United States Department of Agriculture

Animal and Plant Health Inspection Service

Final Report

United States, Mexico, and Guatemala Fruit Fly Emergence and Release Facilities Review

Conducted July 2008

Expert Review Panel Members:

Pedro Rendon, Chairperson
Jorge Hendrichs
Aldo Malavasi
Ed Gersabeck
Wayne Burnett
Bill Macheel
John Stewart
Pablo Montoya

Expert Review Panel Members:

Wayne D. Burnett, Domestic Coordinator
Fruit Fly Exclusion and Detection Programs
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road
Riverdale, Maryland, 20737 USA

Edward Gersabeck, Technical Director
Mexican Fruit Fly Program
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road
Riverdale, Maryland, 20737 USA

Jorge Hendrichs, Head
Insect Pest Control Section
Joint FAO/IAEA Division of Nuclear
Techniques in Food and Agriculture
P.O. Box 100, A-1400 Vienna, AUSTRIA

Bill Macheel, Senior Program Analyst
Policy and Program Development
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road
Riverdale, MD 20737

Aldo Malavasi, Director
Medfly Rearing Facility in Brazil
Juazeiro, Bahia, Brazil

Pablo Montoya, Subdirector
Methods Development Program Moscafrut
SAGARPA-IICA
Tapachula, Chiapas, Mexico.

Pedro A. Rendon, Station Leader
Center for Plant Health Science and Technology
Animal and Plant Health Inspection Service
United States Department of Agriculture
3A. Ave. 12-62 Zona 10.
Guatemala City, Guatemala, C.A.

John Stewart, Senior Program Manager
Animal and Plant Health Inspection Service
United States Department of Agriculture
Eastern Regional Office
920 Main Campus Drive, Suite 200
Raleigh, NC 27606 USA

Cover images of Mexican fruitfly and Meditteranean fruitfly courtesy of USDA ARS Photo Unit

Table of Contents

Executive Summary

This review is a summary of the current status and performance of all APHIS fruit fly emergence and release facilities in the United States, Mexico, and Guatemala. It is based on site visits and discussions with facility staff and program managers. The review contains recommendations for each emergence and release facility (ERF). An international expert panel conducted the site visits to Sarasota, FL; Edinburg and Harlingen, TX; Los Alamitos, CA; Tijuana, Reynosa, and Tapachula, México, and Retalhuleu, Guatemala in July 2008.

The threat from exotic fruit fly (Diptera: Tephritidae) entry and establishment in the United States remains high due to a number of factors. APHIS responds to exotic fruit fly risks with an integrated system incorporating off-shore risk mitigation, surveillance, control, prevention, and regulatory activities. To prevent establishment of the Mediterranean fruit fly (Ceratitis capitata Wiedemann) and the Mexican fruit fly (Anastrepha ludens Loew), APHIS and their cooperators operate domestic and off-shore sterile insect technique (SIT) programs in high risk areas. The transport, emergence, feeding and handling of sterile fruit fly adults and their aerial distribution in the target area constitute the final steps of the SIT process. Therefore, emergence and release operations are critical to the overall success of SIT programs.

Emergence and release facilities operated by APHIS and its cooperators require the following:

- Modernization to implement new technologies, efficiencies, and worker safety.
- Standardization of operating procedures and quality control assessments.
- Periodic review by an independent international panel for quality assurance.

The goal of this review is to maintain sterile fly quality through improvements in the operational efficiency and cost-effectiveness of current fruit fly emergence and release programs. Recommendations in this review were based on quality control guidelines in Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies (FAO/IAEA/USDA 2003) and Guidance for packing, shipping, holding and release of sterile flies in area-wide fruit fly control programmes (FAO/IAEA 2007). A total of 103 recommendations were put forth by the expert panel.

1 Introduction

Fruit flies in the family Tephritidae are among the most destructive, feared and well-publicized pests of fruits and vegetables around the world. The threat from exotic fruit fly introduction and establishment in the United States remains high due to a number of factors:

- Potential for natural spread from infested areas of Mexico and Central America
- High approach rate of fruit fly host material at ports of entry
- Prevailing climatic conditions that are favorable to establishment of reproducing populations
- Availability of host fruits and vegetables

APHIS responds to exotic fruit fly risks with an integrated system incorporating off-shore risk mitigation, surveillance, control, prevention, and regulatory activities. APHIS action programs integrate the sterile insect technique (SIT) with other control methods for eradication and suppression of outbreaks of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) and the Mexican fruit fly (*Anastrepha ludens* Loew). To prevent establishment of introduced Medfly and Mexfly, APHIS and their cooperators also support domestic and off-shore Preventive Release Programs (PRP) based on continuous release of sterile fruit flies in high risk areas.

The transport, emergence, feeding, and handling of sterile fruit fly adults and their aerial distribution in the target area constitute the final steps of the SIT process. As such, emergence and release operations are critical to the overall success of SIT programs. It is essential to maintain not only the quality of pupae received from production facilities, but also to ensure the performance of adult flies derived from those pupae. The sterile fly adults must have sufficient longevity in the field to reach sexual maturity, disperse in the target area, and compete with wild males as mates of wild females. Emergence and Release Facilities (ERF) should provide environmental conditions and handling protocols that maintain the quality of the sterile pupae and adult flies within acceptable parameters. Standardized quality control assessments (FAO/IAEA/USDA 2003) are performed at production and emergence facilities as a measure of adult fly performance.

In July 2008, an expert review panel observed operations at the following eight fruit fly ERFs supported by APHIS and its cooperators in the United States, México, and Guatemala:

- Medfly Preventive Release Programs in Los Alamitos, California, and Sarasota, Florida
- Lower Rio Grande Valley Mexfly Eradication Program in Reynosa, Tamaulipas, México and Edinburg (Mission) and Harlingen, Texas
- Mexfly SIT Suppression Program in Tijuana, Baja California, México
- Moscamed (Medfly) Program in Tapachula, Chiapas, México and Retalhuleu, Retalhuleu Guatemala

The expert review panel assignments were to:

1. Assess operations at each ERF.
2. Examine quality assurance activities conducted by the fly emergence and release programs for consistency with the quality control guidelines in *Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies* (FAO/IAEA/USDA 2003) and *Guidance for Packing, Shipping, Holding and Release of Sterile Flies in Area-wide Fruit Fly Control Programmes* (FAO/IAEA 2007).
3. Present recommendations to maintain sterile fly quality through improvements in the operational efficiency and cost-effectiveness of current fruit fly emergence and release programs.

2 Program Management

Primary program management and administrative support for emergence and aerial release operations are based at the emergence and release facilities (ERF) within the United States and in México along the northern border. Within the Moscamed Program each facility has a director on-site but program managers are off-site at remote locations. The management structure at the other ERFs is based on resident co-directors or a single director. APHIS involvement with daily program management ranges from being the lead agency on-site to support of APHIS cooperators. APHIS State, regional, and headquarter staff work with cooperators to set annual program goals and budgets. In 2006, APHIS also formed the Fruit Fly Program Executive Board, as outlined in the APHIS Exotic Fruit Fly Strategic Plan, as a policy setting and coordination group within APHIS to provide overall leadership for exotic fruit fly safeguarding systems. (USDA, 2006)

Differences in program management decisions between facilities have led to the evolution of inconsistencies in operational and administrative procedures among facilities, including overall management to critical functions, such as quality assurance testing. Efficiencies can be gained in the overall fruit fly safeguarding system by implementing more closely aligned ERF activities. An avenue to achieve this goal is through enhanced communication between ERF managers and program leaders. It was also noted that, with one exception, the involvement of industry in program management is almost nonexistent. Implementing a more aggressive communication plan with industry could lead to a stronger support of the program especially during times of budget shortfalls.

General Recommendations for Program Management

1. Hold an annual open house at each ERF for key cooperators and industry. This will provide a higher visibility and likely generate additional support for SIT programs.
2. Implement consistent management practices and procedures for critical functions across all ERFs, e.g., supply procurement or quality assurance programs.
3. Consider ISO certification for all ERFs.

4. Expand current "FFSIT Quick Place" Internet site as an information dissemination tool to include standard quality control data and other critical information from all production facilities and ERFs. Data should be managed so that comparisions can be made among ERFs.
5. Form an implementation team including APHIS-International Services (IS Guatemala, IS HQ), APHIS-Plant Protection and Quarantine (Western Region, Eastern Region and Headquarters) and cooperators to develop an implementation strategy for review recommendations. This should include a conference call with ERF staffs within 60 days of receiving the final review report.
6. Hold quarterly conference calls between ERF staffs at all locations to discuss operations and quality control data.
7. Hold an annual SIT ERF operational staff meeting to review program accomplishments and needs and to allow for free exchange of ideas between ERF staffs. This should be held in conjunction with other scheduled meetings whenever possible.

Operations Management

Management of daily operations was observed at each ERF. APHIS and the appropriate cooperator co-direct operations in Los Alamitos and Sarasota. In each of these locations the co-directors are stationed on-site. APHIS directors are the only directors on-site to manage operations at Edinburg, Harlingen, Reynosa, Retalhuleu, and Tijuana. There is a single SAGARPA/SENASICA director located in Tapachula. At all locations, oversight for broad strategic and policy program decisions occurs at other locations up the chain of command. The degree of communication and oversight between ERF directors and upper management officials varies from facility to facility.

TABLE 1: Management Structure for ERFs

	Tap	Reu	Sar	Edn	Har	Rey	Tij	Los Al
Executive Oversight	UMT[1]	UMT[1]	APHIS Fruit Fly Program Board and FDACS	APHIS Fruit Fly Program Board and TDA	APHIS Fruit Fly Program Board and TDA	APHIS Fruit Fly Program Board	APHIS Fruit Fly Program Board	APHIS Fruit Fly Program Board and CDFA
Area management	SAGARPA, Mexico City	APHIS-IS Guatemala City	APHIS FL SPHD[2] and FDACS	APHIS TX SPHD and TDA	APHIS TX SPHD[2] and TDA	Absent[3]	Absent[3]	APHIS[2] and CDFA in Sacramento
ERF Direction	SENASICA director	APHIS directors	Co-directors	APHIS director	APHIS director	Absent[3]	APHIS director	Co-directors

1 The Moscamed Unified Management Team (UMT) provides overall policy direction and leadership for Tapachula and Retalhuleu. The UMT's annual work plan is funded through three separate cooperative agreements between USDA/APHIS, MAGA and SAGARPA/SENASICA. The UMT representatives from the United States (APHIS), México, and Guatemala hold formal biweekly management meetings to execute the annual UMT work plan. The APHIS International Services Program Manager in Guatemala is responsible for ensuring that APHIS program budget allocation is aligned with and utilized according to priorities of the APHIS Fruit Fly Executive Board. The UMT retains an international advisory group, the Moscamed Technical Advisory Committee (TAC), for annual program assessment and recommendations.

2 Within APHIS the State SPHD consults with the appropriate APHIS regional office when appropriate for some managerial decisions.

3 APHIS-IS officials in Mexico City are performing this function remotely until the position is filled.

Besides entities designated in **Table 1**, some facilities receive technical policy direction from committees of technical experts. The Florida Fruit Fly Committee provides leadership for the Sarasota operations. This committee meets on an 'as needed' basis. Operations in the Lower Rio Grande Valley (LRGV), including Edinburg, Harlingen, and Reynosa ERFs, are guided by a consortium of local APHIS program managers, APHIS CPHST, the Texas Department of Agriculture, USDA Agricultural Research Service, and Texas citrus industry representatives. APHIS participants at the consortium's monthly meetings may include representatives of the State Plant Health Director, Regional office, and Headquarters. Representatives of SAGARPA and the Mexican citrus industry occasionally participate. The new ERF in Reynosa also benefits from the support of experienced technical and mechanical experts in Edinburg, but this support has recently been hampered by new border crossing restrictions for APHIS personnel. Usually the Los Alamitos ERF receives operational strategies, developed in Sacramento, California through consultation between the State Plant Regulatory Official for the California Department of Food and Agriculture and the APHIS State Plant Health Director. Ad-hoc science advisory panels are convened to provide scientific guidance to the California programs.

Recommendations for Operations Management

1. Prioritize the employment of an Area Director or technical FSO in Reynosa with fruit fly technical experience.
2. Include program officials from the Méxican fruit fly SIT programs in Tamaulipas and Nuevo Leon in regular operational meetings. A regionalized approach would reduce the risk of Mexfly introductions into the LRGV program area and increase cross border support of the eradication program.
3. Schedule periodic visits by program managers from the Edinburg ERF to the Reynosa to assist in program operations and development. This is especially important to fill the interim need until the Area Director has been replaced.
4. Schedule biannual visits of upper management officials to ERF facilities to assess needs and provide oversight.
5. Streamline APHIS border crossing procedures to facilitate technical and maintenance support for the Reynosa ERF.

Resource Management

The resource management systems, in the form of budget development and financial management, are structured similarly to the operational management systems. Resources are managed under a cooperative system between APHIS and the designated cooperator, as is the case of Retalhuleu, Edinburg, Harlingen, Los Alamitos, and Tapachula; or, APHIS leads as is the case in Sarasota, Reynosa and Tijuana. Actual expenditure decisions for most items, except for major equipment purchases, are made by ERF directors. The procurement of supplies, such as diet materials, is typically made at the local level by ERF staff and not as bulk purchases to supply multiple facilities.

Primary responsibility for budget development and decisions regarding the financial management of facilities within the United States usually resides at the area management level (**Table 1**) in consultation with the ERF directors. Budget development and financial management of operations in Reynosa and Tijuana is coordinated and managed by the APHIS IS Associate Deputy for Action Programs and the IS Mexico City Regional Office.

The UMT coordinates budget planning and expenditures for Retalhuleu and Tapachula. Budget development and financial management for Retalhuleu is administered jointly by the Guatemala Ministry of Agriculture (MAGA) Moscamed Program office and the APHIS IS office in Guatemala City, Guatemala. Budget development and financial management in Tapachula is administered through the SAGARPA financial management system. The UMT retains an international advisory group, the Moscamed Technical Advisory Committee (TAC), for annual program assessment and recommendations.

Recommendation for Resource Management

1. Explore the joint purchase of renewed supplies, for example diet materials, for multiple facilities to take advantage of cost savings by purchasing in volume.
2. Determine product technical specifications and establish a minimum of three (3) suppliers for each product.

Program Planning for Sterile Release

SIT is used for the eradication or suppression of fruit flies either in historically infested areas, or as an eradicative tool to eliminate transient populations resulting from an incursion of exotic fruit flies into a free area. SIT is also used as a preventive tool in preventive release programs (PRP) to inhibit the establishment of exotic fruit fly populations in free areas. SIT parameters, such as size of release area and release rate, can vary depending upon the nature of the program. Surveillance records and the nature of the target fruit fly population are critical criteria in determining the scope of the release zone. The criteria used for the designation of release areas and release rates for continual or long-term programs were inconsistent among the program areas observed. The cost efficiency of continual release of SIT in zones that have a single historical incursion of the target fruit fly is questionable, e.g., SIT over. For example, SIT over the SaraMana area (FL) or portions of the Los Angeles Basin which were originally an extension of the PRP to eradicate one incursion of Medfly. Release rate criteria used for the various PRP were also inconsistent.

The PRP perimeter of release areas are based on historical records. These records indicate numerous Medfly detections in the Miami area and a single large incursion in the Tampa Bay area with satellite detections occurring in the Sarasota/Manatee area. Release rates in all the release areas serviced by the Sarasota ERF have been a consistent at 125,000 sterile male Medfly per square mile[1] per wk.

The Lower Rio Grande Valley Mexfly Eradication Program is regional in scope. The Edinburg, Harlingen and Reynosa ERFs coordinate program activities. Sterile Mexfly from Edinburg and Harlingen are released in the two infested Texas counties. SIT functions as not only an eradication tool, but also as a suppression tool in a systems approach. This systems approach is essential for phytosanitary certification of citrus exported from regulated areas. Sterile Mexfly

1 48,100 Medfly per square km

from Reynosa are released south of the Rio Grande over a 25 mile (40 km) wide band that extends through ten 10 municipalities in the State of Tamaulipas. SIT in this generally-infested area is a critical part of the regional eradication effort. The release rate of Mexfly on the Texas side of the LRGV is approximately 160,000 sterile Mexfly (male and female) per square mile[1] per wk for the two counties under eradication.

Sterile Medfly from Los Alamitos is released in a PRP that covers approximately 2,500 square mile[2] of the Los Angeles Basin (CA). The PRP was established in the Los Angeles Basin as a management tool following a series of costly eradication programs. The boundaries of the PRP are based on a long historical record of Medfly detections. The Los Alamitos ERF has the capacity to process Medfly and Mexfly to meet the needs of eradication programs throughout California. The release rates for the PRP fall into two categories: in high risk areas the release rate is 125,000 sterile male Medfly per square mile[3] per wk and 62,500 sterile male Medfly per square mile[4] per wk in other lower risk areas.

Moscamed program managers use detection data integrated into a GIS system to identify SIT target zones within generally-infested and outbreak areas under the scope of the Medfly barrier program. Sterile Medfly from Tapachula and Retalhuleu are released in those zones at the designated densities.

SIT is used to suppress Mexfly populations that enter Tijuana from generally-infested areas of Mexico and acts as a PRP to protect California. The Tijuana ERF has processed sterile Mexfly since 1964 and has the capacity to process Medfly for emergency eradication programs, e.g., during the 2004 Medfly quarantine in Tijuana. The eradication release rate used in California for a Mexfly incursion is 250,000 sterile Mexfly (male and female) per square mile[5] per wk.

Recommendation for Program Planning for Sterile Release

1. Review the designation of release areas and release densities for the all program areas based upon levels of risk.

1 61,540 Mexfly per square km
2 6475 km
3 48,100 per square km
4 24,100 per square km
5 96,150 Mexfly per square km

Emergency Planning

Emergency planning should address two scenarios: continuity of operations and expansion of program scope. Continuity of operations planning considers how to maintain daily operations in the event of a natural disaster, mechanical failure, or other occurrence that has the potential to interrupt normal operations. Expansion of program scope planning considers logistical and resource management to support an emergency response to fruit fly outbreaks. The review team found that the emphasis on emergency planning varied greatly among all the ERFs. Overall there was a general need for more emphasis on a consistent approach to emergency planning.

Sarasota is procuring an emergency generator with the capacity to provide power during frequent outages. This is critical because HVAC and emergence tower fans must cool the Medfly adults housed in the towers or they will die within hours. The ERF has general supplies and space for emergence and knockdown adequate to support an emergency program.

The Reynosa ERF has purchased an emergency generator. However, it has not been installed because funds are unavailable. The sterile Mexfly released from the ERF are used to suppress populations in infested areas of Mexico. At the present time, there are no contingency plans for emergency response to outbreaks.

The Edinburg and Harlingen facilities function as a single unit for emergency contingencies to accommodate expansion of program scope. Both facilities have emergency generators and emergency operation plans. Edinburg is at capacity for emergence, but Harlingen has additional emergence space as a back-up for Edinburg operations and emergency program activities. An evaluation of continued operations in the event of a natural disaster is needed.

The Los Alamitos ERF has a long history of supporting Medfly and Mexfly emergency programs in California and northern Mexico. This ERF has the capacity to process additional flies for emergency programs, if necessary. Los Alamitos has a new emergency generator to maintain operations in the event of power outages.

Operations at the Retalhuleu and Tapachula ERFs are frequently impacted by failures in the electrical supply. Continuity of operations is assured through two emergency generators at Retalhuleu. The plans for the construction of a new Tapachula ERF include an emergency generator. Retalhuleu and Tapachula function under the operational plans that include rapid response to fruit fly outbreaks.

The Tijuana ERF is currently being operated at half capacity. Therefore it is available for emergency response to both Medfly and Mexfly outbreaks. There are adequate refrigerated containers for knockdown and emergence equipment in the form of PARC boxes.

Recommendation for Emergency Planning

1. Conduct a review of emergency preparedness plans for all ERFs for continuity of operations during natural disasters and for emergency action programs.

Scientific Support

SIT is a genetic control strategy whose application to tephritid fruit flies requires continuous monitoring and improvement. Scientific support is integral to improving the efficiency and effectiveness of SIT operations. This is especially true for solving problems of scientific nature and general troubleshooting. The availability of scientific support for the ERFs ranged from essentially no assistance to extensive local resources. It is important to note that scientific advances and troubleshooting at one location can be, and often is, shared with other SIT programs. Except in a few cases it was noted that direct scientific support was lacking for the ERFs and that there should be more networking between ERFs to take advantage of technical advances achieved at individual ERFs.

The Sarasota ERF has been supported by an APHIS scientist in Gainesville, FL. The CPHST scientist worked with two biological technicians hired by operational program on-site at the Sarasota ERF to conduct methods development investigations as required. This scientist retired from APHIS in January 2009. Succession planning for this position is unknown.

Operations at the Edinburg and Harlingen ERF are supported by APHIS CPHST and ARS laboratories located in Edinburg and Weslaco, TX.

There is no scientific support staff at the Los Alamitos ERF. Methods development concerns are currently handled by operational personnel at the ERF with infrequent support from APHIS CPHST.

The Retalhuleu and Tapachula ERFs have strong methods development support groups. Tapachula is supported by scientists from the nearby SAGARPA Moscafrut production facility. Retalhuleu has excellent support from the CPHST laboratory based in Guatemala City and El Piño, Guatemala.

Reynosa and Tijuana APHIS ERFs in México have little scientific support. The Reynosa ERF has had some support from the APHIS scientists in Texas, but this has become difficult due to border crossing issues.

Recommendation for Scientific Support

1. Ensure that provisions are made for regular CPHST or other scientific support for all ERFs with a preference for permanent on-site or near on-site support at larger facilities, e.g., Los Alamitos and Sarasota.

Facility and Equipment Maintenance

The availability of ERF staff to conduct maintenance functions varies from location to location. Some ERFs have no such support, while a few have a strong local support to address facility and equipment needs.

The Sarasota ERF has one full-time maintenance staff. There is access to a small machine shop which has been adequate to meet facility needs.

The Reynosa and Tijuana ERFs use private contractors to perform all maintenance activities. Reynosa has infrequent support from APHIS maintenance staff. Difficulties in crossing the international border have prevented this from occurring on a more regular basis.

Edinburg ERF has excellent support from on-site machine shop and knowledgeable maintenance staff. The Harlingen ERF is supported by the Edinburg staff that travel to that location as required.

Los Alamitos is supported by ten full-time, on-site maintenance staff.

Retalhuleu ERF is supported by an on-site machine shop and maintenance staff. The machine shop staff in Guatemala City provides additional support, as required.

The Tapachula ERF also uses private contractors to perform all maintenance activities.

Recommendations for Facility and Equipment Maintenance

1. At the Los Alamitos ERF, improve operational efficiencies through replacement of the trailers with more permanent modular structures that are more cost-effective to operate and maintain. The long-term plan could focus on a modular facility with one large overhanging roof similar to what is currently in Retalhuleu. This new modular facility could be constructed

gradually in stages with minimal disruption to operations. The most critical need is a large chill room for adult fly knockdown. Additional modules could be built year by year or as the budgets allow. Alternatively, consider other locations with better buildings and infrastructure within close proximity of the Los Alamitos airstrip.

2. Retalhuleu needs to designate an appropriate area to conduct workshop/maintenance activities.

3 Facility Operations and Process Flow

Facility Operations

Buildings

The Sarasota ERF opened in 2002. This ERF is presently in very good condition and offers sufficient space and utilities to process the required number of Medfly. In addition, there is space and equipment to accommodate other fruit fly species if necessary. The building houses administrative offices, a quality control laboratory, diet preparation, pupae loading, tower hold, two chill rooms, a small warehouse, and a large room for tray wash and release box loading. The building under lease to APHIS was originally designed as a dairy processing plant and required minimal renovations to meet the needs of program activities. The utilities in the building appear to be in proper working order. The only observed drawback is the lack of insulation in the tower holding room, making it difficult to maintain the temperature at 74 °F (23 °C). Lack of insulation may reduce the energy efficiency of the air conditioning system. The decision to insulate the room in a leased facility should be based on a cost/benefit analysis.

The ERF in Reynosa, México began operation in May 2005. It is staffed entirely by USDA APHIS personnel. SAGARPA México provided the original building which was totally refurbished by APHIS to meet program needs for fly emergence and trapping activities. There are some minor problems in the building structure that require attention, including: 1) the water drainage system; 2) environmental controls to maintain the required temperature and humidity inside the fly emergence room; 3) required improvements to the water supply of the fly emergence room; and 4) operation and maintenance of the air conditioning system.

The Edinburg ERF is on APHIS-owned space in Edinburg, Texas and the Harlingen ERF is in leased space in nearby Harlingen, Texas. The Edinburg ERF is located in the same complex as the Mexican Fruit Fly Rearing Facility and the Aircraft and Equipment Operations. Adult fly emergence activities are conducted in rooms adjacent to the production facility. The Mexfly production facility operates at its maximum capacity of 180 million Mexfly pupae per week. The 22 year-old buildings require continuous repair and maintenance.

Contingency resources are needed to address unanticipated issues that impact both production and fly emergence activities in the building. In the Harlingen ERF the space was customized for the full range of adult fly emergence activities.

The Los Alamitos ERF operates out of customized trailers. All activities for processing and holding pupae, fruit fly identification (ID), adult fly food preparation, quality control (QC), and administration are conducted in a complex of 43 modified 40-foot trailers. New trailers were added to the complex as the PRP activities expanded, leading to a situation wherein activities are not conducted in the most efficient manner. Materials are transported large distances from one trailer to another, which wastes both time and resources. Further, the complex has different elevations connected by multiple stairways and narrow aisles. This negatively impacts program operations and raises safety concerns. The structure of the trailers and the environmental controls for critical components, e.g., the chill rooms, requires that staff report very early in the morning to turn the equipment on so that the required temperature is reached by 7 A.M. Program managers continue to develop creative solutions that allow the operation to meet program demands. It is highly recommended that program managers re-examine the feasibility of establishing a permanent, more cost efficient ERF for this critical program.

The Tijuana ERF supports APHIS emergence and release operations as well as the APHIS field workers and inspectors working at the Tijuana Airport. Operations are conducted in ten (10) old trailers located on leased property near the Tijuana Airport. These trailers were not designed to accommodate EFR activities that support Medfly and Mexfly PRP and eradication programs. The units are contaminated with mold, are in poor working order, and have precarious installation. Among environmental concerns is the amount of dust in the air and the temperature and humidity controls. It is recommended that risk and cost/benefit analyses be conducted to determine the nature and extent of improvements required for efficient operation of this ERF. It is understood that improvements to the facility must be agreed to by the property owner.

The Tapachula ERF is on space rented by SAGARPA. The facility is located 25 km (15.5 mi) from the production plant. The ERF uses a pre-existing warehouse that has been adapted for emergence and release operations. There are problems in room insulation, space limitations, and poor process flow. Space outside of the main building houses the quality control section and two cold rooms and one thermo King Cab that are used to chill sterile insects and load release boxes. These buildings require continuous repair and maintenance.

The Retalhuleu ERF was constructed in 1999 on land provided by the Guatemalan Government as part of their contribution to the Moscamed Program. This ERF is in very good condition and offers sufficient space and utilities to process the required number of Medfly. The building houses administrative offices, quality control laboratory, diet preparation, pupae loading, six chill rooms, and a small storage area. Tray washing is conducted under a roof and in close proximity to an open drying area. The HVAC system for the building is relatively new and operating well. Utilities in the building are in proper working order.

Recommendations for Buildings

1. Sanitation protocols should be implemented to minimize microbial development. A bacterial or fungal infestation can be very difficult to eliminate once established. Care should be exercised to not only maintain a high level of cleanliness, but also for periodic inspection of equipment.
2. Identify microbial contaminants and target sanitation protocols for these organisms.
3. Redesign and install HVAC systems with the proper temperature and humidity controls. Humidity in the holding rooms should be at 60-70% RH.
4. Spray foam insulation should be added to emergence room ceilings as needed. The initial cost will be offset by lowered overall utility costs.
5. Install emergency generators where needed.
6. Remove unused equipment from work areas. This represents both a hazard and, in the case of large equipment, acts as a heat sink compromising the HVAC systems.
7. Provide adequate warehouse space to avoid clutter and maintain product and equipment quality.
8. Adjustments should be made so that all horizontal surfaces are level. Uneven access ramps and walkways result in tilting of towers to access various locations and puts excessive wear on the wheels and frame attachment points.
9. Review the safety and health procedures in all ERFs.

Facility Location

The Sarasota ERF matches the most important requirement for an SIT ERF. That is, close proximity to the airport where the irradiated pupae arrive and where the chilled adult flies are loaded into airplanes for aerial release. The distance from the facility to the airport is only 2.7 miles (4.3 km), a 4 to 6 minute drive.

The Reynosa ERF is conveniently located 3.7 miles (5.9 km) and a 10 minute drive from the Reynosa Airport, point of departure for aerial release aircraft. In terms of the geographical location, the ERF is directly south of the SIT target areas in Texas.

The Edinburg ERF is located on Moore Air Base. This is quite convenient because of the close proximity, a few hundred feet, to the aircraft operations. The ERF in Harlingen, Texas is adjacent to the Harlingen Airport from which the aircraft departs for aerial releases.

The ERF in Los Alamitos, CA is ideally located to service the Medfly PRP and emergency programs. It is located in the southeast area of the LA basin within an army complex. The adjacent airstrip serves as the departure point of sterile fly release aircraft. The ferry time ranges from 5 and 45 minutes.

The Tijuana ERF is located in the northern part Tijuana, México. It is 13 km (8 mi) from the Tijuana Airport, approximately a 15 minute drive. The SIT release blocks are within the city limits.

The Tapachula ERF is located 9.3 mi (15 km) from the airstrip. Flies are loaded into a release box and taken directly to the airplane. The travel time is approximately 10 minutes. The ferry times to the release blocks are variable. For instance, ferry time to release blocks in the Lagos de Montebello zone is approximately 1 h, but ferry time to the Tacaná volcano zone only 15 to 20 minutes.

The Retalhuleu ERF is located approximately 20 km (12 mi) from the airstrip. Using a refrigerated vehicle flies are delivered in about 25 minutes. Ferry time to the release areas is variable depending on the location, in general round trips are of: 1.) one hour to the most distant blocks in area 1; 2.) 85 minutes to the most distant block in area 2; and 3.) 2 hours to the most distant block in area 3.

Recommendation for Facility Location

1. Implement production of Mexfly in San Miguel Petapa, Guatemala because Edinburg is at full capacity. The release densities could be increased if additional sterile flies are available.

Pupae Supply and Arrival

The El Piño Guatemala Medfly production facility is the only source of Medfly pupae for the Florida PRP. The boxes of sterile pupae from Guatemala City typically arrive four days per week, Monday to Thursday. The boxes leave El Piño at approximately 3 A.M. for delivery to Delta Airlines at the Guatemala City La Aurora Airport before 7 A.M. The Delta flight departs Guatemala City at 12:00 noon for arrival into

Atlanta in the late afternoon. The boxes are held overnight in Atlanta and arrive at Sarasota Bradenton International Airport early the next morning. The boxes are picked up at the airport by APHIS staff and delivered to the ERF between 6 and 7:00 A.M. The average transportation time from El Pino to the ERF in Sarasota is 27 hours. Alternative routes with Continental airlines through Houston or with American airlines through Miami do not appear feasible or would not decrease the total transport time. Any change of the flight schedule would not significantly cut the number of hours in hypoxia. In addition, the present route fits well with the overall work flow and staffing by personnel working from 6:00 A.M and 2:30 P.M.

Mexican fruit fly pupae processed at the Reynosa ERF are from the APHIS Mexfly rearing facility in Edinburg, Texas. Approximately 5.5 million pupae are shipped on each of four days, for a total of 20 million sterile pupae per week. Boxes of sterile pupae are transported by ground and require between one and three hours to reach Reynosa. The drive time from Edinburg to the Mexico border is approximately 35 minutes and from the border to the Reynosa ERF is 10 minutes. Therefore, the total transport time is primarily dependent upon the customs procedures at the border crossing.

Mexican fruit fly released from the Moore Air Base aircraft are from the on-site Edinburg production facility. This rearing facility also provides the sterile pupae to the Harlingen ERF. The distance between Edinburg and Harlingen is 45 miles (72 km) and is approximately 60 minutes travel time.

The boxes of sterile Medfly pupae arrive at LAX from Guatemala and Hawaii seven days a week. The total transport time from point of origin to the ERF is approximately 26 and 23 hours, respectively. This includes a approximately 50 minute drive from LAX to Los Alamitos. Given the large number of flights into LAX from both La Aurora Airport in Guatemala City and Honolulu, it may be possible to reduce the total transport time. The sterile Mexfly pupae that are processed at Los Alamitos for eradication programs in southern California are received from the Metapa Moscafrut facility in Tapachula, Mexico.

The Tijuana ERF receives sterile Mexfly pupae from the Moscafrut facility in Metapa, Tapachula. Pupae boxes are delivered every Tuesday and Wednesday evening and cleared from the Tijuana Airport the following morning. Eight million pupae are processed early each Wednesday and Thursday morning. The total transport time between the Moscafrut facility and the ERF in Tijuana is approximately 27 hours. There is only one daily direct flight from Tapachula to Tijuana and this flight is not reliable.

The Retalhuleu ERF is located 187 km (116 mi) from Guatemala City. The ERF receives sterile pupae produced at El Pino rearing facility. The boxes of sterile pupae from El Pino arrive seven days per week, 52 weeks of the year. The boxes leave El Pino at 9 A.M. and are delivered within 4 hours.

Recommendation for Pupae Supply and Arrival

1. Review the availability of flights from Guatemala to Sarasota and from Honolulu and Guatemala to LAX in order to reduce transportation time.

Pupae Processing

A 4-person team processes the sterile Medfly pupae within a short time after their delivery to the Sarasota ERF. The pupae are loaded into the fly emergence tower trays with specialized equipment designed at the Edinburg ERF. The team loads an entire tower of 50 trays in approximately 5-6 minutes. One tower holds approximately 1.5 million Medfly pupae held in 50 trays with 25,000 pupae per tray. A total of 21 to 22 towers are prepared daily. The process is clean, efficient and smooth.

The temperature of Mexfly pupae is 77 to 80.6 °F (25 to 27 °C) upon arrival at the Reynosa ERF between 9:00 and 10:00 A.M. The boxes are opened and 4.5 kg (9.9 lbs) aliquots are placed in holding trays. Trays are held at 68 °F (20 °C) for 1 to 1.5 hours in order to cool the pupae. After the cooling process, a 3-person team loads the pupae into the tower trays using the same equipment developed at the Edinburg ERF. The number of pupae per tray is 10,300 to 10,400. At a 95% emergence rate, the yield per tray is approximately 10,000 Mexfly adults (male and female) per tray. With 70 trays per tower, adult yield per tower is approximately 700,000. Empty boxes are returned to the Edinburg Mexican fruit fly rearing facility for reuse. Mexfly pupae are processed year-round at the Reynosa ERF which operates daily from 6:30 A.M. to 3:00 P.M.

The Edinburg ERF processes 20 to 25 million pupae per day. A 3-person team takes six (6) seconds to load pupae into each tower tray using the device originally developed at Edinburg. Each tray contains 12,500 pupae, therefore the 80 tray tower contains 1,000,000 pupae. The emergence room can hold up to 164 towers which is sufficient for the present operation.

The Harlingen ERF receives 12 to 15 million Mexfly pupae per day from Edinburg. A 3-person team loads the trays at a rate of one every 6 seconds using the same Edinburg pupae loader. Each tray contains 12,500 pupae, therefore the 70 tray tower contains 875,000 pupae. The emergence rooms have a capacity of 191 towers.

The Los Alamitos ERF processes 325 million sterile Medfly pupae per wk using fly emergence towers. Upon arrival the Medfly pupae are processed by a 5-person team. Pupae are loaded into the emergence towers using two pupae dispensers at a rate of one tower every 6 to 10 minutes. Each tower has 56 trays and each tray holds 300 ml of pupae. The number of trays per tower is restricted to 56 because of the height limit of the trailers. Overall, the process is clean, efficient, and runs smoothly. The Los Alamitos ERF processes sterile Mexfly pupae for emergency programs. Plastic adult release containers (PARC) boxes are used for Mexfly emergence.

A total of 16 million sterile Mexfly pupae are processed per wk at the Tijuana ERF by a 4-person team. PARC boxes are used for Mexfly emergence. Each PARC box is loaded with six paper bags of pupae, 30,000 pupae per bag. Each shipment of 8 million pupae requires approximately 250 PARC boxes.

The Retalhuleu ERF processes 900 million pupae per wk in PARC boxes. PARC boxes hold 6 paper bags each with 7,500 pupae per bag. That is a total of 45,000 pupae per PARC box. The fly density inside PARC boxes should be reduced to allow for improved quality of released insects. The males may need to be held an additional day in order to improve insect sexual maturity prior to release.

The Tapachula ERF processes 530 to 550 million of Medfly pupae per wk. PARC boxes and Guatemala-style emergence towers, with 25,000 pupae per tray, are used for the emergence process.

Recommendation for Pupae Processing

1. Remove pupae from plastic bags immediately upon receipt. Place pupae into fiberglass trays and hold them in a cool room until towers or PARC boxes are loaded.
2. Standardize the use of best practices in handling of pupae by designating equipment type; uniform densities (adults per unit of surface area); food type; holding temperature and RH; and semiochemical and hormone treatments.
3. Evaluate the impact of the length of time pupae are held under hypoxia on field competitiveness of the sterile males.

Towers and Fly Emergence Room

The emergence room at the Sarasota ERF is approximately 30 ft x 100 ft. (9m x 30.5m) and holds a maximum of 140 towers. Currently 84 to 88 towers are being used to process 100 million Medfly pupae per week. Towers are held in the emergence room for 5 to 7 days. Two days are required for adult fly emergence and an additional 3 to 5 days for the males to reach sexual maturity. At the end of this period, the towers are transferred to the chilled room for knocking down the adult flies. Clear separation is maintained between the towers for each week day.

The Sarasota ERF emergence room has a dehumidification system to maintain 62 to 64% RH and 72 to 74 °F (22.2 to 23.3 °C). The ambient humidity is typically at 70%, but may be higher depending on the season of the year. Controlling the humidity within the room and tower trays is critical in order to prevent clumping of the adult flies during the subsequent chilling process. The adult flies produce metabolic heat which must be dissipated. Each tower is equipped with a small, but powerful fan on the top that exhausts the heat from inside the tower. The towers and attached cooling fans are in good working order.

While being held in the Sarasota ERF emergence room, Medfly males are exposed to ginger root oil (GRO) to enhance mating competitiveness. The treatment is accomplished by placing 1 ml of ginger oil on a cotton wick which is held in a Petri dish below each tower. The tower fan pulls air through the tower, thereby moving the ginger oil aroma past the adult flies in the tower.

The Reynosa ERF emergence room has a capacity of 60 towers. The current occupancy is approximately 28 towers per week. The towers are held in the emergence room for 4 to 6 days. Up to two days are required for adult fly emergence and Mexfly adults are 2 to 4 days old when released. Environmental conditions are set for 78.8 °F (26 °C) and 70 to 80% RH. During the visit the temperature was 82.2 °F (28 °C), indicating a possible problem with the air conditioning system. Each tower holds 70 trays, making the overall height of the tower approximately 6.5 feet (2 m). This made it difficult to monitor function of the cooling fans by visual inspection. There were many Mexfly adults observed flying about the emergence room. Sticky traps were deployed to capture adult flies that escape from the tower trays.

The Edinburg ERF emergence room has a capacity of 164 towers and Harlingen 191 towers. This is sufficient for current operations. The towers are kept in the emergence room for 5 to 7 days at which time the adult flies are processed for release. At the time of release, males are 4 to 6 days old.

Each Los Alamitos ERF emergence room is a 40-foot (12m) remodeled trailer that holds 36 towers of 56 trays each. Environmental conditions are set at 78 °F (25.5 °C) and 65% RH; however the actual conditions were 75 to 79 °F (24 to 26 °C) and 62 to 68% RH. This indicates a possible problem with the environmental control systems. Although the ambient environment in California is dry, a dehumidifier is required in the emergence trailers to prevent clumping of the adult flies in the towers and in subsequent chilling procedures. Medfly pupae from Hawaii and Guatemala remain separate throughout the fly Los Alamitos emergence process. Towers containing Hawaii pupae are marked with red tape and those from Guatemala are marked with blue tape. Mexfly pupae in PARC boxes are held in trailers separate from the Medfly towers.

At Los Alamitos Medfly males are exposed to ginger root oil for a 24-hour period prior to knockdown. This treatment is to enhance mating competitiveness. The treatment is accomplished by placing 1 ml of ginger oil on a cotton wick which is held in a Petri dish below each tower. The tower fan pulls air through the tower, thereby moving the ginger oil aroma past the adult flies in the tower.

The Tijuana ERF emergence rooms consist of two old trailers modified to hold 250 PARC boxes each. The temperature is kept between 75 to 80 °F (24 to 26.7 °C) and 65 to 70% RH. The PARC boxes are held in the emergence room for 5 days and then transferred to a refrigerated container to knockdown the adult flies. Up to two days are required for adult fly emergence and Mexfly adults are 3 days old when released.

The Tapachula ERF has two fly emergence rooms with a total capacity of 500 million per week.

The Retalhuleu ERF has 3 rooms of approximately 4,000 square feet (372 m^2) and an additional room of approximately 3,000 square feet (279 m^2). The average temperatures inside the emergence rooms are 73 °F (23 °C) with a range of 70 to 75 °F (21 to 24 °C) and 65 to 70% RH. PARC boxes and emergence towers are held in the emergence rooms for 5 to 6 days. Emergence takes place within 70 hours and flies are released when they are 3 to 4 days old. Accurate and reliable HVAC and humidity control systems are critical to providing high quality sterile insect to operational programs. Priority must be given to evaluation, installation, and maintenance of an adequate temperature and humidity system in critical areas such as the emergence room.

Back up emergency power is a critical element of any fly emergence system. At any point in time, tens of millions of flies could be at risk if there is a power outage. Budgets should set aside sufficient funds to install and maintain backup power.

The fans on top of the fly emergence towers are a critical component without which temperature and humidity within the towers increase due to metabolic activity of the insects. These fans will maintain a constant wattage draw with amperage varying inversely with voltage variations. Although higher voltage results in lower amperage with little impact on the fans, low voltages will result in a higher amperage draw which will burn out the fans. Sites with fan failure should monitor their voltage or install a line conditioner or both to avoid fan loss and resulting mortality in the affected tower.

There were observed variations in the maintenance of the fly emergence tower system depending on location. These variations included both modification to the frames and replacement of the material used for spacers. Beneficial modifications should be shared among all sites, especially with the construction shop at Moore Air Base, so that there is a standardization of the towers. This will have significance during emergencies when it may be necessary to share equipment between sites.

The recommendation is made to limit the tower height to 50 trays. Although this may reduce the number of flies produced per tower, this height ensures that all employees can verify the operation of the fans. This can be accomplished by passing one's hand over the fan to feel for air movement, or a small ribbon can be attached to the fan which will remain vertical as long as the fan is in operation. This is an easily introduced modification given each fan failure could result in the loss of about 1 million flies depending on the species.

In situations where humidity is high, spacers constructed of water absorbing material can result in higher than acceptable levels of humidity within the tower or require excessive times or specialized equipment to adequately dry. All new construction and all repairs should incorporate non-water absorbing material.

Recommendations for Towers and Fly Emergence Room

1. Implement tower system at all ERFs.
2. Limit tower height to 50 trays.
3. Carefully control the humidity in emergence rooms at 60-70% RH.
4. Standardize construction of aluminum tower trays so that parts used at ERFs are interchangeable.
5. Construct tray spacers of non-absorbent materials.

Chilling flies and Loading into Release Boxes

At the Sarasota and Los Alamitos ERF the towers are moved into the chilled rooms and held at 38 °F (3.3 °C) for 30 minutes for the knockdown process. After knockdown the flies are transferred from the tower trays to the release box. A 5 or 7-person team can process two towers simultaneously in 6 to 8 minutes. This is a highly coordinated procedure that includes vacuuming of the pupae cases from the tray well, followed by dumping of the adult flies from the tray into a hopper. It is necessary to hit the tray quite hard in order to dislodge all adult flies from the tray. This process can result in damage to the trays, thereby decreasing their operational use. The process is quite noisy and can be messy as well. It is recommended that an alternative knockdown system be considered.

For loading of mature adult flies into the release boxes in the Reynosa ERF, the towers are moved into the chill room and held at 32 to 39.2 °F (0 to 4 °C) for 20 to 25 minutes for the knockdown process. A 4-person team uses the same process as described for the Sarasota ERF to process the chilled adult flies. Each tower requires approximately 15 minutes for transfer to the release box. Pupae and adult holding trays can suffer from the same damage described above.

For loading of mature adult flies into the release boxes at the Edinburg and Harlingen ERFs, the towers are moved into the chill room and held at 32 to 39.2 °F (0 to 4 °C) for 20 to 25 minutes for the knockdown process. A 4-person team uses the same process as described for the Sarasota ERF to process the chilled adult flies.

For loading of the mature adult flies into release boxes at the Tijuana ERF, the PARC boxes are held in a refrigerated container for 45 to 60 minutes. Each release box holds approximately 2 million adult Mexfly.

The Tapachula ERF uses two emergence systems, PARC boxes and emergence towers. The adult flies are chilled at 5 to 2 °C (35.6 to 41 °F) in cold rooms for one hour. Approximately 85 million flies are processed per day.

The Retalhuleu ERF is operating with both PARC boxes and emergence towers. Adult Medflies are chilled by moving the emergence containers into a cold room maintained at 40 °F (4.4 °C) and held for approximately 40 to 60 minutes for knockdown and loading into release boxes. Approximately 130 million pupae are processed daily.

Recommendations for Chilling and Loading Flies Into Release Boxes

1. Upgrade chilling systems of insufficient capacity to reduce knockdown times. Long knockdown times can negatively affect the quality of the adult flies, e.g., pheromone production in *Anastrepha* males.
2. Replace knockdown hopper metal bars with tygon-covered bars to reduce damage to aluminum tower trays.

Transportation to the Airport

The release boxes used at the Sarasota ERF can hold 7 to 8 million chilled Medfly. Release boxes are typically loaded to 75% capacity. Immediately following loading, the release box is weighted and loaded onto a truck that does not have insulation or a chilling system. The process is well coordinated and the transfer time from the chill room to the airplane takes no more than 10 to 15 minutes, including the 4 to 5 minute drive time to the airport.

One release box at the Reynosa ERF can hold 2.5 million Mexfly (males and females). The first release box is loaded onto the truck, with no cooling system, between 8 and 8:30 A.M., and the second between 10 and 10:30 A.M. The distance from the ERF to the airstrip is 3.7 (6 km) miles and takes approximately 6 minutes. Within 15 minutes of leaving the ERF, the release box is loaded onto the Cessna 206 for aerial releases.

The Edinburg and Harlingen ERFs are in close proximity of their respective airstrips. Transport time is 3 to 5 minutes, respectively. Edinburg conducts 8 to 10 flights per day and Harlingen conducts 2 per day.

The release box used in the Los Alamitos ERF has a capacity of 7 to 8 million Medfly males. The release box is transported to the airstrip in a garden vehicle and reaches the aircraft within 3 to 4 minutes. Empty release boxes, if present in the aircraft, are removed and the full box loaded within 10 to 15 minutes.

The release boxes in Tijuana are transported to the airport in a truck with an air conditioning system. Transport time to the Tijuana airport is approximately 15 minutes. Releases occur on Tuesdays and Wednesdays of each week. Three loads of Mexfly per day are sent to the airport each release day.

The Tapachula ERF uses a band release system provided by the contracted company. This system carries boxes that have a loading capacity of 15 to 20 million Medfly males.

The Retalhuleu ERF uses two types of release machines. The loading capacity of each release machine is approximately 60 million Medfly males. Each machine has 4 boxes with a loading capacity of 15 million Medfly per box. Boxes are loaded into a refrigerated truck and transported to the release strip within 25 minutes. Releases are conducted daily at designated areas.

Fly Release

The Sarasota ERF processes sterile Medfly for releases in Miami, the Tampa area, and Hillsborough County. The ferry time to Miami is approximately 75 minutes and to Tampa and Hillsborough County approximately 30 to 35 minutes. The release time is two hours in Miami and one hour in Tampa and Hillsborough County.

Sterile Mexfly releases from the Reynosa ERF are directed primarily over urban areas located just north of the Reynosa Airport. There is no ferry time. Mexfly are released during morning flights of ca. 1 to 1.5 hr. There are two flights per day, 4 days per week. Each flight releases 2.5 million Mexfly per flight for a total of 20 million per week (2 x 4 x 2.5 =

20 million). The release density is 100,000 flies per square mile (38,500 per square km) which is equivalent to 50,000 males per square mile (19,300 per square km).

Mexfly in the U.S. are released from two sites, Edinburg and Harlingen. Edinburg releases are conducted by APHIS staff and Harlingen releases are conducted under contract by Gulf Aviation. Each release box has a capacity of 2 million Mexfly (male and female). Edinburg flights have one release box loaded with 2 million Mexfly plus 0.5 million loaded on the screw augers, for a total of 2.5 million Mexfly per flight. Harlingen flights carry 2 release boxes each loaded with 2 million Mexfly plus 0.5 million on the screw auger, for a total of 5 million per flight. The two ERFs are well located relative to the Mexfly release areas in Texas so that the ferry time is short.

Sterile Medfly males from the Los Alamitos ERF are released 7 days per week over a 2,500 square mile (6,500 square km) area in the Los Angeles Basin. Flights total 15,000 miles (24,140 km) per week at average altitude of 2,000 feet (610 m). The ferry time between the Los Alamitos airstrip is 5 to 45 minutes, depending on the release run. Each Beachcraft plane conducts two releases per day, one mid-morning and one late-morning. Los Alamitos ERF can service releases to the majority of sites within California.

The sterile Mexfly from the Tijuana ERF are released two days per week via a Cessna 206. The aircraft conducts three flights per day, with departures at 9:30 A.M., 11:00 A.M., and 2:00 P.M. Flight duration is 1.3 hours for a total flight time of 3.9 hours per day or approximately 8 hours per week. The release area is 106 square miles (274.5 square km) at a release rate of 120,000 Mexfly (males and females) per square mile (46,150 per km). The Tijuana Airport has over 300 landing and takeoff operations per day, making it difficult for the release aircraft to obtain flight clearances. The current release flight schedule is based on available windows in the runway and flight patterns. Flies are released over the Tijuana area so that there is essentially no ferry time for the aerial release flights.

Releases are made daily from the Tapachula ERF. Release blocks vary according to the general program strategy. Ferry time to the release area is variable, ranging from 20 to 60 minutes.

The Retalhuleu ERF has a 7-day release schedule, with three releases daily. The ERF uses three LET aircrafts, two with a release machine based on a CO_2 cooling system and an additional unit that carries a compressor system. Releases are conducted in several pre-designated blocks according to the general program strategy. Ferry time to the release areas is variable, but in general round trips are: 1) 60 minutes to the most distant blocks in Area 1; 2) 85 minutes to the most distant block in Area 2; and 3) 120 minutes to the most distant block in Area

3. Release densities are dependent upon the current Medfly population density in the target Area, ranging from 1,500 to 3,000 flies per ha (600 to 1200 per acre).

Although twin engine aircraft give the appearance of increased security, single engine aircraft operate daily without any observed disparity of failure. FAA does not require the use of twin engine aircraft. Unless local authorities require the use of twin aircraft, single engine aircraft should be used for release. Aircraft time is the single most expensive line item in a release budget. Use of twin engine aircraft where a single engine would suffice is a needless waste of limited resources. All aircraft operations should include both single and twin engine aircraft in contracting processes. If local authorities insist on the use of twin engine aircraft where a single engine aircraft would suffice, the federal cost contribution should be based on the contract price of a single engine aircraft.

Information regarding the total weight of the release machine plus insect load should be available for each of the release programs to establish if the use of single engines aircrafts carrying capacity is not exceeded by the carrying capacity of the planes. That not being the case, aerial release should not be considered any different than normal passenger services. Allowances should me made for difficult topographic conditions were those exist.

The Moscamed program uses a significantly different release machine than other locations. A comparison of available release machines should be undertaken to determine the optimal design for program use.

There is a widely held belief that there is a maximum window of 3 hours between knock-down and release beyond which there is a decrease in quality and longevity of the chilled sterile insects. Tests should be conducted to validate this widow and the parameters around which this window is valid.

Although is may seem intuitively obvious that centralization of effort is the most economical management tool, excessive deadhead, especially with twin engine aircraft can eliminate any savings due to the economy of scale. Where long flight distances are involved, satellite installations may prove to be more cost effective.

Recommendations for Fly Release

1. Include single and twin engine aircraft in bids for aerial release contracts.
2. Convene a technical panel to determine the optimal aerial release equipment for program use.

3. Validate the three-hour release window concept based on fly quality vs. time.
4. Evaluate the cost-benefit of distance to release site vs. deadhead costs.
5. Investigate conditions affecting the seasonal quality of sterile flies.
6. Investigate conditions affecting low trap-back of sterile Medfly in Miami releases.
7. Review Mexfly PRP release densities to determine if a higher density is needed.
8. Release Mexfly at seven (7) days of age so that sexual maturity has been reached.
9. Release Medfly at three (3) days of age.

Tray Washing

Tray washers have been installed at different times at different locations. Consequently, the most recent versions incorporate changes that benefit the workers and the system as a whole. For example, the latest configuration eliminates the conveyer chain within the washer and utilizes a higher capacity fan that results in dryer trays. The vender should be contacted to price out upgrading older equipment.

At the Sarasota, Edinburg, Harlingen, Reynosa, and Los Alamitos ERF tower trays are washed in large commercial tray washers. Washing occurs immediately after the adult fly knockdown. The machine speed is approximately 12 to 14 seconds per tray. After washing the trays are dried on racks placed in the direct sun.

PARC boxes are used at the Tijuana, Tapachula, and Retalhuleu ERFs. PARCs are washed manually (Tijuana and Tapachula) or in a commercial washer (Retalhuleu). After washing, PARC boxes are stacked and lids placed on racks to dry in the open air.

Recommendations For Tray Washing

1. Upgrade tower tray washing equipment.
2. Evaluate and improve sanitation procedures.

Process Flow

The process flow in the Sarasota ERF is very efficient. One team conducts the following activities: adult fly knockdown, loading of the adult flies into release boxes, loading the pupae into the trays, diet preparation, and tray washing. The ERF has 25 employees to cover all activities. The staff is dedicated and well prepared for the job.

The process flow in the Reynosa ERF is efficient. All rooms are in the same complex and the flow of towers, diet, and release boxes. No space constraints were observed and all activities occur on the ground level of the building. The ERF has 5 employees to cover pupa processing. The staff seemed to be dedicated and motivated for the job. The office hours are 6:30 A.M. to 3:00 P.M., Monday through Friday.

The process flow in the Edinburg and the Harlingen ERFs is efficient. One team conducts the following activities: adult fly knockdown, loading of the adult flies into release boxes, loading the pupae into the trays, diet preparation, and tray washing. The Edinburg ERF employs 14 persons and Harlingen 7 persons to process pupae.

The use of trailers to conduct all activities at the Los Alamitos ERF results in process inefficiencies. The worksite is spread across a large area which affects process flow and, therefore, operational efficiency. The ERF has 113 employees to cover all activities, processing pupa, chilling adult flies, Quality Control, and identification of flies from 8,000 traps derived from the surveillance program. The staff is dedicated and well prepared for the job. The office hours in the Los Alamitos ERF are 6:00 A.M. to 4:30 P.M., seven days per week.

At the Tijuana ERF, a six-person staff is responsible for all activities: collecting boxes from the airport, PARC box assembly, adult knockdown, release box loading, transport for release boxes to the airport, diet preparation, and PARC box washing. The weekly schedule is very busy except on Mondays. The staff is dedicated and highly motivated for the work and open for changes and suggestions.

The Retalhuleu ERF was designed specifically for emergence activities. The rooms are in the same complex, at ground level and so that movement of equipment is efficient. Cold rooms require readjustments to improve their chilling efficiency.

The Tapachula ERF is an old and temporary building. Activities are located in several disjointed spaces. The building does not run efficiently and requires replacement.

Recommendations for Process Flow

1. Implement the use of mechanical blenders to mix the boiling diet and pumps to transfer the hot emulsion to fiberglass trays.
2. Readjust cold room temperature controls to improve chilling capacity.

4 Quality Control

Routine Quality Control Evaluations

Routine Quality Control (QC) tests are required to determine the effect of rearing, irradiation, handling, shipment duration, holding, and release technologies. Sterile flies should meet the minimum quality requirements as specified in the *Manual for Product Quality Control and Shipping Procedures for Sterile Mass reared Tephritid Fruit Flies* (FAO/IAEA/USDA 2003). Parameters of importance in determining the quality of sterile fruit flies at ERFs include:

- **Pupae weight**—an overall measure of size. Larger male tephritids, in general, are stronger fliers, live longer, have higher mating propensity and produce longer remating refractory periods in females.
- **Percent emergence**—a measure of the percentage of pupae that produce adult flies.
- **Flight ability**—a measure of the percentage of adult flies that have the ability to fly.
- **Longevity under stress**—a relative measure of the nutrient reserves available to the adult fly at the time of emergence.

Average Medfly QC data from Retalhuleu (Guatemala), Tapachula (México), Sarasota, FL, and Los Alamitos, CA were grouped for comparison (**Table 2**). Similarly average Mexfly QC data from Reynosa and Tijuana (México) and Edinburg and Harlingen, Texas were also compared (**Table 2**). At the time of this review Los Alamitos was not processing sterile Mexfly.

TABLE 2: Routine quality control data for Medfly and Mexfly reported by each ERF

QC at arrival	Unit	Reu[1]	Tap[1]	Los Al[1]		Star[1]	Rey[2]	Harl[2]	Edn[2]	Tij[2]
Hypoxia	Hours	4	0.5	26a	23b	27-33	2-3	3	2	29-33
Irradiation dose	Gy	100	120	145	145	145	80	80	80	–
Day of sampling/wk	Days	4	7	7	7	4	4	4	7	2
Size of sample/d	Thousands	225	140	17	17	6	18	15	10.7	1100
Size of sample/wk	Thousands	900	980	119	119	24	74	60	75	2200
Pupal weight	milligrams	7.82	7.2	7.7	7.7	7.3	16	17	17	18
Fly emergence	%	81.1	85.5	76.2	85.7	78	76	85	85	88
Flight ability	%	74.8	75.9	68.4	80.1	79	84	81	81	86
Longevity (48h[1];72h[2])	%	34.5	73	46	63	36	70	60	60	N/A
QC pre-chilling										
Day of sampling/wk	Days	5	7	N/A	N/A	4	4	N/A	N/A	2
Size of sample/d	Thousands	500	60	N/A	N/A	1	50.9	N/A	N/A	90
Size of sample/wk	Thousands	2500	420	N/A	N/A	4	211	N/A	N/A	180
Flight ability	%	71	75.9	N/A	N/A	70.3	85	N/A	N/A	89
QC post-chilling								N/A	N/A	
Day of sampling/wk	Days	5	7	7	7	4	4	N/A	N/A	2
Size of sample/d	Thousands	38.5	60	4	4	1	50	N/A	N/A	90
Size of sample/wk	Thousands	192.5	420	28	28	4	211	N/A	N/A	180
Flight ability	%	95	73.1	60	74.5	N/A	83.3	N/A	N/A	85
QC post-release										
Holding time										
Fly emergence (50%) time	Hours	70	48	48	48	N/A	50	48-72	48-72	55
Fly age at release	Hours	71	72	48	48	72	100	3-5d	3-5d	55

1 Data correspond to *C. capitata*; a= pupa from Guatemala, b = pupa from Hawaii

2 Data correspond to *A. ludens*

Discussion of Routine QC

All ERFs have adequate QC laboratories and qualified personal to perform routine QC tests (FAO/IAEA/USDA 2003). However, some differences were observed among ERFs in the methodologies and type of tests conducted. The following points are observations and recommendations towards standardizing protocols and efficient resource utilization. The goal is to produce the highest quality sterile males for field release:

1. Most QC data in **Table 2** are within the limits established in the standard protocols. However, the number and size of samples used to perform the QC tests is variable. Each ERF uses different sizes of the pupae samples. This should be standardized in order to facilitate interpretation and comparison of the test results and

to optimize sterile insects use. It is recommended that sample size be calculated as outlined in Chapter 2 of the FAO/IAEA/USDA (2003) manual. Another option is to base the sample size on a specific parameter or unit (e.g., per million; per five or per ten million) of pupae received. This would give a variable sample size depending on the quantity of pupae that each ERF receives weekly.

2. Reynosa and Tijuana ERFs are the only ones that perform the test of "viability of pupa" (not reported in **Table 2**). This test consists of dissecting 100 pupae to estimate the percentage that are viable. It is used primarily to adjust the number of pupa that they are loaded into each tray or bag. This parameter is not of high priority and it is recommended that it be discontinued. The facilities could use the historical information on the percent fly emergence of the previous week to load the trays/bags for operational purposes.
3. Pupa received in Sarasota, Los Alamitos and Tijuana ERFs are kept under hypoxia for long periods (> 23 hours). This could result in reduced quality and it is recommended that alternative shipping routes be examined as a way to reduce overall transport and hypoxia time.
4. The results of the longevity under stress test are not reported in a consistent manner. Some ERFs report this as percent mortality, not percent survival. It is recommended that the results be reported as the percent survival at 48 hours for Medfly or at 72 hours for Mexfly.
5. The flight ability evaluation results require standardization. For example the Retalhuleu ERF reports 95% which correspond to the concept of absolute fliers (% of adult flies that can fly after chilling), while in Tapachula the reported data (73%) correspond to the concept of flight ability (% of flying adult flies from the total pupae received at the ERF).
6. The irradiation dose is probably too high, significantly damaging sterile fly quality. It is recommended that a full evaluation be conducted among production facilities to examine the possibility of lowering the irradiation dose.
7. The release age of the sterile males in Los Alamitos and Tijuana is too young. It is recommended that the males are closer to or reach sexual maturity prior to release.
8. There is no QC evaluation of the sterile males post-release.

Recommendations for Routine QC

1. The longevity under stress test (without food and without water) should be implemented at all steps in the packing and release of sterile males. This is critical at the post-chilling and post-release steps because it provides a relative estimate of nutrient reserves of the sterile males post-release.
2. The longevity under stress test with water but without food should be established as an indicator of nutrient reserves of the sterile males. This may provide a means of estimating the quality of the larval and adult diets provided in the rearing and fly emergence processes.
3. The post-release QC evaluations should be established. Parameters should include the sample size, types of tests required, and the frequency of evaluations. It is recommended that: 1. the sample should be taken at the airport from the release machine when the plane returns from the aerial release; 2. the tests should include an estimation of the number of absolute fliers and longevity under stress (with water but without food); and 3. the tests be conducted on a biweekly basis.
4. The mating competitiveness of sterile males should be compared to that of to wild males when competing for wild females a minimum of once per year. The evaluation should be conducted in field cages at or near the rearing facility of origin. Mexfly from Edinburg and Reynosa rearing facilities should be evaluated in Mexico (e.g., Ciudad Victoria, Tamaulipas) in cooperation with SAGARPA.
5. The Reynosa ERF reported a male biased sexual ratio in Mexfly. The biotic and abiotic factors influencing this sex ratio should be determined.
6. QC evaluations conducted in the ERFs should be in compliance with the established protocols (FAO/IAEA/USDA 2003, FAO/IAEA 2007).
7. Hypoxia is critical to reduce irradiation-induced damage during the sterilization process. It is recommended that the potential for use of a plastic sealing machine and time post-sealing be evaluated as a means to standardize the amount of air left in the bags. This may assure the desired uniform and high level of hypoxia as well as limited humidity levels.
8. The irradiation dose is probably too high. It is recommended that a full evaluation be conducted among production facilities to examine the possibility of lowering the irradiation dose while insuring the desired level of adult fly sterility.
9. Standardize the reporting of irradiation dose, e.g., mean dose or minimum dose.

10. The amount of dye added to pupae as an external marker should be standardized among production facilities.

Release of Sterile Flies and Recapture Rates

Aerial release is the most cost-effective means of distributing sterile fruit flies over the target area, especially in large scale operational programs. The release density of sterile males and the frequency of releases during the week should consider the longevity of sterile males post-release (**Table 3**). This is critical to assuring that the required density of sterile males is maintained throughout the target area. QC evaluations post-release are critical because the effectiveness SIT can be strongly influenced by this last step in the process (**Table 3**).

TABLE 3: Sterile fruit fly release parameters for each ERF

Parameters	Unit	Reu[1]	Tap[1]	Los Alam[1]	Sar[1]	Rey[2]	Harl[2]	Edn[2]	Tij[2]
Range – Release density	Flies/mi^2 (x 1,000)	572	516	62.5 to 125	137	100	320	320	120
Release frequency in the same area	Times/wk	2	2	2	2.5	1-2	1	1	1
Distribution (traps with recapture)	%	85	83	N/A	58	60	60-90	60-90	73
Longevity (48h[1]; 72h[2])	Hours	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

1 Data correspond to *C. capitata*

2 Data correspond to *A. ludens*

Discussion of Release and Recapture QC

1. The release density per square mile is variable between programs (**Table 3**). The release rate does not appear to be based on technical or scientific data related to specific patterns or criteria in different control areas. Instead it appears to be dependent upon availability of sterile flies or administrative issues (e.g., Reynosa, Los Alamitos).
2. The percentage of traps in the Florida surveillance program that recapture sterile Medfly males was consistently low in the Miami area. The cause of the low trap-back is not understood and requires some investigation. Possible causes may include: winds patterns, ferry time, and reduced male longevity post-release. There remains concern over the use of Jackson traps baited with Trimedlure in areas under SIT releases using male-only Medfly strains. The use of these traps continues because the distribution of sterile males as indicated by recapture in Jackson

traps is still a parameter of importance. Nevertheless the use of these traps should be reduced in relation to food-baited traps that attract also Medfly females.

3. The percent distribution of sterile Mexfly from Reynosa, Harlingen, and Edinburg was variable as based on a trap-back ranging from 60 to 90%. It is difficult to determine the quality of aerial insect releases and the overall confidence in the SIT program because of this variation. Percent distribution of sterile Medfly from Los Alamitos was not available, since this parameter is apparently no longer measured.

Recommendations for Release and Recapture QC

1. Standardize the application of specific release densities for sterile Medfly males and Mexfly according to the objective of each action program.
2. The frequency of sterile Medfly and Mexfly releases should be twice per week over the same zone, in alternate lines.
3. The percent of distribution of sterile males based on trap-back in surveillance programs should be carefully determined in order to identify problems with the release procedures. The "Detection Guidelines under PRP in the National Detection Trapping Protocol" (USDA 2006) should be updated to include protocols and interpretation of data.
4. Evaluation of Medfly longevity under stress (with water and without food) post-chilling and post-release should be correlated with positive captures in Jackson traps with Trimedlure, as well as food-based traps.
5. In surveillance programs for Mexfly SIT, Multilure trap should be baited with the two component lure (ammonium acetate and putrescine), instead of the three component lure. The three component lure (Biolure) has been shown to include a component that has a repellent effect on Mexfly (trimethyl amine). Use of the two component lure should reduce costs and increase trap efficacy.

5 Adult Diet Formulations and Supplements

Adult Fly Diet Formulations

With the exception of the Tapachula ERF, all ERFs provide sterile males with a wet diet consisting of agar, sugar, water and a preservative. The proportion of diet ingredients varies slightly among the ERFs (**Table 4**). In general, the adult fly diet consists of 81 to 85% water, 14 to17% sugar, 0.6 to 0.9% agar, and 0.001 to 0.1% preservatives.

TABLE 4: Percentage of adult fly diet ingredients in ERFs. Data on the pH of the diet was unavailable

Ingredients	Percentage by ERF				Percentage by ERF			
	Reu[1]	Los Al[1]	Sar[1]	Tap[1, 2]	Harl[3]	Edn[3]	Rey[3]	Tij[3]
Water	84.30	84.39	84.449	N/A	85.25	85.25	84.14	81.18
Sugar	14.92	14.20	14.846	N/A	17.00	17.00	15.05	17.80
Agar	00.77	00.77	00.696	N/A	0.64	0.64	00.74	00.92
Preservatives[4]	00.01S	00.001S	00.01N	N/A	00.07N	00.07N	00.005S	00.10N

1 Data correspond to Medfly, *C. capitata*

2 Dry diet with protein

3 Data correspond to Mexfly, *A. ludens*

4 N=Nipagin; S=Sodium Benzoate

The Tapachula ERF uses a dry adult fly diet, which is unique among the ERFs. The diet ingredients (sugar, corn flour, protein, and no preservative) are brushed onto paper and allowed to dry to provide as a dry mixture. Water is provided to adult flies in reusable sponges; the water contains 5 g (11 pounds) of Guar Gum mixture to improve water retention. The dry food reduces problems with flies sticking to the diet and also any growth of microorganisms. However, a large amount of paper is used in food delivery and the water sponges require sterilization and frequent replacement.

Agars with different water holding capacities are used by all the other ERFs. Several use 1,300 agar and the Tijuana ERF uses 1,000 S agar. The 1,000 agar is associated with sugar/water leaking over time that causes the flies to stick to the screens and enhances the growth of microorganisms. The use of agar as a carrier of water and sugar is costly (approximately 300,000 USD/year combined for all ERFs), particularly when considering that it is discarded at the end of the fly emergence process.

The type and concentration of preservatives vary among ERFs. The more costly Nipagin (methyl paraben) is used in Edinburg, Harlingen, and Tijuana, while the cheaper sodium benzoate is used in Sarasota, Reynosa, Los Alamitos, and Retalhuleu. The advantage of nipagin over sodium benzoate under normal environmental conditions is that it is more effective at inhibiting the growth of yeasts at pHs $\geq$5. It was noted that adult diet blocks become more acidic over time.

The water source and condition may affect the properties of the adult fly diet. In Reynosa well water is used and minerals appear to inhibit the solidification of the agar. A water softener may be required to correct this issue.

Recommendations For Adult Fly Diet Formulations

1. Quality control of adult fly diet ingredients is not established. This should be instituted by all ERFs to ensure the overall quality and consistency of diet ingredients received from the manufacturer. Periodic quality assessments could be assigned and conducted by the ERFs on a rotating basis. This could be done in conjunction with bulk purchases to achieve better prices.
2. The use of preservatives in wet diets needs to be fine-tuned at each ERF based on the diet pH and the type of microorganisms present. Therefore the use of sodium benzoate as a preservative in some ERFs, and Nipagin in others, needs to be reassessed. The amounts included in the diet, which vary over two orders of magnitude, may need to be adjusted.
3. Reassess the types of agars used to minimize costs, leaking, and growth of microorganisms. The possibility of collecting used agar blocks at the end of the process and re-boiling the agar instead of discarding should be investigated.
4. Reassess the need for agar in the adult fly food. Fully reusable water carriers such as Alcosorb should be evaluated as a cost-effective replacement for agar.

Protein Supplements

Male tephritid fruit flies require protein sources in nature as part of their normal biology. Research with several tephritid species generally shows that not only do the males respond to protein traps, but also that sterile *Anastrepha* males provided with protein have increased sexual competitiveness and pheromone production. It remains unclear whether protein provided to male Medfly prior to release confers a significant increase in longevity or sexual competitiveness (Kaspi et al. 2000; Perez-Staples et al. 2007; Shelly et al 2002; Shelly et al. 2006; Taylor and Yuval 1999; Yuval et al. 1998; Yuval et al. 2002; Yuval et al. 2007; but also see Shelly et al. 2008).

The Tapachula ERF has provided hydrolyzed protein to male Medfly for several years as part of a dry diet. The Edinburg and Harlingen ERFs initiated the provision of protein to Mexfly in accordance with scientific studies demonstrating that access to protein accelerates sexual maturation of sterile males by 2 days. In these ERFs, hydrolyzed protein (Lalemmand) is sprinkled as a powder onto the diet blocks with a flour sifter at a rate of 2.5 lbs (1.1 kg) of hydrolyzed yeast per day to supply 1600 trays.

The four Mexfly ERFs in Victoria, Montemorelos, Zacatecas and Sinaloa, Mexico also provide a protein to sterile Mexfly as part of the Mubarqui diet. The Tijuana ERF apparently provided protein for decades to their flies, but this was discontinued some years ago in order to simplify the processing of sterile flies without regard to sterile fly performance.

Recommendations for Protein Supplements

1. Include protein in the Mexfly adult fly diet in order to increase sterile male competitiveness.
2. Continue to evaluate the need for protein in the Medfly adult diet.
3. Optimize the type and amount of protein in adult fly diets based on the nutritional needs and performance of the sterile males.
4. Conduct applied research to determine more efficient methods for providing dry protein; one possibility is dry cakes similar to those used in rearing facilities that are provided separately from water.
5. Assess the type and amount of protein as well as the overall performance of the dry adult fly diet being used in the Mexfly ERFs in Montemorelos, Victoria, and Sinaloa.
6. Provide food and water separately to reduce the sticking of flies to the screens and encourage fly foraging behavior by forcing them to search for these different resources.

Hormone Supplements

Adding methoprene, a juvenile hormone analogue (JH), to the diet of species with prolonged sexual maturation periods such as *Anastrepha* spp. significantly decreases the sexual maturation period. In addition, there is a synergistic effect when JH and protein are jointly provided to *Anastrepha* spp. males (Pereira 2005; Teal and Gómez-Simuta 2002; Teal et al. 2000).

The Edinburg ERF confirmed that methoprene (JH) in the adult fly diet of sterile Mexfly males reduces the sexual maturation period by two days. However, this protocol has not been implemented in the operational program because Methoprene is classified as a restricted use pesticide and is not labeled for application in fruit fly maturation. Clearance from OSHA, EPA, and pertinent State pesticide regulatory agencies would be required before implementation in ERFs.

Recommendations for Hormone Supplements

1. Quality control of the semiochemical and hormonal supplements purchased is recommended. The periodic assessment of quality of these ingredients could be rotated among the ERFs. Bulk purchase could result in cost savings.
2. Obtain OSHA clearance for use of methoprene in the ERFs.
3. A cost-effective, easily provided operational formulation for methoprene delivery is required. Evaluate the use of methoprene in a dry protein cake. A cheaper commercial methoprene needs to be developed.
4. Implement methoprene additives to the sterile Mexfly diet in Edinburg and Harlingen, followed by Reynosa and Tijuana.
5. The Reynosa and Tijuana ERFs should have more direct contact with the Montemorelos, Victoria, Sinaloa, and Zacatecas ERFs. For instance, both protein and methoprene are already being routinely provided in these ERFs as part of the sterile male diet.

Semiochemical Supplements

Aromatherapy, consisting of the provision of ginger root oil (GRO) or citrus oils, has been shown to significantly increase the relative mating frequency of mass reared sterile Medfly males (Katsoyannos et al. 2005; Papadopoulos et al. 2001; Papadopoulos et al. 2006; Shelly, et al. 2001; Shelly et al. 2002; Shelly et al. 2004; Shelly et al. 2008). Approximately 24 hours before sterile males are collected for release they are exposed to GRO (Citrus and Allied Essences, Ltd.).

Aromatherapy is already being applied to Medfly males in Retalhuleu, Sarasota and Los Alamitos, but not in Tapachula. In Los Alamitos and Sarasota 1 ml of GRO is placed on a cotton wick in an aluminum foil-covered small container under each fly emergence tower, while in Retalhuleu 1.35 cc/m^3 are provided per holding room with PARC boxes. Although Los Alamitos and Sarasota apply 1 ml per tower, males are exposed to different concentrations in view of the very different volumes of the holding rooms. For example in Los Alamitos, where each trailer (60 ft x 12ft x 8ft (18.3m x 3.6m x 2.4m)) holds 36 towers, a total of 36 ml of GRO are provided. In Sarasota towers stand in a much larger room, that contains a combination of towers with males of different ages.

Recommendations for Semiochemical Supplements

1. Implement GRO aromatherapy in the new Tapachula ERF.
2. Assess the effect of the holding room volume on application of GRO.
3. Assess the effect of age and duration of treatment on aromatherapy treatment efficacy.
4. Assess the need for protein in the adult Medfly diet.
5. Assess the interaction of GRO aromatherapy and protein for sterile Medfly males.
6. Support basic research on *Anastrepha* spp. to identify semiochemicals with similar aromatherapy effects on males.

Diet Preparation

Wet diet preparation starts by gradually adding the agar and the preservative to cold water so that the agar does not clump. The mixture is brought to a boil in steam kettles, during which the sugar is added gradually. The mixture is manually stirred for 1 to 4 hours in order to obtain the proper emulsion, including completely dissolved sugar and intact agar. This emulsion is poured into the mass rearing fiberglass trays at a rate of approximately 1.5 gallon (5.7 L) per tray to achieve a depth of 0.75 to 1 inch (1.9 to 2.54 cm). The diet cools and solidifies.

With the exception of the large variation in boiling times, the adult fly diet preparation procedures are very similar for all ERFs using an agar diet. The volumes of diet produced vary depending upon the number of pupae being processed and the diet consumption rate of the fly species. For example, in Los Alamitos four-50 gallon (189 L) batches of agar diet are prepared daily, seven days a week by a 2 to 3 person team. In Sarasota the agar diet is prepared twice daily in 40 gallon (150 L) steam kettles. Five diet batches are prepared weekly in Edinburg and two in Harlingen. In Reynosa one daily batch is produced and used on the same day in order to reduce diet desiccation and to delay the growth of microorganisms. In all other ERFs the diet is prepared the day before it is used.

The Edinburg and Harlingen ERFs could not be visited as a result of hurricane Dolly, but it is recognized that many of the procedures used were originally developed by the Edinburg group.

The Tapachula ERF diet preparation procedures are unique because water and diet ingredients are provided separately. Dry diet preparation involves boiling water with the sugar, adding the corn flour as a thickener, and at the end the hydrolyzed protein. The diet is brushed onto paper and allowed to dry. The paper sheets are placed into the tower trays and PARC boxes on the following day. Reusable sponges, routinely disinfected, are used to hold the water that includes 5 g (0.011 lb) of Guar Gum as a thickener to retain the water.

Recommendations for Diet Preparation

1. Produce the diet, as much as possible, on the same day that it is used. This is particularly relevant in places such as Los Alamitos, where rapid diet desiccation is apparent, and in Tijuana where microorganism growth is a serious problem within 24 to 48 hours of fly emergence. The latter situation results in the need for early sterile fly release to avoid mortality in the PARC boxes.
2. Develop formulations for separate delivery of food and water. This should have two advantages, a reduction in the number of flies sticking to the screens and an increase in foraging behavior by flies searching for the two resources.
3. Assess whether providing protein and sugar as dry cakes, without paper, is a suitable delivery method in Tapachula. This would reduce the large volume of paper that is discarded after the fly emergence process.

Amount and Surface of Diet

Once the wet agar diet has solidified and cooled in the fiberglass trays (16 inch x 30 inch x 3 inch (40.6 cm x 76.2 cm x 7.62 cm)), it is cut with a stainless steel blade into diet units or blocks of very different sizes among the ERFs (**Table 5**). In addition, the number of diet units that are provided per tray or PARC box varies significantly among ERFs (**Table 5**). The Retalhuleu, Sarasota and Tijuana ERFs provide only one diet unit per each mesh tray of the tower system (except in the bottom and top trays where 2 are provided by the Sarasota ERF to compensate for desiccation). In other ERFs, two blocks are placed cross-wise on each tray to maximize the available surface area for feeding. In the case of towers, the diet must be placed at the time the towers are assembled with pupae, rather than at the time when flies emerge. In Tapachula 4 units are placed per PARC box.

There are major differences in the numbers of pupae loaded into tower trays or PARC boxes and in the number days the adult flies feed prior to release. As a result, sterile males have access to very different amounts of food and available surface area for feeding (**Table 5**). In Los Alamitos, for example, reducing the number of pupae per tray from 440 ml to 330 ml resulted in more sugar and water per fly. As a consequence there was a significant improvement in the quantity and quality of released sterile males per tray.

After chilling and collecting the sterile males for release, the agar blocks are discarded. The water content, size and weight of these discarded diet blocks varies considerably between ERF, fly emergence systems, and even within towers. This reflects the large differences in environmental conditions and holding periods at the ERFs.

TABLE 5: Diet units and feeding surfaces provided to sterile flies in the different ERFs in relation to pupal density and feeding days.

	Reu[1 2]	Los Al[1]	Sar[1]	Tap[1 2]	Harl[33]	Edn[3]	Rey[3]	Tij[3]
Diet Unit length (inch)	8 (P) (T)	4 (T)	6 (T)	18 (P) (T)	3 (T)	3 (T)	4 (T)	6 (P)
Diet Unit width (inch)	7.5	3	4	9.6[4]	1.5	1.5	2	4
Diet Unit height (inch)	1	1	0.75		0.75	0.75	1	1
Diet Unit number	1	2	1	2 (T) 4 (P)	2	2	2	1
Total Feeding Surface (inch^2)	1 x 151= 151	2 x 38 = 76	1 x 63 = 63	2x173=346 (T) 4x173=692 (P)	2x15.75 = 31.5	2x15.75= 31.5	2 x 28 = 56	1 x 28 = 28
Pupae / Tray or PARC Box (x 1,000)	38.5 (P) 25 (T)	21(T)	25 (T)	60 (P) 21.4 (T)	13 (T)	13 (T)	10.52 (T)	30 (P)
Days Feeding before Release	3	2	3-4	3	3-5	3-5	3-5	2
Surface/Day/ 1000 Pupae	1.31 (P) 2.01 (T)	1.81	0.84 (3d) 0.63 (4d)	3.84 (P) 2.69 (T)	0.81 (3d) 0.48 (5d)	0.81 (3d) 0.48 (5d)	1.77 (3d) 1.06 (5d)	0.47

1 Data correspond to Medfly, *C. capitata*

2 Mainly PARC boxes, different towers being introduced in pilot phase

3 Data correspond to Mexfly, *A. ludens*

4 Paper units with dry food on top side only; water provided separately in sponges with limited surfaces

P = PARC boxes (only one feeding surface/diet unit)

T = Tower trays (6 feeding surfaces/diet unit)

Recommendations for Quantity of Adult Fly Diet

1. Harmonize the amount of food and feeding surface provided per fly for Medfly and Mexfly. This should consider the holding period before release and fly density per tray or PARC box.
2. Increase the feeding surface available to sterile flies by presenting the same amount of food in two separate units. Units should be placed at opposite corners of the screen on alternate trays, thus providing four feeding points (two on the bottom tray and two on upper tray). The size of the unit should be large enough to prevent desiccation.
3. Increase the amount of food provided. This is particularly important where there is a very large differential in development time within towers or PARCs (Edinburg, Harlingen and Tijuana), and where flies are held for 5 or more days after emergence before release (Sarasota).
4. Reduce in some ERFs the amount of pupae per tray or PARC box to optimize the ratio of adult flies to available diet.
5. Increase the number of diet blocks in the lower trays in Los Alamitos. The diet blocks in the lower 8 to 12 trays are progressively smaller and dry at the end of the holding period, indicating that the adult flies in those trays may not receive sufficient resources prior to release.
6. Carefully control the humidity in the holding rooms within the 60-70% RH range. Below this humidity level, the agar blocks dry out. Above this level, the microbial growth and clumping of adult flies increase.
7. Increase the age of release in Los Alamitos and Tijuana to at least three days. Adult diet delivery should be optimized based on the holding period and the ratio of adult flies to available diet. The mortality due to microbial growth and other causes in some ERFs should be seriously considered.

Measuring Food Availability and Reserves

Large variation has been observed in the pupa to adult fly development period within the same batch of sterile Mexfly pupae. Mexfly at the Edinburg, Harlingen, and Reynosa ERFs all receive pupae from the USDA Mexfly Rearing Facility in Edinburg, TX. Mexfly emerge at all of these ERFs over a four day period. The holding period for the adult flies can be up to seven days. During this period, the diet blocks can become desiccated or favor the growth of microorganisms. Actual water and food availability under these conditions needs to be well known because it has important consequences for food availability and reserves post-release. In addition, the environmental controls appear to be insufficient to maintain the proper temperature and RH in emergence rooms. Fly emergence towers may be stacked too high. The measurement of absolute flyers and longevity under stress (with water, but not food) is not used to evaluate the food reserves accumulated during the fly emergence process.

Recommendations for Food and Reserve Measurements

1. Determine the basis of the large variation in pupa to adult Mexfly development time within towers and trays in view of the consequences for food availability and diet consumption for the oldest flies (some over 5 days old).
2. Reduce tower height in ERFs with the space capacity to do so. This may reduce the large variation in development and the resulting differences in access to food and water.
3. Establish QC protocols at all ERFs to evaluate absolute flyers and longevity with water but without food after the emergence, feeding, chilling and release process. This should provide a more accurate measure of food reserves accumulated by adult flies before and during the emergence process as well as their post-release performance.
4. Determine and correct the cause(s) of flies sticking to the tray screens. Among possible causes are the RH of pupae at departure at the production facility and at arrival at the ERF, and the leaking of diet blocks under certain environmental conditions. Adjustments to the composition of the adult fly diet may be made to change the proportion or type of agar to reduce leaking.

Worker Safety

Two important devices significantly increase staff safety at the Sarasota, Edinburg, and Harlingen ERF by avoiding the direct contact with the hot liquid diet during the diet preparation process:

- The use of mechanical blenders that maintain the emulsion in continuous motion within the kettle, thus simplifying the process and reducing labor time.
- Pumping of the liquid diet out of the kettle through a pipe and hose that allows the hot gelatin to be dispensed directly into the fiberglass trays.

Recommendations for Worker Safety

1. Routine handling of the boiling diet manually and transferring it a very hot state in buckets to fiberglass trays puts staff at risk for serious injury. It is recommended that the Retalhuleu, Reynosa

and Tijuana ERF implement the use of mechanical blenders and a pump to transfer the hot diet directly from the steam kettle to the fiberglass trays. The equipment to implement this in the Reynosa ERF has already been purchased, but installation has not been funded.

2. Add a diet dispensing dosimeter to the hose, in order to simplify pouring the hot liquid diet into each of the fiberglass trays.

6 Operational Cost Analysis

APHIS and its cooperators spent about $24.3 million in 2007 on sterile Mexfly and Medfly adult fly emergence and release activities[1] at eight different ERFs (**Table 6**). The bulk of the spending, approximately $22.2 million (91%), went to ongoing Medfly operations at four ERFs. Approximately $2.2 million (9%) of the total was for rearing and release of Mexfly.

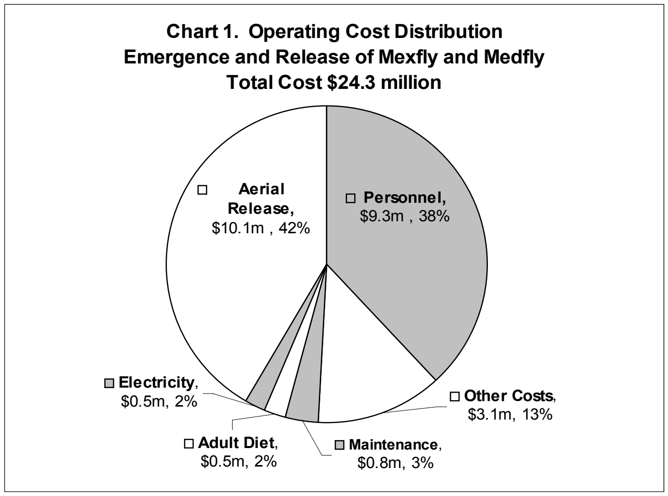
TABLE 6: Emergence and Release Operations Costs in 2007

ERFs	Annual cost	Released per week (millions)	APHIS Share	Cooperator Share
Medfly (*C. capitata*)				
Los Alamitos	$13.3 m	283	40%	60%
Retalhuleu	$4.0 m	700	100%	
Tapachula	$2.7 m	374		100%
Sarasota	$2.2 m	85	100%	
Mexfly (*A. ludens*)				
Edinburg	$0.7 m	95	95%	5%
Harlingen	$0.6 m	36	95%	5%
Tijuana	$0.5 m	14	100%	
Reynosa	$0.3 m	18	100%	
Total	$24.3 m	1,682	100%	

1 This total amount does not include the cost of the sterile pupae supplied to each ERF.

From analysis of the spending data[1] provided, the review team found that:

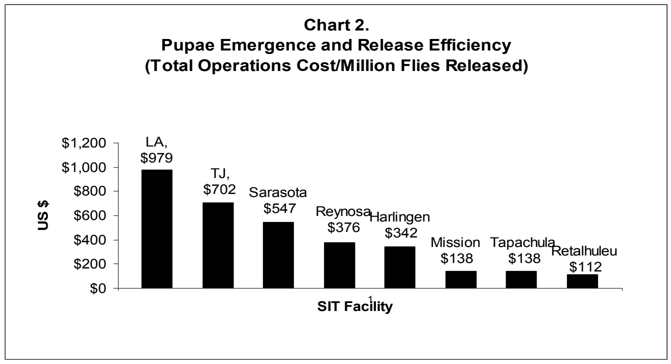
- Labor and aerial release costs made up about 80% ($19.4 million) of overall spending (**Chart 1**).



- The average unit cost for all ERFs to emerge and release Mexfly and Medfly was $417 per million flies released. The average unit cost breakdown by species was $444 per million Medfly emerged and released and $389 per million Mexfly emerged and released.

1 The review team received spending data for six main cost areas (**Table 8**):
Personnel/Labor
Aerial Release
Electricity
Rearing Diet Materials
Maintenance
Miscellaneous items needed to run the operations (facility rents, backup generator fuel, paper bags, etc.)
The personnel/labor spending total included fruit fly identification personnel at Los Alamitos and Tijuana, but not at other facilities. To make an accurate comparison of labor productivity among individual facilities the analysis subtracted out the cost of identification personnel at Los Alamitos and Tijuana.

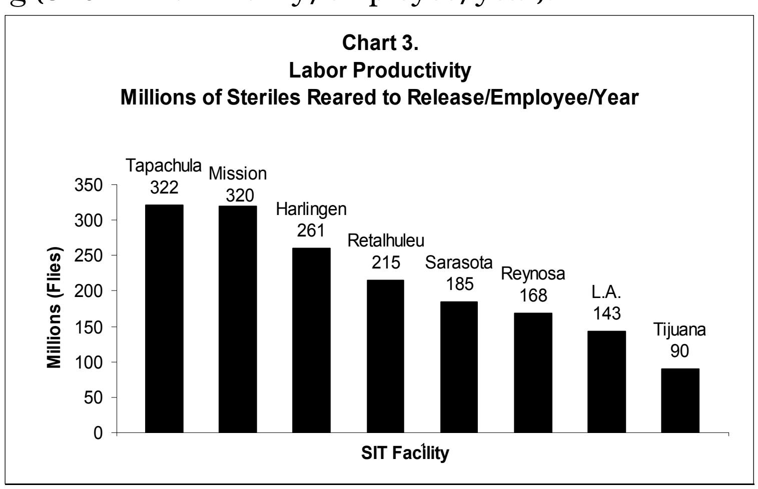
- There were significant differences in overall operating cost efficiencies among the various ERFs (**Chart 2**).



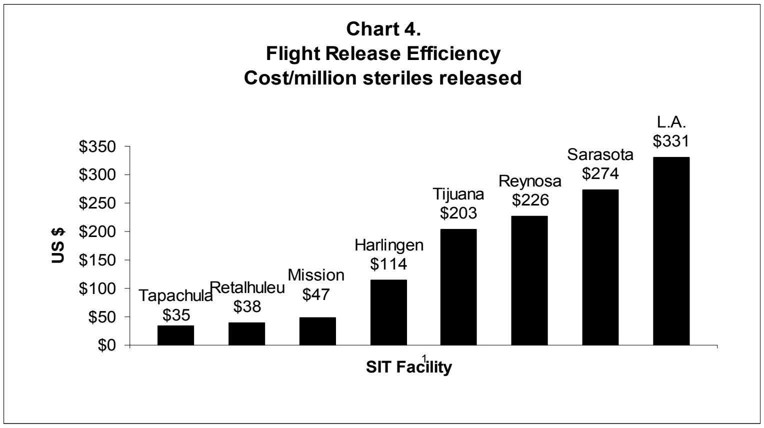
- More detailed analysis of the two most expensive cost areas revealed significant differences in labor productivity and aerial release efficiency among the eight ERFs.

We calculated labor productivity by dividing the total annual number of adult flies released by the number of full-time employees involved in fly emergence and release activities.

Average overall productivity across all eight ERFs was 213 million adult flies produced per full-time worker. The analysis indicated that there were significant differences in labor productivity among the eight ERFs (**Chart 3**). The two most efficient ERFS, in terms of labor productivity were Tapachula (322 million Medfly/employee/year) and Edinburg (320 million Mexfly/employee/year).



Adult fly release efficiency was calculated by dividing the daily cost of adult fly release by the average number of flies released per day. On average overall, it cost $159 to release 1 million adult flies by airplane. The analysis showed that there were large differences in release efficiency among the various ERFs (**Chart 4**). The two most efficient ERFs, in terms of cost to release one million flies, were Tapachula ($35) and Retalhuleu ($38). It was noted that the most efficient ERFs had some of the highest flight-hour costs for their contract, $705/hour at Retalhuleu and $680/hour at Tapachula (See **Table 8**).



Conclusions and Recommendations

There are significant differences in operational efficiencies among the eight ERFs. Some of these differences can be explained by differences in the species characteristics, the volume of fly pupae throughput, and in local costs of labor and fuel. For example, because Mexfly adults are roughly twice the size of Medfly adults it is expected that the per unit cost of emerging and releasing one million Mexfly would be approximately twice that of Medfly at the same ERF. Further, some ERFs appear to be less efficient in their use of labor and aerial release contractors.

The review team recommends that ERF managers continuously analyze potential ways to improve cost efficiencies in all phases of emergence and release operations. Some means for ERF managers to save money in operations without reducing program effectiveness include (**Table 7**):

1. Aerial Release Contracts
   - ❖ Analyze the feasibility of using single engine planes instead of twin-engine aircraft. If possible, implement this cost saving measure at next contract renewal for locations currently using twin-engine equipment.
   - ❖ Maximize the volume of sterile flies in each load to reduce the number of flight hours, including ferry time, needed to release flies at the proper densities.
2. Operations Management
   - ❖ Take advantage of potential savings from volume pricing on contracts, especially for aerial release contracts.
   - ❖ Take advantage of reduced prices with bulk purchase contracts.
   - ❖ Reduce the use of Jackson traps, without reducing recapture monitoring effectiveness.
   - ❖ Adjust release densities in lower-risk blocks, or stop releases completely.

TABLE 7: Potential cost savings measures

Examples	Savings Category	Annual Units of Savings	Unit Cost	Estimated Annual Savings
Florida: Double up on loads in release boxes in plane (Sarasota- Miami Block)	Aerial Release	52 Flights	$3,955	$205,660
Florida: Decrease density in “Sara-Man” block to 62,500/mi2 from 125,000/mi2	Aerial Release	104 flights (1 billion pupae)/year	$2,421	$251,784
California: Reduce Jackson traps for recapture data (Domestic Medfly PRP)	Sterile Release Monitoring and ID	3 identifiers annual salary + 130,000 TML plugs	~$300,000 ~$130,000	$430,000
All Domestic Operations: Purchase agar as a bulk purchase using lowest available price	Diet	7,897 kg	$2	$15,794
TOTAL				**$903,218**

Los Alamitos Facility Design and Infrastructure

The review team was concerned with the high facility operations costs (**Chart 5**) and lower than average labor productivity at Los Alamitos ERF. Some of these costs, e.g., $3.0 million for general operations and maintenance in 2007, were most likely due to the need for constant repair and maintenance of the old mobile trailer units. In addition, the spread-out design of the ERF and lack of protection against inclement weather probably contributed to lower labor productivity. Cooperators should again review the proposal for a new ERF at Los Alamitos. The study should highlight potential cost savings in labor and maintenance with a new ERF, to see if a phased-in construction plan could quickly pay for itself through reduced annual operating expenses.

Chart 5. Los Alamitos Spending
($13.3 million)

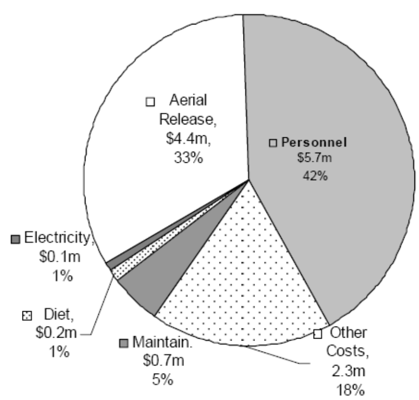


TABLE 8: Cost Data and performance Measure Summary Spreadsheet (Mission = Edinburg)

OPERATIONAL COSTS PER YEAR ($US 000s)	REU		TAPACHULA		TIJUANA		LOS ALAMITOS		HARLINGEN		EDINBURG		SARASOTA		REYNOSA		TOTAL		AVG	MEDFLY AVG	MEXFLY AVG
Account Description	**COST**	**%**	**COST**	**%**	**COST**	**%**	**COST**	**%**	**COST**	**%**	**COST**	**%**	**COST**	**%**	**COST**	**%**		**%**			
Aerial Release	1,963	49	1,900	72	146	28	4,383	33	214	38	234	35	1,067	48	200	63	10,106	42	1,263	2,328	198
Personnel	1,306	32	440	17	234	45	5,653	42	285	48	366	55	935	42	67	21	9,286	38	1,161	2,084	238
Other Costs	293	7	203	8	101	20	2,346	18	59	10	5	1	109	5	0	0	3,118	13	390	738	41
Maintenance	106	3	4	0	6	1	674	5	5	1	5	1	7	0	0	0	807	3	101	198	4
Adult Diet	175	4	94	4	12	2	160	1	12	2	31	5	40	2	6	2	530	2	66	117	15
Electricity	189	5	11	0	16	3	102	1	20	3	20	3	66	3	42	13	466	2	58	92	25
TOTAL ($000s)	4,032		2,655		514		13,318		594		661		2,224		316		24,313		3,039	5,557	521
Performance Measures																					
MILLIONS of Flies Emerged and Release per Month	2,996		1,609		60		1,133		145		380		339		70		6,733		842	1,519	164
Overall Facility Efficiency: COST PER MILLION FLIES EMERGED AND RELEASED ($)	$112		$138		$711		$979		$342		$145		$547		$376				$419	$444	$393
NUMBER OF PERSONNEL FOR THE OPERATIONS	167		80		8		95		7		15		22		5		379		47	86	9
Labor Productivity: MILLIONS EMERGED and RELEASE/ PERSON	215		322		90		143		261		320		185		168				213	216	210
COST PER FLIGHT/HOUR	$705		$680		$350		$596		$245		$258		$774		$350				$495	$689	$301
Aerial Release Efficiency - FLIGHT COST MILLION FLIES RELEASED (US$)	38		35		203		331		114		47		274		226				159	169	148

7 Recommendation Tables

Emergence and Release Facilities

Program Management

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
PM1. Hold an annual open house at each ERF for key cooperators and industry.	X								
PM2. Implement standard management practices and procedures for critical functions.	X								
PM3. Consider ISO certification of all ERFs.	X								
PM4. Expand Quick Place use for QC data of all production facilities and ERFs.	X								
PM5. Develop recommendation implementation strategy with APHIS and cooperators.	X								
PM6. Hold quarterly conference calls to discuss ERF operations and QC data.	X								
PM7. Hold annual SIT ERF operational staff meetings, beginning in 2009.	X								
PM8. Provide program management staff with fruit fly technical expertise.							X		X
PM9. Include SAGARPA management in LRGV operational program planning.					X	X	X		

Program Management (continued)

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
PM10. Provide program operations and development assistance to Reynosa.					X		X		
PM11. Host upper management officials at each ERF on an annual biannual basis for program assessment and oversight.	X								
PM12. Streamline U.S.-Mexico border crossing procedures.							X		
PM13. Purchase supplies in bulk for all ERFs to realize volume pricing.	X								
PM14. Determine technical specifications for diet ingredients and establish a minimum of three suppliers for each product.	X								
PM15. Revise release density and prioritize release zones based on level of risk.	X								
PM16. Review emergency preparedness plans for continuity of operations.	X								
PM17. Provide CPHST technical and scientific support to all fruit fly programs.	X								
PM18. Improve operational efficiencies through replacement of ERFs.								X	
PM19. Convene a technical advisory panel to review and plan LRGV Mexfly SIT.					X	X	X		

Location/Buildings/Process

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
L1. Review the availability of flights from Guatemala to Sarasota and from Honolulu and Guatemala to LAX in order to reduce transportation time.				X				X	
L2. Implement production of Mexfly in San Mogul Petapia, Guatemala, because Mission is at full capacity and the release densities are presently low.					X	X	X		
L3. Review the safety and health procedures in all ERFs.	X								
L4. Redistribute trailers so all towers move over horizontal surfaces.								X	
L5. Carefully control the humidity in the holding rooms at 60-70% RH.	X								
L6. Redesign and install HVAC system with proper heat and humidity controls.							X		
L7. Insulate ceiling of emergence room with spray foam (Fig. 1).				X					
L8. Upgrade chilling system to a higher capacity.				X					
L9. Install emergency generator.							X		
L10. Design and construct warehouse.							X		
L11. Limit tower height to 50 tower trays.					X	X	X		
L12. Monitor voltage of fan system on towers (Fig. 2).				X					
L13. Standardize construction of aluminum tower trays.		X	X	X	X	X	X	X	
L14. Construct tray spacers of non-absorbent materials		X	X	X	X	X	X	X	
L15. Implement the use of mechanical blenders to mix the boiling diet and pumps with the dosimeter to transfer the hot emulsion to fiberglass trays (Fig. 3).			X	X	X	X	X	X	
L16. Complete installation of diet mixing equipment (Fig. 3).							X		
L17. Upgrade tower tray washer (Fig. 4).				X					
L18. Evaluate the impact of the length of time pupae are held under hypoxia on field competitiveness of the sterile males.	X								
L19. Standardize handling of pupae by designating equipment type; uniform densities (adults per unit of surface area); food type; holding temperature and RH; and semiochemical and hormone treatments.	X								
L20. Replace knockdown hoppers metal bars with tygon-covered bars (Fig. 5).				X	X	X	X	X	
L21. Remove unused equipment from work areas.				X					
L22. Evaluate and upgrade sanitation procedures.	X								
L23. Assess microbial contamination within the ERF.L22. Include single and twin engine aircraft in bids for aerial release contracts.	X								

Location/Buildings/Process (continued)

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
L24. Include single and twin engine aircraft in bids for aerial release contracts.	X								
L25. Convene a technical panel to determine the optimal aerial release equipment for program use.	X								
L26. Evaluate distance to release site vs. deadhead without costs.	X								
L27. Validate the three-hour release window concept based on quality vs. time.	X								
L28. Investigate conditions affecting the seasonal quality of sterile flies.				X					
L29. Investigate conditions affecting the low trap-back of sterile Medfly males in the Miami releases.				X					
L30 Review Mexfly PRP release densities to determine if increase is needed.					X	X	X		
L31. Convert to the tower emergence system as soon as possible.									X
L32. Release Mexfly at 7 days of age so that sexual maturity has been reached.					X	X			X
L33. Release Medfly at 3 days of age.								X	X

Quality Control

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
QC1. Compile and update QC manuals with special emphasis on post-knockdown and post-release quality.	X								
QC2. Implement Longevity under Stress test at all steps in the packing and release of sterile males.	X								
QC3. Establish the Longevity under Stress test with water but without food as an indicator of nutrient reserves of the sterile males.	X								
QC4. Establish post-release QC evaluation protocols.	X								
QC5. Evaluate post-release flight QC of adult flies remaining in release boxes.	X								
QC6. Compare the mating competitiveness of sterile males to that of wild males a minimum of once per year.	X								
QC7. Determine the biotic and abiotic factors influencing the male biased sexual ratio in Mexfly observed at Reynosa.					X		X		
QC8. Conduct QC evaluations at the ERFs in compliance with the established protocols (FAO/IAEA/USDA 2003, FAO/IAEA 2007).	X								
QC9. Evaluate the potential use of a plastic sealing machine for pupae bags to assure the desired uniform, high level of epoxy and humidity (Fig. 6).	X								
QC10. Move pupae from bags to trays and place in cool room upon receipt (Fig. 7).	X								
QC11. Conduct a full evaluation of the irradiation dose vs. adult fly sterility for Medfly and Mexfly.	X								
QC12. Standardize the reporting of irradiation dose.	X								
QC13. Standardize the amount of dye added to pupae as an external marker.	X								

Adult Diet Formulations and Supplements

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
A1. Implement quality control of adult fly diet ingredients to insure the overall quality and consistency of diet ingredients received from the manufacturer.	X								
A2. Optimize the type and concentration of preservatives in wet diets at each ERF based on the diet pH and the type of microorganisms present (Fig. 8).		X		X	X	X	X	X	X
A3. Evaluate agars to minimize costs, leaking, and growth of microorganisms (Fig. 9, 12).		X		X	X	X	X	X	X
A4. Assess recycling of agar blocks by re-boiling vs. discarding it (Fig. 10, 11).		X		X	X	X	X	X	X
A5. Evaluate cost-effective replacements for agar in the adult fly diet (Fig. 12).		X		X	X	X	X	X	X
A6. Assess microbial contamination of the adult diet (Fig. 8, 9).		X							X
PROTEIN SUPPLEMENTS									
A7. Include protein in *Anastrepha* adult fly diet in order to increase sterile male competitiveness. This would represent an important improvement in most ERFs					X	X	X		
A8. Continue to evaluate the use of protein in the adult diet of Medfly.		X	X	X				X	X
A9. Optimize the type and amount of protein in adult fly diets based on the nutritional requirements and performance of the sterile males.	X								
A10. Determine more efficient methods for providing dry protein.	X								
A11. Assess overall performance of Mexfly with different types and amounts of protein in the dry adult fly diet used in Montemorelos, Victoria, and Sinaloa (Fig. 13, 14).					X	X			X
HORMONE SUPPLEMENTS									
A12. Establish QC evaluations of semiochemical and hormonal supplements.	X								
A13. Obtain OSHA clearance for use of methoprene in the ERFs.	X								
A14. Develop a cost-effective operational formulation for methoprene delivery.				X	X				X
A15. Implement methoprene additives to the sterile Mexfly diet.				X	X	X			X
A16. Reynosa and Tijuana ERFs should interact with Montemorelos, Victoria, Sinaloa, and Zacatecas ERFs.						X			X

Adult Diet Formulations and Supplements (continued)

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
SEMIOCHEMICAL SUPPLEMENTS									
A17. Implement GRO aromatherapy in the new Tapachula ERF (Fig. 15).			X						
A18. Assess the effect of the holding room volume on application of GRO.		X	X	X				X	
A19. Assess the effect of age and duration of treatment on aromatherapy treatment efficacy.		X	X	X				X	
A20. Assess the interaction of GRO aromatherapy and the increased requirement for protein supplementation in sterile Medfly males.		X	X	X				X	
A21. Support basic research on *Anastrepha* spp. to identify semiochemicals with similar aromatherapy effects on males.					X	X	X		X
DIET PREPARATION									
A22. Reduce the volume of waste paper by providing protein and sugar as dry cakes, without paper, for adult flies (Fig. 14).			X						
A23. Produce the adult fly diet on the same day that it is used.	X								
A24. Develop formulations for separate delivery of adult fly diet and water.	X								
A25. Evaluate the suitability of dry protein and sugar cakes as a diet delivery method in Tapachula.			X						
QUANTITY AND SURFACE AREA OF DIET									
A26. Optimize adult fly diet delivery, the amount of diet, and feeding surface provided per fly for Medfly and Mexfly, based on the holding period and the ratio of adult flies to available diet.	X								
A27. Increase the feeding surface available to sterile flies by presenting the same amount of food in two separate units (Fig. 16).	X								
A28. Increase the amount of diet provided or reduce the number of pupae per unit to account for long holding times (Fig. 16).				X	X	X			X
A29. Increase the number of diet blocks in the lower trays in Los Alamitos.								X	
DIET AND RESERVES MEASUREMENT									
A30. Determine the basis of the large variation in Mexfly pupae to adult fly development within towers and trays.					X	X	X		
A31. Reduce tower height in ERFs with the capacity to do so.				X	X	X	X	X	
A32. Establish QC protocols at all ERFs to evaluate absolute flyers and longevity with water but without food after the fly emergence, feeding, chilling and release process.	X								
A33. Determine and correct the cause(s) of flies sticking to the screens of emergence tower trays.								X	

Operational Costs

	ALL	REU	TAPACHULA	SARASOTA	EDINBURG	HARLINGEN	REYNOSA	LOS ALAMITOS	TIJUANA
OC1. Continuously analyze potential ways to improve cost efficiencies in all phases of emergence and release operations.	X								
AERIAL RELEASE CONTRACTS									
OC2. Analyze the feasibility of using single engine instead of twin-engine aircraft. This should be implemented at the next contract renewal at locations currently using twin-engine equipment.	X								
OC3. Maximize the size of loads to reduce the number of flight hours (including ferry time) needed to release sterile flies at proper densities.	X								
OPERATIONS MANAGEMENT									
OC4. Take advantage of potential savings from volume pricing on contracts, especially for aerial release contracts.	X								
OC5. Take advantage of reduced prices with bulk purchase contracts.	X								
OC6. Reduce the use of Jackson traps, without reducing recapture monitoring effectiveness.								X	
OC7. Adjust release densities in lower-risk blocks, or stop releases completely.	X								
LOS ALAMITOS INFRASTRUCTURE									
OC8. Cooperators should again review the proposal for a new facility at Los Alamitos. The study should highlight potential cost savings in labor and maintenance with a new facility.								X	

8 References Cited

FAO/IAEA/USDA. 2003. Manual for product quality control and shipping procedures for sterile mass-reared Tephritid fruit flies. Version 5.0. International Atomic Energy Agency, Vienna, Austria. 85pp.

FAO/IAEA. 2007. Guidance for packing, shipping holding and release of sterile flies in area-wide fruit fly control programmes. Version 1.0. International Atomic Energy Agency, Vienna, Austria.

IPPC. 2005. Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms, International Standard for Phytosanitary Measures No. 3. Apr. 2005. International Plant Protection Convention. 17 Aug. 2007. https://www.ippc.int/servlet/BinaryDownloaderServlet/76047_ISPM_3_E.pdf?filename=1146657660135_ISPM3.pdf&refID=76047.

IPPC. 2007. Glossary of Phytosanitary Terms, International Standard for Phytosanitary Measures No. 5. Mar. 2007. International Plant Protection Convention. 17 Aug. 2007. https://www.ippc.int/servlet/BinaryDownloaderServlet/184195_ISPM05_2007_E.doc?filename=1180102644600_ISPM_05_2007.doc&refID=184195

Kaspi, R., P.W. Taylor, and B. Yuval. 2000. Diet and size influence sexual advertisement and copulatory success of males in Mediterranean fruit fly leks. Ecol. Entomol. 25: 279-284.

Katsoyannos, B.I., N.T. Papadopoulos, N.K. Kouloussis, and J. Hendrichs. 2005. Effect of citrus peel substances on male Mediterranean fruit fly behaviour. Pp. 13-17. *In* B. N. Barnes (ed.). Proceedings of the 6th international symposium on fruit flies of economic importance. Integ Scientific Publications, Irene, South Africa.

Papadopoulos N.T., B.I. Katsoyannos, N.A. Kouloussis, and J. Hendrichs. 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata*. Ent. Exp. Appl. 99: 253-261.

Papadopoulos N.T., T.E. Shelly, N. Niyazi, and E. Jang. 2006. Olfactory and behavioral mechanisms underlying enhanced mating competitiveness following exposure to ginger root oil and orange oil in males of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). J. Insect Behav. 19:403-418.

Pereira, R. 2005. Influence of a juvenile hormone analog and dietary protein on male Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae), sexual behaviour. Dissertation. U. Florida, Gainesville, Florida, USA.

Perez-Staples, D., V. Prabhu, and P.W. Taylor. 2007. Post-teneral protein feeding enhances sexual performance of Queensland fruit flies. Physiological Entomology 32: 225-232

Shelly, T.E. 2001. Exposure to a-copaene and a-copaene-containing oils enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc.. 94: 497-502.

Shelly, T.E. and D.O. McInnis. 2001. Exposure to ginger root oil enhances mating success of irradiated mass-reared males of Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 94: 1413-1418.

Shelly, T.E., A.S. Robinson, C. Caceres, V. Wornoayporn, and A. Islam. 2002. Exposure to ginger root oil enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae) from genetic sexing strain. Fla. Entomol. 85: 440-445.

Shelly, T.E., S.S. Kennelly, and D.O. McInnis. 2002. Effect of adult diet on signalling activity, mate attraction, and mating success in male Mediterranean fruit flies (Diptera: Tephritidae). Fla. Entomol. 85: 150-155.

Shelly, T.E., D.O. McInnis, E. Pahio, and J. Edu. 2004. Aromatherapy in the Mediterranean fruit fly (Diptera: Tephritidae): sterile males exposed to ginger root oil in pre-release, storage boxes display increased mating competitiveness in field-cage trials. J. Econ. Entomol. 97: 846-853.

Shelly, T.E., J. Edu, and E. Pahio. 2006. Dietary protein and mating competitiveness of sterile males of the Mediterranean fruit fly (Diptera: Tephritidae): measurements of induced egg sterility in large field enclosures. Fla. Entomol. 89: 277-278.

Shelly, T.E. and J. Edu. 2008. Effect of nitrogen-containing dietary supplements on the mating success of sterile males of the Mediterranean fruit fly (Diptera: Tephritidae). Fla. Entomol. 81: 394-399.

Shelly, T.E., M. War, and A. Favela. 2008. Exposing entire adult holding rooms containing sterile male Mediterranean fruit flies to orange oil increases the mating success of those males in field cage trials. Fla. Entomol. 91: 686-689.

Taylor P.W. and B. Yuval. 1999. Postcopulatory sexual selection in Mediterranean fruit flies: advantages for large and protein-fed males. Anim. Behav. 58: 247-254.

Teal P.E.A. and Y. Gómez-Simuta. 2002. Juvenile Hormone: Action in regulation of sexual maturity in Caribbean fruit flies and potential use in improving efficacy of sterile insect control technique for Tephritid fruit flies. IOBC-WPRS Bull. 25:167-180.

Teal P.E.A., Y. Gómez-Simuta, and A. T. Proveaux. 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. Proc. Nat. Acad. Sci. 97: 3708-3712.

USDA. 2007. National Exotic Fruit Fly Trapping Protocol. 80pp.

Yuval, B., R. Kaspi, S. Shloush, and M. S. Warburg. 1998. Nutritional reserves regulate male participation in Mediterranean fruit fly leks. Ecol. Entomol. 23: 211-215.

Yuval, B., R. Kaspi, S. A. Field, S. Blay, and P. Taylor. 2002. Effects of post-teneral nutrition on reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Fla. Entomol. 85: 165-170.

Yuval, B., M. Maor, K. Levy, R. Kaspi, P. Yaylor, and T. Shelly. 2007. Breakfast of champions or kiss of death? Survival and sexual performance of protein-fed, sterile Mediterranean fruit flies (Diptera: Tephritidae). Fla. Entomol. 90: 115-122.

A Appendix A: Definitions, Abbreviations, and Acronyms

APHIS. Animal and Plant Health Inspection Service

ARS. Agricultural Research Service

CDFA. California Department of Food and Agriculture

ERF. Emergence and Release Facility

FSO. Foreign Service Officer

GRO. Ginger root oil

HVAC. Heating, ventilation, and air conditioning system

IS. International Services

MAGA. Ministerio de Agricultura, Ganadería y Alimentación (Guatemala)

Medfly. Mediterranean fruit fly, *Ceratitis capitata* Wiedemann

Mexfly. Mexican fruit fly, *Anastrepha ludens* Loew

MOSCAMED. Joint United States, Mexico, and Guatemala control program to maintain a barrier in Chiapas, Mexico to prevent the northern spread of the Mediterranean fruit fly

OSHA. Occupational Safety and Health Administration

PPQ. Plant Protection and Quarantine

Preventive Release Program (PRP). The prophylactic use of SIT, in an area where the risk of entry of a non-indigenous fruit fly into a free area is high, to thwart any entries of the target fruit fly from becoming an established population.

RH. Relative humidity

SAGARPA /SENASICA. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (México) / Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria

Sterile Insect Technique (SIT). Method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species [ISPM No. 3, 2005]

TAC. Technical Advisory Committee for MOSCAMED program

UMT. Unified Management Team for MOSCAMED program

USD. United States Dollar

USDA. United States Department of Agriculture

B Appendix B: Photo Documentation

FIGURE 1: Insulate the ceiling of emergence rooms to better maintain temperatures and conserve Energy (L7).

FIGURE 2: The voltage of fan systems on towers requires monitoring (L12). Towers with fans are unsuitable where electricity is costly or unreliable; or if emergency generators are unavailable

FIGURE 3: Using mechanical blenders for adult diet preparation maintains the emulsion in continuous motion within the kettle, simplifies the process, and reduces labor time (L15, L16). Pump the liquid from the kettle directly into the fiberglass trays instead of using buckets to pour to reduce risk of serious injury to workers.

FIGURE 4: Upgrade tray washers to incorporate new technologies (L17).

FIGURE 5a: The knockdown hopper has a solid metal bar that can damage the aluminum tray.

FIGURE 5b: Remove flies from the screens by hitting the aluminum trays against the metal bars.

FIGURE 5c: Use of wire covered with tygon tubing reduces the damage to aluminum trays (L20).

FIGURE 6: Hypoxia is critical to reduce irradiation-induced damage during the sterilization process. Use a sealing machine to close plastic pupae ba, and standardize the post-sealing time to assure the desired level of hypoxia and humidity (QC9)

FIGURE 7: To avoid pupal overheating at reception and during processing, remove them from the packing boxes (left), and place them in trays in a cool room (right) (QC10).

FIGURE 8: Optimize the type and concentration of preservatives in the adult diet based on the pH and type of microbes present (A2, A6).

FIGURE 9: Evaluate agars to minimize costs, leaking, and microbial growth (A3, A6).

FIGURE 10: Evaluate the potential to recycle agar blocks to reduce costs and waste (A4).

FIGURE 11: Agar block in good condition after removal from trays (A4).

FIGURE 12: Evaluate different types of agars and cost-effective replacements for agar in the adult diet (A3, A5).

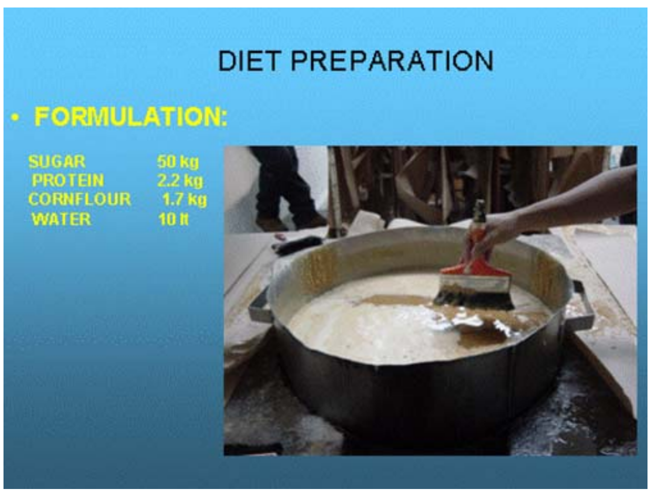


FIGURE 13: Mexfly adult diet formulation used in Tapachula (A11).

FIGURE 14: Mexfly adult diet spread onto paper and dried (A11), which generates a large amount of waste paper (A22).

FIGURE 15: Use ginger root oil (GRO) to improve the mating competitiveness of male Medfly. Implement GRO aromatherapy in all Medfly ERFs (A17).

FIGURE 16: Place adult diet on the tower tray during the pupae loading process. Optimize the amount of diet and feeding surface per fly based on the requirements of the species (A27). Increase the feeding surface by presenting the same amount of diet in two separate units (A28).

Acknowledgements

Mike Arbogast, PPQ Supervisor
Medfly Preventive Release Program
United States Department of Agriculture
Animal and Plant Health Inspection Service
3802 Constitution Ave.
Los Alamitos, CA USA

Eduardo Baltazar, Director
Medfly Preventive Release Program
California Department of Food and Agriculture
3802 Constitution Ave.
Los Alamitos, CA USA

Edward Cusano, Supervisory PPQ Officer
SIT/PRP Sterile Insect Release Program
United States Department of Agriculture
Animal and Plant Health Inspection Service
915 10th Street East
Palmetto, FL 34221 USA

Gary Greene, Regional Director
Moscamed Program
International Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
4a. Avenioda 12-62 Zona 10
Guatemala City, Guatemala City, 01010 C.A.

Susan McCombs, National Bactrocera Coordinator
United States Department of Agriculture
Animal and Plant Health Inspection Service
300 Ala Moana Blvd., Rm 8-120
Honolulu, HI 96850 USA

Terry McGovern, Assistant Regional Director
Moscamed Program
International Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
4a. Avenioda 12-62 Zona 10
Guatemala City, Guatemala City, 01010 C.A.

Pamela Moreno, Program Coordinator
Mexican Fruit Fly Emergence Facility
United States Department of Agriculture
Animal and Plant Health Inspection Service
International Services
Carretera Matamoros Km 79 Brecha 102
Reynosa, Tamaulipas 88501 México

Jose Manuel Ponciano, Facility Director
Programa Moscamed
Estacion Puca
Calzada las Palmas Zona 6
Retalhuleu, Guatemala C.A.

John Renshaw, Facility Director
SIT/PRP Sterile Insect Release Facility
United States Department of Agriculture
Animal and Plant Health Inspection Service
1833 57th Street
Sarasota, FL 34243 USA

Alejandro Rios, Coordinator
Mexican Fruit Fly Emergence Facility
United States Department of Agriculture
Animal and Plant Health Inspection Service
International Services
Carretera Matamoros Km 79 Brecha 102
Reynosa, Tamaulipas 88501 México

Moises Romero, Quality Control Head
Tapachula Release Facility
Programa Moscamed
Tapachula, México

Lucy Tirado Palomeque, Facility Director
Tapachula Release Facility
Programa Moscamed
Tapachula, México

John Worley, Facility Director
USDA APHIS PPQ
Mexican Fruit Fly Rearing Facility
Moore Air Base, Bldg. 6418
22675 North Moorefield Rd.
Edinburgh, TX 78541 USA

Sergio Sanchez, Facility Director
Fruit Fly Release Facility
International Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
Tijuana, México

Rebecca Frasure, Editorial Assistant
USDA APHIS PPQ RPM
92 Thomas Johnson Drive, Suite 200
Frederick, MD 21702 USA