection. Indeed, from the morphometric analysis, we found that eye length was positively associated with copulatory success. Several morphometric studies on C. capitata have shown the importance of this variable on male copulatory success (Vera 1996; Norry et al. 1999; Rodriguero et al. 2002a; Rodriguero et al. 2002b), and it was shown that this selection operated via female choice, probably during close male-female interactions (Norry et al. 1999). A more detailed analysis of the importance of the eye length on copulatory success

TABLE 3. CALLING BEHAVIOR OF MALES THAT ALWAYS, SOMETIMES, AND NEVER CALLED IN THE AREA OF FORMATION OF LEK.

<table>
<thead>
<tr>
<th>Lek participation ( n )</th>
<th>Mean time to call ± SE</th>
<th>Mean calling activity ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always (12)</td>
<td>58.50 ± 31.18</td>
<td>0.44 ± 0.26</td>
</tr>
<tr>
<td>Sometimes (34)</td>
<td>34.44 ± 29.94</td>
<td>0.64 ± 0.25</td>
</tr>
<tr>
<td>Never (81)</td>
<td>63.02 ± 35.25</td>
<td>0.25 ± 0.23</td>
</tr>
</tbody>
</table>
in *A. fraterculus* will probably provide an important tool to evaluate male sexual performance.

We additionally analyzed 2 variables, latency and copula duration, that could be associated with the mating success of males. Latency was expected to be negatively associated with mating success because copulations in *A. fraterculus* are restricted to a narrow time window during the first 3 h after dawn (Malavasi et al. 1983; Petit-Marty et al. 2004a). Copula duration was expected to be positively associated with mating success because sperm transfer could be ineffective in very short copulations (nonetheless it has been shown by Taylor et al. 2001 that long-lasting copulas do not imply sperm transfer, so this variable should be analyzed with care). Our results suggest that morphological traits such as eye length and face width were associated with latency and copula duration, indicating that these traits could have some relationship with the physiological state of the males. Face width was negatively associated with fitness, because it was positively associated with latency and negatively associated with copula duration. On the contrary, eye length was positively associated with copula duration, and therefore -coupled with face width- could be the target of sexual selection. The high correlation between these 2 variables should be taken into account.

Defining the area of lek formation allowed us to analyze where each male called during the hours of sexual activity. We found that a minority of the males called exclusively inside the lek, and most of them attended the lek only occasionally or never. But, from those that remained in the lek, 83% copulated, and only one quarter of the remaining calling males achieved copulation. This result showed the importance of calling inside the leks, and the fact that presence in the lek could result from strong selective pressures to keep these "preferred sites". We could not find any morphological difference among males from these 3 classes. So it seems that overall size and morphometric traits are not requirements to be a part of the lek. Conversely, differences were observed in their calling activity. Males which called only occasionally inside the lek spent more time in calling activity than the rest, and started to call earlier than the others. Thus, another advantage of staying in the lek is that less effort on calling is needed; this is an advantage under the assumption that pheromone calling is an energetically "expensive" activity (Yuval et al. 2007), although joining a lek may also increase the risk of predation (Hendrichs & Hendrichs 1998).

Based on our results, we can propose that *A. fraterculus* males exhibit 3 strategies related to pheromone calling behavior: (1) males that call always in the lek invest a moderate effort in calling and have a high probability of mating (83% in our case); (2) males that call outside the leks invest a moderate effort in calling with the advantage of lacking competitors (even though there were 63% of the males outside the lek, they located in the 23 remaining sectors of the tree) but with the disadvantage of lower probability to copulate (24% in our case) -perhaps these males were less motivated to mate or some undetected trait or interactions impeded them from entering the lek and thus acted as "satellite" males; and (3) males that call inside and outside the lek invest a lot on calling and have a low probability of achieving a copula (21% in our case). Probably these males try to occupy empty sites inside the leks and alternate this behavior with a male satellite strategy. Perhaps they would achieve a copula (as well as the successful males) if females continued arriving at the lek (in our experimental design only 8 females were released, and no more females were added after the males started to copulate). Insightful results would come from studies that register the number of unsuccessful mating attempts made by each type of calling male, to assess if males that call outside the leks attract less females or if they are being rejected more by attracted females.

In summary, the present study showed that, although *A. fraterculus* was considered a lekking species as many other of the same genus, the lek is formed not only by males that remain in it throughout the period of sexual activity, but also by males that call alternatively inside and outside the lek. Also, we found that the area of lek formation was the same in all the trees that we used, indicating that factors other than intrinsic properties of the trees are determining where the males should group to call. In all trees the area of lek formation was the one that received more sunlight during the morning, strongly indicating a major role of this variable. However, as Aluja et al. (1993) noted, the lekking systems in *Anastrepha* are very dynamic and highly influenced by male density, so the present results should be confirmed in nature, where males are allowed to join or leave a tree if it is already occupied by other calling males.

We conclude that the copulatory success of males is associated with morphometric and behavioral traits. Males with longer eyes, spending more time calling, and that call inside the lek, have higher chances of copulating than males with shorter eyes, spending less time calling, and calling outside the lek. Interestingly, those males that call alternatively inside and outside the lek start to call earlier and spend more time calling, and yet obtained less copulas than those inside the lek, and about the same amount as those who call always from outside. This suggests some hierarchical order in the behavioral traits: calling is important, but the males should call from a specific location of the tree. Also, it seems that females are evaluating some morphometric trait of the males, but the distribution of this trait is independent of male behavior (males that always,
never or sometimes called inside the lek do not show differences in morphometric traits). Morphometric traits (in this case EL) could be used by females only after coming in close contact with males. It seems that A. fraterculus follows the pattern found for Anastrepha striata Schiner (Aluja et al. 1993) in that females use a chemical cue (e.g., calling pheromones) to locate males at long distances, and visual, acoustic and/or contact cues (e.g., courtship behavior, morphometric traits) to evaluate males at short distances.

We are still lacking sufficient information on male copulatory success in A. fraterculus. This information is vital within the context of SIT implementation, which has been proposed as a component of an area-wide integrated management of this pest (Guillén & Rodríguez 2007). The results of the present study shed light on several traits of A. fraterculus males that seems to be associated to their copulatory success. In fact, based on our results, morphometric traits such as eye length and face width should be considered in quality control tests rather than weight or overall size. Furthermore, studies or quality control tests directed to assess alterations of behavioral patterns due to mating males that seems to be associated to a lek do not show differences in morphometric traits). Moreover, studies or quality control tests directed to assess alterations of behavioral patterns due to mating males that seems to be associated to a lek do not show differences in morphometric traits). Moreover, studies or quality control tests directed to assess alterations of behavioral patterns due to mating males that seems to be associated to a lek do not show differences in morphometric traits).

ACKNOWLEDGMENTS


STATSOFT, INC. 2000. STATISTICA for Windows (Computer program manual). Tulsa, Oklahoma, USA.


SEXUAL SELECTION ON MULTIVARIATE PHENOTYPES IN ANASTREPHA FRATERCULUS (DIPTERA: TEPHRITIDAE) FROM ARGENTINA

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2Instituto de Genética, Instituto Nacional de Tecnología Agropecuaria, Argentina

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ABSTRACT

Despite the interest in applying environmentally friendly control methods such as sterile insect technique (SIT) against Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae), information about its biology, taxonomy, and behavior is still insufficient. To increase this information, the present study aims to evaluate the performance of wild flies under field cage conditions through the study of sexual competitiveness among males (sexual selection). A wild population from Horco Molle, Tucumán, Argentina was sampled. Mature virgin males and females were released into outdoor field cages to compete for mating. Morphometric analyses were applied to determine the relationship between the multivariate phenotype and copulatory success. Successful and unsuccessful males were measured for 8 traits: head width (HW), face width (FW), eye length (EL), thorax length (THL), wing length (WL), wing width (WW), femur length (FL), and tibia length (TIL). Combinations of different multivariate statistical methods and graphical analyses were used to evaluate sexual selection on male phenotype. The results indicated that wing width and thorax length would be the most probable targets of sexual selection. They describe a nonlinear association between expected fitness and each of these 2 traits. This nonlinear relation suggests that observed selection could maintain the diversity related to body size.

Key Words: sexual selection, morphology, multivariate statistical techniques, sterile insect technique, morphometric analysis, Anastrepha fraterculus

RESUMEN

A pesar del interés por la aplicación de métodos de control de bajo impacto ambiental sobre Anastrepha fraterculus (Diptera: Tephritidae), como la Técnica del Insecto Estéril (TIE), no existe aún información suficiente sobre su biología, taxonomía y comportamiento. Este trabajo tiene como objetivo evaluar el desempeño de moscas en jaulas de campo a través del estudio de la competitividad sexual entre machos salvajes (selección sexual). Para ello, se muestreó una población de Horco Molle, Tucumán (Argentina). En jaulas de campo se liberaron machos y hembras adultos vírgenes para evaluar la competición por el apareamiento. Se midieron ocho rasgos morfométricos en machos exitosos y no exitosos: ancho de la cabeza, ancho de la cara, largo del ojo, largo del tórax, largo del ala, ancho del ala, largo del fémur y largo de la tibia. Se realizaron análisis morfométricos para determinar la relación entre el fenotipo multivariado y el éxito copulatorio. Para evaluar la selección sexual sobre el fenotipo del macho se utilizaron diferentes combinaciones de métodos estadísticos multivariados y análisis gráficos. Los resultados demostraron que el ancho de ala y el largo de tórax serían los blancos más probables de selección sexual, y describen una asociación no lineal entre el éxito copulatorio y cada uno de estos dos rasgos. Dicha asociación sugiere que la selección observada mantendría la diversidad para el tamaño del cuerpo.

Translation provided by the authors.

The South American fruit fly Anastrepha fraterculus (Wiedemann) is an important fruit fly pest which, because of its wide host range, causes major economic losses to Argentina through direct effects reducing total production and indirect effects derived from restrictions to fruit and horticultural exports (Ortiz 1999). The species is abundant in the northwestern and northeastern regions of Argentina, which are characterized by hot and wet subtropical climates and separated from each other by an extremely arid region (Vergani 1956).
In 1994, Argentina embarked on an ambitious National Program for Control and Eradication of Fruit Flies (PROCEM-Argentina), whose strategy is based on integrated pest management. In this context, and with the objective of developing environmentally friendly approaches for their control (Manso & Basso 1999; Ovruski et al. 2003), one of the methods proposed is the sterile insect technique (SIT). Although it is being successfully applied against other tephritid species, most notably *Ceratitis capitata* (Wiedemann), it has not been yet developed for *A. fraterculus*. The SIT relies on reducing the reproductive potential of wild females by inducing egg sterility through the release of mass reared and sterilized males. Such reduction can be achieved only if the released sterile males are successful in mating and transferring sperm to wild females (Lux et al. 2002).

An indispensable requirement for the application of the SIT against *A. fraterculus* is the evaluation of the sexual behavior of wild flies and identification of the factors that affect mate choice, in order to predict the expected performance of laboratory strains under field conditions.Mate choice may be the consequence of 2 different processes: (1) positive assortative mating and (2) sexual selection. The first implies that mating partners are phenotypically more similar to each other than expected by random mating (Falconer & MacKay 2001). Positive assortative mating will place if mate choice preference differs between laboratory and wild females, determining that mass reared males mate preferentially with released females rather than with wild females. This process does not involve selection in natural conditions but may have negative consequences when SIT involves bisexual releases.

Sexual selection implies that certain individuals have advantage over other individuals of the same sex in exclusive relation to reproduction (Darwin 1871). Two different kinds of evolutionary processes can account for the evolution of sexually selected traits (Darwin 1871): (1) “intrasexual selection” or competition for mates between members of the same sex, usually males, and (2) “intersexual selection”, which involves active choice of particular individuals of the opposite sex, usually female choice of mates.

Rodriguero et al. (2002a, b) demonstrated the occurrence of both selective processes operating on several morphological traits correlated with body size in *C. capitata*. Furthermore, body size is usually considered a quality index (Churchill-Stanland et al. 1986; Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi et al. 2000). Because wings, legs, and head are apparently related to courtship behavior in *A. fraterculus* (unpublished results) and thorax length is an index of body size, we compared these traits in mating pairs and nonmated males to identify morphological characteristics involved in the male copulatory success. To understand the process of sexual selection on male morphological traits, different multivariate statistical techniques were used to detect, quantify, and visualize multivariate selection in individuals from a wild population from Argentina.

**MATERIALS AND METHODS**

**Field Cage Experiments**

A wild population from Horco Molle, Tucumán (26°48' latitude South, 68°20' longitude West) was sampled in Feb 2001. Wild flies were obtained from infested *Psidium guajava* L. fruits taken from the tree and the ground. The fruits were sent to the Laboratorio de Insectos, Instituto de Genética “E. A. Favret” at INTA Castelar, where they were kept in plastic trays with a sand layer to allow pupation. Periodically, the sand was sieved to obtain pupae that were maintained under controlled conditions (25 ± 1°C, 50 ± 5% RH and a photoperiod of 12:12 (L:D) until adult emergence. Emerged adults were removed from flasks every 24 h and were separated by sex and kept under laboratory conditions (23-27°C, 50-70% RH and a photoperiod of 12:12 (L:D) until they reached the age of 20 ± 1 d.

Experiments were conducted under outdoor field cage conditions. Screened nylon cages (2.5 m high × 2.5 m diameter) were erected over rooted tangerine (*Citrus nobilis* L.) trees. Mature adults (250 males and 25 females) were released inside each cage. We choose this proportion for 2 reasons: (1) the number of calling males forming a lek in nature is usually much higher than the number of receptive females visiting a lek, and (2) a high number of males results in stronger selection on male mating success.

During observation periods, which ranged from 0800-1400 h, mating pairs were scored and gently removed from the cage by capturing them in a vial. Copulating males were designated “successful”, while those males that failed to mate during the test were designated “unsuccessful”. The whole experiment involved 8 replicates carried out on 12, 13, and 17 of Apr 2001. Although this design measures only mating success, the ability to mate may be considered a key component of sexual selection. In fact, previous studies (Petit-Marty et al. 2004a, 2004b) showed that sperm transfer is verified in virtually all copulations achieved under similar experimental conditions.

**Morphometric Measurements**

A sample of 70 successful and 135 unsuccessful males were selected at random after pooling the data from all replicates. These flies were measured for 8 morphometric traits (Fig. 1): head width (HW), face width (FW), eye length (EL), th-
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rinx length (THL), wing length (WL), wing width (WW), femur length (FL), and tibia length (TIL).

Each fly was dissected on a Petri dish with a paraffin wax layer. Head traits were observed from the front and thorax length was scored from the dorsal view. In the case of bilateral limbs (wings and legs) the measurements were scored on the left side only. To measure HW, FW, EL, and THL, the body part was dissected and placed on a Petri dish with Bacto-agar (DIFCO laboratories, U.S.A.) 1% in H$_2$O. WL, WW, FL, and TIL were measured on a microscope slide (24.4 × 76.2 mm) with a cover glass (24 × 40 mm) sealed with synthetic balsam.

All measurements were made with a stereoscopic microscope, Leitz Wetzler, with a 12.5× ocular provided with a micrometric scale. WL and WW were measured at 1×, HW, EL, THL, FL and TIL at 4×, and FW at 8×.

Data Analyses

In the present work, the term ‘fitness’ was used to mean ‘copulatory success’. Mated males (successful) were assigned an absolute fitness value of 1 ($W = 1$) while unmated males were assigned $W = 0$. Individual phenotypic values for each trait were standardized to have mean zero and unit variance according to the corresponding replicate (to the cage and to the day).

Pearson (1903) showed that multivariate statistics could be used to discriminate the direct and indirect effects of selection to determine which traits in a correlated ensemble are the targets of direct selection (Lande & Arnold 1983).

Multiple linear regression allows multiple traits to be considered simultaneously (Lande & Arnold 1983; Phillips & Arnold 1989; Brodie III et al. 1995). However, some difficulties exist in applying this analytical method to the data from selection studies (Schluter & Smith 1986; Mitchell-Olds & Shaw 1987; Wade & Kalisz 1990; Crespi 1990). To avoid some difficulties such as those derived from assumptions not fulfilled, different complementary statistical techniques were applied in the present study, as follows: (1) multiple logistic regression; (2) multiple stepwise regression; (3) principal components analyses (normalized varimax rotation) coupled with logistic regression; and (4) a nonparametric graphic technique known as ‘cubic spline’ (Schluter & Smith 1986; Schluter 1988).

Multiple logistic regression is a more natural model for studying the relationship between a dichotomous fitness measure and various phenotypic explanatory variables than is multiple lin-
ear regression. It does not rely on assumptions of normality for the predictor variables or the errors, and it allows the selection effect to vary non-linearly (Janzen & Stern 1998). Among different multiple regression methods, stepwise regression analysis was used to find the smallest set of predictor variables that still yields an adequate prediction (Sokal & Rohlf 1979).

Principal component analysis (PCA) of morphological variables was used to eliminate bias due to the correlation among traits. A normalized VARIMAX rotation was applied to extract the first 3 principal components from the standardized phenotypic covariance matrix, because this method insures that components are orthogonal and simplifies interpretation by minimizing the number of variables that have high loadings on each principal component (Norry & Vilardi 1996; Norry et al. 1999; Rodriguero et al. 2002a, 2002b). The logistic regression of relative copulatory success on the rotated principal components estimates the regression of fitness on each of these composite variables (β'). Because copulatory success is a dichotomous variable (W = 1 or 0), the significance of β' was tested by Chi-square test (χ²) (Schluter 1988).

Finally, to reveal the precise shape of the regression of fitness on the multivariate phenotype, we adopted Schluter’s (1988) nonparametric visualizing technique known as ‘cubic spline’. This nonparametric method is not restricted a priori to a particular model of selection and estimates complex functions with multiple peaks and valleys allowing a complete descriptive model of selection pressures on individuals (Schluter 1988; Brodie III et al. 1995), although a shortcoming is the higher sampling variation compared with parametric regression surfaces (Schluter 1988; Schluter & Nychtka 1994).

All statistical analyses were made with the software STATISTICA 6.0 (Statistica Statsoft 1998) and STATISTIX 7 Trial Version (Analytical Software 2000).

Results

For most traits, successful males had on average higher values than unsuccessful ones, but differences were only significant for wing width (P = 0.003) (Table 1). The statistical relationship between fitness and phenotypic characters was evaluated by 3 multivariate methods. First, a multiple logistic regression revealed 3 variables as possible targets of sexual selection: thorax length (β' = -0.59 ± 0.24; P = 0.014), wing width (β' = 0.94 ± 0.32; P = 0.003), and femur length (β' = -1.11 ± 0.50; P = 0.025). Second, the possible correlation among traits was removed by means of a step-wise multiple regression analysis. This analysis identified wing width (β' = 0.32 ± 0.08; P = 0.003) and thorax length (β' = -0.24 ± 0.08; P = 0.002) as possible targets of sexual selection. Both characters were significantly correlated with fitness but with opposite signs. Third, the principal component analysis of morphological characters showed that although PC1 explains most of phenotypic variance, it is not associated with selective differences. Only PC2 and PC3 are significantly related with fitness. PC2 is mainly determined by wing width while PC3 is determined by thorax length (Table 2). Together, the first 3 principal components accounted for nearly 88% of the total variance in traits. The 3 analyses were con-

<table>
<thead>
<tr>
<th>Trait</th>
<th>Unsuccessful (n = 135)</th>
<th>Successful (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW</td>
<td>7.34 ± 0.44</td>
<td>7.41 ± 0.46</td>
</tr>
<tr>
<td>FW</td>
<td>4.60 ± 0.34</td>
<td>4.65 ± 0.34</td>
</tr>
<tr>
<td>EL</td>
<td>5.42 ± 0.36</td>
<td>5.45 ± 0.36</td>
</tr>
<tr>
<td>THL</td>
<td>2.44 ± 0.19</td>
<td>2.41 ± 0.18</td>
</tr>
<tr>
<td>WL</td>
<td>4.80 ± 0.26</td>
<td>4.83 ± 0.42</td>
</tr>
<tr>
<td>WW</td>
<td>2.40 ± 0.15</td>
<td>2.48 ± 0.29</td>
</tr>
<tr>
<td>FL</td>
<td>6.85 ± 0.40</td>
<td>6.85 ± 0.45</td>
</tr>
<tr>
<td>TIL</td>
<td>6.18 ± 0.39</td>
<td>6.22 ± 0.47</td>
</tr>
</tbody>
</table>

1Abbreviations of morphometric traits for this and subsequent tables are given in Fig. 1.
2n, number of individuals.
3Significant differences between successful and unsuccessful males (P = 0.003).

Table 1. Mean values (mm) (± SE) of morphometric traits measured in successful and unsuccessful South American fruit fly males from field-cage mating tests.

<table>
<thead>
<tr>
<th>Trait</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW</td>
<td>0.75</td>
<td>0.50</td>
<td>0.32</td>
</tr>
<tr>
<td>FW</td>
<td>0.81</td>
<td>0.44</td>
<td>0.13</td>
</tr>
<tr>
<td>EL</td>
<td>0.70</td>
<td>0.48</td>
<td>0.42</td>
</tr>
<tr>
<td>THL</td>
<td>0.30</td>
<td>0.25</td>
<td>0.87</td>
</tr>
<tr>
<td>WL</td>
<td>0.81</td>
<td>0.01</td>
<td>0.46</td>
</tr>
<tr>
<td>WW</td>
<td>0.24</td>
<td>0.91</td>
<td>0.23</td>
</tr>
<tr>
<td>FL</td>
<td>0.70</td>
<td>0.32</td>
<td>0.52</td>
</tr>
<tr>
<td>TIL</td>
<td>0.64</td>
<td>0.33</td>
<td>0.54</td>
</tr>
<tr>
<td>% total variance</td>
<td>74.03</td>
<td>82.30</td>
<td>88.05</td>
</tr>
<tr>
<td>Cumulative %</td>
<td>74.03</td>
<td>82.30</td>
<td>88.05</td>
</tr>
<tr>
<td>β'</td>
<td>0.13</td>
<td>0.57</td>
<td>-0.31</td>
</tr>
<tr>
<td>χ²</td>
<td>0.71</td>
<td>8.80</td>
<td>4.29</td>
</tr>
<tr>
<td>P</td>
<td>0.399</td>
<td>0.003</td>
<td>0.038</td>
</tr>
</tbody>
</table>

1Traits with the highest factor loadings on each PC, cumulative variance percentage and where P-value < 0.05, are underlined.
sistent with each other indicating that direct selection favored males with broader wings and shorter thorax.

A 2-dimensional graphical representation of selection surfaces by means of the nonparametric method of cubic spline showed a nonlinear relationship between copulatory success and each of the characters that are probable targets of sexual selection (Fig. 2). Fig. 2a suggests that selection on wing width acts by favoring extreme phenotypes, while Fig. 2b shows a principal and a local maximum in the case of thorax length. Furthermore, a 3-dimensional selection surface plot (Fig. 3) allows visualization of selection on wing width and thorax length simultaneously by considering the loading of these traits on the major axes of the selection surface. This graph showed one absolute and 2 local maxima consistent with Fig. 2. The absolute maximum corresponds to males with the widest wings and large (but not largest) thorax. One local maximum is associated with shortest thorax and medium width wings and the other corresponds to medium-sized thorax and narrowest wings. The nonlinear relationship between expected fitness and the probable targets of sexual selection suggests that observed selection could favor the maintenance of body-size diversity.

**DISCUSSION**

Despite increased interest in applying environmentally-friendly control methods, such as SIT, against *A. fraterculus*, information about its biology, taxonomy, and behavior is still insufficient. Interdisciplinary work is necessary to develop mass-reared strains that mate successfully with wild populations. Some of the key subjects for research involve nutrition to optimize mass rearing methods in the laboratory (Manso 1999; Jaldó et al. 2001), the development of genetic sexing strains, population genetic studies to determine affinity and gene flow among different populations and the routes of colonization (Alberti et al. 1999), studies of sexual behavior, mating systems, and compatibility between wild and mass-reared flies (Petit-Marty et al. 2004a, 2004b), and morphometric studies to determine characters related to fitness (Russo et al. 2002).

In *A. suspensa* (Burk & Webb 1983) and *C. capitata* (Churchill-Stanland et al. 1986; Orozco & López 1993) male size has been shown to be related with mating success. However, this variable itself may not necessarily be the direct target of sexual selection, because such an association could be the result of selection on one or many traits correlated with it (Norry et al. 1995). In *C. capitata* some size-related traits have been detected as targets of sexual selection (Norry et al. 1999; Rodríguez et al. 2002a; Rodríguez et al. 2002b). In this species, the combination of stepwise regression plus principal component analysis, coupled with regression analysis, indicated that eye and thorax length were positively correlated with copulatory success, while face width was negatively correlated, suggesting that those males with largest eyes and thorax, and smallest
faces have the highest copulatory success (Rodriguero et al. 2002a; Rodriguero et al. 2002b). Norry et al. (1999) revealed that copulatory success was also partially determined by intersexual selection on morphology, probably on size or shape of the head capsule. These results suggested that in *C. capitata* discrimination among potential mates on the basis of the male head morphology probably takes place when the female approaches the wing-fanning and head rocking male face to face (Calcagno et al. 1999; Norry et al. 1999; Rodriguero et al. 2002b). In contrast, there are other studies showing no relationship between male size and copulatory success (Whittier et al. 1994; Whittier & Kaneshiro 1995; Vera et al. 1996).

Our results suggest that thorax length, wing width, and femur length are possible targets of sexual selection in *A. fraterculus*. However, when the effect of correlation among traits is removed (stepwise regression and PCA), only thorax length and wing width are significantly correlated with copulatory success. Thus, the selection on femur length would be indirect, due to the correlation of this trait with other size-related traits. According to principal component analysis the PC1, an indicator or overall size, in the present case is not significantly associated with mating success. Our results indicate that wing width and thorax length are selected in different direction. All this evidence leads to the conclusion that under these experimental conditions sexual selection operates on body shape rather than body size level.

Rodriguero et al. (2002a, b) suggested the occurrence of correlational selection on multivariate phenotype as a by-product of directional selection acting on face width and thorax length separately and in the opposite direction in *C. capitata*. The selection surface analyzed in the present study described a nonlinear association between expected fitness and the 2 traits (wing width and thorax length) identified as targets of sexual selection, considered both separately or simultaneously. That nonlinear relation suggests that the observed selection could maintain diversity related to body size or shape. The different multivariate statistical methods and visual analyses yielded consistent results suggesting that sexual selection would affect multivariate phenotype in *A. fraterculus*.

Under natural conditions the adult male:female ratio should be near 1. However, the population of pheromone calling and signaling males is usually several times higher than the number of receptive virgin females that approach the calling males. In the present work, the proportion of males to females released inside each cage (10:1) is probably higher than that in the wild and might increase selective pressure on characters related to mating success. Our experimental design might lead to an overestimation of sexual selection. However, in natural conditions with huge population numbers very slight selective differences will have significant effects that would be impossible to detect under experimental conditions. In our experiment we did not evaluate the whole process of sexual selection because we did not measure post-zygotic components. However, mating success may be considered as the final result of many pre-zygotic components of sexual selection, such as male ability to integrate into leks, initiate pheromone calling, and display successfully the whole sequence of courtship activities that lead to female acceptance. In previous work (Petit-Marty et al. 2004b), we observed that virtually all matings achieved under similar experimental conditions were fertile. This means that an advantage in the ability to mate would be strongly selected under natural conditions.

ACKNOWLEDGMENTS

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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

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 estandarizados de calidad. Machos estériles de 5 días 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 conferió 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 60Co 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 determined 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:

\[
RSI = \frac{LW}{LW + WW}
\]

The data were analyzed by analysis of variance (ANOVA) (Ott & Longnecker 2001). The significance value used in tests was 95% (α = 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 (Fα = 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>
<thead>
<tr>
<th>Outdoor acclimatization</th>
<th>PM (± SD)</th>
<th>RSI (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>0.45 ± 0.13</td>
<td>0.24 ± 0.11</td>
</tr>
<tr>
<td>1 d</td>
<td>0.43 ± 0.11</td>
<td>0.19 ± 0.12</td>
</tr>
<tr>
<td>3 d</td>
<td>0.42 ± 0.15</td>
<td>0.26 ± 0.18</td>
</tr>
</tbody>
</table>
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 as 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**

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MALE COURTSHIP BEHAVIOR IN *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) THAT HAVE RECEIVED AROMATHERAPY WITH GINGER ROOT OIL

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ABSTRACT

The results of previous studies that showed that exposing mass-reared male Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) to ginger root oil ("aromatherapy") increases the likelihood of mating with wild females were confirmed. The increased male success could be due to female responses to changes in male behavior or male pheromones. There were no significant differences in the types of courtship movements executed by males with and without aromatherapy. The durations of movements also did not differ when mass-reared males were paired with mass-reared females; however, when they were paired with wild females, there were a few, small differences. Previous studies indicated that the effectiveness of the male long-distance attractant pheromone is not affected by aromatherapy, but these studies did not consider pheromones released at close range during courtship, which behavioral analyses suggest may be different. We propose the following possible explanation for the different effects of aromatherapy with different females. Selection on males under mass rearing may have altered their close-range pheromones in ways that can be remedied by aromatherapy; and only wild females respond because the pheromonal responsiveness of mass-reared females has also changed. We propose observations that could test these ideas.

Key Words: aromatherapy, *Ceratitis capitata*, close range pheromones, mating behavior, Mediterranean fruit fly, sexual selection

RESUMEN

Los resultados de estudios previos que muestran que al exponer machos criados en masa de la mosca mediterránea de la fruta *Ceratitis capitata* (Wiedemann) al aceite de la raíz del jengibre ("aromaterapia") aumentó la probabilidad del apareamiento con hembras naturales fueron confirmados. El aumento en el éxito de los machos puede ser debido a las respuestas de las hembras a los cambios en el comportamiento o feromonas de los machos. No hubo una diferencia significativa en la clase de los movimientos del cortejo ejecutados por los machos con y sin la aromaterapia. La duración de los movimientos tampoco fue diferente cuando los machos criados en masa fueron apareados con hembras criadas en masa; sin embargo, cuando ellos fueron apareados con hembras naturales, resultaron unas pequeñas diferencias. Los estudios previos indicaron que la eficacia de la feromona atrayente de machos de larga distancia no esta afectada por la aromaterapia, pero estos estudios no consideran las feromonas sueltas en un rango corto durante el cortejo, cuando el análisis de comportamiento sugiere que puede ser diferente. Nosotros proponemos la explicación siguiente para los efectos diferentes de la aromaterapia con las diferentes hembras. La selección de machos bajo condiciones de cría en masa puede haber alterado las feromonas de rango corto de manera que puede ser remediada por la aromaterapia; y solamente las hembras naturales responden por que también ha cambiado la respuesta de las hembras criadas en masa a la feromona. Nosotros indicamos observaciones que pueden probar estas ideas.

The Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) is a serious agricultural pest, and much effort is expended in attempting to control populations in the wild. One important technique involves releasing large numbers of mass-reared sterile males to mate with wild females, thus rendering their eggs inviable. The success of this technique depends on the ability of mass-reared males to successfully mate with wild females. Unfortunately, males from mass-rearing strains are often inferior to wild males, apparently because their courtships are less effective (Lance et al. 2000). Although several aspects of male courtship behavior are known to have changed in at least some mass-reared strains (Liimattainen et al. 1997; Briceño & Eberhard 1998;
Calcagno et al. 1999; Briceño et al. 2002b), it is not clear whether these or other male traits are more important in producing this inferiority (Eberhard 2000). Further understanding of the causes of male inferiority would be useful in designing better systems for mass rearing, and in testing the quality of mass-reared males.

The recent discovery that exposing mass-reared males to certain male pheromone analogues or precursors of pheromone production (“aromatherapy”) greatly increases their ability to compete with wild males for females (Shelly et al. 1996; Shelly 1999, 2001; Shelly & McInnis 2001), may help alleviate the problem of reduced competitiveness. Male ability to copulate with wild females was improved by exposing them to these compounds the day before mating. The mechanism by which this effect occurs is not known, other than that it can be obtained by exposing the male to only the aroma of the substance; feeding is not required. There are several non-exclusive possible explanations for the greater success of treated males, including that they are more motivated and consistent, that their production of attractant substances is somehow altered, or that their courtship behavior is altered. This study focused on possible changes in courtship behavior.

MATERIALS AND METHODS

All observations were made in the Tephritid Fruit Fly Laboratory of the University of Hawaii at Manoa, Oahu, Hawaii. The mass-reared flies used were from a small laboratory colony that was derived in 1996 from the old “HiLab” mass-reared strain (44 years or about 748 generations), and since maintained by D. McInnis. Wild flies were collected from coffee on the island of Kauai. Larvae and pupae were reared in the laboratory, and adults were separated by sex within 24 h of emergence. Flies were held in 5-L buckets (maximum number: 150 flies) topped with screen, with ad libitum access to water and food (hydrolyzed yeast and sugar, 1:3).

Aromatherapy consisted of placing a small vial containing 20 micro liters of ginger root oil (“Oil Ginger Chinese FCC”, Citrus and Allied Essences Ltd. of Lake Success, New York, U.S.A.), on blotting paper (placed in a foil-lined Petri dish) for 7 h in each bucket containing 50 mass-reared males the day before the mating trial. The males could not touch the oil. Mating trials were performed in plastic Petri dishes 13.7 cm in diameter and 1.8 cm deep, and were videotaped from below through a glass table (Briceño & Eberhard 1998) with a Sony Hi8 camcorder equipped with +6 closeup lenses. Flies in mating trials were 5-10 d old, and each fly was used only once.

Durations of different components of courtship behavior in interactions that led to a mounting attempt were determined to the nearest 0.03 s with frame by frame analyses of the videotapes. Only a single mounting was analyzed for each male to avoid pseudoreplication. Male behaviors analyzed were continuous vibration (wings directed postero-laterally and vibrated rapidly dorsoventrally), intermittent buzzing (wings moved periodically from being directed dorsally over to the body to anteriorly while also being vibrated) (for more detailed descriptions see Briceño & Eberhard 2002b), and initial head rock (rotating and lateral movements of the head that occurred just before intermittent buzzing began). Because contact with the male’s sexually dimorphic aristae during buzzing appears to be part of medfly courtship (Briceño & Eberhard 2002a), intermittent buzzing was divided into two parts: prior to full arista contact (the male contacted the female, usually her aristae, with one of his aristae), and after full arista contact (both male aristae contacted those of the female). Also noted was the total time the female had remained immobile before the male launched his mounting attempt, the total number of buzzes, and whether the mount was successful (resulted in copulation). All means are followed by ± 1 standard deviation. Statistical tests were Mann-Whitney U Tests performed by the statistical package “Statistica” unless otherwise specified.

RESULTS

The same behavior patterns (continuous vibration, intermittent buzzing, head rocking) were executed with qualitatively similar movements by both control and experimental males. Aromatherapy did not produce consistent differences in the measured aspects of male courtship behavior (Table 1). There were no significant differences in durations and numbers of repetitions between control and experimental males when courting mass-reared females. There were 2 differences in the small sample of 9 experimental males courting wild females; these differences were not evident, however, in the larger sample of experimental males courting mass-reared females (in fact, the 2 trends involving durations of arista contact were reversed with mass-reared females). Combined data from courtships of wild and mass-reared females showed no significant effects of aromatherapy on male behavior (see discussion of combining data below). Comparisons between courtships preceding successful and unsuccessful mounts also failed to reveal consistent differences (Table 2).

As in previous studies, aromatherapy increased the probability of copulation with wild females (78% of 9 vs. 29% of 34) ($\chi^2 = 6.96, df = 1, P = 0.008$), but did not increase success with mass-reared females (22% of 36 vs. 25% of 40) ($\chi^2 = 0.08, df = 1, P = 0.78$). Aromatherapy resulted in greater likelihood that mounting would be suc-
scessful with wild females than with mass-reared females (78% of 9 vs. 22% of 36) (χ² = 10.0, df = 1, P < 0.0016).

**DISCUSSION**

It is not obvious how to interpret some of these results. Aromatherapy resulted in courtships that were more likely to induce wild females to copulate, but did not affect the receptivity of mass-reared females. What aspects of courtship changed? On the one hand, the durations of nearly all aspects of male courtship of wild-type females were reduced when males had received aromatherapy. Only two aspects (durations of arista contact and total courtship) were statistically significant. However, the number of pairs was small, and most traits were quite variable. On the other hand, there were no indications of corresponding differences in the larger samples of males courturing mass-reared females, or in the totals combining wild and mass-reared females. It is not clear, however, whether combining the data in this way is justified. If the origin of female does not affect male courtship behavior, it is reasonable to combine the samples; but if it does alter male behavior, pooling the data is inadvisable. One previous study showed that, in another pair of strains, the male’s courtship did not change when the strain of the female varied (Briceño & Eberhard 1998), suggesting that combining data from different strains in the present study may be reasonable. However, differences in the cues that affect female behavior in different strains could determine whether or not it is appropriate to combine data. It is possible, for instance, that male pheromones did not vary between strains in the previous study (Briceño & Eberhard 1998) (thus arguing in favor of combining data), but that they did vary in the present study (thus arguing against combination) (see below).

One possible explanation for increased acceptance by wild females of males that received aromatherapy is that some aspect of the complex mix of male sexual pheromones (Millar 1995) is changed by aromatherapy. There are indications that the effectiveness of the male long-distance attraction pheromone mix (Millar 1995) is not affected by aromatherapy (Shelly 2001), but there are no data testing the possibility that it affects the male pheromone(s) released at close range during courtship, after the female has approached the male to within approximately one to five body lengths. Assuming that the male rectal epithelium and his abdominal pleura do not emit the same blend of 90+ components found in the pheromones of this species (Millar 1995), the fact that different male structures are everted at different stages during interactions with females suggests that males release different pheromone mixes at close-range and long-range (Eberhard 2000).

It should be kept in mind that the durations of most of the aspects of male medfly courtship behavior that we measured may be influenced not only by the male’s own tendency to pace his courtship, but also by the female’s responses to his behavior. Courting male medflies are more or less immobile, and movements by the female are usually responsible for bringing the pair of flies close together (Feron 1962), and in orienting the female to face directly toward the male so he can touch her aristae with his and attempt to mount (Briceño & Eberhard 2002a, 2002b). If males use female proximity and orientation to trigger

The following is a table of courtship behavior data:

<table>
<thead>
<tr>
<th>Male behavior duration (s)</th>
<th>Wild females</th>
<th>Mass-reared females</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Aromatherapy</td>
<td>Control</td>
</tr>
<tr>
<td>Continuous vibration</td>
<td>6.20 ± 7.6</td>
<td>3.50 ± 2.1</td>
<td>5.60 ± 6.7</td>
</tr>
<tr>
<td>Intermittent buzzing</td>
<td>12.20 ± 7.9</td>
<td>7.80 ± 2.1</td>
<td>10.50 ± 6.0</td>
</tr>
<tr>
<td>Initial head rocking</td>
<td>1.10 ± 1.0</td>
<td>0.70 ± 0.4</td>
<td>1.50 ± 1.9</td>
</tr>
<tr>
<td>Single arista contact</td>
<td>6.10 ± 4.7</td>
<td>1.00 ± 1.6</td>
<td>3.90 ± 2.5</td>
</tr>
<tr>
<td>Full arista contact</td>
<td>8.50 ± 5.3**</td>
<td>3.60 ± 2.0</td>
<td>6.90 ± 4.5</td>
</tr>
<tr>
<td>Total arista contact</td>
<td>9.30 ± 8.0*</td>
<td>4.30 ± 1.9</td>
<td>6.90 ± 4.8</td>
</tr>
<tr>
<td>Total courtship</td>
<td>17.50 ± 12.7*</td>
<td>9.40 ± 2.1</td>
<td>15.10 ± 10.2</td>
</tr>
<tr>
<td>Number of buzzes</td>
<td>49.00 ± 30.5</td>
<td>34.40 ± 10.5</td>
<td>39.60 ± 21.7</td>
</tr>
<tr>
<td>Frequency of buzzes (number/s)</td>
<td>3.80 ± 0.8</td>
<td>4.35 ± 0.5</td>
<td>3.95 ± 0.9</td>
</tr>
</tbody>
</table>

Female Behavior

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aromatherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>34</td>
<td>9</td>
</tr>
<tr>
<td>Female immobile</td>
<td>13.20 ± 9.2*</td>
<td>6.30 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>8.60 ± 5.3</td>
<td>7.50 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>10.70 ± 7.7</td>
<td>7.20 ± 3.9</td>
</tr>
</tbody>
</table>

**Table 1. Courtship behavior of mass-reared medfly males with and without aromatherapy, when they courted wild and mass-reared females (successful and unsuccessful courtships are combined). Comparisons are between control and aromatherapy males (**P < 0.01, *P < 0.05).**
mounting attempts (Briceño & Eberhard 2002b), then quicker female approaches and alignments could result in shorter durations of several components of male courtship. The shorter durations (if they exist) of the courtship of wild females by males that received aromatherapy could result from more rapid female approaches, perhaps due to improved male short-range pheromone.

The limitation of this effect to interactions with wild but not mass-reared females could be explained if female chemical criteria for accepting males have changed under mass-rearing. Changes in the criteria of mass-reared females for another male courtship trait have been documented in this species (Briceño & Eberhard 2000a, 2002b). It seems undeniable that the pheromonal milieu in mass-rearing cages must be quite different from that under natural conditions, so selection on both male pheromone production and female responsiveness to pheromones is likely to be different.

Whatever the correct interpretation of these aspects of our data, they emphasize the central role of females in determining the effect of aromatherapy on the ability of males to obtain copulations, and focus attention on possible mechanisms responsible for this effect. If aromatherapy causes changes in the durations of different aspects of male courtship behavior, this change occurs only when the male courts wild females, not mass-reared females. If aromatherapy does not directly cause such changes in male courtship behavior, then increased acceptance by wild females is presumably due to responses to other cues from the treated males (probably chemical) that induce the females to accept copulation when mounted.

Future studies could test the possible importance of male and female roles in increased acceptance and courtship duration by concentrating on male and female movements prior to mounting. If changes in duration of male behavior are due to greater female attraction, courtships by males that have received aromatherapy should produce more rapid and extensive female turning to orient toward the male, more frequent female walking movements toward him, and approaches that occur with

<table>
<thead>
<tr>
<th>Table 2. Male courtship behavior in successful and unsuccessful courtships of wild and mass-reared medfly females. Comparisons were between successful and unsuccessful courtships (**P &lt; 0.01, *P &lt; 0.05), for both control and aromatherapy males.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male behavior duration (s)</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>A. Mass-reared males × wild females</td>
</tr>
<tr>
<td>Continuous vibration</td>
</tr>
<tr>
<td>intermittent buzzing</td>
</tr>
<tr>
<td>Initial head rocking</td>
</tr>
<tr>
<td>Single arista contact</td>
</tr>
<tr>
<td>Full arista contact</td>
</tr>
<tr>
<td>Total arista contact</td>
</tr>
<tr>
<td>Total courtship</td>
</tr>
<tr>
<td>Number of buzzes</td>
</tr>
<tr>
<td>Frequency of buzzes (number/s)</td>
</tr>
<tr>
<td>Female Behavior</td>
</tr>
<tr>
<td>Female immobile</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>B. Mass-reared males × mass-reared medly females</td>
</tr>
<tr>
<td>Continuous vibration</td>
</tr>
<tr>
<td>Intermittent buzzing</td>
</tr>
<tr>
<td>Initial head rocking</td>
</tr>
<tr>
<td>Single arista contact</td>
</tr>
<tr>
<td>Full arista contact</td>
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<td>Total arista contact</td>
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<tr>
<td>Total courtship</td>
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<tr>
<td>Number of buzzes</td>
</tr>
<tr>
<td>Frequency of buzzes (number/s)</td>
</tr>
<tr>
<td>Female Behavior</td>
</tr>
<tr>
<td>Female immobile</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td><strong>Note:</strong></td>
</tr>
</tbody>
</table>

**Table 2. Male courtship behavior in successful and unsuccessful courtships of wild and mass-reared medfly females. Comparisons were between successful and unsuccessful courtships (**P < 0.01, *P < 0.05), for both control and aromatherapy males.**
less delay. If, in contrast, aromatherapy changes the male and makes him attempt to court more quickly, then perhaps male orientation and movements toward the female will be more frequent.

A technically more difficult, but more direct test would involve checking whether pheromones differ from courting males that have, and have not, received aromatherapy. Still another test would be to determine both female response behavior and male copulation success for males with aromatherapy when paired with females whose ability to sense pheromone was experimentally altered. This alteration would also be technically challenging, as the chemical sensors on the female antennae would have to be modified without changing their sensitivity to the tactile stimuli that also influence female acceptance (Briceño & Eberhard 2002a).

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