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## 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 desarrollo de protocolos 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](http://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) *Ceratitidis 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. Briceño 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 imple-

mentation 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-

mal 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  $^{60}\text{Co}$  or  $^{137}\text{Cs}$  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

Insect 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  $\text{O}_2$  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 associa-

tion 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; Katsoyannos 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, is essen-

tial 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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