United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs

Final Environmental Impact Statement—October 2008

Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs

Final Environmental Impact Statement—October 2008

Agency Contact:
David A. Bergsten
Biological Scientist
Policy and Program Development
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 149
Riverdale, MD 20737–1238

Table of Contents

Appendices

Tables

Figures

Executive Summary

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), in cooperation with several States and foreign countries, is proposing further development of genetically engineered fruit fly species and pink bollworm for use in various applications of the sterile insect technique (SIT) applied to agency invasive plant pest control programs. There have been laboratory and confined field studies to test the efficacy of certain genetic engineering applications that could provide benefits to these programs, but these techniques have not been applied in agency eradication actions or preventive release program (PRP) strategies.

The plant pests selected for inclusion in this environmental impact statement (EIS) are the pink bollworm (Lepidoptera: Gelechiidae) and three species of tephritid fruit flies (Mediterranean, Mexican, and oriental). These species were selected based upon their ongoing threat to U.S. agriculture and the basic research already conducted to develop genetically engineered strains that can be adapted for use in SIT applications for APHIS' control programs. Although APHIS has existing eradication and PRPs that utilize radiation-sterilized insects for two of these species, the use of genetically engineered insects under consideration applies traits that would provide improved production and quality assurance for separation of sterile insects from wild-type insects through the use of genetic markers for field and facility monitoring, reducing the quantity of insect production through male-only fruit fly mass-rearing, and inducing sterility in released insects without the need for exposure to radiation which damages insects and reduces the mating ability and sexual competitiveness of the insects being released. The actions being considered in the preferred alternative of this EIS provide APHIS plant pest programs with new and potentially more cost-effective methods for SIT. These methods can result in substantial reductions in operating costs and improved efficiency for the ongoing fruit fly and pink bollworm control programs. This would also benefit APHIS by making more effective use of the limited personnel and limited space available at insect rearing facilities for these programs.

The area impacted by the program actions associated with this EIS is limited to those sites within the host ranges of the pest species. The release of sterile insects in this program would diminish the risk of adverse effects to American agriculture from these species. Although the host plants of the pink bollworm are limited to cotton and okra and only in the dry climates of the Southwest, some of the fruit flies have more than 200 susceptible host plants and agricultural crops that occur in both humid and dry climatic areas. The eradication of pink bollworm has progressed to the point where most program applications are likely to be limited to

Arizona, California, and Mexico; however, maintenance of a sterile insect colony for potential use in new introductions or mass releases to prevent infestations (PRPs) is desirable. Most fruit fly outbreaks are expected to occur in California, Florida, and Texas where the most immediate advantages of applications of genetically engineered SIT are anticipated. Nevertheless, future applications for potential oriental and Mediterranean fruit fly eradication efforts in Hawaii and ongoing cooperative control efforts in several foreign countries would also benefit from the use of this technology. The biological fitness of genetically engineered fruit flies and pink bollworm designed for SIT applications in these diverse geographic locations is an important aspect of the successful and environmentally safe use of these insects, and their performance-fitness factors will be assessed for each individual genetic construct or genetically engineered strain before release is considered.

APHIS has an extensive history of using radiation-induced SIT to aid in the timely control and eradication of pest outbreaks. The use of genetic engineering to improve the effectiveness of SIT as a control measure and to minimize program impacts to the environment is a strategic decision that takes advantage of this new technology. Genetically engineered fruit flies and pink bollworm can augment SIT in present control programs by producing: (1) mass-rearing of either male and female or only male fruit flies with a marker gene and are sterilized by radiation exposure and produce practically no offspring, (2) genetically sterilized male-only fruit flies that have a marker gene and that compete more effectively for mates than radiation-sterilized fruit flies, (3) fruit flies that produce only male offspring which carry a heritable sterility gene resulting in only male and no female offspring in the field, thus controlling pest fruit flies through rapid population reduction, (4) mass-rearing of male and female pink bollworm that have a marker gene and are sterilized by radiation before field release, and (5) mass-rearing of male and female pink bollworms that are genetically sterile without radiation exposure and with males that are more competitive in mating with wild female bollworms than radiation-sterilized male bollworms.

The issues regarding the application of this new technology to SIT by APHIS were scoped through a public comment process cited in our notice of intent to prepare this EIS in the *Federal Register* (71 *Federal Register* (FR) 20068–20069, Docket No. APHIS–2006–0166) on December 19, 2006. Comments and information on potential alternatives and substantive issues were provided by the general public, industry, academic, regulatory, and public interest groups. In addition, comments were received during the public comment period on the draft EIS announced by the U.S. Environmental Protection Agency on May 30, 2008 (73 FR 31115, Docket No. ER–FRL–6699–3). This EIS addresses

public comments in appendix E and within the text covering the given issue or alternative.

There are three alternatives and their associated components analyzed comprehensively in this EIS. These alternatives are broad in scope and reflect the need of the program to objectively address potential control and eradication of damaging fruit fly species and the pink bollworm. The alternatives for the use of genetically engineered insects in SIT of APHIS' pest control programs include: (1) no action, (2) expansion of existing programs, and (3) integration of genetically engineered insects into programs (the preferred alternative). The alternatives are presented in a manner that explains the environmental issues and the choices to be made regarding the inclusion or exclusion of insects with specific traits in APHIS' SIT programs. This programmatic EIS is also designed to establish criteria for future decisions regarding use of the genetic engineering technology and to identify the potential impacts to address when documenting these decisions.

The potential consequences from each of the three alternatives have some environmental impacts of concern. The greatest potential impacts occur with the no action alternative, in that potential pest risks are not static and continue to increase with expanding trade and travel. Expansion of existing programs could occur to accommodate the growth in trade and travel, but this expansion is not the most effective or most efficient means to improve control program performance. Although the types of actions in the expansion of existing programs alternative do not differ from those under the no action alternative, their context and magnitude would be expected to increase the species, locations, and size of programs. The preferred alternative of this EIS, integration of genetically engineered insects into programs, incorporates potential impacts of the other two alternatives to the extent that the technology of genetic engineering is not applied independent of other available control methods. The other environmental impacts may also be modified by the degree to which the use of genetically engineered insects—

(1) decrease the need for actions involving insecticide applications,

(2) decrease the need to produce both male and female insects for use in SIT releases,

(3) increase production of males that are more competitive in mating than radiation-sterilized males, and

(4) eliminate the need to use, operate, and maintain strong gamma radiation sources.

Many of the issues of concern from the public comments are also issues of concern to the program, in that genetically engineered traits of insect strains must be maintained and restricted to the mass-reared insects used in SIT to ensure their ongoing effective use in control programs. This requires attention to issues such as the biosecurity of facilities, establishment and adherence to facility containment requirements, comprehensive testing of performance-fitness factors of the reared insects, establishment of effective filters to maintain the desired genetic phenotype within rearing colonies, and monitoring of the facilities and the SIT–release practices for quality assurance. Just as certain measures are already built into our present programs, APHIS will be establishing standard operating procedures and mitigation measures for application of genetic engineering technology to SIT in specific control programs to ensure that potential applications are not compromised. This will require extensive monitoring of strain effectiveness, particularly for new strains that have not been used previously in SIT releases. Incorporation of this new technology into program operations will require an extended commitment by the agency and its cooperators to ensure that pest control expectations are fulfilled.

Review of the pertinent environmental laws and statutes is presented in this EIS on a programmatic basis. Although the findings related to the requirements cover the most likely issues of concern, the application-specific environmental assessments that tier to this programmatic EIS should revisit the issues discussed and any new effect that results from applications or extensions of this technology. The environmental issues of concern are likely to change over time. For example, this EIS arrives at a no effect determination concerning threatened and endangered species and their critical habitat in its assessment completed for compliance with the Endangered Species Act. However, new species of plants and animals are being proposed and added to the lists, so the biological determination will need to be updated to cover any changes. Likewise, other laws, statutes, and executive orders are subject to confirmation that conditions have not changed, and any new issues of concern will need to be addressed.

The rationale to support the preferred alternative relates to the expected potential benefit to the programs and American agriculture from application of this new technology. In particular, its applications to SIT, as described in this programmatic EIS, provide potential for increased program effectiveness with low potential for adverse environmental impacts. Selection of the preferred alternative does not necessarily allow control programs to immediately employ the new technology, but does provide a basis for future decisions about potential environmental impacts for specific uses of genetically engineered strains in SIT that can be tiered to the findings from this EIS. This alternative will assist in facilitating implementation of the technology by providing procedures for application-

specific evaluations of genetically engineered plant pests used to improve SIT release programs.

(This page is intentionally left blank.)

I. Purpose and Need

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) is proposing action as part of an ongoing effort to continually improve the effectiveness of agency invasive plant pest control programs. The increased frequency of travel and trade in recent years has resulted in increased detection of nonnative, invasive pests. The increased introduction rate of these pests, the development by the pests of resistance to conventional chemical control measures, and the commitment to develop and use alternative integrated pest management (IPM) measures have made effective use of sterile insect technique (SIT), an increasingly important component of present suppression, eradication, and exclusion programs at APHIS. SIT involves the release of pest insects that have been artificially mass-produced and then sterilized by gamma radiation so that no offspring result from mating with wild insects in the field, which then provides pest population suppression. It has been used successfully and/or developed as a control method for pink bollworm, Mediterranean fruit fly (Medfly), Mexican fruit fly, oriental fruit fly, Caribbean fruit fly, Queensland fruit fly, and the melon fly. Although APHIS could continue to rely on present methods (the no action alternative in this environmental impact statement (EIS)) and expansion of existing methods (the second EIS alternative), the effectiveness and efficiency of extending the existing methods eventually places the program in a position where costs, personnel, and rearing facilities reach their limits for feasibility. The actions being considered in this document may provide APHIS plant pest control programs with potentially new and more cost-effective methods for sterile insect production, male-only production, and the use of genetic markers that may result in substantial reductions in operating costs for fruit fly and pink bollworm control programs. This would benefit APHIS due to the limited personnel and the constraints on growth of rearing SIT facilities that presently exist.

A. Introduction

Although APHIS could have selected any of a number of pest species for analysis in this programmatic EIS, the potential development of useful genetically engineered strains for control purposes is presently limited to only a few species, mainly due to the available state of the technology. The costs, time, and effort involved in creating and maintaining a colony of insects useful for SIT are of such magnitude that it can be foreseen that only a few major pests are likely to be developed within the next decade. The selection of the pink bollworm (Lepidoptera: gelechiidae: *Pectinophora gossypiella*) and fruit flies (Diptera: tephritidae) as plant pests to document in this EIS was based upon their ongoing threat to U.S. agriculture and the basic research already conducted to develop strains that could be adapted for use in APHIS' SIT and other control programs.

There are six genera of fruit flies that represent a major threat to the agricultural resources of the United States. The genera are the following: *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, *Rhagoletis*, and *Toxotrypana*. Because of their wide host ranges, their abilities to become established or to become more widespread, their potential economic impacts, and their potential ecological impacts (both direct and indirect), species in those genera have been subject to strict quarantines and comprehensive control programs. A brief description of each of the more than 80 fruit fly species subject to control actions was provided in table I–2 of the Fruit Fly Cooperative Control Program Final EIS (USDA–APHIS, 2001a). The 2001 EIS does not provide a detailed analysis of all of those species; however, it selectively analyzes the development of strains of a few representative species. The three representative fruit fly species and the reasons for their more detailed analysis in this EIS are described later in this chapter in section C, where the scope and focus are presented.

1. History of Infestations

a. Mediterranean Fruit Fly

The Mediterranean fruit fly (*Ceratitis capitata*), commonly called Medfly or Moscamed in Spanish, originated in Africa. It has since spread throughout the Mediterranean region, southern Europe, the Middle East, western Australia, South and Central America, and Hawaii. In general, it is found in most tropical and subtropical areas of the world. The Medfly became established in Hawaii in 1910. The first U.S. mainland infestation occurred in Florida in 1929. Several infestations have occurred on the mainland since then; however, State and Federal eradication programs in California, Florida, and Texas have prevented it from becoming established.

The Medfly is a major pest of agriculture throughout many parts of the world. Because of its wide host range (over 250 species of fruits and vegetables) and its potential for damage, the Medfly represents a serious threat to U.S. agriculture. Although it has been introduced intermittently to the U.S. mainland several times since its first introduction in 1929, eradication programs have been implemented to prevent it from becoming a permanent pest on the U.S. mainland.

The Medfly is one of the world's most destructive agricultural pests. The female Medfly attacks ripening fruit, piercing the soft skin and laying eggs in the puncture. The eggs hatch into larvae (maggots), which feed inside the fruit pulp. In the United States, the Medfly could attack peaches, pears, plums, apples, apricots, avocados, citrus, cherries, figs, grapes, guavas, kumquats, loquats, nectarines, peppers, persimmons, tomatoes, and several nuts.

Mediterranean Fruit Fly Cooperative Eradication Programs have been initiated in several States (e.g., California) where Medfly has been detected. In such cases, APHIS and the State departments of agriculture have proposed cooperative programs to eradicate the Medfly infestations. These cooperative programs involve the use of IPM to eradicate Medfly. Specifically, the integrated program allowed the option for use of the following methods: chemical control, sterile insect technique, physical control, cultural control, and regulatory control.

b. Mexican Fruit Fly

The Mexican fruit fly, *Anastrepha ludens* (Loew), is indigenous to Mexico, and is also found in Central America and northern South America. The first detection of Mexican fruit flies in the United States was in 1927, in the Lower Rio Grande Valley of Texas, and resulted in a cooperative program with Mexico designed for control and exclusion. The Mexican fruit fly has since spread into the cultivated citrus sections of the west coast of Mexico, Arizona, and California, resulting in ongoing detection, survey, and eradication campaigns in these areas.

The Mexican fruit fly is a very serious pest of various fruits, particularly citrus and mango. Its natural distribution includes the Rio Grande Valley of Texas, where populations routinely attain pest status if control measures are not practiced. It is a frequent invader in southern California. Mexican fruit fly represents a particular threat to Florida because of its special affinity for grapefruit, of which Florida and the Rio Grande Valley are among the world's leading producers.

Mexican fruit fly larvae are widely transported in infested fruits. In 2003, live larvae were found in Pinellas County, Florida, in manzano peppers that originated in Mexico; however, the discovery of adults in Florida has been surprisingly rare. A single specimen was detected in a multi-lure trap in Orlando in 2003; an extensive survey program yielded no further specimens. Similarly, a single fly was captured in a McPhail trap in Sarasota, Florida, in 1972 (Clark et al., 1996).

Mexican Fruit Fly Cooperative Eradication Programs have been initiated in several States, most recently California and Texas, where Mexican fruit fly has been detected. In such cases, APHIS and the State department of agriculture have proposed cooperative programs to eradicate the Mexican fruit fly infestations. These cooperative programs involve the use of IPM to eradicate fruit fly. Specifically, the integrated program allowed the option for use of the following methods: chemical control, sterile insect technique, physical control, cultural control, male annihilation by use of selective male insect attractants (i.e., methyl eugenol) combined with insecticides or placed in insect traps, and regulatory control.

c. Oriental Fruit Fly

The oriental fruit fly, *Bactroceria dorsalis* (Hendel) (synonym = *Dacus dorsalis* Hendel), has been established in Hawaii since 1948, and damages every commercial fruit crop grown there. Eradication programs have prevented the establishment of oriental fruit fly in the U.S. mainland, where it has been introduced a number of times since 1960. Oriental fruit fly has been detected every year in California since 1966. Because of the species' rapid population growth and potential for damage, a prompt response is desired to contain and eradicate any infestation found in the U.S. mainland. Oriental fruit fly has been introduced into California a number of times through the movement of infested fruits and vegetables into the State. The first eradication project occurred in San Diego in 1974 and, since that time, numerous major infestations have been delimited and successfully eradicated.

Oriental fruit fly is a destructive agricultural pest in many parts of the world. The female has a pointed slender ovipositor to deposit eggs under the skin of host fruit. Eggs are minute cylinders laid in batches. The maggots (larvae) are creamy-white, legless, and may attain a length of 10 millimeters inside host fruit.

Oriental fruit fly has been established in Hawaii since 1946, where it is a major pest of agriculture, particularly on mangoes, avocados, and papayas. Oriental fruit fly also attacks a wide variety of fruits, nuts, vegetables, and berries. Maggots have been found in over 125 kinds of fruit and vegetables in Hawaii alone. A great number of crops are threatened by the introduction of this pest including pears, plums, cherries, peaches, apricots, figs, citrus, tomatoes, and avocados.

Oriental Fruit Fly Cooperative Eradication Programs have been initiated in several States, most recently California, where oriental fruit fly has been detected. In such cases, APHIS and the State departments of agriculture have proposed cooperative programs to eradicate oriental fruit fly infestations. These cooperative programs involve the use of IPM to eradicate the fruit fly. The chemical treatments involved in this integrated approach include soil drenches, foliar sprays, and fruit fly male annihilation spot treatments.

Although Medfly, Mexican fruit fly, and oriental fruit fly infestations are the focus of this EIS, other fruit fly infestations occur and result in crop damage and subsequent monetary losses; however, Medfly, Mexican fruit fly, and oriental fruit fly detections have a higher frequency, broader host range, and pose a greater economic loss to U.S. agriculture than other fruit fly species. In addition, the methods used to eradicate and control infestations of these fruit flies have been developed and proven to be

effective. Considerable work has already been applied to the development of genetically engineered strains for use in SIT against Medfly, Mexican fruit fly, and oriental fruit fly. Thus, these three fruit flies are the selected species of concern in this EIS.

d. Pink Bollworm

The pink bollworm, *Pectinophora gossypiella* (Saunders) was described from larvae recovered from infested cotton bolls in India in 1843 (Noble, 1969). It has become one of the most destructive pests of cotton in many of the major cotton-growing regions of the world. One of the earliest reports of cotton infestations occurred in 1911, in Mexico, presumably from Egyptian cotton seed shipments (Glick, 1967). In the United States, pink bollworm was detected first in Robinson County, Texas, in 1917 (Scholl, 1919). By 1926, the pest had spread from Texas through New Mexico and into eastern Arizona, and had become a major economic pest of cotton in Arizona and southern California by 1965 (Burrows et al., 1982). A 2-year pink bollworm adult detection survey was conducted in Arkansas, Louisiana, Oklahoma, Texas, and New Mexico in 2000 and 2001. Preliminary analysis indicated that no pink bollworm were present in Arkansas, Louisiana, Oklahoma, and most of Texas. Survey results indicated that pink bollworm populations were confined to west Texas and south-central New Mexico. This was confirmed through additional trapping surveys in 2002 through 2004. Trapping surveys conducted in Arizona by the Arizona Cotton Research and Protection Council, and in California by the Imperial Valley Commissioner of Agriculture and California Department of Food and Agriculture (CDFA) continue to indicate wide distributions of pink bollworm in the entire State of Arizona and southern California. The National Cotton Council estimates that U.S. cotton producers' annual losses to pink bollworm are $32 million due to prevention, control costs, and lower yields due to plant damage.

(1) El Paso/Trans Pecos Pink Bollworm Cooperative Eradication Program

In 2001, APHIS initiated participation in a cooperative program among growers, State and Federal regulators for the objective of eradicating pink bollworm from the El Paso/Trans Pecos area of Texas. This program included (1) mapping, to identify cotton acreages and location; (2) detection, by trapping and visual inspection, to identify sites of infestation; and (3) control, using cultural control, mating disruption (pheromone only, or pheromone with permethrin), transgenic cotton, sterile moth releases, and chemical control (aerial or ground applications of chlorpyrifos).

(2) Southwest Pink Bollworm Eradication Program

In 2002, APHIS extended participation in an integrated pink bollworm eradication program to the entire infested part of the cotton belt and Mexico. This program allowed for economy of effort and the reduction of potential environmental impacts through the coordination and minimization of chemical applications. Operational aspects of the program included: (1) mapping to identify cotton field locations, acreage, and genotypes; (2) detection by trapping and visual inspection; and (3) control using a variety of approved methods. Control for pink bollworm included cultural control (uniform planting and harvesting to provide a necessary host-free period), mating disruption (pheromone only or pheromone with permethrin, depending upon population density), use of the transgenic *Bacillus thuringiensis* toxin-expressing cotton, sterile moth releases, and chemical control (aerial or ground application of chlorpyrifos).

In Chihuahua, Mexico, the 2002 eradication program involved the elimination of a few localized bollworm infestations. The procedures and materials used in the Mexico program were identical to those used in the Southwest Pink Bollworm Eradication Program, which was analyzed in an environmental assessment (EA) in April 2002 (USDA–APHIS, 2002a).

2. Fruit Fly Sterile Insect Technique History

Sterile insect technique (SIT) is a method of biological control of pests using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species (IPPC, 2005; IPPC, 2007). Tephritid fruit fly action programs utilize SIT as both an eradication tool and a prevention tool. USDA supports several continuous sterile fruit fly preventive release programs (PRPs), both domestically and offshore. A fruit fly PRP involves the prophylactic use of SIT. This approach is used to inhibit any entries of the target fruit fly from becoming an established population in areas where the risk of entry of a non-indigenous fruit fly into a free area is high.

The idea of the use of SIT was first envisioned, in 1937, by Dr. Edward F. Knipling, a USDA–Agricultural Research Service (ARS) scientist, when studying the mating habits of screwworm flies (*Cochliomyia hominivorax* (Coquerel)) with a USDA-research colleague, Dr. R.C. Bushland. Their observations revealed that, although male screwworms were aggressive and mated repeatedly, female screwworms appeared to mate only once in their lifetime. This observation was the seed for Dr. Knipling's idea: If large numbers of sterile males could repeatedly be released into wild populations, it would eventually eliminate population reproduction and lead to eradication (Adkisson and Tumlinson, 2003).

The successful eradication campaigns against screwworm triggered research, in Hawaii, concerning the potential usefulness of SIT to combat the three exotic pest fruit flies, Medfly, oriental fruit fly, and melon fly infesting Hawaii. Unlike female screwworms, female fruit flies were found to mate frequently. This initial research found that, although there were unfavorable factors which created problems for the suppression of larger populations of fruit flies, SIT had promise as an eradication tool against isolated fruit fly populations (Steiner and Christenson, 1956). Research continued on the application of SIT for the control of oriental fruit fly and melon fly populations through larger field experiments, conducted by USDA, on the island of Rota in the Mariana Islands, with the cooperation of the U.S. Navy in 1962 (Steiner, Mitchell, and Baumhover 1962).

In 1954, efforts were begun to mass-rear sterilized Mexican fruit flies at a USDA lab in Mexico. Besides the use of radiation to sterilize fruit flies, research was also being conducted, within USDA, on the use of chemosterilants to sterilize Mexican fruit flies (Shaw and Sanchez-Riveillo, 1962). A series of field experiments conducted in 1962 and 1963, in Mexico, showed that when sufficient numbers of chemosterilized Mexican fruit flies were released, to overwhelm the natural population in infested mango groves, a high degree of control was achieved for the main crop (Shaw and Sanchez-Riveillo, 1965).

a. Mediterranean Fruit Fly in California

The first known infestation of Medfly, in California, was detected on September 25, 1975, in the Venice area of Los Angeles County. It was reported that authorities believed the outbreak was due to an accidental importation by a yacht that had visited Central America (Rhode, 1976). After much consternation, program officials from USDA, CDFA, and county authorities, in a cooperative effort, agreed to use the release of sterile Medflies as the primary means of control. Since that time, USDA, CDFA, and the county departments of agriculture in California have continued this cooperation with the use of SIT to prevent and combat Medfly infestations in California. The use of SIT in the Venice area was supplemented with ground applications of a malathion/protein hydrolysate bait spray surrounding larval detection sites. Sterile pupae were shipped to Los Angeles from the USDA–ARS facility in Hawaii, with sterile release stations (static release) being set up on October 20, 1975, for the first release of sterile Medflies in California. This was followed on November 10, 1975, with sterile Medflies being released from bags on the back of pickups (roving release). The first aerial release of sterile Medflies occurred on December 15, 1975. The program was a success with the last fertile Medfly being captured on November 14, 1975, and eradication being declared on August 2, 1976.

On June 5, 1980, two separate Medfly infestations were coincidently detected in California. One infestation was in the Northridge area of Los Angeles County. This infestation was controlled in a similar manner as the infestation in 1975, with a combination of SIT and ground bait spray. The sterile flies were released using a combination of three methods: roving release, static release, and aerial spray. Eradication was declared in the Northridge area on December 18, 1980.

A second infestation, detected on June 5, 1980, was in the San Jose area of Santa Clara County; this eventually spread into 10 other counties, including an associated satellite infestation in Los Angeles County. Over 400 adults were captured, and a large number of larval sites were found. The quarantine area was over 4,000-square miles; the treatment area encompassed almost 1,500-square miles. At the peak of the program, there were over 4,000 State, county, and Federal employees working on the eradication project, including the State of California's National Guard.

SIT was used, initially, as the primary control treatment from June 1980 until July 1981. Sterile Medflies used in the eradication campaign were obtained from several sources including production facilities in Peru, Costa Rica, Mexico, and Hawaii; however, the effective use of SIT was limited because of the need to have continuous adequate release of sterile Medflies over such a large area of infestation, and, the poor reliability of the sourcing of a consistently large number of sterile Medflies. The program suffered from the inconsistent weekly releases of sterile Medflies over the entire treatment area. There was even an attempt to remedy this situation by sending a team to Hawaii to put together, on an emergency basis, a sterile fruit fly rearing facility capable of producing 100 million additional sterile Medflies per week. Although this emergency facility started producing sterile fruit flies in April of 1981, it was hampered by mold contamination in its mother colony which caused a severe reduction in its production capability. Consequently, in July of 1981, the aerial application of malathion bait spray replaced SIT as the primary eradication control treatment. Eradication was finally declared on September 21, 1982, following an outbreak that lasted more than 2 years.

From 1987 through 1994, there were several outbreaks of Medfly in the Los Angeles basin area of California. In 1991, the largest of the outbreaks began with the detection of a mated female in the Country Club Park area of the city of Los Angeles. By 1993, the infestation had grown to include 400 wild Medflies detected in 39 cities in 5 southern California counties, with 35 discrete core infestation areas. The quarantine area included 1,576-square miles; the treatment areas surrounding each core infestation area were merged together. Program officials used SIT as the primary treatment option focusing on each core infestation, coupled with ground bait spray applications. In response to the total size of the infested areas,

and the fact that there were multiple points of infestation within the Los Angeles Basin, USDA formed an international science advisory panel charged with making recommendations for the development of a proactive approach to control the Medfly infestation in the area. The science advisory panel recommended an area-wide approach using SIT continuously for a 2-year period over an area of 1,464-square miles. This recommendation was adopted and resulted in decreases of wild Medfly captures in the area from about 400 in 1993 to none in 1995. Eradication was declared in the area on June 15, 1996.

Based on the success of the area-wide concept, a new, proactive use of SIT was initiated by the establishment of a Medfly PRP in the Los Angeles Basin area on July 10, 1996, in another cooperative effort between CDFA and USDA. The initial program included the eclosion of 450 million sterile pupae, to produce sterile adults, to be evenly dispersed over the Los Angeles Basin. This Medfly PRP has remained in operation since its initiation in 1996, and was expanded from a 2,155-square mile area to the current 2,500-square mile area. Sterile Medflies are supplied to the Medfly PRP in California by two production facilities, a CDFA-Medfly rearing facility in Hawaii and a USDA-Medfly rearing facility in Guatemala. From 1990 until 2002, USDA also operated a Medfly rearing facility in Hawaii to support the Medfly PRP in California; however, this facility was closed in 2002 due to the discovery of a mold that created an unhealthy working environment. Today the sterile eclosion and release facility located in Los Alamitos, California receives, incubates, and emerges over 350 million sterile pupae per week. Emerged adults are prepared and loaded onto aircraft from the same facility for weekly dispersion in the Los Angeles Basin.

The area-wide Medfly PRP in Los Angeles Basin has proven to be a great success. Compared to the yearly and sometimes multiple outbreaks of Medfly in the Los Angeles Basin area, in the early 1990's, prior to the initiation of the area-wide Medfly PRP, there have been only three outbreaks of Medfly in the PRP area as of the summer of 2007. The release of sterile Medflies remains the primary prevention and eradication control tool for Medfly outbreaks throughout California. SIT has been successfully implemented as a control tool on periodic Medfly eradication projects outside of the Los Angeles Basin, including the 2005/2006 eradication campaign in Tijuana, Baja California (BC), Mexico, by capitalizing on the eclosion and aerial distribution infrastructure used by the Los Angeles Basin area-wide PRP.

b. Mediterranean Fruit Fly in Florida

Medfly first gained a foothold in the continental United States in Florida in 1929. The infestation was detected on April 6, 1929, in Orlando, Florida, and grew to involve 20 counties of central Florida. The primary control tool during the eradication was the ground spraying of lead arsenate mixed with brown sugar molasses and water. Copper carbonate was substituted for lead arsenate later in the campaign. Eradication was declared in July of 1930.

Subsequent to this major incursion, a larger infestation, including 28 counties in Florida, was detected on April 13, 1956, in Dade County. Malathion bait spray was used for the first time, applied both on the ground and in the air from fixed-winged aircraft. Eradication was declared 18 months later on November 26, 1957. From 1962 until 1984, Medfly infestations occurred several times in Florida. The primary control tool during these Medfly eradication campaigns was the aerial spraying of malathion bait spray, supplemented with ground applications.

On February 25, 1985, a single Medly was detected in the Opa Locka area of Dade County. With the deployment of delimitation surveillance, two more Medflies were detected on April 9, 1985, triggering control actions to be implemented. SIT was used in this campaign, following four aerial applications of malathion. Both aerial and ground release of sterile Medflies was intitiated on May 7, 1985, marking the first time SIT was used in Florida to control tephritid fruit flies. Eradication was declared on August 27, 1985. A similar combination of the use of aerial applications of malathion and SIT was implemented in a subsequent Medfly eradication campaign in the Hialeah area of Dade County in 1987.

On May 28, 1997, a Medfly was detected in the Tampa area of Hillsborough County. This single detection, and the subsequent delimiting survey, led to the discovery of a large infestation. Six hundred and sixty one adults were eventually discovered in Hillsborough County, and the infestation spread to four other counties, including some satellite infestations in Florida counties non-contiguous to the large core infestation.

Due to the breadth of the infestation, program officials announced the planned implementation of an eradication plan using at least eight aerial applications of malathion, augmented with ground bait spray treatments around positive finds in environmentally sensitive areas; however, in response to local opposition to the use of the aerial bait sprays over the metropolitan area of Tampa, Florida, in Hillsborough County, SIT was used to combat the fruit fly infestation in conjunction with fewer pesticide treatments. An eclosion facility was built on the MacDill Air Force Base

in Tampa; dispersal aircraft were loaded there with sterile Medflies for release. The initial source of sterilized pupae for the SIT program was Hawaii; however, this was quickly replaced by the USDA rearing facility in Guatemala as the program became fully operational and the needs for more sterile pupae increased. SIT in the Tampa area began on July 25, 1997, and ended on November 24, 1997.

On April 1, 1998, a Medfly infestation not associated with the previous huge Tampa infestation was detected in the Miami Springs area of Dade County. For the first time in Florida, SIT was used as the exclusive primary control tool for a fruit fly infestation, supplemented by ground bait sprays surrounding detection sites. The choice to use SIT as the primary tool was predicated by the fact that the infestation was small and in an urban area which made the use of aerial bait sprays undesirable. The SIT program was still based out of Tampa, thereby proving the ability to use SIT as an effective eradication tool throughout Florida. Eradication was declared on August 24, 1998.

Also in 1998, three separate latent infestations were detected that were concluded to be associated with the large Tampa infestation of 1997–1998. These infestations were detected in the Umatilla area of Lake County on April 27, 1998, the Bradenton area of Manatee County on May 12, 1998, and Sebring area of Highlands County on July 9, 1998. In the Umatilla area, 1,315 adult Medflies were detected; in the Bradenton area, 660 adult Medflies were detected; and in the Sebring area, 134 adult Medflies were detected. In Umatilla and Sebring, due to the size of the infestation and the rural nature of the treatment area, aerial bait spray (eight applications) was used as the primary control tool, followed by varying degrees of the use of SIT. In Bradenton, sterile Medflies were used, in conjunction with only three aerial applications of malathion bait sprays, as part of the eradication control efforts. The Umatilla infestation was declared eradicated on August 7, 1998; both the Sebring and Bradenton infestations were declared eradicated on October 2, 1998.

In September 1998, a group of international fruit fly experts convened as a Medfly assessment panel to investigate the history of Medfly in Florida, and to specifically review the recent major campaigns, conducted in 1997 and 1998, with the charge of offering recommendations for future actions and improvements to fruit fly programs in Florida. This international panel recommended that Florida maintain a continual Medfly SIT program to both initiate and maintain a PRP in areas subject to infestations, including the high risk areas of Tampa, Sarasota/Bradenton, and Miami. The panel also recommended extension of the ability for a quick response with SIT to any Medfly infestations detected in any other part of the State. This recommendation was adopted and the release of sterile Medflies in the Tampa and Sarasota/Bradenton areas was continued non-stop in 1998,

switching from eradication mode to PRP upon the declaration of eradication on October 2, 1998. The sterile release of Medflies was reinitiated in the Miami area in March of 1999, officially beginning the Medfly PRP in that area.

As a direct result of the terrorist attacks on September 11, 2001, the increased security and military activities on MacDill Air Force Base required the eclosion and distribution facilities to move off-base. SIT operations on MacDill Air Force Base were closed down in October 2001 and, subsequently, moved to Sarasota, Florida, with the additional benefit of moving from a trailer operation to a permanent facility. Since the initiation of the PRP in late 1998, Florida has not had another Medfly infestation.

c. Medfly in Tijuana, Baja California, Mexico

On September 16, 2004, APHIS' International Services fruit fly surveillance officials' staff reported the detection of five adult Medflies in a trimedlure Jackson trap in Tijuana, BC, Mexico. The detection site was located 7 miles south of the California border. The size of the infestation grew to include several larval and adult detection sites.

In response to this major Medfly infestation just south of the California border, CDFA, USDA, and Mexico cooperated to implement a 251-square mile sterile Medfly PRP in the San Diego area of California. It was initiated on September 22, 2004, and supported from the sterile Medfly eclosion facility in Los Alamitos, California. In Tijuana, the aerial application of spinosad bait spray was initiated as the primary control tool on September 26, 2004, supported by ground foliar bait spray applications and fruit stripping. Following the completion of eight aerial bait spray treatments, SIT was utilized as a control tool. The aerial release of sterile Medflies over Tijuana began on November 22, 2004. The sterile adult Medflies were obtained from the sterile Medfly eclosion facility in Los Alamitos, California and flights were begun after over-flight permission was obtained from Mexican authorities, and the U. S. Department of Homeland Security, to fly over the international border and return. Sterile Medflies were released over an area of 110-square miles in Tijuana. Through the coordination of USDA and the Mexican Government, the Mexican fruit fly SIT eclosion facility in Tijuana was expanded to accommodate the Tijuana Medfly eradication campaign. After the expansion was complete, the eclosion and release activities for sterile Medflies were switched from Los Alamitos, California, to Tijuana, BC, Mexico. The last aerial release flights were flown from Los Alamitos on January 27, 2005. Both the aerial release of sterile Medflies over Tijuana and the aerial release sterile Medflies in the San Diego PRP area stopped on May 28, 2005. Eradication was declared in Tijuana on July 16, 2005.

d. Programa Moscamed

Medfly was first detected in Central America in Costa Rica in 1955. By 1967, Medfly had spread to Nicaragua; by 1975, Medfly was detected in El Salvador near the southern border of Guatemala. These detections prompted Mexico to enter into an agreement with Guatemala, in 1975, to prevent the spread of Medfly north, but to no avail; in 1976, Medfly populations spread across the breadth of the southern coast of Guatemala and entered Mexico. At that time, USDA entered into a cooperative agreement with the government of Mexico to prevent the further spread of Medfly north. The economic consequences posed by the pest threat of Medfly to Mexico and the United States precipitated the creation of the "Programa Moscamed," otherwise known as Moscamed. Through the creation of Moscamed, by cooperative agreement, a large-scale area-wide program was initiated, including the construction of a cooperative U.S.-Mexico sterile Medfly production facility in Metapa de Dominguez, Chiapas, Mexico, with the capacity of producing 500 million sterile pupae per week. Moscamed conducted the first release of sterile Medflies in 1978. Through cooperative efforts, the spread of Medfly north was stopped, Medfly populations were pushed back to within the Guatemalan border, and eradication of Medfly in Mexico was declared in 1982.

Since the successful eradication efforts in 1982, Moscamed has successfully used SIT as the primary control tool in an area-wide campaign to maintain a barrier which has kept the northern parts of Guatemala, Belize, the majority of Mexico, and the United States free of Medfly even though Medfly detections have occurred in Chiapas, Mexico, since 1983.

In 1983, to support the Moscamed Program, USDA constructed a sterile Medfly production facility with a capacity of producing 150 million bisexual pupae per week in San Miguel Petapa, Guatemala. In 1996, to further support the program, USDA constructed another larger sterile Medfly production facility, El Pino (in Guatemala), with a current capacity of producing 3.5 billion temperature sensitive lethal (TSL) male pupae per week. The facility in San Miguel Petapa was later used to support production of the El Pino facility, and is currently being renovated to support the production of other fruit flies of the genus *Anastrepha*, and as a center for SIT methods development work. In addition to producing sterile pupae for Moscamed, the El Pino facility now also supplies sterile Medfly pupae for the Florida and California sterile Medfly PRP programs.

e. Mexican Fruit Fly in Tijuana, Baja California, Mexico

Sterilized fruit flies were first used by USDA in a cooperative fruit fly program with the government of Mexico, in 1964, to control populations

of Mexican fruit fly in Tijuana, BC, Mexico. Sterile Mexican fruit flies have continued to be eclosed and released from a USDA-supported facility in Tijuana, BC, Mexico since the initial release on April 23, 1964. In 1964, the Mexican fruit fly puparia were sterilized using the chemosterilant tepa (as opposed to the radiation methods used today by USDA) to sterilize fruit fly puparia. The initial release of sterile Mexican fruit flies was in response to rising fly populations, from the 1950's through the early 1960's, along the U.S./Mexican border in both the Tijuana and Mexicali areas of Baja California. This included the single detection of one female Mexican fruit fly in San Ysidro, California, in 1954 (Dutton, 1968, Shaw et al., 1966). The successful eradication of Mexican fruit fly from the northwestern part of Mexico has led to the expansion of the release program in Tijuana into a PRP to prevent any Mexican fruit fly populations established there from spreading across the international border into California.

f. Mexican Fruit Fly in California

In 1990, wild Mexican fruit flies were detected in several areas of California. Some of the areas were already located in an aerial Medfly bait spray treatment area, and no further control actions were prescribed. For other areas outside of the ongoing Medfly control area, again through the cooperative efforts of USDA and CDFA, a combination of aerial malathion bait spray treatments, followed by a Mexican fruit fly SIT control regime, was adopted following the recommendation of a science advisory panel formed to investigate the recent occurrences of Mexican fruit fly infestations in California. Sterile Mexican fruit flies were first released in an eradication campaign in California on June 19, 1990. Both the aerial release and roving release methods were used in the two working campaigns in Los Angeles and San Diego Counties. The source of the sterile Mexican fruit flies for these initial releases was the USDA–Mexican fruit fly rearing facility in Mission, Texas. Eradication was successfully declared for both areas in the fall of 1990.

With the success of the partial substitution of the release of sterile Mexican fruit flies, as an alternative to aerial bait sprays as the primary eradication control tool for subsequent Mexican fruit fly eradication campaigns, the release of sterile Mexican fruit flies was used as the sole primary eradication tool in line with the movement to use alternatives for malathion in fruit fly control programs. The aerial release of sterile Mexican fruit flies was supplemented with localized ground applications of bait spray surrounding detection sites. When Mexican fruit fly detections in California prompt a control action, the eclosion and distribution infrastructure, used for the Medfly PRP in the Los Angeles Basin, can be adapted for Mexican fruit fly eradication campaigns in California. The eclosion and distribution of sterile Mexican fruit flies is

incorporated, sometimes as a simultaneous occurrence, into the operation of the ongoing eclosion and distribution of sterile Medflies. This control strategy has been proven successful for all subsequent Mexican fruit fly eradication campaigns in California with the exception of a large infestation in a commercial production area of the Valley Center area of San Diego County. The size of the infestation (7 adults and 75 larvae) in a commercial setting, detected on November 21, 2002, made aerial release of spinosad bait spray followed by the aerial release of sterile Mexican fruit flies the best option for control. Eradication was finally declared in Valley Center on September 25, 2003.

g. Mexican Fruit Fly in the Lower Rio Grande Valley, Texas

The date of the first detection of Mexican fruit fly in the Lower Rio Grande Valley of Texas is debatable but is thought to be some time in the early 1900's; however, the first infestation of economic significance in commercial citrus groves occurred in the spring of 1927. Adult Mexican fruit flies have been detected annually in the Lower Rio Grande Valley since 1927, with the quarantine areas sometimes extending outside of the Lower Rio Grande Valley. From the 1940's through the 1970's, Mexican fruit fly populations spread north and west, extending the quarantine area to include the Laredo area and approached San Antonio. The quarantine area was reduced, in 1991, to three counties in the Lower Rio Grande Valley, after a period of negative detection in the northern and western areas. This is attributed more to the reduction of host material after several major freezes in Texas in the 1980's, rather than any program activities.

To facilitate the interstate movement of host material out of infested areas of Texas, with minimal risk of spreading Mexican fruit fly, postharvest treatments have been developed. The most prevalent postharvest treatment used was fumigation with ethylene dibromide (EDB) until the U.S. Environmental Protection Agency (EPA) announced the prohibition of its use because of suspected carcinogenicity in 1984. The loss of the use of EDB prompted the development of alternative postharvest treatments and systems to certify host fruit for movement.

Some alternatives to the use of EDB fumigation for certification, which did not include the use of SIT, were either currently available, for example cold treatment, or developed because of the eventual loss of the use of EDB. The cold treatment option was not utilized by industry because of the duration of time for the treatment (18 to 22 days). Another alternative offered to producers to certify fruit for interstate movement to citrus-producing States was a preharvest production site bait spray regimen. Similar to cold treatment, this option was rarely used and not preferred by

industry due to the 30-day preharvest treatment requirement and the need to continue the treatments throughout the harvest season.

Due to the need by industry for a quick and effective postharvest treatment similar to fumigation with ethylene dibromide (EDB), a fumigation treatment using methyl bromide (MB) was developed; however, the dosage rate of MB needed to achieve the same efficacy as EDB resulted in fruit damage. Consequently, a 2-stage certification process was developed to allow for a fumigation régime which was not known to damage the fruit, thereby affecting marketability, but was still efficacious to meet phytosanitary security needs. Fruit to be fumigated with MB would first have to pass a qualifying biometric sampling plan to ensure that the infestation rate of the harvested fruit was below a specific infestation threshold prior to fumigation. This extra requirement reduced the pest risk to an acceptable level; however, industry did not prefer this option.

The preferred option supported by the citrus industry was encouraged from the positive results demonstrated by a pilot program using a combination of SIT and fruit fly trapping monitoring system conducted from 1981 until 1984. The certification option that was developed involved the use of an area-wide SIT program combined with an underlying Mexican fruit fly surveillance monitoring trapping array. USDA had opened a sterile Mexican fruit fly production facility in March of 1966 in Monterrey, Nuevo Leon, Mexico, which supplied the initial sterile Mexican fruit flies for the pilot program and was subsequently closed when the production facility in Mission, Texas, was opened. The Mission facility supported the implementation of the SIT/fruit fly monitoring surveillance certification system. In 1984, the area-wide release of sterile Mexican fruit flies in Texas was begun and continues in operation in the Lower Rio Grande Valley to facilitate the certification of host commodities without fumigation. Certification is approved under this system as long as SIT is applied in the harvest areas and the monitoring trapping array indicates that the infestation level is below a designated threshold.

In 2006, the overall goal of the Mexican fruit fly SIT program in the Lower Rio Grande Valley changed from suppression to eradication with an expansion of the program. The release rate of sterile Mexican fruit flies was increased in the Lower Rio Grande Valley, and the release area was increased to include parts of the Lower Rio Grande Valley in Mexico in the northern part of the State of Tamaulipas, Mexico, including the opening of a new USDA-supported eclosion facility in Reynosa, Tamaulipas, Mexico.

3. Fruit Fly Control Methods Development

a. Temperature Sensitive Lethal Strain

Given that the concept of SIT is dependent upon the over-flooding of male insects into a population to achieve suppression, the development of a genetic sexing strain that eliminates females of the species early from the production system could result in cutting the rearing costs by half. The search for a genetic sexing strain for sterile Medfly production began in earnest in the early 1980's (Saul, 1984; Busch-Petersen, 1989). Early in the 1990's, a genetic sexing strain, the temperature sensitive lethal (TSL) strain, was developed by the Joint Division Food and Agriculture Organization/ International Atomic Energy Agency Laboratories at Seibersdorf, Austria, which could eliminate females during the egg stage of production (Rendon et al., 1996). It was not until the late 1990's that a filter system was developed to successfully maintain the integrity of the TSL strain within a mass-production system (Fisher and Caceres, 2000). With its successful incorporation and maintenance into the rearing system and proven effectiveness, the TSL strain has been incorporated into all of the USDA Medfly SIT programs.

b. Worley Eclosion Towers

The adoption of Worley eclosion towers (WETs) into eclosion operations, for both Mexican fruit flies and Medflies, demonstrated another cost-saving technological development. WETs are used to efficiently replace the Plastic Adult Rearing Container (PARC) system for the eclosion of sterile fruit flies. The Florida Sterile Insect Release Facility in Sarasota, Florida, was the first facility to fully adopt the use of WETs into operation. The savings accumulated by the elimination of paper bags used in the emergence process, less trash and waste disposal, ease of cleanup, and a significant reduction in utility expenses amounted to a yearly savings of approximately $1 million in operational costs.

c. Other Methods For Development Initiatives

Other examples of research and development initiatives that either led to program efficiencies or an increase in program effectiveness include the adaptation of aerial release machines to accommodate more than one species, and the use of aromatherapy to increase the mating performance of sterile male fruit flies (Briceno et al., 2007). Double release machines are now used in California to release both sterile Medflies and sterile Mexican fruit flies simultaneously. The exposure of ginger oil vapors during the emergence period of sterile fruit flies has been proven to increase the mating competitiveness of released sterile fruit flies.

4. Pink Bollworm Sterile Insect Technique History

SIT has been used successfully for over 30 years to keep a large cotton growing area in the Central Valley of California free of the pink bollworm. A sterile pink bollworm moth release program was initiated in California's San Joaquin Valley in 1970, and has protected the region's cotton acreage for 30 years. The annual program costs to California growers are about $6 million and costs to USDA are about $1 million. Releases of sterile pink bollworm, as part of an IPM program, were recently carried out on 36,400 hectares of cotton in Texas, New Mexico, and northern Mexico for the eradication of this pest. To achieve eradication and continue to effectively operate the Central Valley containment program, a more effective and lower cost program is needed. The irradiation of pink bollworm with a sterilizing dose in the present program greatly reduces mating competitiveness. The development of a conditionally lethal genetically engineered strain of pink bollworm, as an alternative or supplement to sterilization by irradiation, may allow a more effective and less costly program.

One of the most successful SIT programs involves pink bollworm in the San Joaquin Valley of California (Staten et al., 1993). This cooperative grower/State/Federal effort began in 1968. Sterile pink bollworm adults, produced at the pink bollworm rearing facility in Phoenix, Arizona, have been released each day of the cotton-growing season on approximately 1 million acres of cotton. This program has proven successful in preventing the high populations of pink bollworm occurring in the adjacent regions of southern California, Arizona, and northern Mexico, from becoming established in the San Joaquin Valley (Staten et al., 1993). Genetically engineered SIT field tests have been performed in efforts to express enhanced green and fluorescent protein (EGFP and DsRed) genes. The purpose of EGFP or DsRed is for use as a marker for confirming SIT. The fluorescent marker genes will assist in ensuring that SIT procedures are successful and that only sterile insects are released from rearing facilities.

5. Cooperative Control Programs

There are a number of cooperative programs that APHIS has established with States and foreign countries to manage the pest risks associated with invasive fruit fly pests and pink bollworm. These programs are intended to increase cooperation and communication among the cooperating agencies for coordinated control of these plant pests. Any development of genetically engineered insects for application to SIT will inherently involve the cooperation of all regulatory agencies that are likely to use the technology. General descriptions of the ongoing cooperative efforts are described in the strategic plan for exotic fruit flies (USDA–APHIS, 2006a) and the eradication plan for pink bollworm (El-Lissy et al., 2005a).

The National Exotic Fruit Fly Detection Program is a cooperative program between APHIS and several States that have a relatively high risk of

invasive fruit fly species becoming established due to climate and production of vulnerable crops. This program employs a network of traps and attractants to detect Mediterranean, Mexican, oriental, and other exotic fruit flies. APHIS and State officials developed the "National Exotic Fruit Fly Trapping Protocol" (NEFFTP), which includes a set of guidelines that provide information on fly biology, traps to use, types and dosages of attractants, trap density, trap inspection frequency, baiting interval, trapping season, selection of trap site, and host plants. The guidelines are comprehensive and considered adequate by most experts. To the extent that genetically engineered fruit flies could be used in SIT releases, these guidelines are likely to be subject to minor revision in regards to identification of markers (fluorescent protein genetic markers) to differentiate mass-reared sterile insects from wild-type insects in field monitoring.

There are several cooperative SIT PRPs for fruit flies in different States. These programs apply area-wide preventive or prophylactic releases of mass-reared irradiated fruit flies to prevent any introductions that occur in high risk locations from becoming established infestations. The largest programs involve area-wide releases of sterile Medflies in cooperation with California and Florida. These cooperative programs operate mass-rearing and irradiation facilities on Oahu, Hawaii, and in El Pino, Guatemala. The weekly demand for production of Medflies for PRPs is expected to continue to exceed 400 million sterile pupae during each crop growing season. There is also a large, ongoing cooperative PRP in the Lower Rio Grande Valley of Texas to suppress Mexican fruit fly introductions. The weekly production from the mass-rearing facility in Mission, Texas, is at capacity of 150 million sterile Mexican fruit fly pupae per week.

The largest international cooperative control program for fruit flies is the ongoing cooperative effort between APHIS and the governments of Mexico and Guatemala to maintain a control barrier to stop the natural spread of Medfly further into Mexico. This program was initiated in 1982 as the Guatemala Moscamed Program. The operational effectiveness and interim objectives of the Moscamed Program have been influenced greatly by available resources, political change, and environmental issues. The ongoing improvements in control technology are the result of methods development research in Guatemala. Any potential application of genetically engineered Medfly to the Moscamed Program will require a cooperative effort and adaptation of the methodology to the unique environmental, economic, and social conditions in Guatemala.

The pink bollworm eradication program is a cooperative program involving the cotton producer communities, APHIS, the Government of Mexico, and the States of Texas, New Mexico, Arizona, and California.

The mass-rearing facility in Phoenix, Arizona, provides sterile moths for use in the eradication program. The average weekly production of moths from this facility, in 2006, was 154 million sterile adults; however, the production capacity is about 210 million sterile moths per week. The program goal is to increase average weekly production to 196 million sterile adult moths in 2008. There are three geographical phases (increments) to the present eradication program in the United States (El-Lissy et al., 2005a), of which the eradication efforts against pink bollworm are essentially completed in the increment for west Texas and New Mexico.

B. Purpose and Need for Action

1. Purpose

APHIS, in cooperation with other Federal and State organizations, is evaluating the potential environmental effects of using genetically engineered insects in invasive plant pest control programs against fruit fly species of the family tephritidae and pink bollworm. This technology is under consideration for application to SIT used in preventive area-wide release programs, suppression programs, and emergency eradication programs. The genetically engineered traits introduced into the mass-reared confined insects prior to release could be marker genes designed for ease of identification of released insects in field monitoring, sterilization to stop production of offspring, and gender selection or separation to produce males-only for irradiation and release. The continuing or expanded use of SIT in agency programs within the next decade is anticipated to occur at sites where there is a high risk of introduction, at sites of historically occurring infestations, and at locations of insect rearing facilities (see section A, above). This includes potential locations within the States of Arizona, California, Florida, Hawaii, Texas, and Washington. APHIS cooperates with the State governments and grower group associations in these States for control and eradication of invasive fruit flies and pink bollworm. In addition, the countries of Mexico and Guatemala are presently cooperating with APHIS in eradication programs for pink bollworm and for Medfly, which could also benefit from the use of genetic engineering technology. As with other EISs prepared for compliance with NEPA, this EIS is intended to present and describe the preferred alternative and other reasonable alternatives, along with any anticipated effects on the human environment from each alternative for the purpose of informing and obtaining input and comment on the alternatives and their potential environmental impacts to assist the Agency in making the best possible decision to benefit the public and the environment.

2. Need

There is an impending need for the development of more efficient, lower cost, and effective methods for control and eradication of pink bollworm and the invasive fruit fly species because of the continuing and increasing frequency of detection of invasive insects (see the infestation history in

section A). Although APHIS has existing control and eradication programs for some of these species, the use of genetically engineered insects would provide biological traits of value for use in the SIT control methodology. These traits could provide quality assurance for separation of sterile insects from wild-type insects for field monitoring, reduce the need for insect production through male-only fruit fly mass-rearing or production, and confer sterile characteristics of no offspring without radiation to insects in a manner that does not reduce their ability to compete with wild-type insects for mates. Radiation sterilization injures insects in a manner that reduces their ability to compete with wild-type males for mates. This program is necessary because of the destructive potential of these exotic pests and the serious threat that they pose to U.S. agriculture (USDA–APHIS, 2001a).

3. Authority to Take Action

APHIS' authority for action and cooperation with other agencies in these control programs is based upon Title IV of the Agricultural Risk Protection Act of 2000—Plant Protection Act, Public Law 106–224, 114 Stat. 438–455, which authorizes the Secretary of Agriculture to carry out operations to eradicate insect pests and to use measures to prevent the dissemination of plant pests that are new or not known to be widely prevalent or distributed within or throughout the United States.

4. Statutory Authority

The documentation prepared in this EIS is designed specifically to address the requirements of the National Environmental Policy Act of 1969 (NEPA), 42 U.S.C. 4321, et seq. It is prepared to comply with APHIS' NEPA Implementing Procedures (7 CFR 372), USDA's NEPA Regulations (7 CFR 1b, 3100), and the President's Council on Environmental Quality (CEQ) NEPA Regulations (40 CFR 1500, et seq.). To the extent that some program actions are likely to occur in Mexico, Guatemala and other countries working with APHIS in cooperative pest control programs, this EIS also fulfills the requirements of Executive Order 12114—Environmental Effects Abroad of Major Federal Actions.

C. Scope and Focus of the Environmental Impact Statement

The framework for decisionmaking at APHIS, regarding the use of genetically engineered insects in plant pest control and eradication programs, has involved documentation of ongoing efforts to develop and test strains before applying the technology to actual program actions. The present EIS limits agency consideration of applications of genetic engineering to SIT release, a method with which APHIS has many years of experience and effective program use. On February 4, 2002, APHIS published a notice of intent in the *Federal Register* to prepare an EIS for release of genetically sterile pink bollworm into the environment (Docket No. 01–124–1, 2/4/2002, V. 67, No. 23, p. 5086). The research associated

with that announcement, and subsequent work on the EIS, has been expanded to other plant pest species and to broader program applications of SIT than had previously been envisioned. This expanded scope requires that APHIS make a programmatic review of the environmental impacts associated with the broader applications being contemplated.

Part of the documentation of research and development efforts involved the preparation of EAs for agency decisions to issue permits for confined field release studies of genetically engineered pink bollworm. On January 11, 2002, APHIS published a notice (67 FR 1434–1435, Docket No. 01–024–2) announcing the availability of the final EA and a finding of no significant impact (FONSI) in response to a permit request by APHIS' Plant Protection and Quarantine, Center for Plant Health Science and Technology, Decision Support and Pest Management Systems Laboratory in Phoenix, Arizona, for a confined field study and field performance tests of genetically engineered pink bollworm. APHIS published another notice in the *Federal Register* (71 FR 20068–20069, Docket No. APHIS–2006–0015) on April 19, 2006, announcing the availability of a final EA and FONSI for field release of genetically engineered pink bollworm in Pima County, Arizona. An addendum to the April 2006 EA was prepared and made available on July 26, 2006 (71 FR 42348–42350), to cover field release trials in Yuma County, Arizona. These EAs covered the confined field studies; however, the use of genetic engineering technology in a larger pest control program was not addressed.

APHIS announced the intent to prepare this EIS in the *Federal Register* (71 FR 75933–75934, Docket No. APHIS–2006–0166) on December 19, 2006. That notice identified potential issues and alternatives, requested public comment to further delineate the scope of the issues and alternatives, and provided notice of public meetings. The public meetings were held at five different locations within the 60-day comment period. The sites for those meetings were Washington, DC, Ontario, Canada, Tempe, Arizona, Weslaco, Texas, and Tampa, Florida. There were four formal comments provided at the public meeting and six written comments received by APHIS on the docket during the comment period. The responses included some input from the general public, industry, academia, regulatory authorities, and public interest groups. APHIS was aware of most of the issues of concern to the respondents.

EPA announced the availability of the draft EIS and the public comment period from May 30 to July 14, 2008, (May 30, 2008, 73 FR 31115, Docket No. ER–FRL–6699–3). There were seven public comments submitted to APHIS on the draft EIS. Those comments and APHIS' responses are proviced for the reader in appendix E. This EIS is designed to address those issues raised during the comment period, as well as other

potential environmental effects related to the alternatives that were not mentioned in any of the comments received.

1. Hosts and Potential Hosts

A great number of crops in California would be threatened by the introduction of Medfly including apricot, avocado, grapefruit, nectarine, orange, peach, and cherry. It has been estimated that the permanent presence of this pest in California would result in yearly losses of over $205 million in crop damages, additional pesticide use, and quarantine requirements. In addition, the Mexican fruit fly is an important agricultural pest and has potential to threaten a large number of commercially grown crops in California including peach, avocado, orange, grapefruit, and pear. Mexican fruit fly adults have been trapped a number of times in California, and two infestations have been eradicated from the State. Likewise, a great number of crops in California are threatened by the introduction of oriental fruit fly including pears, plums, cherries, peaches, apricots, figs, citrus, tomatoes, and avocados. It has been estimated that the cost of not eradicating oriental fruit fly in California would range from $44 million to $176 million in crop losses, additional pesticide use, and quarantine requirements. The need to eradicate Medfly, Mexican fruit fly, and oriental fruit fly in California alone demonstrates the need for the proposed action for which this document will assess.

Since its discovery in 1843, pink bollworm has become one of the most destructive pests of cotton in many of the major cotton-growing regions of the world. Control costs for pink bollworm in southern California and Arizona were estimated to exceed $1.2 billion over the past 30 years (USDA–APHIS, 2004). Yield losses caused by pink bollworm ranged from $85 to $170 per acre (USDA–APHIS, 2004). Most recently, the National Cotton Council estimated that cotton producers' annual losses to pink bollworm are about $32 million due to prevention, control costs, and lower yields resulting from plant damage (NCC, 2001). As with the need to eradicate and prevent future detections of fruit fly, it is equally as necessary to continue prevention and eradication methods for pink bollworm.

The species of concern for this EIS are, therefore, Medfly, Mexican fruit fly, oriental fruit fly, and pink bollworm. As previously mentioned, the selection of Medfly, Mexican fruit fly, and pink bollworm as pests of concern in this EIS was based upon their ongoing threat to U.S. agriculture, economic losses resulting from pest damage, and the basic research already conducted to develop strains that could be adapted for use in APHIS' control programs. This programmatic EIS focuses specifically on impacts associated with the use of SIT and the potential benefits and environmental impacts from incorporating genetically engineered traits into fruit flies and pink bollworm used for releases in SIT programs or in alternate biotechnological control programs. Current use of SIT for

control of fruit flies and pink bollworm have proven effective; however, there are enhancements that have been developed to further assist SIT applications (marker genes), thereby also improving strategies for eradication of these important agriculture pests.

This document will evaluate the potential environmental effects of using genetically engineered insects in invasive plant pest control programs against pink bollworm and fruit fly species of concern. This technology is under consideration for application to SIT used in preventive area-wide release programs, suppression programs, and emergency eradication programs. The continuing or expanded use of SIT in agency programs within the next decade is anticipated to occur at sites where there is a high risk of introduction, and at locations of insect rearing facilities (see section A, above). This includes potential locations within the States of Arizona, California, Florida, Hawaii, Texas, and Washington. In addition, the geographical scope of this document will be consistent with infestation patterns of the species of concern.

2. Potential Locations for Program Activities

The potential locations for program actions discussed within this EIS are consistent with areas most affected by or at highest risk for infestations by the species of concern. For the Mexican fruit fly, the highest risk appears to be the southern United States from western Texas to North Carolina, as well as southwest Arizona, Hawaii, and the interior of northern California down to central California where the remaining coastal and interior areas would also be considered high risk. Within these areas, Hawaii showed the highest risk of Mexican fruit fly establishment. Medfly, based on historical data regarding known infestations in the United States, has high risk areas in California, Florida, and Texas. The greatest numbers of fruit fly infestations have occurred in California. Oriental fruit fly, to date, has only been detected in isolated incidents in California and Florida, and is not thought to have become established in the United States, with the exception of Hawaii.

The geographic scope of the pink bollworm program is based on factors related to host range, climate, and potential avenues of introduction. Pink bollworm hosts are limited to cotton and okra; therefore, the geographic areas will be limited to States where cotton and okra are grown. Cotton is commercially grown in the following States: Alabama, Arizona, Arkansas, California, Florida, Georgia, Kentucky, Kansas, Louisiana, Mississippi, Missouri, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia. Unlike cotton, okra is not a major commodity for any one State. California, Georgia, and Florida are the leading okra-producing States; however, it is grown throughout the South in Louisiana, Arkansas, Alabama, Texas, North Carolina, Oklahoma, Tennessee, South Carolina, and Mississippi, and Kansas, Arizona, and Virginia (Izekor and Katayama, 2007). Currently, there are

no States that grow okra that do not grow cotton. The humid climatic conditions of the southeastern United States are not favorable to the survival of pink bollworm, and recent infestations have been limited to cotton-growing areas from west Texas to central California and adjacent parts of Mexico.

3. Potential Sites for Rearing Facilities

The potential sites for rearing facilities for fruit fly species and pink bollworm considered in this EIS are those sites that currently handle mass-rearing of sterile insects. These rearing facilities apply area-wide preventive or prophylactic releases of mass-reared irradiated fruit flies to prevent any introductions that occur in high risk locations from becoming established infestations. The potential sites for rearing facilities considered in this document are Oahu, Hawaii, El Pino, Guatemala, and Mission, Texas. As new facilities are identified as capable to handle the mass-rearing of genetically engineered SIT insects, as assessed in this document, they will be added to the list of those facilities currently recognized by APHIS.

4. Potential Actions to Consider

The program actions considered in this EIS are the following: (1) no action (continuation of cooperative eradication programs as they currently exist), (2) expansion of existing programs, and (3) integration of genetically engineered insects into programs (the preferred alternative). The alternatives and their components vary with regard to their practicality or feasibility based upon environmental, scientific, regulatory, economic, and logistical perspectives. They may also vary considerably with regard to their effectiveness, capability to attain program objectives, and immediate applicability for large-scale programs. Some potential actions and components for the proposed program may lack the funding, resources, level of development, and logistical capability to be considered feasible for the reasonably foreseeable future. Although technological development of these program actions may eventually be possible, present circumstances do not warrant intensive study of potential implementation at this time; thus, such potential actions were considered but dismissed from further consideration in this EIS.

D. Programmatic Analysis and Application-specific Review

This EIS is designed to be a broad, programmatic analysis of new and existing alternatives for fruit fly and pink bollworm programs that use SIT in their control and eradication efforts. It focuses on available program control methods related to SIT and their environmental consequences, and is not intended to serve as an encyclopedic compendium of information about specific pest programs. Instead, it provides an overview of the programs and their methodology. Much of the program methodology has already been described and analyzed in previous documents; findings and

program descriptions from those previous documents are summarized and incorporated by reference into this EIS. Specific documents that are incorporated by reference include the Fruit Fly Cooperative Control Program Final Environmental Impact Statement—2001 (USDA–APHIS, 2001a) and the Southwest Pink Bollworm Eradication Program Environmental Assessment, April 2002 (USDA–APHIS, 2002a). There have also been site-specific EAs prepared for the ongoing development of genetically engineered pink bollworm (USDA–APHIS, 2001b; USDA–APHIS, 2005a). Although much of the description of methods and impacts from these EAs will be repeated within this EIS, the findings and descriptions from those documents are also incorporated by reference into this EIS.

In addition to providing a broad overview, this EIS also presents the specific procedures which APHIS would follow prior to release of any genetically engineered insects in control programs to ensure that site-specific characteristics of the program area and application-specific characteristics are considered. For example, prior to release of any genetically engineered insect in a SIT program, APHIS will consider site-specific characteristics such as the following: (1) unique and sensitive aspects of the proposed program area, (2) applicable environmental documentation including the programmatic EIS, (3) applicable program mitigations, and (4) applicable new developments in environmental science or plant pest control technologies. To the extent possible, when separate Federal and State site-specific environmental reviews are conducted, they will be coordinated. Such site-specific reviews will summarize and incorporate, by reference, all programmatic analyses contained in the EIS.

Application-specific review for the program areas will consider such things as the following: (1) land usage patterns, (2) unique or sensitive areas, (3) water bodies, (4) threatened and endangered species, (5) human population density, (6) cultural factors, and (7) unique human health issues including homeless people, people with special medical conditions, or ethnic groups that require special notification procedures. Also, after publication of the EIS, APHIS will consider new developments in the science of genetic engineering, new findings related to potential risk to humans or other nontarget species, and further developments in control technology as they may be applied to SIT.

The use of application-specific review will be deemed appropriate based upon the circumstances, issues, and timeframe of need for the program. Generally, the application-specific assessment prepared for a program will be adequate to analyze and disclose new and important information relative to a specific site and genetic construct. In cases where major changes are apparent, a supplement to this EIS or a new EIS may be

required. Specific procedures for application-specific evaluations are included within this EIS (see appendix K).

(This page is intentionally left blank.)

II. Alternatives

A. Introduction

APHIS and its cooperators have analyzed three alternatives and their associated components in this EIS. These three alternatives are broad in scope and reflect the need of the program objective to address potential control and eradication of damaging fruit fly species and pink bollworm. Previous analyses of many control actions used by APHIS for applications against those species have been presented in broad review (USDA–APHIS, 2001a; USDA–APHIS, 2002a). Findings from those documents are summarized and incorporated, by reference, into this EIS, as applicable; however, this programmatic EIS focuses specifically on impacts associated with the use of SIT and the potential benefits and environmental impacts from incorporating genetically engineered traits into fruit flies and pink bollworm used for releases in SIT programs or in alternate biotechnological control programs. Methods, other than genetic engineering, which provide control in eradication and suppression programs, are summarized as part of the alternatives of no action and expansion of existing programs. This EIS is not intended to provide a detailed analysis of the methods of insect pest control, such as chemical pesticides; however, it will present a comprehensive review of those potential future uses of genetically engineered traits in fruit flies and pink bollworm in APHIS' control programs.

The alternatives for the use of genetically engineered insects in APHIS' pest control programs are presented in a manner that clarifies the environmental issues and the choices that are to be made regarding the inclusion or exclusion of insects with specific traits from usage in APHIS' SIT programs. The alternatives considered in this EIS are the following: (1) no action, (2) expansion of existing programs, and (3) integration of genetically engineered insects into programs (the preferred alternative). The alternatives and their components vary with regard to their practicality or feasibility based upon environmental, scientific, regulatory, economic, and logistical perspectives. They may also vary considerably with regard to their effectiveness, capability to attain program objectives, and immediate applicability for large-scale programs. Selection of specific applications for control programs will require further documentation (see section IV.B.7. regarding Federal permits and approvals required for implementation). This EIS is designed to establish criteria for future decisions regarding use of the genetic engineering technology and to identify the potential impacts to address when documenting these decisions.

B. Description of Alternatives

Analysis has determined that there are potential environmental consequences for each of the alternatives, including the no action alternative. Environmental consequences result from APHIS program activities and capabilities to exclude, detect, protect from, and control fruit flies and pink bollworm. Those consequences from control actions, other than from the use of genetic engineering, have been described in detail in previous documentation in an EIS for fruit flies (USDA–APHIS, 2001a) and in an environmental assessment (EA) for pink bollworm (USDA–APHIS, 2002a). The primary control method associated with adverse environmental impacts in these documents was the program usage of chemical pesticides. There is potential to replace or limit the need for chemical controls when SIT is applied through prophylactic releases to prevent invasive plant pests from becoming established. The continuing usage of chemical control actions, in the absence of alternate control measures, is anticipated to pose greater potential consequences than would occur with nonchemical control measures.

The fruit fly EIS analyzed classical SIT and the use of biotechnological control; however, the document did not analyze genetically engineered insect strains for applications to SIT (USDA–APHIS, 2001a). This fruit fly EIS found that SIT poses minimal environmental consequences, and that biotechnological control poses unknown environmental consequences for the limited applications analyzed. There are two EAs that analyze potential impacts from the testing of genetically engineered pink bollworm (USDA–APHIS, 2005a; USDA–APHIS, 2001b). These documents consider confined and limited field studies with mitigations built into the tests. Unlike the environmental documents for control programs, these EAs for field tests analyze genetically engineered strains that are being developed through research for use in control programs. The analysis of potential impacts reached a finding of no significant impact (FONSI) for each series of tests. The intent of this EIS is to compare potential environmental impacts from the use of genetic engineering technology incorporated into SIT as a broad control measure in invasive fruit fly and pink bollworm suppression and eradication programs to those impacts resulting from present control programs. This EIS compares alternatives consisting of (1) no change in present control programs (no action alternative) with (2) an expansion of present program measures in the absence of development of genetically engineered insects, and with (3) the integration of genetically engineered plant pest strains into SIT programs. Although some control measures used in the present program (no action) are anticipated to continue with each of the other two alternatives, their environmental impact is expected to be decreased as new methods are used to decrease adverse effects. The use of mitigation measures to reduce potential risks is critical to the analysis of impacts from

implementation of each alternative. This chapter describes the available program components for each alternative and each of the alternatives in detail. Citations of overall descriptions from previous documentation are provided, as appropriate, to minimize repetition, particularly for those control methods which are not the focus of this EIS.

1. No Action

This alternative is characterized as no change to the existing plant pest control programs that use SIT based upon irradiation of mass-reared insects. Although continuation of this approach does not contribute to the further mitigation of plant pest risks, the analysis of the no action alternative provides a baseline for comparison to the other alternatives, and is required by NEPA and its implementing regulations. This alternative involves cooperative efforts to control (suppress, eradicate, or otherwise manage) invasive exotic fruit fly pests and pink bollworm. Such programs use (singly, or in combination) exclusion, detection, prevention, nonchemical control, and chemical control. The continuation of the present program does not provide any flexibility for the application of new methods or new technologies to the control of fruit flies or pink bollworm. This approach would exclude the use of genetically engineered insects for SIT and new biotechnological control measures in control programs. The selection of other control measures to be used would take into consideration several factors, including economic (the cost and cost effectiveness of various methods in the short- and long-term), ecological (the impact on nontarget organisms and the environment), and sociological (the acceptability of various control methods to cooperators or the potential effects on land use).

Program managers can vary their use of control measures to protect human health, nontarget species (including threatened and endangered species), sensitive areas, and other parts of the environment within the potential program area. They can utilize specific protection and mitigation measures, in combination with their selection of control methods, to maximize efficacy and minimize environmental effects. This provides considerable flexibility to the program managers; however, it does not take advantage of the potential benefits from development of genetically engineered plant pest strains for use in SIT. In particular, this alternative lacks clear options to expand the use of irradiation, to expand the use of fluorescent dye, to expand development and use of the classical gender selection processes, and to increase the overall fitness of released radiation-sterilized insects. Also, any improvement of the insect mass-rearing colony production as a result of genetic engineering would not occur.

This alternative does not utilize genetically engineered insects that are potentially more able to mate than radiation-sterilized insects that are damaged by their irradiation exposure. Usage of this methodology could

diminish the need for program use of chemical pesticides. The public is informed of the times and areas of pesticide applications in these programs, and can, therefore, take the precautions to minimize or avoid exposure.

Under the no action alternative, the constraints to available control measures would continue to meet APHIS program needs as long as the frequency of infestation occurrence and associated control actions do not increase. Pink bollworm eradication could proceed at the present pace, and there would be sufficient mass-reared flies for use of SIT in fruit fly program control efforts and prophylactic area-wide releases as long as the present demand does not increase substantially; however, the increased frequency of introductions of invasive fruit flies relates partly to recent increases in trade and travel. The need to maintain large ratios of sterile flies to prevent establishment of introduced fruit flies requires that SIT numbers greatly exceed the introduced wild-type insects. It can, therefore, be anticipated that greater production will eventually be required to meet program needs. This alternative does not fulfill the purpose of the EIS, but it does present the current program as a baseline for comparison. Although it is feasible to proceed with this alternative presently, the long-term effectiveness is anticipated to diminish, especially if the need for sterile insects increases.

2. Expansion of Existing Programs

This alternative involves an increase in the present plant pest control actions and inputs to improve the effectiveness of SIT currently used in APHIS control programs against invasive plant pests, particularly fruit flies and pink bollworm. This could include expansion of the following—

- pest insect mass-rearing operations,
- irradiation treatment capacity,
- development of classical genetic selection methods for separation of insect sexes for more fruit fly species,
- the use of SIT for more plant pest species than in present programs,
- additional sterile insect dispersal capacity,
- additional monitoring and surveillance capacity, and
- additional pest mitigation capacities which could include the use of chemical pesticides.

Under this alternative, the expansion of existing programs has the advantage of meeting increases in the demand for sterile insects. In addition to meeting the demand for sterile fruit flies, this approach could increase the rate of eradication of pink bollworm from the Southwest. The research, time, facility improvements, and costs involved in this type of expansion are considerable. Although this approach could be taken, the integration of genetically engineered insects into SIT programs could meet these needs at reduced cost, reduced new construction, and in a shorter

timeframe. This alternative would most likely be limited by anticipated program needs and available funding. Implementation of this alternative does not expressly address the purpose of this EIS; however, it does provide another reasonable alternative to compare to the integration of genetically engineered insects into the programs. Although there is ongoing research and development work with the use of genetic markers and various genetic strains at APHIS, this alternative and the no action alternative do not consider application of this technology to any of the control or eradication programs. It could be feasible to pursue this alternative to meet the long-term effective control or suppression; however, ultimately, the costs would be greater than those from integration of genetically engineered insects due to the need for greater mass-rearing production and the lower cost-effectiveness of this alternative.

3. Integration of Genetically Engineered Insects into Programs

Implementation of this alternative involves the use of genetic engineering to improve the effectiveness of APHIS' invasive plant pest control programs, and minimize the impact of these programs to the environment. Specific methods for risk reduction would be applied to releases in APHIS' plant pest control programs to ensure that the program goals are met in an environmentally safe and efficient manner. Genetically engineered fruit flies and pink bollworm could augment SIT in present control programs by producing the following—

- mass-rearing of only male fruit flies that have a marker gene and that are subject to sterilization by radiation,
- genetically sterilized male fruit flies that have a marker gene and that compete more effectively for mates than radiation-sterilized male insects which produce practically no offspring (thus reducing the number of insects that need to be reared and released),
- fruit flies that produce only male offspring which carry a sterility gene resulting in only males and no female offspring, thus controlling pest fruit flies in the field through rapid population reduction,
- mass-rearing of male and female pink bollworm that have a marker gene and that are subject to sterilization by radiation, and
- mass-rearing of male and female pink bollworm that are genetically sterile without radiation, and are more competitive in mating with wild bollworms than radiation-sterilized bollworms.

It would be desirable to mass-rear only male pink bollworm; however, technology to achieve this is not yet feasible. The components designed to achieve this augmentation of the program are presented in section C of this

chapter. This alternative expressly addresses the purpose of this EIS and can fulfill the need to the extent that funding allows the development of the component methods. The benefits to fruit fly programs are long-term with the continuing introductions that occur, and there are also long-term benefits to cotton growers from successful eradication of pink bollworm that may incorporate this technology into their program actions.

a. Fruit Fly Control

The cooperative Medfly SIT program mass-rears these flies in Hawaii and in Guatemala for release in several areas. Mexican fruit flies are mass-reared by APHIS in Mission, Texas; there is no current mass-rearing SIT program for oriental fruit fly. Part of the present Medfly mass-rearing process involves production of only males through a TSL strain of this fruit fly in SIT programs. Females die at a temperature above 29 °C. By putting the fruit fly eggs in a water bath at the threshold temperature, the females are killed and only the males survive to later be irradiated and released to mate with wild-type females, which then produce no offspring. This has been achieved through selective breeding over many years and is not a result of genetic engineering.

Genetic engineering of a marker gene trait into fruit flies, in the absence of any other genetically engineered traits and without significant fitness costs or harm to the insects, is of immediate value for surveillance and monitoring of radiation-sterilized fruit flies in APHIS SIT cooperative control programs. Currently, dyes are primarily used to identify mass-reared fruit flies that are subject to irradiation treatment; however, the verification of mass-reared status through detection of dyes has not been consistent enough for adequate differentiation of wild from irradiated fruit flies. Marker traits developed from mutations and a long selection process are also possible, but at a higher cost and time than genetically engineered marker traits.

Genetically engineered Medflies, as well as Mexican fruit flies and oriental fruit fly, may be designed to produce only male insects, as with the TSL strain; however, genetic engineering could greatly shorten the research time and cost needed to achieve the desired result of producing only males. These males could then be sterilized by irradiation and released to mate with wild-type females. The genetically engineered and gamma radiation-sterilized male fruit flies may also be provided with a marker gene trait, such as green fluorescent protein derived from a jellyfish or DsRed fluorescent protein derived from a coral, which would greatly facilitate surveillance of released SIT fruit flies in the field and monitoring the effectiveness of the SIT program. The disadvantage of this strategy is that irradiated male fruit flies will be injured by the radiation and not as competitive as wild-type males in successfully mating with

wild-type females. This lack of competitive mating potential of the irradiated male fruit flies results in the continuing need to mass-rear, irradiate, and release much larger numbers of irradiation-sterilized insects than wild-type insects to overwhelm the wild population with radiation-sterilized males.

Genetically engineered Mediterranean, Mexican, and oriental fruit flies may also be designed to produce males that do not need to be irradiated to be sterile. These genetically engineered sterile males are not exposed to the effects of gamma radiation and, therefore, are not damaged by it. They would be much more competitive with wild-type males in mating with wild-type females than would the irradiated males. Therefore, smaller numbers of the genetically engineered sterile fruit flies would be needed for release than radiation-sterilized males to achieve suppression or control of the wild population of pest fruit flies.

Genetically engineered sterile male fruit flies may also have another major advantage over radiation-sterilized male fruit flies—these fruit flies could carry an introduced gene that is lethal to the development of their female fruit fly offspring, but are capable of passing this gene on to male-only fruit fly offspring. The result would be that a genetically sterilized male mates with a wild-type female, who then produces no female fruit fly offspring and only male offspring, which carry the gene that is lethal to the development of female fruit fly offspring. This reproductive obstacle to the production of female fruit flies would rapidly reduce a fruit fly pest population within only a few generations, and likely within one growing season. Fruit flies genetically engineered for sterility would also be engineered with a marker gene trait for monitoring dispersion and program effectiveness in the field.

b. Pink Bollworm Eradication

Pink bollworm adults used in the APHIS SIT program are released as both irradiation-sterilized males and females, mainly due to lack of an efficient way to separate out the males from the females in the rearing process. The mass-rearing facility is in Phoenix, Arizona, but serves to supply sterile insects for several States.

Genetic engineering of a marker gene trait into pink bollworm would be of great benefit to the SIT eradication program. In the absence of any other genetically engineered traits, and without significant fitness costs or harm to the insects, this technique would be of immediate value for surveillance and monitoring of radiation-sterilized pink bollworm.

Pink bollworm has been developed and field tested with a green fluorescent protein marker gene from a jellyfish and DsRed fluorescent

protein marker gene from a coral. Currently, the insects are mass-reared and irradiated with a high-dose of gamma radiation (20 kilorad (kR)) to produce bollworm adult moths that mate with wild-type insects resulting in no or very few offspring, thus causing rapid pest population control or reduction. Dyes are used to identify mass-reared pink bollworm that are subject to irradiation treatment; however, the verification of mass-reared status through detection of dyes is marginally adequate for differentiation of wild from irradiated bollworms. The presence of a marker gene will facilitate monitoring of released sterilized bollworms in the field and program effectiveness.

Mass-reared pink bollworm may be irradiated with a lower dose of radiation, such as 7 to 10 kR, resulting in offspring that are able to pass on sterility to the next generation. This is named "F_1" Sterility and may be as effective, or possibly more effective, than using the high dose of 20 kR gamma radiation to cause immediate sterility to the pink bollworm. Also, the bollworm is less damaged by the lower dose of radiation. This F_1 sterility method may be used without genetic engineering or with genetically engineered pink bollworm that have a fluorescent protein marker gene.

As with irradiated fruit flies, pink bollworm is damaged by a high exposure to radiation, resulting in reduced mating efficiency compared to that of the wild-type insects. Many more irradiated bollworms have to be released than the number of wild-type pink bollworm in the field. Genetically engineered pink bollworm are designed to be sterile and would not have to be irradiated. They would be healthier than irradiated bollworm and better able to compete with wild-type bollworms for mating in the field. Fewer genetically engineered sterile pink bollworm would have to be mass-reared and released to achieve bollworm pest population reduction or control in the field compared to radiation-sterilized bollworms that are weaker competitors. Genetically engineered pink bollworm designed to be sterile would also have a marker gene to facilitate monitoring of releases in the field and of program effectiveness.

C. Component Methods of the No Action and Expansion of Current Program Alternatives

The component methods for the no action alternative have been reviewed and described for fruit fly control programs (USDA–APHIS, 2001a) and the pink bollworm eradication program (USDA–APHIS, 2002a). The components of fruit fly control programs include quarantine and inspection activities for exclusion, detection activities for monitoring, preventive actions, chemical control, and nonchemical control. Most of these components were determined to pose minimal impacts to the human environment. Some chemical insecticide control methods were

determined to pose higher risks that would require mitigation measures to lower potential environmental or health impacts. The actual pest risk from outbreaks of the invasive fruit flies that were not prevented by exclusion or prophylactic practices were also determined to be an effect that required program actions to mitigate impacts on the environment. The Fruit Fly Cooperative Control Program EIS provides a more detailed review of potential impacts in table 3–2, and in the text of chapter III (USDA–APHIS, 2001a). Although this EIS did consider impacts from all potential program components, the review did not rate the use of biotechnological control, and did not analyze SIT using genetically engineered insects. Therefore, that document does provide a good background and analysis on the impacts from components of the no action alternative for fruit fly programs and, to a certain extent, for any expansion of the present program; however the impacts from specific components to integrate the use of genetically engineered fruit flies into these programs are not addressed.

Likewise, the impacts from components of the ongoing eradication of pink bollworm in the Southwest were analyzed in an EA (USDA–APHIS, 2002a). That document addressed impacts from program components such as exclusion, monitoring, preventive actions, chemical control, and nonchemical control activities. Other than mitigation of the continuing pest risk to the environment posed by pink bollworm infestation of cotton, program-specific mitigation and protection measures are required to minimize impacts from chemical control actions. Although the EA does discuss the releases using SIT and the mass-rearing of pink bollworm, the development of genetically engineered pink bollworm to enhance the effectiveness of SIT is not addressed in the EA. The components of the no action alternative and the alternative for expansion of existing programs have been described in previous documentation as cited above; therefore, those details will not be repeated here. Nevertheless, the description of the components of the preferred alternative were not presented in the above referenced NEPA documentation for previous program actions, therefore, the next section of this chapter will describe those component methods being considered for application in the fruit fly control and pink bollworm eradication programs.

D. Component Methods of the Preferred Alternative

The preferred alternative consists of the following five components, which may be adopted in APHIS cooperative SIT fruit fly and pink bollworm control and eradication programs singly, multiply, or with minor variations—

(1) mass-rearing of either male and female or male-only fruit flies with a marker gene and that are sterilized by radiation exposure and produce practically no offspring;

(2) genetically sterilized male-only fruit flies that have a marker gene, that compete more effectively for mates than radiation-sterilized male insects, and that produce practically no offspring;

(3) fruit flies that produce only male offspring, which carry a heritable sterility gene resulting in only males with that trait and no female offspring in the field;

(4) mass-rearing of male and female pink bollworm that have a marker gene and that are sterilized by radiation before field release; and

(5) mass-rearing of male and female pink bollworms that are genetically sterile without radiation exposure and that results in males that are more competitive in mating with wild female bollworms than radiation-sterilized male bollworms.

A description of the genetic engineering of these pests and risk assessment are presented in appendices C and D of this EIS.

APHIS cooperative eradication and control programs would use these five components in the following manners—

(1) mass-rearing of either male and female or only male fruit flies with a marker gene and that are sterilized by radiation exposure and produce practically no offspring,

It may be feasible to introduce a fluorescent protein marker gene into the conventionally bred and selected TSL strain of Medfly that produces male-only flies. Those flies would then be sterilized by radiation prior to release.

The genetically engineered fluorescent marker could be used alone or in combination with existing chemical dyes and other methods to facilitate rapid and positive identification in traps baited with Trimedlure synthetic pheromone Medfly attractant. Trimedlure is registered by EPA. The baited traps are used to detect the occurrence of Medflies resulting from accidental importation, monitor new and existing infestations, and determine the ratio of released marked sterile Medflies to Medflies that are wild-type pests and not marked. When there are no more unmarked Medflies caught in the traps, it indicates that infestations have been controlled or eradicated.

With the Mexican fruit fly, the different trap lures include BioLure® (Suterra, Inc., Bend, Oregon); AFF Lure® (Advanced Pheromone Technologies, Inc., Marylhurst, Oregon); AMPu, ammonium carbonate, torula yeast, methylamine HCL and putrescine; and CEHO from the fruit of chapote amarillo, but no pheromone or lure is EPA registered yet. The oriental fruit fly is attracted to methyl eugenol, which was EPA registered in 2007. As with the Mexican fruit fly, baited traps are used to detect infestations and monitor the control performance of SIT release programs. Genetic markers will improve the accuracy of distinguishing irradiated released flies from those that occur as wild-type pests in the field.

(2) genetically sterilized male-only fruit flies that have a marker gene, that compete more effectively for mates than radiation-sterilized male insects, and that produce practically no offspring,

There are no TSL strains for the Mexican and oriental fruit flies, as there are with the Mediterranean fruit fly, but it is feasible to produce male-only strains by genetic incorporation of a lethal genetic construct whereby the females die in an early stage of development during the last cycle of production before release, thus resulting in only males. These males could be sterilized by radiation or by genetic engineering as further described in appendix C of this EIS.

The genetic sexing mechanism could also be applied to the Mediterranean fruit fly if it is found that the male insects produced are heartier and more reproductively competitive than males produced by the TSL method. TSL Medflies are not as healthy or competitive as wild-type pest fruit flies.

The potential advantages to the APHIS cooperative fruit fly eradication programs of genetically sterilized males are that the insects are sexually more competitive and heartier than those sterilized by radiation. Gamma radiation injures the insects. Production efficiency and economy would be achieved with male-only strains in the last large-scale production cycle by less use of diet and rearing facilities; reducing the overall number of insects that need to be reared by about one-half; and comparable reductions in numbers released to achieve effective control and eradication. In addition, since no radiation sterilized females would be released in the field, there would be fewer females for the sterilized males to mate with, which would increase their frequency of sterile matings with wild-type pest females.

All genetically engineered male fruit flies sterilized genetically or by radiation will most probably have a genetic fluorescent marker for the positive identification advantages described above.

(3) fruit flies that produce only male offspring, which carry a heritable sterility gene resulting in only males with that trait and no female offspring in the field,

Genetic engineering of male-only fruit fly strains, which carry a heritable sterility gene resulting in only males and no female offspring in the field after mating with wild-type pest females would be used by the APHIS cooperative fruit fly eradication programs to greatly increase efficiency of the program by vastly reducing the number of insects that need to be mass-reared and released. These male-only flies will not need to be sterilized by gamma radiation, thus reducing the costs and biologically injurious effects of irradiation. Therefore, they would be heartier and more sexually competitive than radiation sterilized males. However, the greatest advantage is that when they mate with female wild-type field pest insects, there will be no female offspring produced and only male offspring produced, which then carry the gene for female lethality to the next generation. This will be an extremely effective and efficient method to induce pest population collapse and achieve eradication with the greatest economy of effort and expense. These fruit flies would also bear a genetic fluorescent protein marker for monitoring program effectiveness in insect traps baited with pheromones or other lures.

(4) mass-rearing of male and female pink bollworm that have a marker gene and that are sterilized by radiation before field release,

Gossyplure pheromone baited traps are used to detect the presence of pink bollworm, monitoring new and existing infestations, and determine the ratio of released marked sterile pink bollworm to pink bollworm that are wild and not marked. When there are no more unmarked pink bollworm caught in the traps, it indicates that infestations have been controlled or eradicated.

The most progress to date with genetic engineering of the pink bollworm has been with the DsRed fluorescent protein marker. In 2007, extensive testing was done in Arizona, under an APHIS permit in Arizona, which resulted in findings that demonstrated genetically engineered DsRed pink bollworm was suitable for larger-scale field-testing and subsequent full-scale incorporation by the SIT pink bollworm cooperative eradication program.

Current plans are to use the DsRed genetic marker in combination with the Calco chemical red dye to verify identification of the irradiated moths compared to wild-type pest moths collected in pheromone traps baited with EPA registered Gossyplure.

Development of a rapid screening technique using the fluorescent marker can lead to more efficient processing and possible automation of trap reading, resulting in significant savings in labor costs for the pink bollworm SIT program. Genetically marked pink bollworm can also be read with PCR techniques to provide a backup means of identifying the released insects. Another advantage of genetically marked pink bollworm is F_1 sterility, unique to Lepidoptera, which allows lowering the irradiation dose. In F_1 sterility, the surviving offspring of partly sterilized moths are also sterile, thus increasing the efficiency of SIT. Moths treated with a lower sterilizing radiation dose live longer, are stronger fliers, and obtain more matings than moths produced with a high radiation dose. This results in biologically better quality moths, allowing lower release rates in program areas.

A quarantine moth production and collection system was constructed for 2007 field experiments and weekly production exceeded 600,000 moths/week. The maximum production capability is estimated to be 1.7 million moths/week, which is sufficient production for future trials of up to 1,000 acres. Releases were made in three cotton fields in Arizona, totaling 100 acres for 10 weeks at rates of up to 500 moths/acre/week. Recapture rate in pheromone monitoring traps were approximately equal with a slight trend for higher recapture rates for the genetically engineered DsRed pink bollworm moth. Results from detecting DsRed moths in traps with light and PCR indicate that a DsRed pink bollworm can add a robust new tool that will increase accuracy in counting sterile release moths. The DsRed moth coloration is very durable and can be seen in moths in traps that have been in the field for as long as 2 weeks and stored in the laboratory for as long as 3 months.

Two environmental assessments have previously been prepared for genetically engineered pink bollworm that express fluorescent protein markers, including DsRed. Analysis of each of these documents arrived at a finding of no significant impact (FONSI). These tests were conducted to develop this component for potential field application.

Although the present eradication program has succeeded in eliminating the pink bollworm from most of its range in the United States, reintroductions will occur from across the border in Mexico, where it still is a cotton pest. It will be necessary to continue monitoring for the pink bollworm with gossyplure pheromone traps and to release SIT pink bollworm to eradicate reinfestations. Preventative releases of SIT pink bollworm may also be employed in strategic locations to preclude reinfestation of cotton in the USA.

(5) mass-rearing of male and female pink bollworms that are genetically sterile without radiation exposure and that results in males that are

more competitive in mating with wild female bollworms than radiation-sterilized male bollworms.

It is not yet feasible to mass-rear only male pink bollworm, so both males and females are mass-reared. Sterile strains are produced by genetic incorporation of repressible sterile construct in which sterility occurs during the last cycle of production before release as a result of deletion of a repressive agent in the diet at the final insect mass-rearing cycle. The genetic sterilization mechanism provides insects that are heartier and more reproductively competitive than irradiated insects because of the debilitating effects of a high dose of gamma radiation. All pink bollworm produced through genetic engineering sterilization would also include the genetic fluorescent protein marker construct, DsRed, for the identification advantages described.

E. Potential Alternatives and Components Not Analyzed In Detail

Some potential alternatives and components for the proposed program may lack the funding, resources, level of development, and logistical capability to be considered feasible for the reasonably foreseeable future. Although technological development of these program actions may eventually be possible, present circumstances do not warrant intensive study of potential implementation at this time. Although three action alternatives are proposed and discussed in detail in this EIS, other alternatives have been suggested for consideration. The potential alternatives and component methods not considered in detail in this EIS include those discussed below. It is anticipated that further comment regarding this EIS, when it becomes available for public comment in the *Federal Register*, will also result in additional proposed alternatives, which will be considered and discussed accordingly.

1. Use of Genetically Engineered Transgenes

This alternative involves the use of genetically engineered transgenes that confer traits preventing the survival of the progeny (dietary, climatic, and other survival limitations). This is discussed in appendix C, Repressible Lethal and Marker Genetic Engineering Analysis of Issues Pertaining to Transposon Mobility and Potentiation of Horizontal Transfer for Technology Under Development by APHIS in those sections of appendix C on repressible vs. inducible lethal systems and repressible lethal dominant constructs.

The examples that were given included a lethal system in which a gene is released to introgress into a wild population through breeding and survival of offspring, which will then become lethal under some subsequent physiological or environmental triggering mechanism or circumstance. One example is a “diapause lethal” which kills the insects when they

attempt to enter diapause at the onset of winter or other diapause-inducing environmental circumstance. Another example is the low temperature sensitive *Notch*^{cs} gene discussed by Fryxell and Miller (1995), and later modeled more extensively by Schliekelman and Gould (2000a). It would have a similar lethal effect, accumulating in the population during the summer due to mass-release of insects carrying it, breeding with wild-type relatives, and then killing all the insect progeny with the lethal gene when the temperature falls below a certain level.

In yet another system described by USDA–ARS researchers Xavier and Handler (2006), lethality would result from antimorphs that disrupt proteasome function. As with the system described by Fryxell and Miller (1995), this system is temperature-dependent; however, the system of Xavier and Handler would require increased rather than reduced temperature to induce lethality.

A potential difficulty with temperature as the lethal condition, particularly for field use, is the lack of control over ambient outdoor temperatures and daily temperature fluctuations that may vary greatly in some areas and in some seasons. For example, an unusually mild winter might render a cold-sensitive lethal ineffective, or low summer nighttime temperatures might inadvertently trigger a lethal condition before the expected seasonal low temperatures needed for optimal lethal effects.

The technologies for these and other methods of preventing survival of progeny using climatic factors is neither as promising nor as well developed as the repressible lethal technology in which a dietary factor, such as presence of tetracycline, is used to suppress the lethal trait in the mass-rearing larval diet, but is not sufficiently present in the environment to continue suppression of the lethal trait, thus triggering a lethal expression. It is anticipated that the development costs and associated potential risks are significantly greater for temperature dependent lethal systems than for the repressible lethal system presently being researched by APHIS as a cost-effective adjunct to SIT fruit fly and pink bollworm cooperative eradication and control programs.

The next three potential alternatives (2, 3, and 4), not considered in detail in this EIS, were submitted by the Center for Food Safety on February 20, 2007, as a comment letter regarding APHIS' Notice of Intent to prepare an EIS on the proposed use of genetically engineered fruit flies and pink bollworm (see: http://www.regulations.gov/fdmspublic/component/main; search for Dockets, Docket ID: APHIS–2006–0166–0007.1).

2. Nonuse of Autocidal Gene for Pink Bollworm

The suggestion was made that APHIS consider "at least one alternative involving a different approach to genetic engineering of the pink bollworm not involving use of an autocidal gene. . ." APHIS is currently exploring the use of genetically engineered pink bollworm, with independent marker genes and no repressible lethal genes, which would greatly expedite the deployment of the APHIS gamma radiation induced SIT cooperative pest eradication and control programs. Fluorescent protein marker genes, such as green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP) and DsRed, originally derived from jellyfish and coral, would allow the APHIS cooperative programs to distinguish insects that are mass-reared and sterilized with radiation from their wild-type relatives in pheromone attractant baited monitoring traps in the field. The proportional numbers of fluorescent marked insects to unmarked insects would allow APHIS cooperative programs to plan and conduct SIT releases more strategically and economically. This would improve cost effectiveness and target efforts in accordance with the most critical program needs. The use of marker gene traits in these insect pests will also help to more definitively evaluate success of the programs.

Genetically engineered repressible lethal insects, as a replacement for gamma radiation, would also contain a marker gene trait to facilitate pheromone trap monitoring and measurement of program success. Due to limited resources and lack of applicable available genetic engineering technologies, APHIS is not currently considering other alternatives involving a different approach to genetic engineering of either fruit flies or pink bollworm other than that which is discussed in this EIS.

3. Pink Bollworm Program without Genetic Engineering

Another suggestion was that APHIS consider "one alternative involving improvement of the sterile pink bollworm release program through approaches not involving genetic engineering. . ." The suggestion of improvement of the sterile fruit fly or pink bollworm SIT release programs through approaches not involving genetic engineering is accommodated by EIS alternative number two, which is expansion of existing programs for control of fruit flies and pink bollworm. This alternative could also mean increased insecticide reliance and use, which is the component method of most significant hazard in the present APHIS fruit fly and pink bollworm cooperative programs. This approach is clearly not the optimal method available to promote environmental risk reduction. Alternative two, expansion of existing programs and insecticide use activities and hazards, is described in detail elsewhere in this EIS.

4. Pink Bollworm Control Without Sterile Insect Technique

The suggestion was made for APHIS to consider "one alternative approach to pink bollworm control not involving sterile insect release." The suggestion of an approach to fruit fly or pink bollworm control not involving sterile insect release, either through use of gamma radiation or genetic engineering, is accommodated in the no action alternative of this EIS, which is described in detail elsewhere in this EIS.

If APHIS' SIT cooperative programs were not conducted to intercept, control, and eradicate invasive plant pests, such as fruit flies and pink bollworm, the following are potential impacts that could be of economic and environmental concern:

a. reduction of some fruit and vegetable availability in the U.S. marketplace;

b. increase in cost of some fruit and vegetables;

c. decrease in quality of some fruit and vegetables;

d. increase in insecticide use to control pests that are inadvertently imported;

e. increase in potential environmental hazards arising from more insecticide use;

f. increase in potential human health hazard from more insecticide use;

g. search costs for specific biocontrol agents for fruit flies and pink bollworm;

h. biocontrol agent risk assessments;

i. loss of export markets due to introduced fruit or vegetable pests; and

j. increase of genetic engineering to introduce genes for pest resistance in crops.

(This page is intentionally left blank.)

III. Affected Environment

A. Host Plant Ranges

1. Mexican Fruit Fly (*Anastrepha ludens*)

The Mexican fruit fly (*A. ludens*) is considered a serious pest in areas where it has become established. Populations have been identified in areas of South America, Central America, and Mexico. Within the United States, the Mexican fruit fly has been collected in the Lower Rio Grande Valley of Texas, and has been detected both in California and Florida where it was subsequently eradicated (Sequeira et al., 2001). The host species for Mexican fruit fly includes a wide variety of crops including citrus, pome fruit, mango, and avocado. Some of the host plants for Mexican fruit fly, such as apples, peaches, and pears, have a wide distribution throughout the United States suggesting Mexican fruit fly could become established over a large part of the United States; however, Mexican fruit fly will be limited by its northern spread due to environmental factors (figure 3–1).

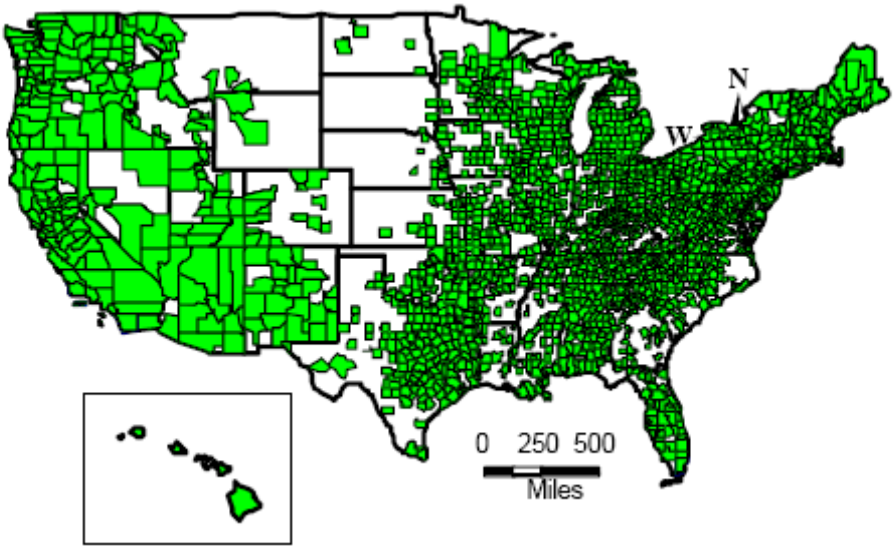

Figure 3–1. Distribution of commercially produced hosts of *Anastrepha* spp. in the United States. Hosts included are citrus, avocado, mango, peaches, plums, apples, pears, guavas, and figs. (Source: USDA–APHIS, 2001e).

Most flies within the *Anastrepha* genus are considered tropical, therefore, their spread northward into the United States is limited; however Mexican fruit fl*y* is considered a subtropical species and poses a larger threat to U.S. fruit production as compared to other fruit flies in the same genus. A developmental temperature threshold of 50 ^{o}F has been reported for Mexican fruit fly, with reports of high mortality occurring at temperatures above 100 ^{o}F (Sequeira et al., 2001). Using degree days per year, host

availability, and generation potential as primary factors that would dictate where Mexican fruit fly could spread in the United States, the highest risk appears to be in the southern United States, from western Texas to South Carolina, as well as southwest Arizona, Hawaii, and the interior of northern California down to central California, where the remaining coastal and interior areas would also be considered high risk (figure 3–2). Within these areas, Hawaii showed the highest risk of Mexican fruit fly establishment (Sequeira et al., 2001). The low area of risk is based on several factors including air temperature below freezing greater than 3 weeks per year, less than two fly generations per year, and host availability equal to or less than 6 months per year.

Due to the large geographic area that could be impacted by Mexican fruit fly, the focus of the affected environment discussion will be directed at those areas that pose a high risk for establishment. Site characteristics are based on descriptions provided by USDA regarding land resource and major land resource areas (USDA–NRCS, 2006).

The area of California considered high risk is characterized as having low mountains and broad valleys, with low annual precipitation and warm, long growing seasons. Average rainfall ranges from a low of 6 inches per year in the San Joaquin Valley, to a high of 40 inches per year along the coastal area north of San Francisco. The average annual temperature ranges from 41 to 67 ^{o}F, with the lower temperatures occurring at the higher elevations. The area is a major agricultural area with a variety of fruits and vegetables being grown in the area under irrigation, as well as dairy and beef cattle production.

Moving eastward from the coastal area of California, another high risk area exists in the extreme southeast portion of California and the southwestern part of Arizona, extending to the south-central part of the State. The area is primarily composed of the Sonoran Basin which has sloping valleys, as well as abrupt mountain ranges that can range in elevation from 980 to 4,590 feet. Rainfall is low with an average range of 3 to 10 inches per year for most of the area, with occasional 22 inch annual rainfall events in the mountains. Average annual temperatures range from 58 to 74 ^{o}F with freeze-free periods ranging from 205 to 365 days, with the lower values occurring at higher elevations. Greater than 80 percent of the land use is in grassland management with little crop production. Crops grown in the area are citrus, melons, cotton, alfalfa, small grains, and a variety of vegetables. Crop location is based on the presence of favorable water supplies for irrigation.

The next area of high risk is in southwestern and southern Texas which has a diverse topography ranging from mesas, ridges, and canyons in the northern part of the area, to gently rolling hills in the southern part. The

area has low annual precipitation with a range of 20 to 29 inches per year. The average annual temperature will range from 66 to 70 ^{o}F with a freeze-free period ranging from 265 to 320 days per year. A majority of the land use in the high risk area of Texas is privately managed grassland for cattle production with minor crop use. The exception is the extreme southern tip of Texas where citrus, cotton, sorghum, and vegetable production is common.

The remaining high risk areas, within the contiguous United States, occur from east Texas through a portion of each Gulf State, and include coastal areas of North and South Carolina. With the exception of the coastal areas of the Carolinas and most of Florida, this area contains Atlantic and Gulf Coast marine terraces and the hilly piedmont area. Elevation ranges are 80 to 655 feet on the coastal plains, while in the piedmont areas, the elevation can range from 330 to 1,310 feet. Along the coast, the region is mostly flat with little elevation relief. This area, unlike the western high risk areas, is characterized by hot and humid growing seasons with comparatively higher annual rainfall. Average annual rainfall will range from 44 to 63 inches per year, with a mean annual temperature range of 59 to 66 ^{o}F, and a freeze-free period ranging from 225 to 290 days per year. Within this high risk zone, the large area supports a variety of land use activities. Native vegetation is comprised mostly of oak-pine forests with a large amount of crop production focused on cotton, soybeans, peanuts, corn, rice, and sugarcane. Agricultural production that could be affected by the introduction of fruit flies include strawberries in Louisiana, as well as apple, peach, grape, and melon production in parts of Georgia, Alabama, and South Carolina. Within central and southern Florida, the predominant geography is low, flat, coastal plains with average annual rainfall ranging from 44 to 59 inches per year, and average annual temperatures of 70 to 75 ^{o}F. For most of the area, the freeze-free period is longer compared to other coastal areas ranging from 325 to 365 days. Approximately half of the area is swamp and marsh with agricultural activities consisting of grassland management for cattle, forestry, and crop production. Approximately 10 percent of the area is cropland, with citrus being a major crop for the area. Other crops grown are winter vegetables, avocado, papaya, and sugarcane.

The area that poses the greatest risk of Mexican fruit fly introduction is Hawaii. Compared to the high risk areas identified in the contiguous United States, Hawaii has the highest average rainfall. The average rainfall can vary widely from 60 to 220 inches on the windward side of the islands, and from 30 to 60 inches on the leeward side, with average annual temperature ranges from 56 to 75 ^{o}F, and a typical 365 day freeze-free period on all islands. The topography is diverse consisting of coastal plains, upland slopes, mountain ranges, and summits. Due to the unique climatic and environmental conditions, a diversity of unique crops is

grown on the islands. Pineapple, coffee, macadamia, papaya, and floral products are major export items. Other crops grown include tomatoes, cucumbers, and a variety of vegetables and other minor use crops. Cattle production is also important.

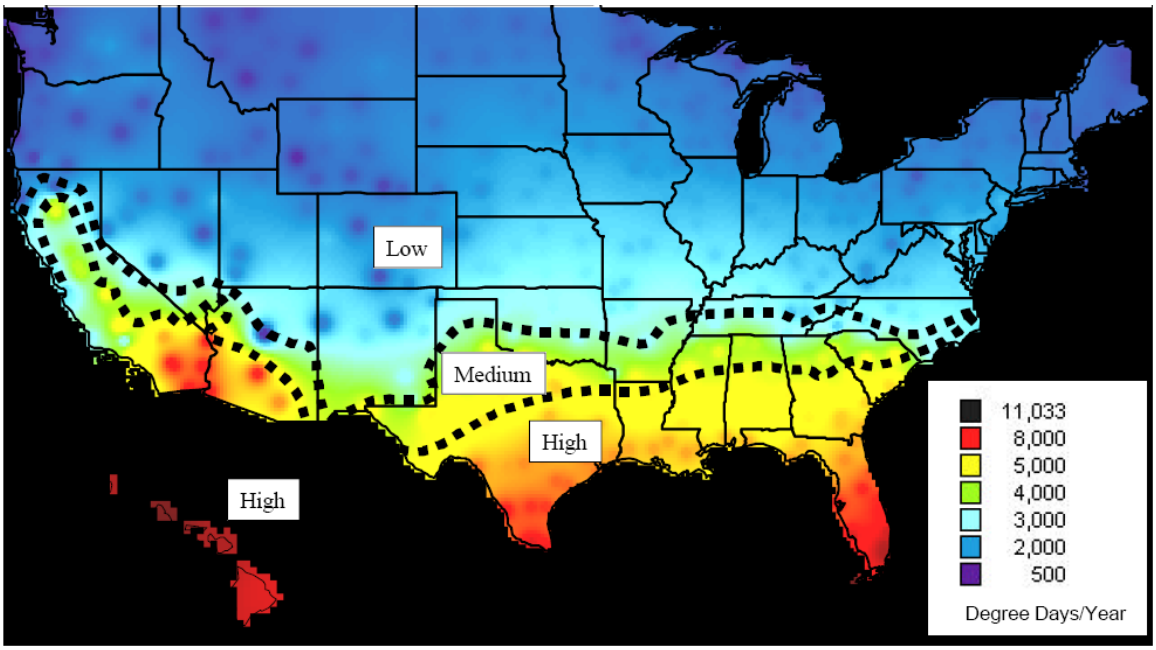

Figure 3–2. Potential risk of Mexican fruit fly establishment in the United States. (Source: USDA–APHIS, 2001e.)

2. Mediterranean Fruit Fly (*Ceratitis capitata*)

The Medfly is a subtropical tephritid fly that is native to Africa and has spread to all countries bordering the Mediterranean Sea, Australia, Central America, South America, Europe, and various Pacific Islands, including Hawaii (USDA–APHIS, 2003). The host plant range for Medfly is large and varied, and includes over 200 fruits and vegetables (USDA–APHIS, 2003; Thomas et al., 2006). As with Mexican fruit fly, the potential spread of Medfly within the United States could be widespread based on host plant distribution alone; however, due to a short period of time for development and lethal low temperatures, its northern expansion is expected to have a similar geographic risk profile, as outlined for the Mexican fruit fly (figure 3–2) (USDA–APHIS, 2001e; Vera et al., 2002).

Based on historical data regarding known Medfly infestations in the United States, the high risk areas identified for the Mexican fruit fly support the view that those areas are also high risk for Medfly (Carey, 1996; USDA–APHIS, 2007c). Based on infestation data collected since the 1920's, Medfly has occurred in California, Florida, and Texas. The greatest number of infestations that have occurred within the three States has occurred in California. Within California, the counties in and

surrounding Los Angeles have had the largest number of infestations beginning in 1975. The northernmost extent of known infestations in California has occurred in San Joaquin County, which was part of a larger infestation that included San Jose, Alameda, Contra Costa, Los Angeles, Monterey, San Benito, San Mateo, Santa Clara, Santa Cruz, and Stanislaus Counties (USDA–APHIS, 2007c). This area is known as the central California coast range with an average annual temperature of 51 to 66 ^{o}F, decreasing from south to north, and a freeze-free period average of 275 days. The freeze-free period can range widely from 180 to 365 days due to latitude and elevation changes (USDA–NRCS, 2006). A majority of the infestations have occurred in the southern California coastal range, with the Los Angeles area being an area of multiple infestations. The southernmost extent of known Medfly infestations, within the southern coastal range, has occurred in San Diego County. The freeze-free period in this southern coastal area is much longer with an average of 310 days, ranging from 255 to 365 days (USDA–NRCS, 2006). Although agriculture is not a dominant land use, a variety of agriculture production occurs in the area including irrigated subtropical and deciduous fruits, grains, truck crops, grapes, hay, and pasture.

In Texas, infestations have been confined to the extreme southern portion of the State, near Brownsville in the Lower Rio Grand Plain area, where agriculture predominates as a land use. A majority of the crops are grown under irrigation and include cotton, sorghum, citrus, onions, cabbage, and other truck crops.

In Florida, Medfly has been found in Miami and surrounding Dade County on multiple occasions, and represents its southernmost extent in the State. Infestations have also been detected in the more central part of the State, with detections in and around the Tampa area, including Hillsborough and Polk Counties. Further north, detections have occurred around Orlando in Orange and Lake Counties, with the northern extent being Marion County. The areas of infestation in Florida represent the southern Florida flatwoods and everglades area. Both areas have little elevation change with high rainfall, and an average freeze-free period ranging from 335 to 355 days. Cropland use is approximately 10 percent of total land use, with subtropical fruits grown in the northern area and a wide variety of fruits and vegetables grown in the south.

3. Oriental Fruit Fly (*Bactrocera dorsalis*)

The oriental fruit fly is a tropical species that has been introduced into the United States from Asia, and is known to attack over 230 fruits and vegetables (USDA–APHIS, 1989). To date, it has only been identified in California and Florida in isolated incidents, and is not thought to have become established in the United States, with the exception of Hawaii. Because oriental fruit fly is a tropical fruit fly, its ability to become established within the United States is expected to be more limited than

the areas identified for Medfly and Mexican fruit fly since it will be more sensitive to cold climate conditions. Current cold treatment measures for oriental fruit fly require temperatures to be maintained at 35 ^{o}F for 14 days to kill eggs and larvae (USDA–APHIS, 2005c). Based on temperature alone, using an average monthly temperature of 35 ^{o}F, the potential for introduction of oriental fruit fly into the United States is similar to those areas that were defined as high and medium risk for Mexican fruit fly. However, this is a conservative estimate since other factors, such as generation potential and host availability, were not considered. The affected environment for populations of oriental fruit fly is similar to those areas described above in Hawaii, the extreme southern area of California, Texas, and Florida where any freezing temperatures would not be likely occur.

Data on known infestations of oriental fruit fly in the continental United States supports the above assertion that its distribution will be more limited when compared to subtropical fruit flies. With the exception of an infestation in Santa Clara and Contra Costa Counties, all remaining infestations (35 total) occurred between the Los Angeles and San Diego surrounding areas in the southern coastal area of California. These infestations were focused in the Los Angeles, San Bernardino, San Diego, and Orange Counties of southern California. In Florida, infestations are only known to occur as far north in the State as the Tampa area of Hillsborough County (USDA–APHIS, 2007c). Limitations in potential oriental fruit fly infestations have also been validated based on climatic conditions, host susceptibility, and generation potential in the United States (USDA–APHIS, 2007c). Based on these range limiting factors, the highest potential for infestation is in southern Florida, the extreme southern tip of Texas, and those parts of California where it has previously been reported.

4. Pink Bollworm

The geographic scope of the pink bollworm program is based on factors related to host range, climate, and potential avenues of introduction. Pink bollworm hosts are limited to cotton and okra; therefore, the geographic areas will be limited to States where cotton and okra are grown. Cotton is commercially grown in the following States: Alabama, Arizona, Arkansas, California, Florida, Georgia, Kentucky, Kansas, Louisiana, Mississippi, Missouri, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia. USDA–Economic Research Service (ERS) divides the cotton production by geographic region: Southeast, Delta, Southwest, and West. The Southeast area includes Virginia, North Carolina, South Carolina, Georgia, Alabama, and Florida, and accounts for 25 percent of the production of cotton (USDA–ERS, 2007). The Delta region includes Kentucky, Tennessee, Mississippi, Arkansas, Louisiana, and Missouri and accounts for 35 percent of the nation's cotton (USDA–ERS, 2007). The Southwest region includes

Kansas, Oklahoma, and Texas, and accounts for 25 percent of the production of cotton (USDA–ERS, 2007). The West region includes New Mexico, Arizona, and California, and accounts for 16 percent of total production; however, this region has the highest yield of cotton (USDA–ERS, 2007). Of the cotton-producing States, Texas, Georgia, Mississippi, and Arkansas are traditionally the states that harvest most of the cotton in the United States (USDA–NASS, 2006). Texas, Mississippi, and Georgia rely on cotton as one of their top commodities (USDA–NASS, 2006).

Unlike cotton, okra is not a major commodity for any one State. California, Georgia, and Florida are the leading okra-producing States; however, it is grown throughout the South in Louisiana, Arkansas, Alabama, Texas, North Carolina, Oklahoma, Tennessee, South Carolina, Virginia, Mississippi, Kansas, and Arizona (Izekor and Katayama, 2007). Currently there are no States that grow okra that do not grow cotton.

The affected environment is limited because of meteorological effects, such as colder temperature and humidity levels, which limit the establishment and survival of pink bollworm populations. Colder temperatures decrease the likelihood of establishment in the northern States, and excessive moisture lessens the chances for establishment in parts of the southeastern United States such as Florida, Georgia, North Carolina, South Carolina, Virginia, Mississippi, Alabama, and parts of Louisiana and Arkansas (Venette and Hutchison, 1999).

The most likely introduction of pink bollworm to the southeastern United States is through the arrival of larvae transported to noninfested areas in cotton lint, trash, or harvesting equipment (Venette et. al, 2000). In a study done by Venette et al., (2000), different phases of the larvae were tested to see how cold temperatures and humidity levels affected their survival. The results revealed that colder temperatures, over a certain time period, had the most direct effect on mortality (Venette et. al, 2000). Humidity levels in the southeastern United States, although limiting the survival, did not affect survival rates significantly enough to prevent the permanent establishment of pink bollworm in the southern United States (Venette et. al., 2000). Although such introduction is possible, quarantine restrictions associated with the Pink Bollworm Eradication Program preclude the transport of cotton, cotton seed, lint, waste, cotton gin trash, equipment, and any other product that presents a risk of spread of pink bollworm without a certificate or permit.

A 2-year pink bollworm detection survey was conducted in Arkansas, Louisiana, Oklahoma, Texas, and New Mexico in 2000 and 2001 (Grefenstette et al., 2007). Preliminary analysis indicated that no pink bollworm populations were present in Arkansas, Louisiana, Oklahoma, or most of Texas (Grefenstette et al., 2007). It appeared that pink bollworm

populations were confined to west Texas and south central New Mexico (Grefenstette et. al., 2007). Additional trapping surveys from 2002 to 2004 confirmed these findings. Trappings in Arizona and Southern California have confirmed wide distributions of pink bollworm in those areas (Grefenstette et. al., 2007).

Eradication efforts have been successful in parts of Texas and New Mexico. Several finds have occurred at sites in a small area of Corpus Christi, Texas, after eradication efforts; however, this area is generally not an issue because pink bollworm finds have been too few and inconsistent to suggest that a population still exists. APHIS is also aware of subeconomic populations in other parts of Texas and New Mexico outside the eradication zones.

B. Comparative Wild Nongenetically Engineered Insect and Genetically Engineered Insect Biology

Comparison of the biological characteristics of genetically engineered fruit flies has not yet undergone testing by APHIS; however, there has been considerable fitness or performance testing of a pink bollworm strain expressing the DsRed protein fluorescence in the laboratory and field. The genetically modified fruit fly and pink bollworm biological characteristics and life table attributes that would be of importance, if the technology was not autocidal or self-mitigating would be related to fitness factors, which are those aspects of the biology, physiology, or behavior of the genetically modified insects that would allow them to have a selective advantage in the environment over its wild-type or sylvan strain. (See appendix D of this EIS for a more detailed discussion and some references about biological fitness factors.) However, those fitness factors that pertain to establishment, persistence, and growth of genetically engineered animal populations in the environment, when they can reproduce, do not apply to conditional lethal autocidal fruit flies and the pink bollworm strains that may be used in APHIS' cooperative SIT programs. In these programs, the released insects die with no offspring. Any kind of a fitness advantage that might conceivably exist would have to compete for survival against overwhelming reproductive sterility, even if the penetrance or successful expression of the sterility trait is less than 100 percent.

Biological fitness for transgenic fruit flies and pink bollworm, in this EIS, primarily relates to biological performance factors for use in APHIS' cooperative SIT control programs and not to establishment, reproduction, and persistence in the environment, because the insects are intended to be sterile and, therefore, unable to reproduce. Biological fitness would be 0 percent for male and female sterile transgenic insects because neither gender would be able to produce offspring and, thus, they would be

biologically unfit. This fitness would be theoretically 50 percent for the first generation of a 100 percent female-lethal system, in which all of the daughters die, but the male offspring live on to reproduce only males. However, a female-lethal system would also soon lead to population collapse because these males produce no female offspring to bear young.

1. Pink Bollworm Fitness or Performance Testing in 2007

Fitness or performance testing was conducted in 2007 and previous years under APHIS permits to compare APHIS mass-reared nonengineered pink bollworm to a pink bollworm strain genetically engineered to express DsRed fluorescent protein marker. Results in 2007 showed that the DsRed strain of the pink bollworm was comparably fit or performed comparably well to the APHIS mass-reared strain used for SIT.

APHIS mass-reared and the DsRed strains were released three to four times per week on about 100 acres of conventional, nonBt cotton in three fields for 10 weeks. The target release rate was 500 moths/ac/day. Pheromone traps baited with 2 mg Gossyplure were set out every 3 to 7 days. To estimate if there were differences in dispersal for one field, additional traps were set up outside the field at 200-m intervals along each cardinal direction up to 1 km from the field edge.

2007 research and development objectives were the following:

1. Test field performance of genetically modified market strain of pink bollworm in regard to—
   - Mass-rearing
   - Moth collection
   - Release by aircraft
2. Evaluate recapture rate;
3. Evaluate dispersal;
4. Evaluate longevity;
5. Conduct mating studies;
6. Monitor marker function; and
7. Confirm PCR identification of genetic marker.

a. Mass-rearing

A quarantine moth production and collection system was constructed for this experiment, and weekly production exceeded 600,000 moths/week. The maximum production capability is estimated to be 1.7 million moths/week, which is sufficient production to run future trials of up to 1,000 acres.

The DsRed and APHIS mass-reared strains of the pink bollworm had statistically similar rearing success and equivalent pupal sizes and moth weights.

b. Moth Collection/Recapture Rates

Throughout the experiment, recapture rate on pheromone monitoring traps were approximately equal with a slight trend for higher recapture rates for the DsRed moths. The number and proportion of male moths of the two strains caught in the pheromone traps was also practically identical. The DsRed moth visible marker is durable as fluorescence was still seen from moths in traps that were in the field for as long as 2 weeks and stored in the laboratory for at least 3 months.

c. Dispersal Evaluation

Dispersal occurred out to 800 m and 1,000 m for one DsRed moth edge with no significant difference between the conventional APHIS mass-reared and the DsRed strains.

d. Longevity/Mortality

Moth mortality measured at collection time was low for both types with a nonsignificant trend for the APHIS strain mortality to be higher than the DsRed strain.

e. Mating Studies

Comparison of female and male mating data suggest that the DsRed moth is highly competitive with the APHIS moth. Sterile female matings were designed to occur in the field. Results indicated that little difference occurred between the APHIS mass-reared and DsRed strains. Mating success for the DsRed strain ranged from 20 to 65 percent, and APHIS mating success ranged from 26 to 61 percent, with no statistical difference.

f. Monitor Marker Function/Confirm PCR Identification of Genetic Marker

Comparison of detection by DsRed fluorescence and PCR was virtually identical.

2. Research and Development Plans

Research and development plans include a season-long operational trial. This trial could lead to incorporation of the DsRed strain into the Pink Bollworm Cooperative Eradication Program. The DsRed marker adds an additional and possibly the best method over Calco Red dye and PCR to detect sterile moths.

Repressible lethal fruit flies and pink bollworm have not been tested by APHIS long enough, under laboratory or field cage conditions, to evaluate the applicable performance or fitness factors. However, because these

performance factors are directly linked to the successful and environmentally safe use of genetically engineered insects to improve APHIS' SIT cooperative programs, their application would be assessed in the process of evaluating the potential for each individual genetic construct or genetically engineered strain to improve APHIS' SIT cooperative programs. This testing would be conducted upon decision and funding, by APHIS and its collaborators, to proceed with the preferred alternative of the EIS to develop and expand research and development of repressible lethal and marker genetic engineering constructs for use in APHIS' SIT cooperative fruit fly and pink bollworm control programs.

These performance-fitness factors are relevant to genetic engineering of fruit flies and pink bollworm for use in SIT because the insects must be fit enough to be amenable to the mass-rearing and handling conditions, and be able to mate successfully with wild-type pest populations of the same species. SIT males must be able to live long enough and be sexually competitive with wild males to be able to ensure enough reproductive failure to significantly reduce the pest population. Description of the performance-fitness factors of importance for genetically engineered fruit flies and pink bollworm, in comparison to its nonengineered mass-reared cohorts and wild-type plant pest insects, is provided in appendix D (pages D–14 to D–15).

C. Description of the Affected Environment

1. Ecoregions of the Potential Program Area

The geographic area most at risk for future programs falls within the boundaries of eight ecoregions. (Refer to figure 3–3 for a general map of the eight ecoregions and the States included in each.)

Northwestern Forest, Forage, and Specialty Crop ecoregion includes the potential program areas in the State of Washington and adjacent areas of Oregon. The program areas are primarily east of the Cascades in the Columbia River Basin. For the purposes of this EIS, the mountainous areas of the Cascades (usually considered part of this ecoregion) have been omitted because these areas are unlikely to continuously support fruit fly populations or cotton fields where pink bollworm could establish.

California Subtropical Fruit, Truck, and Specialty Crop ecoregion includes southern coastal and south-central valley areas of California. For the purposes of this EIS, the Sierra Nevada range (usually considered part of this ecoregion) has been omitted because it is an area unlikely to continuously support fruit fly or pink bollworm populations.

Western Range and Irrigated ecoregion spans potential program areas in Arizona, New Mexico, and southestern California.

Lower Rio Grande Plain ecoregion in Texas is bounded on the east by the gulf coastal plain and the south by the Rio Grande River. It marks the southern terminus of the central Texas plains and includes potential program areas in South Texas.

Mississippi Delta Cotton and Feed Grains ecoregion includes potential program areas in the Mississippi River Delta areas of Louisiana and Mississippi.

South Atlantic and Gulf Slope Cash Crops, Forest, and Livestock ecoregion consists of generally smooth marine terraces and the hilly piedmont area in Louisiana, Mississippi, Alabama, and Georgia. Although this ecoregion also includes parts of Tenessee, Arkansas, South Carolina, North Carolina, and Virginia, these have been omitted because the area is unlikely to continuously support fruit fly or pink bollworm populations.

Atlantic and Gulf Coast Lowland Forest and Crop ecoregion is a low-lying area bounded by the Atlantic Ocean, the rolling hills of the southeastern plains, and the Gulf of Mexico.

Floridian ecoregion includes most of peninsular Florida. Potential program areas are found throughout the State.

2. The Physical Environment

A general description of the physical environment of the potential program areas (climate, land resources, water resources and quality, and air quality) follows. More detailed information on the physical characteristics of the area may be found in tables 3–1 through 3–8, for each ecoregion, according to major land resources subregions.

a. Climate

The climate of the potential program areas varies considerably. The cool, wet marine climate of the Pacific Northwest differs from the warm Mediterranean climate of southern California. The hot climate of the southwestern desert and Lower Rio Grande Valley contrasts with the cooler climate of the mountains and foothills of the West.

Annual precipitation varies from less than 6 inches in the Sonora Basin and Imperial Valley in Arizona and California, to 87 inches in the Cascade Mountains, Eastern Slope, and Siskiyou-Trinity areas. The climate affects soils, vegetation, and wildlife that are indigenous to individual areas, as well as land resources, socioeconomics, and human populations in potential program areas. Degradation of residues from potential program pesticide applications generally would be greater in areas with higher rainfall and temperatures. In general, warmer temperatures and longer

freeze-free periods allow fruit fly populations to increase more rapidly with resultant increased potential for spread.

b. Land Resources

The topography of the potential program area varies from the slightly rolling gulf coast to the steep regions of the Cascades and Sierra Nevada. Elevations range from 275 feet below sea level in the deserts of California to about 7,000 feet in the Arizona and New Mexico mountains. Soil reaction ranges from predominantly acid in the East to alkaline in the West. Introduced fruit fly populations would not be expected to survive or get established at high elevations. Degradation of residues from potential program pesticide applications would be expected to occur more rapidly at lower elevations. Varied topography and cropping patterns provide more host crops and microclimates that contribute to enhanced fruit fly survival and spread.

c. Water Resources and Quality

Water availability varies greatly across the potential program area, ranging from very abundant in Florida and the eastern gulf coast, to extremely scarce in the desert regions of the West. The more mountainous areas are characterized by natural lakes and large, deep reservoirs. Groundwater is abundant in the valleys and is used for irrigation and livestock production. Water supply is low to moderate in the prairie subregions. Surface lakes, shallow wells, and streams in these areas are used for irrigation and watering of animals. Intermittent waters, such as seasonally flooded impoundments, are important breeding grounds, as well as migration stops for waterfowl and other wetland species. The southwest, intermountain areas, Sacramento Valley, and San Joaquin Valley are characterized by low precipitation and constant water sources. Water for irrigation and livestock comes primarily from the few reservoirs and large rivers. Although the annual precipitation east of the Cascades in Washington is low, there is a constant source of available water from the mountains. Potential contamination of surface water and groundwater resources by program pesticides could pose a hazard to both wildlife and human populations. Because of agricultural and other uses, low-level background residues of certain pesticides in water are common in some areas. Therefore, cumulative effects of the program use of pesticides must be considered.

d. Air Quality

In general, the air quality of most of the potential program area is good. Most air pollution problems occur in industrialized and urban areas, particularly in the Eastern States. The air quality of most of the Western

Figure 3–3. Principle Ecoregions of the Cooperative Control Program

Table 3–1. Land Resources and Characteristics
Northwestern Forest, Forage, and Specialty Crop Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Northern Pacific Coast Range, Foothills, and Valleys	Mostly densely forested; timber production is the major industry; recreation and wildlife habitat are also important land uses.	100 to 2,500 ft; peaks in southern end range to 400 ft.	60 to 200 in, increasing with elevation. --- Precipitation is evenly distributed throughout fall, winter, and spring; summers are cool and dry.	40 to 55 °F, decreasing with elevation --- Avg. 200 days, ranging from 150 to 280 days, decreasing with elevation.	66% surface water, 34% ground water sources; precipitation and perennial streams fed by springs provide abundant surface water; The Oregon – Washington River Basin.	Andisols, Inceptisols, and Utisols.	Small number of Washington and Oregon State parks. Indian Reservations: Grande Ronde and Siletz
Williamette and Puget Sound Valleys	Nearly 1/3 of the land is forested—timber production the major industry; agriculture highly diversified (deciduous fruits, berries, vegetables, seed crops, and grains; wine grapes increasing); urbanization increasing in much of the area.	Sea level to 1,640 ft.	Avg. 30 to 60 in—down to 17 in on lee side of western border, 60 to 90 in (highest average) along eastern border. --- Precipitation evenly distributed fall, winter, and spring; dry summers.	42 to 54 °F --- 190 days average and ranges from 165 to 220 days.	80% surface water, 20% ground water sources; moderate precipitation and abundant stream flow provide water; surface water supplies often short in summer. Rivers: Columbia and Willamette.	Alfisols, Inceptisols, Mollisols, and Ultisols.	Cities: Seattle, Tacoma, Olympia, and Vancouver, WA; Portland, Corvallis, and Eugene, OR. Indian Reservations: Lummi, Tulalip, and Nisqually..
Olympic and Cascade Mountains	Densely forested with timber the major industry; mining, recreation and wildlife habitat; at high elevations, alpine meadows provide summer range.	660 to 5,600 ft, as high as 14,400 ft on mountain peaks.	Avg. 60 to 140 in, as much as 280 in on Mt. Olympus. --- Most rainfall occurs during the fall, winter, and spring.	27 to 53 °F, decreasing with elev. --- Avg. 189 days, ranges from 72 to 307 days.	85% surface water, 15% ground water sources; precipitation and perennial streams fed by glaciers and springs. Rivers: Columbia.	Andisols, Inceptisols, Spodosols, and Ultisols.	No major cities. National forests: the Olympic, Mt. Baker-Snoqualmie, Gifford Pinchot, Mt. Hood, Willamette, and Umpqua; national parks.
Sitka Spruce Belt	Farms, ranches, or forests; major industry is lumbering; vegetables and fruits (apples), specialty crops (cranberries and lily bulbs).	Sea level to 1,800 ft.	52 to 60 in near the beach, up to 191 in at higher elevations. --- Evenly distributed precipitation throughout fall, winter and spring; dry and cool summers.	45 to 55 °F --- Avg. 290 days, ranging from 220 to 365 days.	78% surface water, 22% ground water sources; abundant precipitation, many perennial streams; Rivers: Columbia.	Andisols, Inceptisols, Spodosols, and Entisols.	Cities: Aberdeen, Hoquiam, and Forks, WA, Astoria, Tillamook, and Coos Bay, OR. Indian Reservations: Quinault, Quileute, Ozette, Hob, and Makah.

Table 3–1, continued.

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Coastal Redwood Belt	Privately owned farms, ranches, or forests; grasslands for grazing; farming (vegetables, fruits (apples), and lily bulbs); the major industry is lumbering.	Sea level to 2,600 ft, some Coast Range peaks are 3,940 ft.	23 to 98 in, incr. with elevation inland. --- Evenly distributed precipitation throughout fall, winter, and spring; dry summers.	49 to 59 °F --- Avg. 300 days and ranging from 230 to 365 days, decreasing with inland elev.	84% surface water and 16% ground water sources; abundant precipitation, many perennial streams; surface water supply often short in summers. Rivers: Smith, Klamath, Mad, Eel, Mattole, Noyo, Navarro, Chetco, Winchuck, and Garcia. Redwood Creek.	Alfisols, Entisols, Inceptisols, and Ultisols.	Cities: Crescent City, Arcata, Eureka, and Fort Bragg, CA, and Bookings, OR. Redwood Natl. Park, numerous CA State Parks.
Siskiyou-Trinity Area	Coniferous forests important for wood products, wildlife habitat, and recreation; 1/10th of area grazed, a smaller acreage is cropped; raising livestock is the principal farm enterprise.	330 to 6,000 ft, some mountain peaks 8,850 ft.	Lower elevations 14 to 20 in, mts. as much as 200 in. --- Most precipitation occurs between November and April, very little precipitation in summers.	40 to 62 °F, decr. with elevation. --- Avg. 240 days and ranges from 110 to 365 days; shorter freeze-free periods at higher elevations.	75% surface water and 25% ground water sources; moderate to high precipitation and mountains supply water. Rivers: Rogue, Eel, Trinity, and Klamath.	Alfisols, Inceptisols, Ultisols, and Xerolls.	Cities: Grants Pass, Medford, and Roseburg, OR; Weaverville, CA. Many national forests incl. Siskiyou, Klamath, Trinity, Shasta, and Mendocino. Indian Reservations: Hoopa Valley and Round Valley.
Cascade Mountains, Eastern Slope	Primarily coniferous forest, timber production important industry; grassland for grazing and woodland grazed by cattle. Recreation and wildlife habitat. Irrigated cropland produces tree fruits, small grains, and forage crops.	900 to 8,000 ft, some mountain peaks approach 10,000 ft.	12 to 87 in. --- Precipitation occurs during winter, spring, and fall; summers are relatively dry.	32 to 53 °F --- Averages 145 days and ranges from 0 to 250 days.	71% surface water, 29% ground water sources; precipitation and perennial streams; surface runoff is dominated by snowmelt. Rivers: Columbia, Klamath, and Yakima.	Alfisols, Andisols, Inceptisols, and Mollisols.	Cities: Levenworth, Washington; Bend, and Dalles, Oregon. Indian Reservations: Yakama and the Warm Springs.

Source: USDA–NRCS, 2006.

Table 3–2. Land Resources and Characteristics
California Subtropical Fruit, Truck, and Specialty Crop Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Central California Coastal Valleys	Farms and ranches with urban development increasing rapidly. Crops include wine grapes, strawberries and other fruits, cut flowers, small grains, hay, and pasture; dairy farming; livestock grazing.	Sea level to 1,970 ft, mostly less than 985 ft	11 to 66 in --- Low to moderate rainfall; Pacific frontal storms in winter; the area is very dry from mid-spring to mid-autumn	56 to 61 °F --- Averages 315 days and ranging from 265 to 365 days.	56% of water is from ground water sources, 44% from surface water sources; low or moderate rainfall and local streamflow (inadequate for needs)	Alfisols, Entisols, Mollisols, Vertisols	Cities: Ukiah, Santa Rosa, Napa, San Francisco, Berkeley, Vallejo Oakland, San Jose, Santa Cruz, Monterey, and Carmel.
Central California Coast Range	Farming and ranching; dry-farmed grain; native grasses and brush. Open woodland used for grazing; small acreage for urban development.	Sea level to 2,650 ft, 4,950 ft in some of the mountains	South of San Francisco—6 to 20 in; north of San Francisco—18 to 20 in; far north—40 to 79 in. --- Precipitation evenly distributed throughout fall, winter, and spring; low in summer.	51 to 66 °F --- Averages 275 days and ranging from 180 to 365 days.	22% of water is from ground water sources, 78% is from surface water. Low or moderate rainfall, moderate streamflow.	Alfisols, Entisols, Mollisols, Vertisols	Towns of Clearlake, Suisun City, Benicia, Martinez, Concord. Pleasant Hill, Alamo, Atascadero, Paso Robles. Santa Ynez Indian Reservation.
California Delta	Farming; most important crops: asparagus, sugar beets, potatoes, corn, grain, and hay, pear trees, grapes.	Below sea level to slightly above sea level.	12 to 21 in --- Dry summers	59 to 61 °F --- Averages 345 days and ranging from 330 to 360 days.	About 20% of water is from ground water, 80% from surface water sources; almost all of it comes from sloughs and waterways. Rivers: Sacramento and San Joaquin.	Entisols, Histosols, Mollisols	No cities or large towns.

Table 3–2, continued.

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Sacramento and San Joaquin Valleys	Farms and ranches, rapidly incr. urban development; irrigated cropland incl. products of cotton, nuts, grapes, hay, grain, pasture, rice, alfalfa, citrus, and truck crops incl. tomatoes. Grazing and dry-farmed grain on nonirrigated cropland.	Sea level to 660 ft.	San Joaquin Valley—5 to 12 In; at southern end of area, Tulare Basin—less than 6 in. Avg. ann. precip. most of Sacramento Valley 12 to 30 in; 40 in higher elevations. --- Long, hot, dry summers; cool and rainy winters.	59 to 67 °F --- Avg. 325 days, ranging from 280 to 365 days.	Abt. 47% from ground water sources, 53% surface water sources; Rivers: Sacramento and San Joaquin.	Alficols, Aridisols, Entisols, Mollisols, and Vertisols.	Cities: Redding, Red Bluff, Chico, Yuba City, Davis, Sacramento, Stockton, Modesto, Fresno, Hanford, and Bakersfield.
Sierra Nevada Foothills	Rangeland used for livestock; hardwood forest; cropland for nuts, grapes, and other fruits grown using irrigation.	656 to 1,641 ft, up to 3,937 ft on mountain peaks	18 to 45 in --- Hot and dry summers, cool and moist winters.	47 to 67 °F --- 275 days ranging from 180 to 365 days.	Abt. 12% from ground water sources, 88% surface water; moderate rainfall, intermittent streamflow; numerous stock ponds.	Alfisols, Entisols, Inceptisols, and Mollisols.	Auburn, Folsom, Cameron Park, Oroville, Ione. Tule Indian Reservation.
Southern California Coastal Plain	Urban areas; brushland used for watershed protection; irrigated crops such as subtropical fruits, deciduous fruits, grain, truck crops, grapes, hay, and pasture; dairy farming, flower seed production, some livestock.	Sea level to 1,970 ft	10 to 29 in --- Dry summers, fog provides moisture along the coast.	55 to 66 °F --- 310 days ranging from 255 to 365 days.	Abt. 35% from ground water sources, 65% surface water resources; low rainfall and intermittent streamflow.	Alfisols, Entisols, and Mollisols.	Cities: Ventura. Los Angeles, and San Diego.
Southern California Mountains	Urban development, farms, ranches; open woodland and brushland for grazing; dry-farmed grain and hay; some irrigated fruit crops.	1,000 to 7,900 ft in most of the area.	8 to 51 in --- Dry summers, some snow in winter	41 to 66 °F --- 245 days ranging from 125 to 365 days.	Abt. 61% from ground water sources, 39% surface water; Rivers: Santa Clara, Los Angeles.	Alfisols, Entisols, Inceptisols, and Mollisols.	Ciies: Santa Barbara, Fillmore, Ramona, and Banning. Numerous Indian reservations.

Source: USDA–NRCS, 2006.

Table 3–3. Land Resources and Characteristics Western Range and Irrigated Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Mojave Desert	Mostly a cover of desert vegetation used only locally for grazing; irrigated cropland, where adequate water supply available, undergoing urbanization.	282 ft below sea level in Death Valley to 3,950 ft above sea level.	2 to 8 in—some scattered areas, in higher elevations exceed 37 in. --- Most rainfall occurs in winter months; avg. snowfall ranges from nearly 0 in (deserts) to more than 30 in (highest elevations).	43 °F in the highest mountains to 76 °F in areas along Colorado River. --- 200 to 330 days in desert areas; higher mountains and valleys 150 to 180 days.	Abt. 14% from, ground water sources, 86% surface water; water is scarce. Rivers: Colorado, Armagosa, and Mojave.	Aridisols, Entisols.	Lancaster, Palmdale, Victorville, Apple Valley, and Barstow, CA; Bullhead City and Kingman, AZ; Las Vegas, NV. Mojave National Preserve, Joshua Tree and Death Valley National Parks.
Lower Colorado Desert	All agricultural crops grown under irrigation: cotton, alfalfa, hay, small grain, row crops (lettuce, melons, onions, sweet corn, grain sorghum, squash, sugar beets); table grapes, citrus fruit, winter vegetables, dates. Warm-season pasture grasses, winter pasture for sheep is provided by alfalfa.	Approx. 275 ft below sea level to 1,650 ft above sea level.	3 to 22 in --- Summer precipitation makes up 20 to 35% of total annual precipitation.	53 to 74 °F --- Avg. 290 days, ranges from 220 to 365 days.	2% ground water sources, 98% surface water; water is scarce. Salton Sea. Rivers: Colorado, New, Alamo.	Entisols, Aridisols.	Blythe, El Centro, Indio, and Oasis, CA; Cabezon, Augustine, Torres-Martinez, and Fort Yuma Indian Reservations in CA; the Cocopah Indian Reservation in AZ; Colorado River Indian Reservation between CA and AZ.
Colorado Plateau	Rangeland grazed by sheep and cattle; irrigated cropland—alfalfa, small grains for hay and corn for silage are chief crops. Some dry-farmed areas (corn). Desert shrub and woodland vegetation (juniper and pinyon-juniper woodland) producing firewood and pinyon nuts; recreation; being converted to housing developments.	Most areas 4,250 to 4,950 ft; Mt. Trumbull 8,028 ft; Navajo Mt. (on UT-AZ State line) 10,388 ft.	Almost all areas—6 to 18 in but la few basins have less than 5 in. --- Abt. half precipitation falls from July through September; April, May, June are driest months.	36 to 66 °F --- Avg. 215 days, ranges from 105 to 320 days.	Abt. 35% ground water sources, 65% surface water; water is scarce—ephemeral streams and rivers. Navajo Reservoir Rivers: Colorado, Little Colorado, Mancos, McElmo	Alfisols, Aridisols, Entisols, and Mollisols.	Kingman and Winslow, AZ; Gallup and Grants, NM; Kanab and Moab, UT. Navajo, Hopi, Zuni, Havasupai, Hualapai and Kaibab Nations.

Table 3–3, continued.

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Mogollon Transition	Most area used for livestock grazing; many tracts of rangeland subdivided for community development.	3,000 to 5,500 ft in most areas, 5,100 to 7,500 ft in the mts.	10 to 37 in for most of this area. --- More than half precip during July, August, September; 2nd rainy season December to March.	47 to 70 °F --- Avg. 255 days, ranges from 145 to 365 days.	Abt. 22% ground water sources, 78% surface water; much water stored and used for irrigation; small natural and artificial lakes.	Aridisols, Alfisols, Mollisols.	Globe and Prescott, AZ; Silver City, NM; Hualapai, Yavapai, Camp Verde, Lower Camp Verde, and San Carlos Indian Reservations.
Arizona and New Mexico Mountains	Most area used for timber production or livestock grazing; many tracts of rangeland subdivided for community development.	4,000 to 7,000 ft in southern half of area, north rises to more than 7,500 ft and drops northward to 5,000 or 6,000 ft.	Avg. 15 to 30 in for most of the area, a few of the lower areas avg. 9 to 15 in. --- More than half precip. during July, August, September; 2nd rainy season December to March.	36 to 55 °F --- Avg. 135 days with range of 60 to 205 days.	Abt. 70% from ground water sources, 30% surface water; several lakes and reservoirs. Rivers: Black, White, Verde, and Salt.	Inceptisols, Mollisols, Alfisols, Entisols.	Flagstaff and Springerville, AZ; Reserve, Ruidoso, and Cloudcraft, NM; includes large part of the Fort Apache Indian Reservation.
Sonoran Basin and Range	Desert land for limited grazing during times of favorable moisture; irrigated areas/crops—cotton, alfalfa, barley, other small grains; where water supplies are favorable—lettuce, carrots, cabbage, cauliflower, melons, other market vegetables and citrus. Rapid urbanization.	980 to 3,600 ft in most of this area, as high as 4,590 ft in the mts.	3 to 10 in for most of this area; rainfall can avg. 22 in per yr in mountain ranges. --- Most rainfall July to September and December to March.	58 to 74 °F --- Avg. 285 days, ranges from 205 to 365 days.	40% ground water sources, 60% surface water; alluvial aquifers with deep wells; ground water table continually drops. Rivers: Colorado, Salt, and Gila.	Aridisols, Entisols.	Yuma, Tucson, Phoenix, AZ; Tohono O'Odham, Colorado River, Salt River, and Gila River Indian Reservations.
Southeastern Arizona Basin and Range	Most of the area used for livestock grazing; some areas used for cotton, corn, alfalfa, small grains, or other farm crops; tracts of rangeland and cropland subdivided for community development.	2,620 to 4,590 ft in most areas, gen. ranges from 4,920 to 5,900 ft in mts.; some peaks almost reach 8,900 ft.	Avg. 9 to 20 in for most of the area but as much as 45 in at higher elev. --- More than half precip. In July to September; 2nd rainy season from December to March.	47 to 68 °F --- Avg. 245 days, ranges from 160 to 335 days.	75% ground water sources, 25% surface water. Rivers: San Pedro	Aridisols, Entisols, Alfisols, and Millisols.	Nogales, Bisbee, and Sierra Vista, AZ; eastern edge of Papago Indian Reservation and southern part of San Carlos Indian Reservation.

Source: USDA–NRCS, 2006.

Table 3–4. Land Resources and Characteristics Lower Rio Grande Plain Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation ---------- Rainfall Distribution	Avg. Annual Temperature ---------- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Lower Rio Grande Plain	Most area extensively irrigated cropland or improved pasture; major crops: cotton, grain sorghum, citrus, onions, cabbage, other truck crops; hunting.	15 to 600 ft, mainly less than 275 ft.	22 to 27 in ---------- Most rainfall during winter; heavy rainfall late summer and early fall.	72 to 74 °F ---------- Avg. 350 days and ranges from 330 to 365 days.	Abt. 43% ground water sources, 57% surface water; International Amistad and Falcon Reservoirs. River: Rio Grande	Alfisols, Mollisols, Vertisols, Inceptisols	Brownsville, Edinburg, Harlingen, McAllen, and Raymondville

Source: USDA–NRCS, 2006.

Table 3–5. Land Resources and Characteristics
Mississippi Delta Cotton and Feed Grains Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Southern Mississippi River Alluvium	Farming (cotton, soybeans, milo, corn, sugarcane rice); catfish and crawfish produced commercially on farm ponds contained in levees; migratory waterfowl are harvested throughout the area; hardwood timber is harvested on most forested areas and are managed for wildlife.	Sea level to 330 ft.	46 to 60 in, can be as high as 65 in --- Most of the rainfall occurs during late fall, winter, and early spring.	56 to 69 °F --- Avg. 285 days and ranges from 210 to 355 days	Abt. 58%ground water sources, 42% from surface water; high amounts of precipitation, stream-flow, oxbow lakes, bayous, canals, and rivers. Mississippi River	Alfisols, Vertisols, Inceptisols, and Entisols	Morgan City and Houma, LA

Source: USDA–NRCS, 2006.

Table 3–6. Land Resources and Characteristics
South Atlantic Gulf Slope Cash Crops, Forest, and Livestock Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation ---------- Rainfall Distribution	Avg. Annual Temperature ---------- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Southern Coastal Plain	Timber production, cash-grain crops, forage production; crops: soybeans, cotton, corn, and wheat are the major crops; pastures are grazed by beef cattle; some dairy cattle and hogs.	80 to 655 ft	41 to 60 in ---------- Max precipitation occurs during midsummer in eastern part and during winter and spring in western part.	55 to 68 °F ---------- Avg 250 days and ranges from 200 305 days, increasing in length from north to south	Abt 18% ground water sources, 82% from surface water; precipitation and perennial streams; large reservoirs, shallow wells, aquifers.	Ultisols, Entisols, and Inceptisols	Tallahassee, FL
North-Central Florida Ridge	Wooded farms, forestry; pulpwood and lumber, crops: corn, peanuts, tobacco, soybeans, vegetables, and melons; some hay and feed grains grown for livestock.	80 to 165 feet	53 to 60 in ---------- Max precipitation occurs in summer; min in winter and late autumn.	67 to 69 °F ---------- Avg 295 days and ranges from 280 to 305 days.	Abt 82% is from ground water sources, 18% from surface water; abundant rainfall and Floridan aquifer	Ultisols, Entisols, and Alfisols	No large cities in area

Source: USDA–NRCS, 2006.

Table 3–7. Land Resources and Characteristics
Atlantic and Gulf Coast Lowland Forest and Crop Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Gulf Coastal Prairies	Farmland—rice, soybeans, grain sorghum, cotton, corn, and hay. Hardwoods from forested areas. Urban development rapidly expanding.	Sea level to about 165 ft.	45 to 63 in --- Fairly evenly distributed precipitation over the year.	66 to 72 °F --- Avg. 325 ranging from 290 to 365 days.	70% from surface water, 30% ground water sources; rainfall and perennial streams. Sabine River. Urbanization and industrial wastes are threatening water supplies.	Alfisols. Mollisols, and Vertisols.	City and Port of Houston.
Gulf Coast Saline Prairies	Urban and recreational developments are expanding; Ranching—beef cattle, rice, grain sorghum, wildlife refuges.	Sea level to 10 ft, occasional coastal dunes to 25 ft.	45 to 57 in --- Abundant rainfall spring and fall.	68 to 74 °F --- Avg. 340 ranging from 315 to 365 days.	1% from ground water, 99% from surface water sources; freshwater streams and rivers, many bays and small entrapments of salty water. Sabine River.	Alfisols, Entisols, Inceptisols, Mollisols, and Vertisols.	Galveston
Gulf Coast Marsh	Wildlife refuges and State parks; livestock and cattle, rice farming.	Sea level to 7 ft, 10 ft on beach ridges.	60 to 65 in --- Precipitation occurs during growing season	67 to 69 °F --- Avg. 325 ranging from 290 to 365 days.	93% from surface water, 7% ground water sources. Lakes and bayous, tidal channels and manmade canals. Rivers: Mississippi, Sabine, and Vermillion.	Entisols and Histosols.	New Orleans, Ft. Jackson, Jean Lafitte National Historic Park and Preserve.
Eastern Gulf Coast Flatwoods	Pulpwood and lumber; State and national forests; game refuges, military training sites. Small percentage of acreage is crops: corn, peanuts, tobacco, soybeans.	Sea level to 80 ft	60 to 68 in --- Precipitation usually occurs in summer.	64 to 71 °F --- Avg. 300 ranging from 250 to 350 days.	24% ground, 76% surface water sources; abundant rainfall and perennial streams. Rivers: Escambia, Yellow, Choctawhatchee, Suwannee.	Alfisols, Ultisols, Entisols, Spodosols, and Histosols.	Gulfport and Biloxi, MS; Mobile, AL; Pensacola and Panama City, FL

Table 3–7, continued.

Subregion	Land Use	Elevation/ Topography	Annual Precipitation --- Rainfall Distribution	Avg. Annual Temperature --- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
Atlantic Coast Flatwoods	Farms, national forest, game refuges; crops—melons, sweet potatoes, Irish potatoes, corn, soybeans, wheat, tobacco; poultry farming.	25 to 165 ft	44 to 57 in --- Maximum in summer	58 to 69 °F --- Avg. 290 days, ranging from 210 to 365 days	24% from ground water sources, 76% surface water; rainfall, perennial streams Rivers: Ogeechee, Suwannee, Savannah.	Spodosols and Ultisols.	Florence, Summerville, Orangeburg, SC; Ft. Stewart, GA; Lakeside and Jacksonville, FL
Tidewater Area	National forests, game refuges, urban development, farmwood lot, croplands—corn, soybeans, tobacco, and vegetables; recreational enterprises.	Sea level to 25 ft.	40 to 58 in --- Maximum precipitation occurs in summer.	58 to 69 °F --- Avg. 295 days, ranging from 230 to 360 days.	Abt. 8% ground water and 92% surface water sources; rainfall, perennial streams, wells and estuaries. Rivers: Cooper. Edisto, and Coosaw	Alfisols, Entisols, and Histosols.	Mt. Pleasant and Charleston, SC; Savannah and Brunswick, GA; Yulee, FL.

Source: USDA–NRCS, 2006.

Table 3–8. Land Resources and Characteristics Floridian Ecoregion

Subregion	Land Use	Elevation/ Topography	Annual Precipitation ---------- Rainfall Distribution	Avg. Annual Temperature ---------- Freeze-free Period	Freshwater Resources	Soils	Representative Sites
South-Central Florida Ridge	Agriculture (livestock/beef cattle, major citrus-producing area, and winter crops); forest products (pulpwood and lumber); dairying near some large cities; large amount of urban land.	80 to 165 ft, ranging from sea level to 330 ft on some hills.	46 to 56 in --- About 60% precipitation occurs from June through September; late autumn and winter are relatively dry.	68 to 73 °F --- Averages 335 days and ranges from 300 to 365 days.	21% surface water sources, 79% ground water; rainfall; many lakes and few perennial streams. Rivers: Withlacoochee	Entisols and Ultisols	Parts of east side of city of Tampa Bay and west half of Orlando; Ocala Natl. Forest and Withlacoochee State Forest.
Southern Florida Flatwoods	Forestland grazed extensively; improved pasture or native range grazed by cattle; winter vegetables, some citrus fruits, and other subtropical fruits.	Ranges from sea level to less than 80 ft.	44 to 60 in --- 60% precipitation occurs June through September; late autumn and winter are relatively dry.	68 to 75 °F --- Averages 335 days and ranges from 300 to 365 days.	60% ground water sources, 40% surface water; rainfall. Rivers: St. Johns, Kissimmee, and Caloosahatchee.	Alfisols, Entisols, and Spodosols.	Cities: Gainesville, Ocala, Daytona Beach, West Palm Beach, Fort Lauderdale, St. Petersburg, Fort Meyers, and most of Tampa Bay.
Florida Everglades and Associated Areas	Abt. 1/3 of area is Indian reservations;,national parks, game refuges or other large holdings; cypress and mangrove forests; recreation; crops (winter vegetables, citrus fruits, avocado, papaya, sugarcane); pasture for beef cattle and dairying; urban development.	Sea level to less than 80 ft.	40 to 62 in --- 60% precipitation occurs June through September; late autumn and winter are relatively dry.	73 to 78 °F --- Averages 355 days and ranges 345 to 365 days.	Abt. 53% surface water sources, 47% ground water; these plus rainfall provide abundance of water. Half of Lake Okeechobee	Entisols and Histosols	Miami; Everglades Natl. Park; Big Cypress Seminole Indian Reservation.
Southern Florida Lowlands	Largely farms and ranches; citrus crops are the chief crops; rangeland vegetation is native grasses, forbs, sedges and a few scattered pines. Forestland is mixed palms, cabbage palm, hardwoods.	85 ft—mostly flat area.	46 to 60 in --- Abt. 60% precipitation occurs from June through September; spring, fall, and winter are relatively dry.	71 to 74 °F --- Averages 360 days and ranges 360 to 365 days.	Abt. 52% surface water sources, 48% ground water; these plus rainwater provide abundance of water.	Alfisols, Entisols, and Histosols.	No major towns; between Lake Okeechobee and the eastern coast.

Source: USDA–NRCS, 2006.

States is relatively good because of low population densities and lack of polluting industries. The major air quality problems that do occur in the West are confined to the urban areas of California (e.g., the Los Angeles Basin, the San Francisco Bay area, and Sacramento) and the smelter industrial areas of southeastern Arizona. Some undesirable conditions are also associated with agricultural activities and urbanization in central California. Because of agricultural and other uses, low-level background residues of certain pesticides in air are common in some areas. Consequently, cumulative effects of the program use of pesticides must be considered.

Reduced air quality (smog) affects visibility, which is especially valued for some areas. EPA has identified special Class I areas (national parks and wilderness areas) and vistas outside class I areas where visibility is an important value. The best visibility (more than 113 kilometers (km) or 70 miles (mi)) exists in the mountainous Southwest, while the Pacific coastal regions have the worst visibility (16 to 40 km or 10 to 25 mi). The potential for toxic air pollution resulting from agricultural and urban pesticide use remains a concern for the general public.

3. The Human Population

The human population of the potential fruit fly program area is extremely diverse (see table 3–9). The metropolitan areas are not homogeneous, but include human subpopulations with dissimilar compositions and social structures. That diversity is apparent, for example, when comparing the retirement communities of Florida, the Mexican-American communities of southern Texas, and the Asian-American communities of California. In addition, communities adjacent to metropolitan areas may include Native Americans, suburban families, and farmers. Depending on the locale of future programs (hence, also community structure and activity), the exposure to control activities could vary considerably.

The economic levels vary widely across the potential program areas, as well. Within the potential program areas, the lowest per capita incomes are in Mississippi, Louisiana, and Alabama. Although per capita income in metropolitan areas is higher than statewide averages, every large city contains at least one area characterized by low-income residents; homeless people are more numerous in cities than in rural areas.

The general health of a human population may be influenced by the population's economic status in that low-income people are often not able to afford nutritious food and good health care. Studies have demonstrated that liver disease and protein or thiamine deficiency can increase sensitivity to the effects of organophosphate pesticides (Casterline and Williams, 1969; Cavagna et al., 1969). Thus, populations prone to these conditions may be at greater risk than the general population. In general, differences in populations that influence individual's risks are generally

Table 3–9. Demographics of Potential Program Areas by State

Statewide Data				Metropolitan Area Data			
State	% <5 years old	% >65 years old	% population in metropolitan areas	Major city or metro area(s)	% Hispanic	% Asian	Per[1] capita income
AL	6.7	13	55.4	Mobile	1.4	1.5	18,072
AZ	7.5	13	88.2	Phoenix	34.1	2	19,833
				Tucson	35.7	2.5	16,322
CA	7.3	10.6	94.4	Los Angeles-Riverside-Orange County	40.3	10.4	21,170
				San Francisco-Oakland-San Jose	19.7	18.4	30,769
				San Diego	25.4	13.6	23,609
				Sacramento	21.6	16.6	18,721
FL	5.9	17.6	89.3	Miami-Ft. Lauderdale	40.3	1.8	20,454
				Tampa-St. Petersburg-Clearwater	10.4	1.9	21,784
				West Palm Beach	18.2	1.5	23,188
				Orlando	17.5	2.7	21,216
GA	7.3	9.6	71.6	Savannah	2.2	1.5	16,921
LA	7.1	11.6	72.6	New Orleans	3.1	2.3	17,258
MS	7.2	12.1	48.8	Biloxi	3.6	5.1	17,809
OR	6.5	12.8	78.7	Portland	6.8	6.3	22,643
SC	6.6	12.1	60.5	Charleston	1.5	1.2	22,414
TX	7.8	9.9	82.5	Brownsville-Harlingen-San Benito	84.3	0.5	10,960
				Houston	37.4	5.3	20,101
WA	6.7	11.2	82	Seattle	5.3	13.1	30,306

Source: U.S. Census Bureau, 1991.
[1] Data from 1988, in dollars.

compensated by the EPA's use of "ten-fold child safety factors" in pesticide risk assessments.

The diverse demographic and economic characteristics of the potential program area indicate the need for special considerations in carrying out

program activities. These considerations relate primarily to issues related to environmental justice for minority and low-income populations. Notification of treatment, an important aspect of the program, can be complicated by language differences. The higher percentages of Hispanic or Asian Americans in cities such as Brownsville, Texas, and San Francisco, California, suggest that notification and other public communication may need to be presented in languages other than English.

Other human factors such as age, income, health, and culture may pose problems that will require special program considerations in order to minimize exposure to pesticides and resultant risk. Certain segments of the population (such as some of the elderly and children) will be more sensitive to the program activities than the majority of the population.

Generally, metropolitan areas can be expected to include populations with a lower-than-average income and, therefore, with less health care, as well as more homeless people. Nonurban populations with low income might have more reliance on backyard fruits and vegetables as a food source. Cultural practices are another consideration if the program expands beyond metropolitan areas into Native American lands (such as those surrounding San Diego, California, or Phoenix and Tucson, Arizona); program activities could affect a population of low-income sustenance farmers whose exposure might be greater because of their cultural practices (i.e., use of wild food).

a. Cultural Resources

Cultural resources (see table 3–10) are those resources that contribute to intellectual or aesthetic education. Cultural resources include historic sites, archaeological sites, Native American lands, religious sites, zoos, and arboreta. Many such sites exist within the potential program area. Cultural resources of special concern, with respect to pest eradication programs, include zoos, arboreta, and gardens because they contain nontarget species. The Floridian and California Central Valley and Coastal ecoregions have a large number of such sites.

Historic, archaeological, and Native American sites are protected by the National Historic Preservation Act, the Archaeological and Historical Preservation Act, and the Native American Graves Protection and Repatriation Act. Furthermore, many Native American reservations are considered as sovereign nations and, therefore, program activities would have to be coordinated with their councils or the equivalent.

Table 3–10. Representative Cultural Resources of Potential Program Areas

City and State	Representative Cultural Resources
Los Angeles-Anaheim-Riverside, CA	University of California Botanical Gardens, Los Angeles Zoo, Los Angeles Arboretum
San Diego, CA	Quail Botanical Gardens, San Diego Zoo, Indian Reservations
Phoenix, AZ	Westward Expansion historical sites, Indian reservations, Phoenix Zoo, Desert Botanical Garden
Superior, AZ	Boyce Thompson Southwestern Arboretum
Tucson, AZ	Spanish historical sites, Indian reservations, Desert Museum, Tucson Botanical Gardens
Brownsville, TX	Palo Alto National Historic Site
Charleston, SC	Magnolia Plantations, Cypress Gardens, Fort Sumter and other Civil War historical sites
Savannah, GA	Colonial and Civil War historical sites
Mobile, AL	Historical sites
Biloxi, MS	Historical sites
Houston, TX	Houston Zoological Gardens
New Orleans, LA	French historical sites, Longue Vue House and Gardens, Louisiana Nature Center
Miami-Ft. Lauderdale, FL	Metro Zoo, Orchid Jungle, Fairchild Tropical Garden, Seminole Indian Village reconstruction, Butterfly World
Tampa-St. Petersburg, FL	Gamble Plantation, Yulee Sugar Mill, De Sota National Monument, Weedon Island Indian Mounds
Orlando, FL	Fort Mellon, Mead Botanical Gardens
Portland, OR	Portland Zoo, Forest Hills Park
Seattle, WA	Seattle Zoo, botanical gardens, parks and trails

b. Visual Resources

Visual resources (see table 3–11) consist of the landscapes and wildlife of a particular area. Natural visual resources are preserved in parks, forests, and wilderness areas. Most scenic areas are located some distance from urban centers; however, a few are near major cities in the potential program areas, and could be affected by program activities. For example, traps placed in city parks could detract from the appearance of blossoms or foliage; equipment noise (trucks, airplanes, or helicopters) could intrude upon otherwise peaceful areas; and bird watchers or other visitors to natural areas could become upset if wildlife species are affected by program activities or treatments.

Table 3–11. Representative Visual Resources of Potential Program Areas

City and State	Representative Visual Resources[1]
Los Angeles-Anaheim-Riverside, CA	Cucamonga WA, San Gabriel WA
San Diego, CA	Sweetwater Marsh NWR, Tijuana Slough NWR, Agua Tibia WA, Hauser WA, Pine Creek WA, San Mateo Canyon WA
Phoenix, AZ	Tonto NF
Tucson, AZ	Saguaro WA, Coronado NF
Brownsville, TX	Laguna Atascosa NWR
Charleston, SC	Cape Romain WA, Little Wambaw Swamp WA, Wambaw Creek WA
Savannah, GA	Savannah NWR, Tybee NWR
Mobile, AL	Bon Secour NWR
Biloxi, MS	Deer Island
Houston, TX	Sheldon WMA, Armond Bayou WMA
New Orleans, LA	Bayou Sauvage NWR, Bohemia State Park WMA
Miami-Ft. Lauderdale, FL	Biscayne NP, Everglades NP and WA, Hugh Taylor Birch SP
Tampa-St. Petersburg, FL	Weedon Island Preserve, Pinellas NWR, Caladesi Island SP
Orlando, FL	Clear Lake, Lake Fairview, other lakes
Portland, OR	Columbia River, Williamette Valley, Mt. Hood
Seattle, WA	Puget Sound, Lake Washington, Pacific Cascades, San Juan Islands

[1] Abbreviations: NF = National Forest, NP = National Park, NWR = National Wildlife Refuge, SP = State Park, WA = Wilderness Area, WMA = State Wildlife Management Area.

4. Nontarget Species

The nontarget species of the potential program area include the plants, animals, and microorganisms that are found there. These organisms exist as individuals, populations, and multispecies communities. They are dynamic, interactive components of their ecosystems which undergo structural and functional change and vary with location and over time. A broad consideration of the biological environment promotes understanding of the biological systems which are exposed to program operations and facilitates a more detailed analysis of the organisms or systems which might be at risk from those operations.

a. Domestic Animal and Plant Species

Eradication efforts typically occur in urban, suburban, and agricultural areas. Domesticated species that may be exposed to program operations include dogs, cats, tropical pet birds, and in some locations, livestock and poultry. Goldfish or koi ponds and stock ponds occur in some locales. Commercial aquaculture enterprises may rear fish or crustaceans in natural or artificial impoundments and are of major regional importance.

Backyard gardens occur throughout the program area. Annuals (such as peppers and tomatoes) as well as perennials (such as citrus and avocado

trees) are commonly grown. Many of these are fruit fly hosts. Commercial groves of host plants such as apricots, apples, peaches, pome fruits, and citrus are found throughout the program area. There are organic growers found at certain locations within the program area, and their needs are important program considerations.

b. Wild Animal and Plant Species

The numbers and kinds of wildlife associated with particular habitats depend on the season and on land resources. Typical species include a variety of invertebrate fauna, birds (American kestrels, European starlings, barn swallows, meadowlarks, and other songbirds), mice and other rodents, rabbits, raccoons, skunks, opossums, foxes, bats, and in some areas, coyotes.

Throughout the program area, soil and sediment support a great diversity of organisms which may inhabit the surface layer, occur beneath leaf litter or detritus, or are distributed throughout several layers. Earthworms and microorganisms inhabit the soil, and many insects spend portions of their life cycle as larvae or pupae in soil and sediments. These species provide food for a variety of fish, birds, and small mammals.

Water birds, including ducks, frequent lakes, ponds, and reservoirs throughout the program area. Introduced and native fish (including shiners, sunfish, bass, and catfish) occur in these water bodies as well as canals. Commercial and sport fishing occur throughout the program area.

Representative species for each ecoregion are presented in tables 3–12 through 3–19. A sampling of typical species is analyzed in the nontarget risk assessment (incorporated by reference). The assessment serves as the basis for an evaluation of potential environmental consequences of the eradication programs.

c. Habitats of Concern

Aquatic habitats within the program area are of special concern because of the vulnerability of aquatic species to program pesticides. These habitats support a variety of threatened and endangered species, particularly in the more arid program areas. Estuaries are spawning and nursery grounds for many marine and anadromous fish, as well as crustaceans and mollusks. They support a high density and diversity of birds, as well as plankton, which provides the base for many food webs. Sediments contain a variety of macroinvertebrate species, many of which are sensitive to program pesticides. In addition, intermittent streams and ponds are seasonally important as breeding and egg development habitat for amphibians, and as

reservoirs for migratory waterfowl. These areas often contain a variety of rare plants.

There is some disagreement as to the precise definition of a jurisdictional (regulated) wetland. Whether broadly or narrowly interpreted, there is a consensus that wetlands are extremely valuable ecosystem components. They provide wildlife habitat, flood control enhancement, water quality improvement, sediment stabilization, nutrient transformation, and groundwater recharge/discharge. Degradation of water quality in any aquatic or wetland habitat could disrupt food webs and have serious implications for composition, density, and diversity of invertebrate, fish, and bird species.

The Eastern coastal plain wetlands have been designated by the U.S. Department of the Interior's Fish and Wildlife Service (FWS) as habitats of special concern because of their value to migrating birds and as breeding grounds for shorebirds. As a whole, the Mississippi Delta is adversely affected by the high rates of erosion and submergence caused, in part, by human alteration of the natural drainage systems. The wetlands of the delta are designated as habitats of special concern for waterfowl.

Much of the southern tip of Florida is occupied by Everglades National Park, Big Cypress National Preserve, and several smaller State and private wildlife refuges. The Everglades' ecosystem is unique in North America and many species are threatened or endangered. Water management projects have altered the timing and quantity of freshwater flow, and preservation of the Everglades' ecosystem relies on the supply of high-quality water from the north. Runoff from adjacent agricultural and urban areas can enter the water conservation areas and contaminate water in the park with high concentrations of nutrients and pesticides.

Wildlife refuges and other land preserves are also areas of potential concern. These lands have been set aside to protect wildlife resources and often become islands surrounded by altered, intensely managed land. Generally comprised of many habitat types, they serve as refuges for less common species, provide wildlife corridors, and are important habitats for migratory birds. Nature Conservancy lands are protected because they contain unique features, which often includes rare plants. Impacts to these habitats could affect many species.

The Laguna Atoscosa National Wildlife Refuge in eastern Cameron County, Texas, on the gulf coastal plain, is the southernmost waterfowl refuge in the central flyway, and is a primary overwintering area. It is the focal point for the recovery of the endangered northern aplomado falcon. FWS has issued a Biological Opinion that the use of chlorpyrifos, diazinon, and several other pesticides will jeopardize the continued

Table 3–12. Biological Resources
Northwestern Forest, Forage, and Specialty Crop Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Grassland	Needle and thread grass, bunchgrass, wheatgrass, downy brome	Mule deer, rabbits, coyote	Western meadowlark, grouse, mourning dove, American kestrel, western kingbird, killdeer	Gopher snake, grasshoppers, spiders	Valuable for wintering birds; introduced grasses predominate; converted to agriculture and range land.
Woodland	Western redcedar, hemlock, Douglas fir	Western gray squirrel, opossum, white-tailed deer, deer mouse, bobcat, wolves	Western bluebird, American crow, scrub jay	Western rattlesnake	Variety of wildlife foods; strong lumber industry
Alluvial and floodplain	Willow, cottonwood, cattail, sedge, bulrush	Muskrat, beaver, mink	Great blue heron, mallard duck, red-winged blackbird	Garter snake, Western toad, Pacific tree frog, bluegill, mosquitofish, rainbow trout	Especially valuable for wintering waterfowl; coastal marshes near urban areas.

Source: USDA–NRCS, 2006

Table 3–13. Biological Resources
California Subtropical Fruit, Truck, and Specialty Crop Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Grassland	Brome, fescue, wild oats	Pocket gopher, California vole, mule deer, coyote, California ground squirrel, black-tailed jackrabbit	Western meadowlark, savannah sparrow, American kestrel, horned lark, western kingbird, killdeer	Gopher snake, grasshoppers, spiders	Valuable for wintering birds; introduced grasses predominate; converted to agriculture and rangeland.
Scrubland	Interior: chamise, California lilac, toyon Coast: coyote brush, purple and black sage, coastal sagebrush, scub oak	Brush rabbit, brush mouse, dusky-footed wood rat, bobcat, gray fox	California quail, California thrasher, rufous-sided towhee, sage sparrow, wrentit	Western rattlesnake, coast horned lizard, alligator lizards, common kingsnake	Interspersed with urban areas near coast; development threatens southern sage scrub.
Woodland	Valley oak, interior live oak, blue oak, coastline oak, California buckeye, Engelmann oak	Mule deer, raccoon, striped skunk, bobcat, western gray squirrel, deer mouse	Acorn woodpecker, plain titmouse, western bluebird, American crow, scrub jay	Arboreal salamander, slender salamanders, alligator lizards, western fence lizard, ring-necked snake	Variety of wildlife foods; some southern woodlands reduced by development.
Aquatic	Fresh marsh: cattail, sedge, bulrush. Salt marsh: salt grass, pickleweed, frankenia	Muskrat, beaver	Great blue heron, red-winged blackbird, marsh wren, mallard, Virginia rail	Garter snakes, red-legged frog, western toad, Pacific tree frog, California newt, mosquitofish, California killifish, bluegill	Especially valuable for wintering waterfowl; coastal marshes sometime near urban areas.

Source: USDA–NRCS, 2006

Table 3–14. Biological Resources
Western Range and Irrigated Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Mojave and Sonoran Deserts	Joshua tree, ocotillo, Mojave yucca, California juniper, saltbush, spiny sage brush, creosote bush, saguaro, cholla cactus, burro bush	Antelope squirrel, kangaroo rats, black-tailed jackrabbit, round-tailed ground squirrel, kangaroo rats, cactus mouse, desert mule deer, coyote, desert pocket mouse	Scott's oriole, white-winged dove, greater roadrunner, Gila woodpecker, cactus wren, LeConte's thrasher, common poorwill, Gambel's quail, elf owl	Chuckwalla, fringe-toed lizards, zebra-tailed lizard, side-blotched lizard, shovel-nosed snake, glossy snake, western whiptail	Slow to recover from disturbance, e.g., off-road vehicle use
Wash	Mesquite, catclaw acacia, smoke tree, blue palo verde, ironwood	Bailey pocket mouse, white-throated woodrat, javelina, mule deer, coyote	Black-throated sparrow, verdin, black-tailed gnatcatcher	Red-spotted toad, spadefoot toads, desert spiny lizard, brush lizard, horned lizards, tiger rattlesnake	Desert wildlife concentrates here
Riparian/aquatic	Willow, sycamore, cottonwood, saltcedar	Striped skunk, ring-tailed cat, raccoon, deer mouse	Summer tanager, Lucy warbler, ladder-backed woodpecker, yellow-billed cuckoo, green-backed heron, mallard	Western diamondback rattlesnake, spiny soft shell turtle, Colorado River toad, red-side shiner, Gila topminnow, bluegill	Little woodland remains--invaded by saltcedar; heavily used by wildlife; often near agricultural and urban areas

Source: USDA–NRCS, 2006

Table 3–15. Biological Resources
Lower Rio Grande Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Mid-grass Grasslands	Grama, three-awns, bluestems, curly mesquite, buffelgrass (introduced)	White-tailed deer, cotton rat, coyote, least shrew, Mexican ground squirrel, Eastern cottontail	Turkey, turkey vulture, bobwhite, scaled quail, mourning dove, great horned owl, meadowlark	Grasshoppers, spiders, Texas ratsnake, bullsnake	Little native grassland remains; converted to agriculture or rangeland uses; brush encroachment
Shrublands	Blackbush (acacia), mesquite, guajillo, granjeno, pricklypear, ceniza	Javelina, raccoon, white-tailed deer, Mexican spiney pocket mouse, striped skunk, jackrabbit, bats	Harris' hawk, scaled quail, white-winged dove, mourning dove, mockingbird, lesser nighthawk	Spotted whiptail, rose-bellied lizard, reticulate collared lizard, diamondback rattlesnake, Texas tortoise	Many community types—largely fragmented, some threatened; nesting sites; used by migratory raptors; wildlife corridors; refugia from disturbed sites; native citrus thicket (Starr County)
Riparian woodlands	Mesquite, granjeno, cedar elm, hackberry, acacias, many fruiting species	Bobcat, ocelot, raccoon, bats, white-footed mouse	Ferruginous pygmy owl, orioles, mourning dove, chachalaca, green jay, kingfishers, warblers, boat-tailed grackle	Giant toad, Rio Grande leopard frog, Texas indigo snake, blue tilapia (introduced), killifish, catfish, green sunfish	Variety of wildlife foods; roosting and feeding areas; only occurrence of many species in the United States; unique biota in aquatic habitats
Seasonally wet basins and potholes	Granjeno, huisache, mesquite, pricklypear, Texas persimmon	Ocelot, jaguarundi	White-winged dove, white pelican, sandhill crane, black-bellied tree duck	Reticulate collared lizard, Texas tortoise	Wintering waterfowl habitat; habitat for many Texas rare and threatened species

Source: USDA–NRCS, 2006

Table 3–16. Biological Resources
Mississippi Delta Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Salt marsh	Smooth cordgrass, wire grass, salt grass, black rush	Muskrat, otter, Norway rat	Marsh hawk, pintail, common loon, white pelican	Gulf salt marsh snake, gulf coast toad, diamondback terrapin	Feeding grounds for nesting and migrating birds; fish nursery
Fresh/brackish marsh	Maidencane, bulltongue, spike rush, alligator weed	Nutria, harvest mouse, rice rat	Scaup, teal, widgeon, gadwall, shoveler, mottled duck	Green treefrog, green anole, green frog	Feeding grounds for nesting and migrating birds
Bottomland hardwood	Water oak, overcup oak, bitter pecan, green ash, hawthorns	White-tailed deer, opossum, Cottontail	Wood duck, red-shouldered hawk, turkey vulture	Three-toed box turtle, Mississippi ring-necked snake	Very high nesting density; habitat for large mammals
Swamp	Southern cypress, bald cypress, pond cypress, tupelo, black willow, swamp gum, cottonwood, button bush, swamp privet	Mink, bobcat, swamp rabbit, red bat	Great blue heron, great egret, anhinga, white ibis, Louisiana heron	Western cottonmouth, green anole, bronze frog, alligator	Rookeries for herons and egrets
Levee	Water oak, live oak, hackberry, American elm, honeylocust, hawthorn, marsh elder, groundsel bush	Rice rat, fulvous harvest mouse, least shrew		Bronze frog, ribbon snake, narrow-mouthed toad	Refuge during flooding; dry land corridors

Source: USDA–NRCS, 2006

Table 3–17. Biological Resources
South Atlantic and Gulf Slope Cash Crops, Forest, and Livestock Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Alluvial and Floodplain	Bald cypress, swamp gum, tupelo, swamp nettle	Otter, muskrat, raccoon	Red-eyed vireo, wood duck, pied-billed grebe	Many insects, eastern mud turtle, marbled salamander, ratsnake	Flood control; high density of nesting birds and amphibians
Marsh	Cordgrass, rushes, sedges, wild rice, some shrubs	Muskrat, marsh rice rat	Herons, egrets, ducks, common gallinule	Many insects and other invertebrates	Rookeries, fish nurseries
Pine Forest	Species of pine, bay, blueberry, spicebush, hydrangea	Opossum, white-tailed deer, gray squirrel, short-tailed shrew, striped skunk, raccoon, big-eared bat, red fox	Long-eared owl, pine warbler, red-cockaded woodpecker	Tiger salamander, box turtle, coral snake, gopher tortoise	Cover and nesting sites; few old growth forests remain, most are intensively managed
Hardwood forest	Species of oak, gum, hickory, elderberry, greenbriar, ferns	Opossum, white-tailed deer, gray squirrel, short-tailed shrew, striped skunk, raccoon, big-eared bat, red fox	White-eyed vireo, blue jay, great-crested flycatcher, wood duck, red-tailed hawk, cardinal		
Grassland	Species of bluestem or panic grass	Ground squirrel, cottontail, plains woodrat	Common nighthawk, eastern meadowlark, bobwhite, killdeer, scissor-tailed flycatcher, mockingbird	Many insects	Undisturbed grasslands very rare

Source: USDA–NRCS, 2006.

Table 3–18. Biological Resources
Atlantic and Gulf Coast Lowland Forest and Crop Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Alluvial and Floodplain	Bald cypress, swamp gum, tupelo, swamp nettle	Otter, muskrat, raccoon	Red-eyed vireo, wood duck, pied-billed grebe	Many insects, eastern mud turtle, marbled salamander, ratsnake	Flood control; high density of nesting birds and amphibians
Marsh	Cordgrass, rushes, sedges, wild rice, some shrubs	Muskrat, marsh rice rat	Herons, egrets, ducks, common gallinule	Many insects and other invertebrates	Rookeries, fish nurseries
Pine Forest	Species of pine, bay, blueberry, spicebush, hydrangea	Opossum, white-tailed deer, gray squirrel, short-tailed shrew, striped skunk, raccoon, big-eared bat, red fox	Long-eared owl, pine warbler, red-cockaded woodpecker	Tiger salamander, box turtle, coral snake, gopher tortoise	Cover and nesting sites; few old growth forests remain, most are intensively managed
Hardwood forest	Species of oak, gum, hickory, elderberry, greenbriar, ferns	Opossum, white-tailed deer, gray squirrel, short-tailed shrew, striped skunk, raccoon, big-eared bat, red fox	White-eyed vireo, blue jay, great-crested flycatcher, wood duck, red-tailed hawk, cardinal		
Grassland	Species of bluestem or panic grass	Ground squirrel, cottontail, plains woodrat	Common nighthawk, eastern meadowlark, bobwhite, killdeer, scissor-tailed flycatcher, mockingbird	Many insects	Undisturbed grasslands very rare

Source: USDA–NRCS, 2006

Table 3–19. Biological Resources Floridian Ecoregion

Habitat	Dominant Vegetation	Representative Mammals	Representative Birds	Other Nontarget Species	Significance/Status
Cypress swamps	Cypress, longleaf pine, slash pine, sabal palm	Cotton mouse, raccoon, shrews	Wood stork, herons, Everglades snail kite, turkey, warblers, bald eagle	Alligators, spiders, aquatic invertebrates	More rare or endangered species found in Cypress Swamps than any other Florida swamp; Florida panther habitat
Freshwater marshes	Pickeral weed, beakrush, maidencane, sawgrass	White-tailed deer, Florida water rat	Egrets, wood stork, ducks, Florida sandhill crane	Apple snail, amphipods (scuds), prawns, catfish, alligator	
Lakes, rivers, canals	Water hyacinth, cattails, eelgrass, pondweed	Raccoon, river otter, manatee	Kingfisher, herons, egrets, anhinga	Zooplankton, snails, clams, gar, catfish, suckers, silversides, minnows, sunfish	
Mangroves	Black mangroves, red mangrove, white mangrove, buttonwood	Raccoon, river otter, striped skunk, black bear, manatee	Brown pelican, spoonbill, wood stork, egrets, herons	Tarpon, mullet, snappers, shrimp, sea turtles, American crocodile	Nursery area for many commercial fish species
Salt marshes	Saltmarsh cordgrass, saltbush	Raccoon, marsh rabbit, cotton rats, bottlenose dolphin, rice rat	Cattle egret, swallows, marsh wren, seaside sparrow	Fiddler crab, shrimp, marsh crab, grasshoppers, plant hoppers, spiders, diamondback terrapin	Nursery area for many fish species
Pine flatwoods	Longleaf pine, slash pine, wax myrtle, saw palmetto	White-tailed deer, cotton mouse, cotton rat, gray fox, fox squirrel	Brown-headed nuthatch, pine warbler, great horned owl	Box turtle, black racer, pinewoods snake, anoles	
Scrub	Scrub oak, saw palmetto, myrtle oak, sand live oak, Florida rosemary	Flying squirrel, Florida mouse, cotton mouse, bobcat, gray fox, white-tailed deer	Florida scrub jay, bobwhite, common nighthawk, palm warbler, woodpeckers, screech owl	Florida scrub lizard, blue-tailed mole skink, gopher tortoise, sand skink	40 to 60% of the species are endemic
Dry prairies	Switch grass, saw palmetto, wiregrass, gallberry	Cotton rat, nine-banded armadillo, Eastern harvest mouse, Eastern spotted skunk	Florida sandhill crane, common nighthawk, vultures, burrowing owls, crested caracara	Box turtle, black racer	
Rocklands	Gumbo limbo, pigeon plum, royal palm, live oak, strangler fig, wild coffee	Opossum, key deer, Florida mastiff bat, mangrove fox squirrel, white-tailed deer, raccoon	Northern cardinal, gray kingbird, Carolina wren, red-bellied woodpecker, pine warbler	Florida tree snail, Schaus swallowtail, anoles	Many tropical species only found in this habitat of the United States
Coastal dunes	Sea oats, sea lavender, saltbush	Marsh rabbit, rice rat, raccoon, cotton rat	Seaside sparrow, marsh wren, cattle egret, wading birds, fish crow	Sea turtles, diamondback terrapin, marsh crab, fiddler crab, grasshoppers, mollusks	

Source: USDA–NRCS, 2006.

existence of this species. As a result, FWS has recommended a 20-mile prohibited-use zone around the refuge for these pesticides.

In addition to national- and State-protected areas, many areas of considerable importance are not afforded protection. An example of an unprotected area is the Colorado River in Yuma County, Arizona, which is known internationally as a prime bird watching location. Many such locations occur throughout the program area.

The Columbia River Basin and the tributaries of Puget Sound, in Washington State, are also important wildlife habitats. The damming and diversion of water on the Columbia River have threatened the survival of several species of anadromous fish, particularly salmon.

d. Threatened and Endangered Species

Various species of fish, wildlife, and plants in the United States are so few in number that they are in danger of or threatened with extinction. The decline of most of these species is directly related to loss of habitat, however, it may also be the result of other factors including hunting, collecting, pollution, road kills, interspecies competition, or pesticides. (See appendix L for a listing of species in potential program areas.) More than 200 federally listed species are found within the potential program area; they include plants, birds, fish, mammals, amphibians, reptiles, and at least one insect.

The Endangered Species Act of 1973 (ESA), as amended (16 U.S.C. 1531 et seq.), mandates the protection of federally listed threatened and endangered species and their critical habitats. It also requires Federal agencies to consult with FWS or the U.S. Department of Commerce's National Marine Fisheries Service (NMFS) to ensure that any actions they authorize, fund, or carry out are not likely to jeopardize the continued existence of a listed species or a species proposed for listing, or result in the destruction or adverse modification of its critical habitat or its proposed critical habitat.

IV. Environmental Consequences

The environmental consequences of APHIS' plant pest control and eradication programs result from or are related to control actions. Although this chapter focuses on the potential environmental effects from the use of genetically engineered insects in these programs, it also summarizes and updates information related to environmental impacts from other aspects of the programs. This chapter includes an analysis of potential effects on the physical environment, human health and safety, and biological resources. Control methods are individually analyzed and discussed within each alternative; however certain topics (socioeconomics, cultural and visual resources, and unavoidable effects) are described based upon their potential cumulative effects from the combined use of all control methods. (See also chapter II, Alternatives, which characterizes program alternatives and control methods in more detail.)

A. Program Alternatives

Environmental consequences of the no action alternative have largely been described in previous documentation, which will be summarized briefly. There have been a few changes to the environmental documentation and some potential changes to the programs since the previous documentation that will be discussed in this EIS. The section on the alternative covering expansion of existing programs considers impacts associated with circumstances that could require increased use of methods that are presently used and those program adjustments that could be designed to mitigate the resulting pest risks. Although the types of actions would not differ from those under the no action alternative, their context and magnitude could differ in terms of species, location, and size of each program. Description of environmental impacts from the preferred alternative (integration of genetically engineered insects into APHIS programs) includes potential impacts of the other two alternatives. The technology of genetic engineering would not be used alone, and certain environmental impacts from the other two alternatives apply. The other environmental impacts may also be modified by the degree to which the use of genetically engineered insects—

(1) decrease the need for actions involving insecticide applications,

(2) decrease the need to produce both male and female insects for use in SIT releases,

(3) increase production of males that are more competitive in mating than radiation-sterilized males, and

(4) eliminate the need to use, operate, and maintain strong gamma radiation sources.

This EIS focuses on the effectiveness and efficiency of methods using genetically engineered insects. These methods are compared to present mass-reared insects that are not genetically engineered.and to other control methods used in present cooperative plant pest control programs.

1. No Action

The environmental consequences of no action have largely been described in detail in APHIS' plant pest control and eradication program documents incorporated by reference in this EIS. This documentation includes information from the EIS for fruit fly cooperative control programs (USDA–APHIS, 2001a), and information from the EA for the Southwest Pink Bollworm Eradication Program (USDA–APHIS, 2002a). The impacts described in these two documents address effects associated with the use of specific methods; however, those impacts are not generally site-specific. Each of the documents considers impacts to the potential habitats and segments of habitats affected within the control program action areas. Although the programmatic descriptions provided here do not assess individual actions, this EIS characterizes the types of impacts, their relative intensity, and the context in which those impacts are likely to pose adverse effects when applied to site-specific program actions. This provides information to allow the decisionmaker to select those methods which will best meet the program goals for site-specific program actions with the least potential impacts to the human environment.

a. Fruit Fly Control Programs

The environmental consequences section in the EIS for the Fruit Fly Cooperative Control Programs (USDA–APHIS, 2001a) describes the potential impacts to the physical environment, human health and health and safety, and biological resources for each control method for the present fruit fly control programs. This fruit fly EIS addresses program methods involving nonchemical and chemical control. The nonchemical control methods analyzed in the fruit fly EIS included SIT, physical control, cultural control, biological control, biotechnological control, cold treatment, vapor heat treatment, and irradiation treatment. The chemical control methods analyzed in the fruit fly EIS included bait spray applications using malathion, spinosad or phloxine B, soil treatments using chlorpyrifos, diazinon or fenthion, fumigation using methyl bromide, mass trapping using borax, dichlorvos, malathion, naled, phloxine B and/or lures, fruit fly male annihilation technique using malathion, naled and lures, and the use of cordelitos consisting of lengths of 6-ply cotton string of about 30- to 45-centimeters long impregnated with a mixture of naled and lure. Each of the methods was rated for relative potential for adverse environmental consequences in table 3–2 on

page 15 of the fruit fly EIS. The methods with higher potential impact were those involving aerial pesticide applications of bait spray and ground pesticide applications to treat soil. There is an extensive section in chapter VII of the fruit fly EIS that describes standard operating procedures, mitigation measures, and risk reduction strategies used to minimize those potential impacts in actual programs.

Much of the basic information about potential environmental effects of the chemical control methods in the fruit fly EIS (USDA–APHIS, 2001a) has not changed; however, there have been important developments since that time. Some chemicals were in various stages of development and not in operational use at the time of publication. One chemical (phoxine B or SureDye) was subject to substantial efficacy research at the time of the EIS preparation; however, that pesticide is not currently being used. Another chemical (spinosad) was in early stages of development and is now used more extensively.

There was limited research completed on environmental fate, toxicity, and potential environmental impacts of spinosad at the time of publication of the fruit fly EIS. The nontarget risk assessment for spinosad use against fruit flies has been updated to include more recent research (USDA–APHIS, 2003a). Much of the more recent research cited in the risk assessment is related to field studies of the impacts on nontarget invertebrates, such as honey bees. The manner of application and limited routes of exposure preclude impacts to some nontarget species. Spinosad intoxication to insects occurs primarily through the route of ingestion, unlike the adverse effects from malathion that include dermal and inhalation routes of exposure. Pollinators, such as honey bees, were not found to be affected by aerial bait spray applications of spinosad due to a lack of attraction or stimulation to feed on the spinosad bait. The adverse effects on nontarget species from spinosad bait spray applications have been found to be limited largely to those nontarget invertebrate species feeding on treated surfaces of leaves or attracted to feed on the bait spray itself (USDA–APHIS, 2003a).

SIT and biotechnological control are the only nonchemical control methods with substantial new developments since the completion of the fruit fly EIS (USDA–APHIS, 2001a) in 2001. In that document, the section on SIT considers only the release from conventional mass-reared and irradiation-sterilized flies. The use of genetically engineered strains is not addressed. The section on the use of biotechnological control discusses the development and early implementation phase of a TSL strain of Medfly for use in SIT programs. As noted in the alternatives section, this strain is now regularly used in SIT programs. The TSL strain has a recessive mutant TSL gene that causes death to female flies at temperatures above 29 ºC. The male flies are heterozygous, therefore, the

presence of this recessive gene is not expressed and their survival is not temperature-limited, unlike the female flies. This genetically modified strain has been developed through classical genetic selection and does not involve genetic engineering of traits from other species to the fly strain.

The TSL-sexing method has benefited the SIT program in the following ways: (1) it avoids ovipositional "sting" fruit damage from sterile females, (2) it avoids matings between sterile males and sterile females, (3) it substantially reduces SIT production costs by eliminating females in the egg stage, (4) it uses a relatively stable strain under mass-rearing conditions, and (5) it improves the overall efficacy of SIT. The fruit fly EIS determined the relative environmental consequences from the use of SIT to be minimal and the relative consequences of biotechnological control to be unknown (table 3–2). The fruit fly program experience with the use of the recessive mutant TSL strain in SIT has shown that this biotechnological control has been both an efficacious and cost effective alternative to the use of strains lacking the gender selection capability. The relative environmental consequences from the use of this technology are considered to be negligible because these fruit flies are not environmentally or reproductively fit. The fruit fly EIS (USDA–APHIS, 2001a) did not consider the potential use of genetic engineering of other traits into fruit fly strains for use in control programs, as is contemplated in the preferred alternative of this EIS.

The use of exposure to gamma radiation (irradiation) emitted by radioisotopes, Cobalt 60, or Cesium 137 is the only nongenetic method available by which to sterilize mass-reared insects effectively (IAEA, 1999); other nongenetic methods of sterilization have not been consistently effective. Chemosterilants carry a high risk for environmental contamination and pose serious health concerns. Linear accelerators have not demonstrated sufficient applicability to consistently achieve the desired level of sterility. The minimum dose of radiation to achieve sterility in exposed insects varies with the species being sterilized. The minimum absorbed dose to Medfly pupae at the Honolulu, Hawaii, facility is 120 Gray (IAEA, 1999) or 12 kilorad (kR). The minimum absorbed dose to Medfly pupae at the El Pino facility in Guatemala is 145 Gray (IAEA, 1999) or 14.5 kR. The minimum absorbed dose to Mexican fruit fly pupae at the Mission, Texas, facility is 70 Gray (IAEA, 1999) or 7 kR.

The unique design and shielding of the irradiation equipment at fruit fly rearing facilities prevents workers from being accidentally exposed to the radiation used to sterilize the fruit flies (USDA–APHIS, 2001a, 1997). There have been no reported problems of radiation exposure associated with use of irradiation equipment at APHIS facilities. The irradiation equipment at APHIS facilities is inspected regularly to ensure that all facilities are in compliance with the stringent environmental protection

requirements set by the Nuclear Regulatory Commission for facility use of radionuclides (10 CFR Parts 20, 30, 51, and 71). APHIS also complies with the requirements of Department Regulation 4400–5, Radiation Safety Program and the USDA Radiation Safety Handbook.

The present uses of mass-reared fruit flies in SIT release programs include the preventive release programs (PRPs) and in detected outbreaks of Medfly and Mexican fruit fly. The PRPs involve release of sterile flies on a continuous basis at locations that are at high risk of Medfly introductions. PRPs are established in the States of California and Florida. Since the institution of the PRPs in Florida and California, there have been no Medfly outbreaks in the PRP areas in Florida and only two Medfly outbreaks in the PRP area in California (USDA–APHIS, 2006a).

It is clear that the use of PRPs has resulted in considerable benefit over responding to introductions of Medfly with emergency eradication programs. It is, however, also clear that PRPs do not protect all potential sites of introduction—outbreaks occurred outside the program area. The average cost of each of the three outbreaks of Medfly that did occur in California outside the areas protected by the PRP since 1996 was $2.52 million. Although it may not be cost-effective to protect these areas at present, increasing amounts of travel and trade are elevating the risk of introduction of Medfly to locations outside the PRPs in both California and Florida. In Florida, interest has been expressed in extending PRP coverage to include parts of the Orlando area. There are 378 million sterile Medfly pupae processed by California per week for aerial release over 2,489-square miles in its PRP. Florida processes 100 million pupae per week to protect the 600-square miles in their PRP. Independent of consideration for the cost and efficiency savings, the environmental impacts of the PRPs are considerably less than those from eradication programs that use insecticides and other control measures that pose greater risks to the physical environment, human health and safety, and nontarget species.

Production of the sterile flies used in release programs is accomplished through mass-rearing at three facilities. These facilities are designed to produce flies with a minimum of waste, and to recycle or reuse materials to the extent that the production equipment and supplies provide viable conditions for the production process. Disposal of wastes from the production process are subject to compliance with local laws and regulations and do not pose substantial risks to the physical environment, nontarget species, or human health (USDA–APHIS, 2001a). Other than the potential risk of aircraft or motor vehicle accidents, which would involve emergency response rather than risk assessment, the present SIT production and release programs pose negligible risks of adverse environmental impacts to any part of the human environment.

APHIS and CDFA partner to produce sterile Medfly pupae for the California PRP. CDFA operates a rearing facility in Hawaii that produces 180 million male pupae per week (USDA–APHIS, 2006a). These pupae are then sterilized in an APHIS irradiation unit nearby before being transported to California for release. In addition, other sterile male Medflies for the California PRP are produced by APHIS at the El Pino, Guatemala, facility. The El Pino mass-rearing facility also supplies the 100 million sterile Medfly pupae for the Florida PRP and the pupae for any other emergency resulting from introductions that may arise (USDA–APHIS, 2006a). The approximate weekly production of the El Pino facility is 1.6 billion pupae with a capacity to produce between 3 to 4 billion pupae. The development of the TSL strain has greatly assisted in increasing the fly production at these facilities and the sterile insects needed for PRP and emergency programs.

The other mass-rearing facility for processing sterile fruit flies is in Mission, Texas, and supports the Mexican fruit fly program actions. The Mission facility is producing at capacity levels of 150 million sterile pupae (males and females) per week. This facility operates with financial support from APHIS, the Texas Department of Agriculture, and the Texas Valley Citrus Committee. Unlike sterile Medfly production, the Mexican fruit fly does not yet have a TSL strain, or comparable strain, to limit sterile insect production to only males. This results in the need for a considerably greater production capacity to achieve the same suppression or eradication results that occur with Medfly.

Both the Medfly and Mexican fruit fly sterile production units depend upon irradiation to induce sterility in the fruit flies. This radiation exposure weakens the male flies, causing them to be unable to compete for mates as effectively as the wild-type male fruit flies can; therefore, an overflooding, or much higher ratio of sterile flies to wild flies, has to be released to achieve the desired control objective.

In addition to the program not utilizing genetic engineering to develop more competitive sterile males, selection of the no action alternative would not include development or use of classical genetic strains, such as the TSL strain of Medfly. A comparable selection tool for males-only in Mexican fruit fly would be very useful to increase production of male-only insects.

At present, there is no mass-rearing production of oriental fruit fly or other invasive *Bactrocera* sp. in the United States; however, there was mass-rearing production of oriental fruit fly, up to 5 million pupae per week in Honolulu, Hawaii, in the past (IAEA, 1999). The potential development and use of TSL or genetically engineered male-only strains of these fruit fly species in SIT could become important with the increased risks of their

introduction associated with increasing travel and trade. Should the need arise due to unforeseen introductions and infestations, the no action alternative would not provide the flexibility to develop other invasive fruit fly mass-production for use in future SIT release programs,.

Although the potential environmental effects of the no action alternative (the present program) have been thoroughly analyzed in those documents incorporated by reference in this EIS, a comprehensive analysis has not been prepared for future actions to address unforeseen outbreaks. Currently, the mass-production of sterile flies is sufficient to cover anticipated introductions and the ongoing need for preventive releases of sterile flies in California and Florida; however, there are resource constraints on the total production needed to maintain the present capacity.

The no action alternative does not address the potential increase in demand for sterile insects that could occur with multiple outbreaks in a short timeframe. Although APHIS programs could continue to utilize control measures applying insecticide until the sterile fly production reaches the capacity needed for SIT release, the potential adverse environmental impacts of this approach would exceed most control methods. The no action alternative would meet minimum program needs; however, the potential environmental impacts would most likely be greater than the other alternatives, and the long-term implementation would be less cost-effective than the other two program alternatives analyzed in this EIS.

b. Pink Bollworm Eradication Program

The potential environmental impacts from the ongoing pink bollworm eradication program have been assessed in an EA (USDA–APHIS, 2002a). The EA analyzes potential environmental effects from cultural control; mechanical control including usage of chlorpyrifos, dichlorvos, and propoxur in pheromone-baited traps; SIT; and chemical control methods using chlorpyrifos and permethrin insecticides. As with the fruit fly control programs, the relative potential for adverse impacts from program actions is low except from the aerial applications of pesticides. In this program, aerial applications are applied to a limited number of fields where monitoring indicates that at least 5 percent of the cotton is infested with pink bollworm larvae. Although this level of infestation has only occurred in a relatively small number of locations, the environmental impacts are higher to those sites treated with pesticides.

There are a number of additional protective measures employed by the program when pesticide applications are required. Worker protection provisions are required, and notification procedures for potentially affected residents are conducted to minimize the potential human pesticide

exposure and associated effects (pages 25 to 26 of the EA cited above). A finding of no significant impact to the human environment was determined for the implementation of the eradication program analyzed in the 2002 EA.

The basic information regarding potential environmental effects of chemical pesticide control in the pink bollworm eradication program EA (USDA–APHIS, 2002a) has not changed; however, there has been recent evidence of pesticide resistance that could limit potential future progress of the present eradication program. In particular, in parts of Arizona, pink bollworm is showing signs of resistance to applications of chlorpyrifos. Although use of alternate chemicals is being considered by program officials, no decisions have been made yet. The environmental effects from the use of other pesticides by the program would require a revision to the EA.

The effectiveness of SIT releases in the pink bollworm eradication program requires considerably more mass-reared and radiation-sterilized moths than the population of wild-type moths present in the fields. Although SIT release can lower populations, successful eradication is generally dependent upon population reductions prior to sterile insect release to ensure effective elimination of the population in the field. Higher production of sterile moths from the production facility could help with this; however, physical and economic constraints on production limit the quantities available for release. The no action alternative does not provide the benefits that an expansion of the present program alternative or the development of genetically engineered strains alternative could provide. This includes the use of genetically sterile moths with greater mating ability.

The use of SIT in the pink bollworm program has similar environmental concerns to those for the fruit fly programs. A sterile pink bollworm moth release program was initiated in California's San Joaquin Valley in 1970 (National Cotton Council, 2001), and has protected the region's cotton acreage from introductions of pink bollworm in a manner similar to the PRPs for Medfly. In 2001, APHIS proposed to cooperate with the Texas Department of Agriculture and the government of Mexico in a pink bollworm eradication program for the El Paso/Trans Pecos region, an EA was prepared for that increment of the program (USDA–APHIS, 2001c). That EA and the EA for the subsequent Southwest Pink Bollworm Eradication Program (USDA–APHIS, 2002a) analyzed the environmental effects associated with SIT, as well as the other eradication methods.

Aerial release of sterile moths in the Pink Bollworm Eradication Plan occurs at a rate of 100 moths per acre per day, beginning at the four-leaf stage of cotton until defoliation or harvest (El-Lissy et al., 2005a). As

with the Mexican fruit fly, pink bollworm lacks a strain that can be used to easily separate the mass-reared males from females. Irradiation sterilization does result in effective matings that produce no progeny for either gender. The lack of a TSL strain, or the equivalent, for selection of males-only results in the sterilization and field release of both genders. This type of release is not as effective as release of males-only in eradication efforts because it requires release of at least twice as many insects, or more, to achieve the high or overflooding ratio of released sterilized insects to wild insects, considering that some matings between only sterile insects occur.

As with the fruit fly species, sterilization of mass-reared pink bollworm is accomplished through exposure of the insects to gamma radiation (irradiation) emitted by radioisotopes (Cobalt 60 or Cesium 137). The review of environmental effects and safety requirements (discussed above in the paragraph on use of irradiation in fruit fly programs) applies to the pink bollworm eradication program. There are two different doses of irradiation applied to pink bollworm pupae, depending upon the desired outcome. Mass-reared pink bollworm are sterilized at a dose of 20 kilorad (kR) or 200 Gray, which results in adult bollworm moths that mate with wild-type insects to produce no or virtually no offspring. Experimentally, a lower dose of gamma radiation (7 to 10 kR (70 to 100 Gray)) is used to sterilize offspring of released insects; this technology is referred to as F_1 sterility.

Although the potential environmental effects of the no action alternative (the present program) have been thoroughly analyzed in the EA for the pink bollworm eradication program, the present program would not benefit from the use and release of genetically engineered pink bollworm moths to improve SIT in the no action alternative. The rearing facility has a production capacity of 210 million sterile moths per week; however, the actual average weekly production has been closer to 154 million sterile moths. The present mass-production of sterile moths is sufficient to continue to support the ongoing eradication effort; nevertheless, the completion time and costs for eradication could exceed those anticipated from the use of more effective mating strains expected to become available through genetic engineering.

2. Expansion of Existing Programs

The expansion of existing programs alternative extends those methods described in the no action alternative to more plant pest species and more applications; however, this alternative does not incorporate the continuing research and development of genetically engineered strains for use in SIT. This alternative could include expansion of the following activities: rearing operations, irradiation treatment capacity, classical genetic selection methods for separation of insect sexes, insecticide use, physical

or cultural control, trapping, pheromones, other attractants, monitoring, and the use of SIT releases for more insect plant pest species. The impacts from nonchemical control and chemical control methods for this alternative would be similar to those from the no action alternative, and those impacts would increase commensurate with the expansion. Most of those impacts are not repeated in this section; however, impacts associated with expansion of rearing operations, irradiation treatment capacity, classical genetic selection methods for separarion of insect sexes, and the use of SIT releases for more insect plant pest species are analyzed in relation to the present program and the preferred alternative.

a. Fruit Fly Control Programs

The potential effects on the human environment, from expansion of existing fruit fly control programs, are comparable to effects from the no action alternative, but involve increased impacts commensurate with the increased use of available methods other than genetic engineering. Although this expansion would likely increase waste from rearing facilities, any changes in those impacts from disposal or recycling are not expected to be substantial. The extent to which expansion of the existing program would increase or decrease the need for chemical control measures would determine the extent to which this alternative would increase or decrease potential environmental impacts associated with the insecticide use that could arise.

Expansion of existing rearing operations is limited to the size of the rearing facilities available for this purpose. The Mexican fruit fly production facility in Mission, Texas, is operating at capacity and any expansion of that program is not possible unless another source of sterile Mexican fruit fly pupae is found. Likewise, this facility cannot provide supplies of sterile Mexican fruit fly pupae for emergency actions outside of Texas without detracting from the existing Mexican fruit fly contol efforts in the Lower Rio Grande Valley.

The supply of sterile Medfly pupae may not be adequate to meet total program needs in the case of any expansion. The production of sterile pupae in the El Pino mass-rearing facility is limited by the irradiation capacity at the facility.

Production is now at maximum irradiation capacity of 1.6 million pupae irradiated weekly with the majority of those designated for use in the Moscamed Program. In the case of an emergency Medfly program within the United States, sterile pupae must be diverted from the Moscamed Program to address the emergency. In particular, there has been interest in Florida to extend their PRP coverage for Medfly to include parts of the Orlando area. This potential increase in coverage would require additional

sterile fly production. Likewise, there are areas of California with host plants susceptible to Medfly that are not part of the current PRP there. Although the present risk may not be considered to be high enough to justify this increased production, those risks are increasing with the increase of trade and travel of recent years. Ongoing assessment of plant pest program needs may eventually justify expansion of existing rearing operations or construction of new facilities to fulfill an increasing need for sterile flies in control and eradication programs.

Expansion of irradiation treatment capacity could assist with the timely release of more sterilized flies. The safety requirements and potential environmental risks from this use of irradiation technology would not differ from those associated with the no action alternative. The shielding and other protective measures preclude radiation exposures of concern; therefore, the potential impacts of this expansion would not differ substantially from the usage of irradiators under the no action alternative.

Expansion of classical genetic selection methods for production of males-only is a development that is beneficial because it allows considerably less production of flies to maintain present program levels of activity. The TSL strain of Medfly was developed using classical genetic selection methods, and has resulted in a considerable increase in capacity for sterile male fly production; however, the time and effort required to develop the TSL strain were extensive. Although similar developments using classical genetic selection methods might be utilized for other plant pest species, based upon program experience with the TSL strain, development of each new strain could be anticipated to take about 20 years,. The research and development of these new strains pose no greater expected risks to the human environment than those strains currently used in the mass-rearing of flies at existing facilities. It is feasible to achieve mass-production of other male-only fruit fly strains using genetic engineering in only 2 to 3 years at a far lower cost. The efficiency and effectiveness of gender selection through genetic engineering would not be realized through selection of the EIS alternative for expansion of existing programs.

Extension of the use of SIT releases to other invasive fruit fly species could provide another control measure for some invasive fruit fly species. The present facilities limit mass production only to the Medfly and the Mexican fruit fly; however, there are also frequent periodic outbreaks of oriental fruit fly in the United States. Since 1981, there have been at least 15 economically significant outbreaks of oriental fruit fly that required APHIS' environmental documentation. In addition, there have been a number of smaller outbreaks that did not require Federal involvement. This frequency of Federal program action against oriental fruit fly is

comparable to Mexican fruit fly (17 outbreaks) and Medfly (38 outbreaks).

Other introductions of invasive fruit fly species have occurred at lower frequencies than Medfly, Mexican fruit fly, and oriental fruit fly. The lower frequency of introductions of other invasive fruit flies does not provide economic incentive to develop mass-rearing and SIT release programs for these species at this time. Production and release in PRPs must be timed and placed so that the sterile insects can effectively mate with any wild-type insects that are introduced. Establishing successful criteria for PRPs is a difficult, complex, and costly process.

Introductions of species, such as oriental fruit fly, occur almost every year within their potential host range in California; however, the sites of introduction vary considerably. Even with timely mass-rearing of flies and irradiation, the acreage needed and program location is unpredictable. Currently, the cost effectiveness, compared to present costs for emergency response to individual detections of oriental fruit fly, does not warrant ongoing mass-production for use in SIT releases.

The status of SIT development and potential delivery in programs for different fruit flies was reviewed recently (IAEA, 1999). The review indicated that mass-production methods, sterilization methods, and release methods are available for oriental fruit fly; however, research and development is continuing towards genetic methods or marker gene traits for identification and monitoring purposes. In the United States, the unpredictable occurrence of the other invasive fruit fly species does not provide enough justification for development and maintenance of ongoing SIT programs. There have only been infrequent outbreaks of the peach fruit fly (*Bactrocera zonata*) and the West Indian fruit fly (*Anastrepha obliqua*) since 1981; therefore, the logistics and cost of developing mass-rearing facilities and SIT release for these species are unlikely to be undertaken. Should the frequency and predictability of introductions increase for these species, the incentive for SIT program development may be justified.

The impacts to the physical environment, human health and safety, and biological resources from expansion of the existing program would not be much different from those effects anticipated for the no action alternative. The increased disposal of rearing media, other waste products from production and release of flies, and general maintenance byproducts from increased production would not contribute significantly to overall impacts. The impacts from the construction of new facilities or construction of major additions to current facilities would require further environmental review before such efforts could be considered to be undertaken. If pesticide use increases were to result from expansion of existing

programs, pesticide impacts to the human environment would correspondingly increase.

b. Pink Bollworm Eradication Program

The potential effects on the human environment from expansion of the existing pink bollworm eradication program are similar to the no action alternative, nevertheless, involve correspondingly increased impacts from more use of available methods other than genetic engineering. Although this expansion would likely increase production waste from the rearing facility in Phoenix, the changes in those effects are not substantial. If expansion of the existing program control options decreases the need for use of pesticides in the eradication program, then benefits to the human environment would result which correspond to the reduced amounts of pesticides applied.

Expansion of existing rearing operations is limited by the size of the rearing facilities available for this purpose. The current rearing facility for the pink bollworm eradication program is in Phoenix, Arizona. The rearing facility has a production capacity of 210 million sterile moths per week; however, the actual average weekly production has been closer to 154 million sterile moths. The program goal for 2008 is an average weekly production of 196 million sterile moths. Although the current rate of production is considered adequate to meet the program goal of eradication, the ongoing pest risks from pink bollworm remain high. More rapid elimination of this invasive pest by expansion of the existing program would decrease the potential for adverse impacts to the environment. Ongoing assessment of program needs may justify expansion of existing rearing operations to increase production of sterile moths in the future.

Expansion of the irradiation treatment capacity is less limiting for sterile pink bollworm production than constraints on mass-rearing because the sterilization process is limited by the availability of pupae to treat. The safety requirements and potential environmental risks from this use of irradiation would not differ from those associated with the no action alternative or current circumstances. The shielding and other protective measures preclude radiation exposures of concern; therefore, the potential impacts of this expansion do not differ substantially from the use of irradiators under the no action alternative.

Expansion of classical genetic selection methods for the separation of pink bollworm sexes would be very helpful to the program because fewer moths would be needed to maintain present program efforts if only sterile male moths were released. However, an effective gender selection process does not exist for pink bollworm, therefore, mass-rearing does not separate

males from females. This limitation results in the need for large-scale production of sterile insects to achieve the high ratio of released insects to wild insects in the field required to achieve successful SIT. Although development of useful gender-specific mass-rearing may not double the production capacity of the Phoenix facility, it would be expected to substantially increase production. As with the fruit flies, the development of classical genetic selection methods may require more time and resources than would be required for genetic engineering of a male-only trait into a strain of pink bollworm; however, the potential economic benefits of being able to separate the moth sexes effectively during mass-rearing for SIT are considerable, and could help shorten the time needed to achieve eradication of pink bollworm in the United States and parts of Mexico.

The potential impacts to the physical environment, human health and safety, and biological resources from the expansion of the existing program alternative of this EIS would not be substantially different from those effects of the no action alternative, except for the benefits from more rapid eradication of pink bollworm. The disposal of rearing media and other waste products from increased production and general maintenance would not contribute significantly to overall impacts. Any impacts from construction of major additions to the present facility in Phoenix would require further environmental review before such efforts could be undertaken.

Although the potential environmental effects of expansion of the existing pink bollworm eradication program alternative of this EIS have clear benefits to the human environment over the no action alternative, the expansion alternative would not provide the benefit of lower research and development costs and less time associated with genetically engineered pink bollworm moths. This expansion alternative does not incorporate the use of marker gene traits into SIT for the pink bollworm eradication program for monitoring purposes as has been subject to ongoing confined field tests. Production of genetically engineered sterile moths would be expected to increase effectiveness of the eradication program. The amount of time needed to achieve eradication of pink bollworm could be shortened with more competitive and effective mating strains derived through genetic engineering.

3. Integration of Genetically Engineered Insects into Programs

The integration of genetically engineered fruit flies and pink bollworm into APHIS' plant pest control programs is the preferred alternative of this EIS. This approach is intended to provide more flexibility, better performance, and cost reductions for SIT releases. This alternative incorporates methods previously discussed in the other two alternatives and their associated impacts, which will not be repeated in this section. The specific component methods considered under this alternative include the following:

(1) development of genetically engineered fruit flies and pink bollworm with marker gene traits for use in SIT programs that allows these insects to be distinguished under ultraviolet light from wild fruit flies and wild pink bollworm caught in insect monitoring traps,

(2) development and use of genetically engineered male-only production for sterilization by irradiation in fruit fly control programs,

(3) development and use of genetically engineered male-only production with field release of males that produce only male offspring, which then pass on an inherited trait that prevents female offspring from occurring, and

(4) development and use of genetically engineered sterile insects without irradiation for the pink bollworm eradication program.

These components are described in greater detail in chapter II.D. (Component Methods of the Preferred Alternative). Other potential components derived from genetic engineering of these pest species are not considered to be at a stage of development where they can be adequately analyzed.

a. Fruit Fly Control Programs

The adverse environmental consequences of using genetically engineered fruit flies with a marker gene trait, such as a protein that fluoresces under ultraviolet light, would be no more significant than the continuation of the present SIT fruit fly programs (as described in the no action alternative of this EIS) because the mass-reared fruit flies would continue to be sterilized by radiation and produce practically no offspring. Their release also results in elimination of the invasive pest fruit fly population in the area of release. The marker trait would replace other means of marking the flies, such as temporary dyes or other non-genetically engineered heritable traits that require development by selection over many generations. The genetically marked fruit flies are easily distinguished under ultraviolet light from wild fruit flies caught in insect traps used to

monitor the dispersal of the SIT fruit flies and to evaluate program effectiveness.

The adverse environmental consequences of using genetically engineered fruit flies that produce only males and no females in the mass-rearing process would also be of no more significance than the continuation of current SIT fruit fly program practices because the mass-reared fruit flies would be sterilized by radiation and produce practically no offspring. Production of males-only is achieved by using an agent, such as tetracycline or a threshold temperature, in the diet or rearing conditions that initially results in both males and females, but when the agent is withdrawn or conditions are changed, only male flies continue to develop. The resulting male fly pupae would then be sterilized by radiation and used in SIT fruit fly control and eradication programs. It is also desirable that these male fruit flies have a genetically engineered marker trait to monitor their dispersal and overall effectiveness of the program.

The adverse environmental consequences of mass-rearing genetically engineered male fruit flies that produce only male insects upon release, and mating with wild fruit flies would also have no greater significance than the continuation of the present SIT fruit fly programs because the genetically engineered male fruit flies would produce only male offspring that carry the male-only trait and no females. The male offspring of these genetically engineered mass-reared and released fruit flies would inherit the trait for producing no female offspring. As a result, the wild population would soon collapse because of the elimination of female flies, thus providing control and eradication of the pests. It is also desirable that these male fruit flies have a genetically engineered marker trait to monitor dispersal and effectiveness.

The production of genetically engineered sterile males would be expected to increase the effectiveness and efficiency of the control and eradication programs. The amount of time needed to achieve control of an infestation of invasive fruit flies could be shortened, and associated costs of the control or eradication effort could be diminished with the more competitive and effective mating strains anticipated to be made available through genetic engineering. The availability of genetically engineered flies with the marker gene trait would be expected to provide a more consistent and more readily discernable tool for differentiation of mass-reared flies from wild fruit flies caught in traps used for monitoring sterile fly dispersal and program effectiveness against the invasive pest flies.

b. Pink Bollworm Eradication Program

The adverse environmental consequences of using genetically engineered pink bollworm with a marker gene trait, such as protein that can be

detected in tissues by ultraviolet light, would be no more significant than the continuation of the present pink bollworm eradication program (as described in the no action alternative of this EIS) because the mass-reared bollworms would be sterilized by radiation and produce practically no offspring. The marker trait would replace other means of marking the bollworms, such as dyes, which are much less effective. They could be used with either a high dose of 20 kR radiation for conventional exposure that results in immediate sterilization, or a lower dose of 7 to 10 kR for F_1 sterility, in which their offspring inherit a high degree of sterility. The genetically marked bollworm moths are easily distinguished under ultraviolet light from wild moths caught in insect traps used to monitor their dispersal and overall program effectiveness.

The adverse environmental consequences of using genetically engineered pink bollworm that are sterile and do not require radiation sterilization would be no more significant than the continuation of the current pink bollworm eradication program because the mass-reared bollworms would be genetically sterilized and produce practically no offspring. This can be achieved by using an agent, such as tetracycline, in the diet that initially results in both fertile males and females; however, when the agent is withdrawn, the insects mass-reared for field release are reproductively sterile. The current state of the technology would result in mass-rearing and release of both sterile males and females; however, it would be more efficient and cost effective to improve the technology so that only sterile males are produced. It is also desirable that pink bollworm carry a genetically engineered marker trait to monitor dispersal and program effectiveness.

The production of genetically engineered sterile moths would be expected to increase effectiveness of the eradication program. The amount of time needed to achieve eradication of pink bollworm could be shortened, and associated costs of the eradication effort could be diminished with the more competitive and effective mating strains anticipated to be made available through genetic engineering. The availability of genetically engineered pink bollworm with the marker gene trait would be expected to provide a more consistent and more readily discernable tool for differentiation of mass-reared moths from wild moths caught in traps used for monitoring sterile moth dispersal and program effectiveness against the pink bollworm.

4. Potential Cumulative Impacts

The potential cumulative impacts are those effects that result from the incremental impact of a program action when added to other past, present, and reasonably foreseeable future actions. Cumulative impacts are associated with long-term quality of environmental and human resources. Although many program actions only result in temporary effects, their

interactive relationships with other actions, over time and space, can be important to the human environment and affected resources.

Although this EIS is focused on nonchemical control alternatives and methods, the integrated control applied in invasive pest programs for fruit flies and pink bollworm requires the use of both chemical and nonchemical control methods. Issues, such as the following, may lead to cumulative effects related to control applications in these programs:

(1) development of pest resistance,
(2) bioaccumulation,
(3) chemical synergism,
(4) aggregate risk of pesticides with similar modes of action, and
(5) contribution of program chemical applications to chronic health conditions (e.g., hypersensitivity).

These cumulative effects relate to ongoing chemical control applications. Other possible effects to the human environment require this broad cumulative review to provide meaningful input for program decisions to be made. This includes socioeconomic considerations, impacts to cultural and visual resources, unavoidable effects from program applications, and irreversible and irretrievable commitments of resources to pest control research and program control measures.

a. Chemical Control

The chemical control measures used in fruit fly and pink bollworm programs are generally applied with the intent of eradication of the extant infestation; nevertheless, the focus of this EIS is on nonchemical control or eradication of the Medfly, Mexican fruit fly, oriental fruit fly, and pink bollworm. Other than the ongoing efforts against Mexican fruit fly in the Lower Rio Grande Valley in Texas, chemical eradication efforts have not required continuing applications to the same sites over an extended number of years. Even the Mexican fruit fly program in Texas seldom requires chemical applications to the same orchards every season. This eradication strategy for controlling these invasive species minimizes the chance that the pest population will receive sublethal doses from program chemical applications that could lead to the development of resistance of the pesticides used. However, the application of pesticides in the program areas is often done by growers or homeowners in a manner that could result in pesticide resistance or tolerance to pesticide residues. Although occurrence of pesticide resistance may not prevent eradication, it can complicate the program strategy and increase the time and associated costs.

The only example where the cumulative use of a pesticide has potentially affected an eradication effort is the recent evidence of resistance to chlorpyrifos in pink bollworm in Arizona, which may limit the use of chlorpyrifos there. Although no program decisions have been made about how to manage this resistance in the pink bollworm population, the eradication program approach has generally been to seek efficacious pesticides with different mechanisms of toxic action to minimize the potential for resistance development, and to lower populations to a level where nonchemical methods, such as SIT, can complete the goal of eradication. The application of pesticides in this program is limited to those cotton fields with infestations above a certain threshold population. The frequency of chlorpyrifos-resistant pink bollworm occurrence in Arizona is not expected to be substantial, and this resistance is expected to be ultimately eliminated when the Arizona increment of the pink bollworm eradication program is completed. The limited program applications of chlorpyrifos are not expected to contribute to the inherent resistance already present in the pest population, and the projected rapid completion of the Arizona increment of the beltwide eradication program is not expected to expose enough generations of pink bollworm to sublethal levels to select for resistance traits within the wild population.

Most of the cumulative impacts associated with chemical control actions against fruit flies and pink bollworm have been discussed in previous documentation (USDA–APHIS, 2001a; USDA–APHIS, 2002a). The frequency and manner of application of pesticides used in these programs is designed to preclude bioaccumulation of pesticide residues in animal tissues, as well as preclude accumulation in abiotic media (i.e., soil, water, air). Most of the chemicals are not persistent, and those with greater persistence are applied with less frequency to avoid accumulation of pesticide residues. The potential for nontarget organisms or individual persons to receive multiple chemical exposures depends upon site-specific conditions and persistence of the chemical. Although it is possible to receive exposure to more than one pesticide, the programs coordinate with growers and notify the public in advance of pesticide applications to avoid exposure to humans, livestock, and domesticated animals.

Synergistic effects are adverse effects that result from exposures to more than one chemical with sufficient frequency to have greater adverse effects than would be expected from simply adding the effects from exposure to each chemical. The potential for multiple chemical exposures depends on site-specific circumstances and the program application of pesticides. However, the standard operating procedures and mitigation measures for these programs are designed to prevent such exposures (See chapter VI of the fruit fly EIS (USDA–APHIS, 2001a) and pages 25 to 26 of the pink bollworm eradication program EA (USDA–APHIS, 2002a)).

The overall aggregate risk of pesticides is subject to ongoing review by EPA in their compliance with the Food Quality Protection Act (FQPA). Their aggregate chemical risk measure is generally referred to as the "risk cup" and sets exposure limits for all pesticides with a common mechanism of toxic action (same class of pesticides). EPA has completed its review of the organophosphate pesticide class for regulation under FQPA guidelines. Several program chemicals (malathion, chlorpyrifos, diazinon, fenthion, naled, dichlorvos) are in this class. The mitigation measures of the fruit fly and pink bollworm programs mentioned in the previous paragraph are designed to mitigate any potential for adverse human health effects and exceed the usage restrictions established by EPA under FQPA.

Exposure to pesticides and other chemicals can be a contributing factor to adverse cumulative effects for some highly chemical sensitive individuals. Chronic conditions, such as allergy and hypersensitivity, can result from even very low chemical exposure for a small percentage of the general population (Calabrese, 1991). As part of the program mitigations, those people who are registered as having multiple chemical sensitivities with the appropriate State health agency are notified at least 24 hours in advance of program pesticide applications scheduled near their residence. This allows them to take appropriate steps to avoid exposure. To the extent that nonchemical control methods (such as more effective use of SIT) can replace pesticide applications, the potential for cumulative effects from chemical applications can be reduced further.

b. Nonchemical Control

The nonchemical control methods used in fruit fly and pink bollworm programs have been evaluated previously (USDA–APHIS, 2001a; USDA–APHIS, 2002a) and found to have minimal cumulative environmental impact. Some of the nonchemical control methods may cause temporary disturbances to nontarget organisms and their habitats, but the effects are of short duration and reversible, so long-term or cumulative effects on populations are highly unlikely. Previous documentation did not consider the use of genetically engineered insects in SIT and biotechnological control applications.

The cumulative impacts of developing, mass-rearing, and field release of genetically engineered fruit flies and pink bollworm in SIT plant pest control and eradication programs are expected to be negligible primarily because of the sterility of the insects produced and their inability to sustain a population of genetically engineered insects in the environment.

It has been found that most genetically engineered insects are not as environmentally fit as wild-type insects. Should any genetically engineered insects survive the very effective irradiation treatment or

genetically engineered sterility, those insects will be eliminated by the natural selection process or other physical and chemical pest control measures that are typically used against fruit flies and pink bollworm. (See appendix D for more information.)

The quality control, biosafety, physical containment, and security measures used at the present fruit fly and pink bollworm mass-rearing facilities are not expected to pose any new or novel risks as a result of the development and potential use of genetically engineered fruit flies and pink bollworm. The mass-rearing mitigation measures will be reevaluated before mass-production and field release commences.

Horizontal transfer of genes to other species has emerged as a theoretical risk for genetically engineered insects that use mobile transposable elements (transposons) as the means to insert a novel gene into an insect genome. However, the transposable elements used to genetically engineer the fruit flies and pink bollworm in APHIS programs have been disabled to prevent them from making the transposase enzymes needed for their mobilization, and they are, therefore, rendered incapable of moving themselves and any other genes. Self-induced horizontal transfer by transposons is very difficult to scientifically establish in the laboratory, may take millions of individual insects over innumerable generations, and may be difficult to differentiate from the normal evolutionary selection process that occurs over long periods of time. (See appendix D for more information.)

c. Socioeconomic Effects

People potentially affected by fruit fly or pink bollworm infestations and the actions associated with control programs may belong to any of the following social groups: agricultural producers (producers of host crops, home gardeners, organic farmers, and beekeepers), pesticide applicators, residents, and consumers. Many other groups may be indirectly affected; however, this discussion will be restricted to those groups immediately impacted. The program will result in both benefits and risks for people within these social groups.

The impact of a plant pest control program on agricultural producers is primarily beneficial. The individual growers benefit from the decrease or elimination of the pest problem. Successful eradication can dramatically reduce the need to use pesticides for crop protection. Although there are risks to organic farmers from the drift of pesticides in chemical control applications, the increased use of SIT in preventive releases reduces the need for future pesticide applications. The potential mortality to predators and parasites of plant pests and to pollinators, due to pesticide use, as well as the potential loss of "pesticide-free" status, is critically important to

organic farmers. The mitigation measures for pesticide applications are designed to minimize exposure to bees, through advanced notification to beekeepers, which allows them to move their hives away from exposure to pesticides. The use of nonchemical control methods, including SIT, precludes concerns of organic farmers and beekeepers.

Pesticide applicators for these plant pest control or eradication programs have various benefits, as well as risks. The work may provide vital income; however, there are health and safety risks related to handling and application of pesticides. The programs continue to seek less toxic and less persistent pesticide applications to reduce risks to human health and the environment. The standard operating procedures and mitigation measures of these programs require work practices and personal protective equipment that greatly decrease the likelihood of adverse exposures.

The residents of a program area will receive both benefits and risks. The protection of garden and backyard fruits and vegetables and ornamental host plants from invasive fruit flies is a benefit to the residents. The risks associated with pesticide use are the most frequent concern of residents, even though pesticides are used safely according to label precautionary statements approved by EPA. Pesticide use is not an issue with SIT releases; however, the noise and other disturbance from aerial release of sterile insects could frighten or irritate some residents. The program chemical applications and SIT releases are timed to minimize disturbance of residents, and minimize human exposure to chemicals or contact with sterile insects.

The largest group to benefit from these programs is the consumer of produce or products from host plants or crops that are subject to infestation by the invasive fruit flies and pink bollworm. These programs help to maintain the availability and low cost of fruit, vegetables, cotton, and other crops by protecting against invasive plant pests. The pesticides used in the program are applied in a manner that protects crops and ensures that crops lack harmful pesticide residues. The rapidly invasive and destructive nature of the pests controlled by these APHIS programs requires a timely and coordinated response to prevent major crop losses. The consumer benefits most from these programs because produce availability and low cost throughout the United States are maintained by continuous program monitoring and emergency pest response to control invasive plant pest introductions.

d. Cultural and Visual Resources

Nonchemical control methods are expected to have minimal or negligible effects on cultural and scenic resources of the potential program areas. Equipment (aircraft or trucks) used to release sterile insects may affect

these kinds of resources only to the extent that the activity or noise may disturb visitors to these resources. Physical control methods may affect the appearance of public and private gardens. Stripping of fruit from trees would not result in harm to plants; however, removal of vulnerable host plants could change the appearance of gardens. Cultural control occurs on agricultural lands where the use of this control method should not affect the cultural resources. Neither physical control nor cultural control is practiced in scenic areas, such as national forests or wilderness areas.

The potential effects of biological and biotechnological control on cultural resources would depend on the specificity of the controls to the target pest, the relative cultural and visual contribution of affected nontarget species to the particular resource, and the degree of damage caused by the pest species. Mortality of insects is not likely to directly affect the value of a cultural resource; however, adverse effects on plants and pollinators could change the appearance of gardens or natural scenic areas. The establishment of quarantine checkpoints under regulatory control and the associated traffic, noise, and signboards may affect nearby cultural or scenic resources. The effect of IPM on these kinds of resources would depend on the component control methods selected for use.

Aerial pesticide applications have potential to adversely affect cultural and visual (scenic) resources through direct or indirect effects on nontarget species. The effect of aerial applications on cultural and scenic resources (such as gardens, parks, zoos, arboreta, forests, and wildlife refuges) will depend to a large extent on the animal and plant species they contain. Standard operating procedures (such as notification of residents within a spray area and avoidance of recognized major bodies of water) help to limit the exposure of wildlife in zoos, arboreta, gardens, and water. Most pesticide applications in these programs occur at locations in agricultural fields, orchards, residential areas, and similar locations where cultural or scenic resources are not likely to be treated.

Some pesticide applications are known to mark surfaces. Malathion bait spray is known to affect some types of car paint. SureDye bait spray is known to leave red or brown marks on external surfaces of some buildings. No data exist about the potential effects of pesticide formulations on the types of exteriors found on historical buildings or Native American petroglyphs. However, archaeological sites are not likely to be treated, and the vertical walls and exposures of petroglyphs would minimize impingement of pesticide residues. Cultural practices, such as wild food gathering by Native Americans on reservations, could be temporarily halted by advanced notification of pesticide applications; however, applications to reservation lands are not likely.

Other chemical control methods have little to no effect on cultural or scenic resources. Soil treatments and ground applications may affect those resources if substantial mortality of nontarget species were to occur as a result of treatment. However, these applications are applied to limited areas, and any resulting impacts would be minimal and localized. Methyl bromide fumigation should not have any impact on cultural or scenic resources. The use of insect monitoring traps, in gardens or around historic sites, may temporarily detract from their appearance.

e. Unavoidable Environmental Effects

The extent of unavoidable environmental effects depends upon the ability to assess and delimit the control effort needed. The unavoidable environmental effects from pesticide applications against established pests, such as pink bollworm, are minimized by coordinated completion of incremental geographic areas subject to eradication. This approach makes it less likely that eradication efforts for completed increments will need to be repeated. With detections of new introductions of fruit flies, the unavoidable environmental effects can be minimized through continuous insect trap monitoring, followed by rapid implementation of eradication measures in local areas where infestations are detected. However, if an infestation of fruit flies covers a large geographic area, multiple control techniques may have to be employed for a longer time period, with commensurate increase in unavoidable adverse effects, particularly when pesticides are needed.

Use of nonchemical control methods may result in localized unavoidable environmental effects, such as causing flight of some birds due to the movement and noise from vehicles and personnel. Minimal physical habitat disturbance is anticipated from the personnel, vehicles, and equipment employed to implement program treatments. Some soil compaction, erosion, and aquatic habitat disruption could result from intensive physical control measures. Biological control agents are usually specific to the target-pest species; however, some predators and parasites could have unintended effects on nontarget species. The current programs for fruit flies and pink bollworm do not employ these organisms and, therefore, this is not expected to be an issue of concern.

Genetically engineered insects are intended to be used to improve the efficiency and cost effectiveness of the plant pest control and eradication programs that employ SIT for control or eradication of the Medfly, Mexican fruit fly, and pink bollworm. In the future, this technology may also be applied to SIT-based programs for other invasive plant pests, such as oriental fruit fly, as needs arise to protect America's agriculture. Therefore, the use of genetically engineered insects in SIT programs is expected to cause no more adverse impacts than present SIT programs.

There are no foreseeable unavoidable environmental effects expected from this technology that would be different from those of the current SIT programs; however, if this technology led to significant expansion of APHIS' SIT programs, to include other plant pests and much larger SIT program operations, the present unavoidable environmental effects that result from mass-rearing operations, such as the amount of waste produced, would increase accordingly. Prior to implementation of this technology, research testing and strain development will also involve unavoidable environmental effects that are comparable to those from the classical genetic selection methods currently used to develop new strains.

Regulatory control measures will result in noise and air pollution, and will add to the waste stream. Pesticides used in regulatory control programs will have the same or similar effects to those from the use of the same pesticides in the program control and eradication applications.

Chemical control methods pose various unavoidable environmental effects. Their magnitude varies with the physical and chemical properties, the toxic mode of action, the application method and rate, the size of the treatment area, site-specific environmental factors, timing of applications, and length of the treatment program. Program pesticide use will increase pesticide load to the environment, as does pesticide use for other purposes. Effects may vary according to pesticide persistence and its movement in the environment. Other than the specificity of insect pheromones and other attractants used in insect traps, the pesticides used in the programs are broad in spectrum and are not insect-specific. Consequently, some nontarget species, particularly arthropods, will be exposed and affected by the application of these pesticides.

Humans exposed to pesticides vary with respect to individual response. People who are chemically hypersensitive could be affected by exposure to even small quantities of pesticides in the environment if they do not take measures to minimize their exposure. Similarly, applicators that do not follow established pesticide use safety procedures and do not use appropriate personal protective equipment could be affected by repeated exposures.

Aerial applications of pesticides have the most potential for unavoidable effects due to broad-scale application. Many invertebrate species may suffer high mortality. Secondary pest outbreaks can occur when their parasites and predators are killed by pesticides intended for other pests. Secondary pest outbreaks have occurred in the past and are an unavoidable impact anticipated with future aerial applications. Insect species diversity will be reduced in the treatment areas and some pollinators, such as honey bees, are likely to suffer losses. An indirect effect from the loss of pollinators could be decline in fruit and seed production from crops that

depend upon insect pollinators. Vertebrate insectivores may also be affected due to loss of food supply from insecticide applications. Although larger bodies of water are avoided during aerial applications, smaller ponds and riparian zones are often sprayed or receive drift. Depending upon the amount of spray reaching these aquatic habitats, the invertebrates, fish, and amphibians may be affected.

The physical aspects of aerial application, including noise, disrupt activities of some nontarget species. Although the effects are usually temporary, nest abandonment by sensitive avian species could affect bird hatching or fledging success. People can also be disturbed by the noise and vehicular combustion emissions.

Although ground treatments, such as soil drenches, are hazardous to some ground-dwelling vertebrate species, the number of exposed organisms is considerably less than are exposed from aerial applications because of the small and limited treatment sites of these applications. Localized populations of soil invertebrates and susceptible microorganisms are certain to be adversely affected by soil drench pesticide applications.

Methyl bromide fumigations will release bromine to the atmosphere, which contributes to the stratospheric ozone depletion. Organisms that are inside or enter fumigation chambers during treatments will suffer mortality.

There are some nontarget invertebrates that will be attracted to insect pheromones and other attractants in insect traps and bait treatments; however, most nontarget species are not attracted by these control techniques. Only minor unavoidable environmental effects are expected from employment of traps and bait stations in the control programs.

B. Special Programmatic Considerations

1. Applicable Environmental Statutes

In the planning and implementation of its programs and actions, APHIS complies with a variety of environmental statutes and regulations. Most of those statutes and regulations have the objective of requiring Federal managers to comprehensively consider the environmental consequences of their actions before making any firm decisions. In addition, the statutes and regulations provide guidance about the procedures that must be followed, about the analytical processes involved, and about how to best obtain public involvement. This EIS is prepared specifically to meet the requirements of the National Environmental Policy Act of 1969 (NEPA) 42 United States Code (U.S.C.) 4321 et seq.

APHIS complies with environmental regulations and statutes as an integral part of the decisionmaking process to identify and consider

available alternatives that lead to more successful programs. NEPA is the origin of current APHIS environmental policy. It requires each Federal agency to publish regulations implementing its procedural requirements. APHIS originally published the "APHIS Guidelines Concerning Implementation of NEPA Procedures" (44 CFR 50381–50384, August 28, 1979). Subsequently, the APHIS "National Environmental Policy Act Implementing Procedures" (7 CFR 372) were published to supersede the earlier guidelines, and most recently revised on March 10, 1995. APHIS bases its environmental compliance on NEPA; the CEQ's "Regulations for Implementing the Procedural Provisions on the National Environmental Policy Act," 40 CFR 1500, et seq.; the U.S. Department of Agriculture's "NEPA Regulations," 7 CFR 1b, 3100; and the APHIS "National Environmental Policy Act Implementing Procedures."

a. The National Environmental Policy Act

NEPA requires Federal agencies to consider potential environmental consequences in planning and decisionmaking processes. It requires the agencies to prepare detailed statements (EISs) for major Federal actions which significantly affect the quality of the human environment. These documents must consider—

- the environmental impact of the proposed action (i.e., adverse effects which cannot be avoided should the proposal be implemented),
- alternatives to the proposed action,
- the relationship between local and short-term uses of the human environment,
- the maintenance and enhancement of long-term productivity, and
- any irreversible and irretrievable commitments of resources necessary to implement the action.

NEPA provides the basis for many other statutes and environmental regulations within the United States.

NEPA established the President's CEQ, which published regulations for the implementation of NEPA that became effective in 1979 (40 CFR Parts 1500–1508). Those regulations were designed to standardize the process that Federal agencies must use to analyze their proposed actions. Those regulations have been the models for the NEPA implementing regulations that have been promulgated by Federal agencies.

b. The Endangered Species Act

The Endangered Species Act of 1973 (ESA), 16 U.S.C. 4332 et seq., was passed to provide a Federal mechanism to protect threatened and endangered species. Compliance with this act involves an analysis of the

impact of Federal programs and actions upon listed species. Under ESA, animal and plant species must be specifically listed in order to gain protection. Federal agencies that propose programs and actions, which could have an effect on threatened and endangered species that are listed or proposed to be listed, or on designated or proposed critical habitat must prepare biological assessments for those species potentially affected by their programs and actions. Those biological assessments analyze potential effects and describe any protective measures the agencies will employ to protect the species or habitat. A consultation process in compliance with section 7 of the ESA is employed, as needed. Consultation under ESA occurs with the U.S. Department of the Interior's FWS and/or U.S. Department of Commerce's National Marine Fisheries

Service. Such consultation is important to APHIS' environmental process and then becomes an integral part of the proposed program. Details of compliance with ESA, in regard to the use of genetically engineered fruit fly and pink bollworm in APHIS' plant pest control programs, are provided in the next section under Special Concerns and in appendix L.

c. Executive Order 12114––Environmental Effects Abroad of Major Actions

Executive Order (EO) 12114, "Environmental Effects Abroad of Major Actions," was written to require Federal officials to become informed of pertinent environmental considerations and to take them into account, along with other national policy considerations, when making decisions regarding certain kinds of Federal actions, generally those that would have significant effects outside the jurisdiction of the United States. The EO specifically covers major Federal actions that significantly affect—

(1) the global commons (environment outside the jurisdiction of any nation),
(2) the environment of nations not participating in or involved in that action,
(3) the environment of a foreign nation by providing to that nation a product that is toxic or radioactive and prohibited or regulated in the United States, and
(4) natural or ecological resources of global importance designated by the President.

EO 12114 (section 2–4) specifies the kinds of documents to be used for each class of actions listed above. The types of documents include EISs (generic, program, or specific), bilateral or multilateral environmental studies, and concise reviews (including EAs, summary environmental analyses, or other appropriate documents). For some actions, EO 12114 stipulates NEPA-type documents be prepared; however, NEPA procedures

do not apply. Although EO 12114 states that nothing contained in it invalidates any existing regulations of an agency under NEPA and other environmental laws, it explicitly states that it "…represents the United States government's exclusive and complete determination of the procedural and other actions to be taken by Federal agencies to further the purpose of NEPA, with respect to the environment outside the United States, its territories and possessions" (section 1–1). Because of its specificity on the type of document to be prepared (based upon class of action), it should be regarded as the exclusive procedural guidance for that determination.

Details of compliance with EO 12114, in regard to the use of genetically engineered fruit fly and pink bollworm in APHIS' plant pest control programs, are provided in the next section under Special Concerns.

d. Executive Order 12898––Environmental Justice

EO 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations," focuses Federal attention on the environmental and human health conditions of minority and low-income communities, and promotes community access to public information and public participation in matters relating to human health or the environment. This EO requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participating in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

e. Executive Order 13045––Protection of Children from Environmental Health Risks and Safety Risks

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and appropriate, and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. It also establishes a task force, requires the coordination of research and integration of collected data, gives guidelines for the analysis of effects, and directs the establishment of an "Interagency Forum on Child and Family Statistics." Both EO 12898 and EO 13045 call for special environmental reviews in certain circumstances. No circumstance that would trigger the need for

special environmental reviews is involved in implementing the preferred action considered in this document. No disproportionate adverse effects are anticipated to any minority, low-income population, or particular subgroup of the U.S. population.

f. Executive Order 13112––Invasive Species

EO 13112, "Invasive Species," directs Federal agencies to use their programs and authorities to prevent the spread or to control populations of alien species that cause economic or environmental harm, or harm to human health. Alien species are, with respect to a particular ecosystem, any species including its seeds, eggs, spores, or other biological material capable of propagating that species that is not native to that ecosystem. The fruit fly species and pink bollworm considered for use in APHIS' control programs, which are being analyzed in this programmatic EIS, are all classified as invasive, alien species. In this EIS, identification of these species and the proposed alternatives for control and containment of these invasive species serves to fulfill obligations under NEPA and under this EO.

g. Miscellaneous Federal and State Environmental Statutes

APHIS complies with a number of other environmental acts, statutes, and regulations. Examples of these include the—

- Migratory Bird Treaty Act;
- Bald and Golden Eagle Protection Act;
- Federal Insecticide, Fungicide, and Rodenticide Act;
- Toxic Substances Control Act;
- Resource Conservation and Recovery Act;
- Comprehensive Environmental Response, Compensation, and Liability Act of 1980;
- Clean Air Act; Clean Water Act; and,
- the Food Quality Protection Act.

Environmental compliance with these statutes must be verified before any program, rulemaking, or action is undertaken.

The potential program States all have various environmental statutes and regulations. Many of the regulations and regulatory organizations that enforce them are direct parallels of the Federal regulations and regulatory organizations. California, for example, has the California Environmental Quality Act and has formed the California Environmental Protection Agency.

For parallel programs and initiatives, APHIS will work with State and/or other Federal agencies to implement site-specific actions. APHIS will rely on its State cooperators to identify applicable State environmental regulations to take the lead for their procedures, and to ensure full compliance with State laws.

2. Special Concerns

There are a number of issues that relate directly back to the applicable environmental statutes described above, or to potential impacts generally associated agency program actions. These special concerns are discussed in this section.

a. Endangered Species Act Compliance

USDA, Departmental Regulation, Fish and Wildlife Policy No. 9500–4, dated August 22, 1983, sets forth the purpose, policy, and responsibilities of USDA with respect to fish and wildlife. Agencies of USDA will not fund or take any action that is likely to jeopardize the continued existence of threatened or endangered species or destroy any habitat necessary for their conservation. USDA will coordinate with the Secretaries of the Department of the Interior and the Department of Commerce in the administration of the ESA and animal and plant quarantine laws.

b. No Action and Expanded Program Alternatives

APHIS, or cooperators, in Florida, Texas, and California have conducted ESA section 7 consultation with FWS and/or NMFS since the 1990's when outbreaks of fruit flies have occurred and the eradication program required Federal involvement. The first biological assessment for fruit fly species was prepared for the Mediterranean Fruit Fly Cooperative Eradication Program (USDA–APHIS, 1993). Since that time, protection measures developed from that consultation have been refined and built upon primarily through discussions with NMFS on site-specific programs, rather than through broad programmatic reviews. Timely consultation is important to the rapid response required for the emergency actions of most fruit fly cooperative control programs. Section 7 consultation generally occurs through contacts made informally with local offices of NMFS. The State of California has developed a Natural Diversity Data Base that assists programs in accessing the location of threatened or endangered species and their critical habitats. In addition, programmatic consultations in California regarding specific methods for fruit fly control programs have been conducted to establish those techniques that are compatible with (have no effect upon) threatened or endangered species located in or near control or eradication program areas. This information allows the program to readily determine whether given actions are likely to have an effect or no effect on these species before deciding how to handle the pest risk from the infestation. As fruit fly infestations occur, consultation will

occur with NMFS on a site-specific basis to ensure that the appropriate protection measures are in place so that program activities will have no effect or are not likely to adversely affect listed species or their habitats.

Since 2005, APHIS has conducted ESA section 7 consultations with FWS for the National Pink Bollworm Eradication Program. APHIS prepared and submitted a biological assessment to FWS in Albuquerque, New Mexico, that analyzed the effects of program activities occurring in certain counties in Arizona, New Mexico, and Texas. Activities considered in that biological assessment included mapping, trapping, cultural control, SIT, use of *Bacillus thuringiensis* (Bt) cotton, and chemical control. APHIS determined that none of these activities, except insecticidal control, would have an effect on threatened or endangered species or their habitats. Measures to protect listed species from exposure to program insecticides were put in place and these measures are used by the program. Since 2005, APHIS has reinitiated consultation each year to consider newly-listed or proposed species or proposed or designated critical habitat in the program area. As new chemicals or techniques are added to the program, or the program area is expanded, APHIS will continue to consider the effects of those actions on threatened and endangered species and their habitats, and will enter into section 7 consultation with NMFS, as necessary.

c. Integration of Genetically Engineered Insects Into Programs Alternative

APHIS has determined that implementation of integration of genetically engineered insects into programs and, specifically, genetically engineered insects for sterility for use in SIT, will have no effect on federally listed threatened and endangered species (listed), species proposed for listing (proposed), or proposed or designated critical habitat (see appendix L for federally listed species occurring in pink bollworm eradication areas and fruit fly PRPs). Genetic engineering, when used as a component of SIT, is self-mitigating in respect to most of the possible theoretical hazards and risks that may be associated with arthropod genetic engineering (see appendix D of this document). However, the potential effects considered below include the transfer of transgenes to listed insects, the toxicity of genetically engineered insects, and the potential for genetically engineered insects to attack/feed on listed plants.

Reasons for the no effect determination include:

- Transfer of modified genetic material via mating between genetically engineered fruit flies or pink bollworm and listed insects will not occur because these species are not closely related. Currently, there are 57 insects federally listed as threatened or endangered and none proposed for listing. Most are beetles (order Coleoptera) or moths, butterflies, and skippers (order Lepidoptera). There are also 13 federally listed flies (order Diptera), 1 dragonfly (order Odonata), 1 grasshopper (order Orthoptera), and 1 naucorid (order Hemiptera). There are no federally listed insects that occur in the same family as fruit flies (Diptera: Tephritidae) or pink bollworm (Lepidoptera: Gelechiidae); thus, transfer of modified genes to listed insects by mating will not occur.

- The *piggyBac*-derived transposable elements (transposons) used to genetically engineer fruit flies and pink bollworm have been disabled to prevent them from making the transposase enzymes needed for their mobilization; thus, they are rendered incapable of moving themselves, or any other genes, to listed insects (Thibault et al., 1999; Peloquin et al., 2000; Gomulski et al., 2004; USDA–APHIS, 2005a; appendices C and D of this document).

- Genetically engineered sterile insects, gender selection genes are not toxic to listed animals and may even provide a food source to insectivorous wildlife. Ingestion of fluorescent marker proteins is not expected to be toxic or serve as allergens, if eaten, by listed or proposed animals (Richards et al., 2003; USDA–APHIS, 2005a). In addition, persisting residues from the dead insects in the environment contain no toxic compounds and consist only of ubiquitous proteins, nucleic acids, carbohydrates, naturally occurring minerals, fats, and other organic compunds (see appendix D of this document).

- The use of SIT is compatible with protection of threatened and endangered species and their habitats (USDA–APHIS, 2002a). Use of SIT may result in a decrease in the use of program insecticides (Nagel and Peveling, 2005).

- Most genetically engineered insects are not as environmentally fit as wild-type insects (USDA–APHIS, 2005a). Should any genetically engineered insects survive the very effective irradiation treatment or genetically engineered sterility, those insects will be eliminated by the natural selection process or other physical and chemical pest control measures that are typically used against fruit flies and pink bollworm (see appendix D for more information.)

- Although rearing facilities are a potential source of unintentional release of fertile insects into the environment, these facilities are located in the vicinity of where the pest insect already occurs, thus, no new effects on any species are expected. In addition, sterile pink bollworm adults are produced at the pink bollworm rearing facility in Phoenix, Arizona. The CDFA Medfly rearing facility is in Honolulu, Oahu, Hawaii. The other fruit fly facilities are located in California, Florida, Texas, Guatemala, and Mexico. (See III.B.6—Irreversible and Irretrievable Commitment of Resources, Fruit Fly Exclusion and Detection Programs, for a list of fruit fly rearing and eclosion facility locations.)

- Listed plants susceptible to fruit flies will not be affected by release of genetically engineered sterile insects since no progeny will be produced from sterile flies. The following threatened and endangered plant species are congeners of known hosts of the Medfly in the continental United States and Puerto Rico: *Eugenia haematocarpa*, *Eugenia woodburyana*, *Juglans jamaicensis*, *Opuntia treleasei*, *Prunus geniculata*, *Solanum drymophilum*, and *Ziziphus celata*. Potential hosts of oriental fruit fly include *Prunus geniculata* and *Ziziphus celata*. Both of these plant species are present in the oriental fruit fly predicted climatic range in the continental United States and are congeneric with plant species reported as hosts of this species. A potential host of the Mexican fruit fly is *Prunus geniculata.*

- No listed plants serve as hosts of pink bollworm and, thus, release of sterile pink bollworm will have no effect on listed plants regardless of whether conditional lethal pink bollworm strains are developed or where they are released.

- No specific flower-pollinator relationships exist for the Medfly, oriental fruit fly, (Nagel and Peveling, 2005), Mexican fruit fly, or pink bollworm. None of the targets are native to the United States, and none are known to be involved in pollination of listed plants.

In the future, if genetically engineered traits other than sterility or fluorescent marking are considered for use in APHIS programs, or species other than fruit flies or pink bollworm are proposed for engineering with these genes, APHIS will consider potential direct, indirect, and cumulative effects on listed and proposed species and their habitats as a result of these actions. Section 7 consultation will be conducted with NMFS, as necessary.

d. Anaylsis in Compliance with Executve Order 12114

EO 12114, "Environmental Effects Abroad of Major Federal Actions," was written to require Federal officials to become informed of pertinent environmental considerations and take them into account, along with other national policy considerations, when making decisions on certain kinds of Federal actions (generally those that would have significant effects outside the jurisdiction of the United States). The EO specifically covers major Federal actions that significantly affect (1) the global commons (environment outside the jurisdiction of any nation), (2) the environment of nations not participating in or involved in the action, (3) the environment of a foreign nation by providing to that nation a product that is toxic or radioactive and prohibited or regulated in the United States, and (4) natural or ecological resources of global importance designated by the President.

EO 12114 (section 2–4) specifies the kinds of documents to be used for each class of action above. To the extent that the actions considered in this EIS include cooperative work in foreign nations and potential effects to the global commons, this EIS addresses those environmental effects abroad. The potential environmental consequences for rearing facilities and SIT release programs in these nations are expected to be comparable to those for such actions in the United States. This EIS addresses the broad issues related to these topics, programmatically, and cites site-specific or facility-specific concerns abroad, where possible.

e. Hypersensitivity

Hypersensitive humans experience toxicological symptoms and signs at dosage levels much lower than those that are required to produce the same symptoms in the majority of the population. Hypersensitive individuals constitute only a small portion of the total population. If the response of the population being studied follows the varying doses in a normal distribution (bell-shaped curve), the hypersensitive individuals would be expected to be on the left side of the curve. The increased genetic susceptibility of these individuals is quite variable. Although a margin of safety factor of 10 (uncertainty factor) has traditionally been used by regulatory agencies (National Academy of Sciences, 1977) to account for intraspecies variation or interindividual variability, human susceptibility to toxic substances has been shown to vary by as much as three orders of magnitude (Calabrese, 1984). Individual sensitivity to effects from chemical exposure is known to be strongly influenced by several factors including age, nutritional status, and disease status. Individuals with immune systems that are less developed or that are compromised physically are more likely to be more hypersensitive. The hypersensitive individuals, therefore, would be expected to include larger proportions of

the populations of elderly and young children than other subgroups of the population. Calabrese (1984) examined several studies of human responses to chemicals and found that a safety factor of 10 was useful for predicted effects in 80 to 95 percent of a population. In APHIS fruit fly programs, pesticide rates and protection measures would result in a safety factor much greater than 10 for the general population. Similar safety factors are also applied in APHIS' pink bollworm programs.

There is no single established mechanism or measurable biological marker that is associated with the reported reactions of individuals who purportedly suffer from multiple chemical sensitivities. Thus, there is no chemical identity or established physiological relationship to individual chemical exposure. The etiology of multiple chemical sensitivity is, therefore, very subjective. The reactivity of this group of individuals cannot be effectively evaluated because there are no objective criteria to use to evaluate specific chemical agents.

Based upon the current state of knowledge, individual susceptibility to toxic effects of the chemicals used in the Fruit Fly Cooperative Control Program cannot be specifically predicted. The approach used in this risk assessment takes into account much of the variation in human response (Calabrese, 1984); however, unusually sensitive individuals may experience effects even when the hazard quotients indicate that there are no unacceptable risks. An association may exist between exposure to the protein bait and resulting dermal, respiratory, and other immunological responses. The program will ensure that residents are notified if bait spray applications are made in their neighborhood to allow sensitive individuals to avoid exposure and the possibility of adverse effects. Only limited amounts of the soil drench chemicals are permitted to be applied to specific areas (to the drip line under infested trees) so that potential exposure is minimized. Exposures from trap chemicals, fruit fly male annihilation treatments, cordelito applications, and wood fiberboard square applications are expected to be minimal due to the limited usage and placement of chemicals. Because an extra effort is made to contact individuals on the lists of registered hypersensitive persons, those individuals can take extra precautions to avoid exposure to residues from program pesticide applications. In addition to chemical hypersensitivity, some individuals are highly sensitive to exposure to insect parts from moths or flies. In particular, the scales from moths are known to induce allergic reactions.

f. Psychological Effects

Program actions, including pesticide applications, may elicit psychological effects in some members of the general population. During an eradication effort, the public is notified of anticipated pesticide applications and

informed of when the personnel and equipment are likely to be in their neighborhoods to make those applications. Nevertheless, individuals are generally uncomfortable with actions that are not under their direct control. Literature from environmental and citizen groups that disapprove of the use of pesticides may influence attitudes of the public and cause additional concern.

Some individuals have expressed a fear of malathion, branding it as a nerve gas. This fear stems from information about a German company, I.G. Farben, whose organophosphate pesticide development led to research into the development and production of nerve gases for the Nazi government during the World War II. Private individuals have circulated literature to a wide segment of the populations in program areas, implying that malathion is a nerve gas or can have the same effects as a nerve gas. Malathion and other organophosphate pesticides in this program are not nerve gases. Instead, there are chemical differences in the classes of compounds, and there are vast magnitudes of difference in their effects. Nevertheless, misinformation or misperception could lead to unfounded distrust of the fruit fly and pink bollworm programs.

Some people may be disturbed by the sight of the helicopters overhead during aerial applications of bait spray. Some individuals who have not seen the notifications may not be aware of the program and may wonder what the helicopters are for and what is being sprayed. Concerns have been raised on behalf of Vietnam veterans, especially those who have been diagnosed with post-traumatic stress disorder, regarding the use of helicopters in the program. Some have speculated that the use of helicopters may trigger uncontrolled behavior because of memories of fighting in the jungles of Vietnam; however, no evidence exists to indicate this has happened in previous programs.

The notification sent out to the affected population states that the public should remain indoors during spraying operations, cars should be covered, and pets should be taken indoors. Adequate notification and education of the public should minimize the risk of individuals developing psychological traumas from the fruit fly and pink bollworm programs.

Should a substantial infestation or establishment of fruit flies or pink bollworm occur in the United States, it could result in psychological effects on farmers, farming communities, and consumers. Farmers, farming communities, and consumers could suffer psychologically from the loss of crops, the loss of control over their business due to product movement restrictions, disruptions in community life, increased pricing as a result of limited product availability, and from stress over their financial future.

Some environmentalists are concerned about what they refer to as the "Frankenbug" or genetically modified organisms and potential adverse impacts on the ecosystem as a result of releasing genetically modified organisms. These concerned environmentalists define a "Frankenbug" as a genetically modified organism containing a modified gene that has unpredictable effects on an ecosystem, and once the organism is released, there is no way to get the modified gene into a controlled (safe) state. Further, environmentalists warn that genetic modifications could cross into related species, or lead to new diseases (Benner, 2001). However, with proper adherence to mitigations and standard operating procedures, adverse environmental impacts are not anticipated in APHIS' programs.

g. Noise

The effects of noise from the application procedures of program pesticides have been considered. Aircraft noise and ground application equipment noise occur for only short durations of time and at low frequencies of repetition, so that disturbances to humans from program actions are likely to be minimal and temporary. The potential use of large aircraft in fruit fly and pink bollworm programs could increase the noise level, particularly close to the airport where loading, take-offs, and landings could occur at late hours in the night. Soil drench applications should not cause any noise disturbances other than minimal equipment noise and conversation of hand applicators. Noise is also expected to be minimal from conversation and use of equipment for fruit fly male annihilation treatments, trapping, cordelitos applications, and applications of wood fiberboard squares.

h. Socioeconomics

People potentially affected by fruit fly and/or pink bollworm infestations or resulting fruit fly and/or pink bollworm eradication efforts may belong to any of several major social groups: agricultural producers (producers of host crops, home gardeners, organic farmers, and beekeepers), pesticide applicators, residents, and consumers. Many other groups may be indirectly affected; however, this discussion will be restricted to those groups immediately impacted. The program will result in both benefits and risks for people within these social groups.

The impact of a program on agricultural producers will be, for the most part, beneficial. Fruit flies and pink bollworm represent a threat to numerous crops, and their establishment could lead to substantial losses of produce, income, and export markets. These losses could be most serious for small farmers and people dependent upon gardens for a substantial portion of their food. Fruit fly and pink bollworm eradication programs will protect crops and income, as well as alleviate the need for (and cost

of) uncoordinated farm-by-farm control programs. The use of PRPs and more extensive use of SIT releases benefit the producers, including organic farmers.

There are, however, some risks for agricultural producers from a program, particularly a program which uses pesticides. These risks include the potential mortality of biological control agents, the loss of "pesticide-free" status (and, thus, certain markets) for organic farmers, and potential mortality of honeybees. The risk to honeybees can be substantially reduced by early notification of beekeepers so that they can take precautions to protect their hives. With proper precautions there should be no loss of hives due to pesticide use (see program mitigative measures).

A program using pesticides will create both benefits and risks for pesticide applicators. The timely nature of an eradication program and its intensive work schedule will probably create additional income for pesticide applicators. There are some health risks for pesticide applicators, although use of protective clothing greatly reduces these risks.

The residents of an area infested with fruit flies and pink bollworm will receive both benefits and risks from eradication programs. The benefits will include the protection of backyard and ornamental host plants from fruit flies, as well as cotton crops from pink bollworm. The risks will be those associated with pesticide use, although only certain subpopulations of the area residents are at risk due to program pesticide use.

The largest groups of beneficiaries include cotton growers and anyone who consumes produce that is a host of fruit flies. Because commercial farms and orchards ship produce to other States and countries, this group encompasses a wide spectrum of people. The proposed program changes benefit this group by preserving the current availability and cost of certain products. Federal regulations governing pesticide residues on produce protect the general public from any risks associated with pesticides used in a program.

The potential for the rapid spread of fruit fly and pink bollworm infestations requires that programs be initiated as preventive release efforts or shortly after infestations are detected. Fruit fly outbreaks often occur first in urban/residential areas, thus, nonagricultural areas are involved. Under these conditions, the distribution of benefits and risks of the program among various social groups can be somewhat inequitable. Because the potential distribution inequity of the program is unavoidable, every effort is made to reduce risks from the program to all social groups.

i. Cultural and Visual Resources

(1) Nonchemical Control Methods

Nonchemical control methods are expected to have minimal effect on cultural and scenic resources of the program area. Equipment (aircraft or trucks) used to release sterile fruit flies may affect those resources only to the extent that the activity or noise may disturb visitors to these resources. Physical control methods may affect the appearance of public and private gardens; fruit stripping would not result in harm to plants, but host removal could change the appearance of gardens. Cultural control should not affect cultural resources because it involves agricultural lands that generally are not considered cultural resources. Neither physical control nor cultural control will be applicable in scenic areas, such as national forests or wilderness areas, because of the resources' large sizes and nonagricultural nature. The potential effects of biological and biotechnological control on cultural resources would depend on the species-specificity of the controls, the relative contribution of nontarget species to the particular resource, and the effect on the species. Mortality of insects is not likely to directly affect cultural resources; however, adverse effects on plants could change the appearance of gardens. The establishment of quarantine checkpoints under regulatory control, and the associated traffic, noise, and signboards, may affect nearby cultural resources such as Indian reservations. The effect of IPM on cultural or scenic resources would depend on the component control method used.

(2) Chemical Control Methods

Aerial bait spray and pesticide applications have the potential to adversely affect cultural and visual (scenic) resources through direct or indirect effects on nontarget species that are associated with or comprise the resources. The effect of aerial applications on cultural and scenic resources (such as gardens, parks, zoos, arboreta, forests, and wildlife refuges) will depend, to a large extent, on the animal and plant species they contain. Aerial applications of malathion bait spray tend to have more adverse effects on the desired wildlife than some other bait sprays. Standard operating procedures (such as notification of residents within a spray area and avoidance of recognized major bodies of water) generally help to limit the exposure of wildlife in zoos, arboreta, gardens, and the major bodies of water.

Bait spray applications are known to mark some surfaces. No data exist about the potential effects on bait spray formulations on the types of paint or exteriors found on historical buildings or Native American petroglyphs. However, archaeological sites are not likely to be treated, and the vertical walls and exposures of the petroglyphs would serve to minimize exposure to any bait spray. Cultural practices, such as wild food gathering by

Native Americans on Indian reservations, could be temporarily halted due to aerial applications of bait spray.

The soil treatments and ground applications of bait spray may affect those resources if substantial mortality of nontarget species were to occur as a result of treatment. However, these applications are applied to limited areas and any resulting impacts would be minimal and localized. Methyl bromide fumigation should not have any impact on cultural or scenic resources because fumigation generally is not conducted in or near cultural or scenic resources. The use of traps in gardens or around historic sites may temporarily detract from the appearance of cultural and scenic resources. Use of fruit fly male annihilation technique, cordelitos, and wood fiberboard squares are generally not applied to areas of cultural or visual resources; however, their limited application to specific areas ensures that any impacts would be minimal and localized.

3. Logistical Considerations for Mass-rearing and Quality Control

The logistical considerations for mass-rearing facilities for SIT can be broadly divided into (1) general facility sanitation, (2) disinfection of rearing media and supplies, (3) maintenance of optimal environmental conditions for insect growth, (4) management of facilities to exclude nontarget arthropods and rodents and to contain reared insects, and (5) maintenance of desired traits of reared insect colonies within the facility. Each of these logistical considerations requires consistent and deliberate practices to ensure optimal production is achieved.

Insect production can be greatly reduced by the presence of molds, bacteria, protozoa, viruses, mites, and rodents. The effort to preclude these organisms can be greatly enhanced by good sanitation, disinfection, and control over facility access. Although individual rearing facilities may not be required to meet specific National Institutes of Health (NIH) guidelines (NIH, 2002) for recombinant organisms (http://www4.od.nih.gov.oba/rac/guidelines/guidelines.html), each facility must meet certain sanitary criteria to maintain adequate quality and quantity of insect production for program needs and to ensure that virile mass-reared pest insects are not released from the facility into habitats suitable for population establishment. Independent of whether the insects are conventionally selected or genetically engineered, many of these practices are required in the facilities to ensure production is adequate. To the extent that the genetically engineered strains require additional tests to ensure that the strain maintains the desired fitness, genotype, and genetic marker, the rearing protocols and strain filters are even more comprehensive.

a. Guidelines, Policies, and Procedures

Many of the facilities described earlier in this EIS involve cooperative rearing, production, and research with USDA–ARS. These facilities are designed to meet the expectations of both APHIS and ARS. Any facilities that handle known biohazardous agents or their toxins are subject to compliance with USDA Security Policies and Procedures in DM 9610–001 (USDA, 2002b) and DM 9610–002 (USDA, 2003c). However, the insect rearing facilities considered in this EIS are not subject to these requirements. Many of the guidelines applied to containment of genetically engineered plants and microbes (Adair et al., 2001) are pertinent to genetically engineered insects. Likewise, the NIH guidelines for research involving recombinant DNA molecules (NIH, 2002) provide applicable information; however, the mobility of insects requires special considerations.

Several guidelines have been developed that apply directly to containment of pest arthropods and specifically genetically engineered arthropods. The most recent is the North American Plant Protection Organization (NAPPO) Regional Standards for Phytosanitary Measures (RSPM) No. 27, "Guidelines for Importation and Confined Release of Transgenic Arthropods in NAPPO Member Countries" (NAPPO, 2007). In addition, there are two other guidelines that pertain to SIT programs involving plant pests. There are also "Guidelines for Construction and Operation of Containment Facilities for Insects and Mites Used As Biological Control Agents" in RSPM No. 22 (NAPPO, 2004) and "International Guidelines for the Export, Shipment, Import, and Release of Biological Control Agents" in ISPM No. 3 (IPPC, 2005). Each of these guidelines applies to various aspects of the SIT release programs for fruit flies and the pink bollworm. In addition, the APHIS–PPQ "Containment Guidelines for Nonindigenous, Phytophagous Arthropods and their Parasitoids and Predators, Nonindigenous Snails, Plant Pathogenic Nematodes, and Other Organisms" sets standards for rearing and containment. In that the eradication programs for Mediterranean fruit fly, Mexican fruit fly, and pink bollworm all have international components, the NAPPO and IPPC standards relating to exportation apply. To the extent possible, all programs and rearing facilities adhere to these international guidelines. However, program adjustments to new guidelines and revisions to the guidelines require time and effort to initiate, and there is often some delay between guideline publication and program implementation. The guidelines provide some flexibility in their interpretation to facilitate compliance with the standards set, but implementation procedures for integrating them into PPQ programs have not yet been developed by USDA or APHIS for all of these guidelines.

In general, each facility sets up its own protocols to ensure the sustenance of adequate production to meet program needs. For example, the Phoenix Pink Bollworm Rearing and SIT Facility has standard operating procedures designed to eliminate or keep pest organisms from entering the facilities. The document associated with the Phoenix facility protocols is referred to as the "General Facility Sanitation and Pest Management" manual and is available from that facility. Some of the other facilities have not formalized their protocols; however, all employ similar measures to maintain optimal rearing conditions.

b. General Facility Sanitation

Routine removal of dust, insects, insect parts, diet, and other debris from all surfaces is generally achieved by sweeping and vacuuming, followed by wiping or mopping surfaces with disinfectant. This is particularly important for floors and counter tops. An electric floor scrubber may be used for rough surfaces of the floor where dirt and insect debris can collect. All cleaning equipment is cleaned, rinsed, and disinfected daily or weekly, based upon the need for sanitary conditions.

The filters used to remove particulates and dust from the external air ducts supplying air exchange for the facilities are regularly cleaned and replaced to prevent introductions of molds, bacteria, protozoa, viruses, and other potential airborne pathogens. The entry of such organisms is also prevented by the use of positive pressure, HEPA-filtered rooms in critical-production areas of the facilities.

Ultraviolet lights are used extensively to exclude pathogenic microorganisms at specific locations in rearing facilities. This helps to prevent cross-contamination of insect broods and growing media.

Critical production areas require the workers to wear clean uniforms and head cover. Authorization of personnel is often limited to working only certain areas of the facility at certain times. This is designed to prevent transfer of potential pathogens between different life stages and different broods. The use of sterile gloves is required for many critical operations.

c. Disinfection of Life Stages, Rearing Media, and Supplies

The disinfection of mass-reared insect life stages is generally limited to the egg stage. At the egg stage, disinfection and cleaning of scales and wings can be accomplished effectively and safely. Elimination of pathogens from later life stages (larval, pupal, and adult stages) can generally only be achieved by eliminating all potentially infected insects. This destruction of brood is not desirable when seeking to optimize

production at the facilities, however, it may be necessary to eliminate microbial contamination.

The disinfection of all contact surfaces and supplies is critical to maintaining production in these facilities. Some supplies can be cleaned and used repeatedly. Some may require autoclaving or other sanitizing treatments. All components of the rearing media must be carefully checked to prevent contamination by microorganisms or toxins that may affect the insects. The water used in media preparation for certain steps may require autoclaving, boiling or otherwise treating to ensure purity of diet and growing media. Materials are reused or recycled, to the extent possible; however, there is still considerable disposal of waste from the mass-production of insects.

d. Maintenance of Optimal Environmental Conditions for Insect Growth

The conditions in each room of the rearing facilities are set for optimal growth of the mass-reared insects at that stage of life. The temperature and humidity are carefully regulated to ensure healthy conditions. These parameters are monitored by hydrothermographs to track the conditions within the facility. Different life stages are placed in different rooms that meet optimal conditions for that particular stage. Broods are physically separated to maintain scheduling the time for collection of insects for ultimate release. Light schedules are set for different life stages, and entry to rooms is restricted during dark room hours to avoid upsetting insect development.

e. Management of Facilities to Exclude Intrusion of External Arthropods and Rodents

An arthropod is any invertebrate animal with jointed legs and a segmented body. Ants, cockroaches, crickets, mites, and spiders are the most common arthropods to establish a colony, nest, or any foothold within the rearing facilities. Use of the following methods may be employed to manage arthropods in rearing facilities—

- Use of pesticides and pesticide-bait combinations may be applied selectively to eliminate species such as ants, crickets, and roaches. However, care must be taken to avoid effects to the colonies being mass-reared. Traps with contained lures and pesticides are preferred.
- Use of “bug lights” to attract, collect, and remove some insects is also employed.
- Good housekeeping techniques such as meticulous cleaning and sanitation of floors, walls, and equipment reduce the available food sources for many arthropods in these facilities.

- The use of a steam-wand has been used for effective control of spiders, cockroaches, and other arthropods in cracks and crevices of carts, racks, facilities, and equipment.

The exclusion of rodents is best achieved by securing against potential entry. Keeping external areas of rearing buildings clear of debris and trash that attract rodents is practiced at these facilities. Routine maintenance of doors, windows, and air ducts also helps to minimize the entry of rodents by making sure there are no openings that allow their entrance. Selective use of rodenticide, in bait form or tracking powder, may be employed if an ongoing problem with entry by mice or rats develops.

f. Management of Facilities to Contain the Mass-reared Insects

The containment of the mass-reared insects is not only critical to protection of the environment from outside pest risks, but also to successful production in these facilities. Proper dating and identification of brood stocks is critical to this process. The physical separation of genetically engineered strains from the conventional mass-reared strains in the facility is required to maintain the desired traits within a given line.

The conventional sterilization process for fruit flies involves irradiation treatment of an immature stage (pupae) and, therefore, it is important to keep track of those flies irradiated for SIT use and those not irradiated (virile) flies used to maintain the colony. The irradiated flies can be readily transported in the pupal stage to eclosion facilities or transported to release sites. Prevention of the escape of adult flies from the oviposition chambers to other parts of the facility is a constant challenge; security against inadvertent release from the facility is a source of constant review. This issue is expected to become more important when conventional strains and genetically engineered strains of fruit flies are reared in the same building or adjacent buildings within the same facility.

The conventional sterilization process for pink bollworm involves irradiation treatment of the adult moths just prior to packaging for transport to release sites. The emergence boxes, trays, and collector require special attention to sanitation and cleaning due to the abundance of moth scales. The maintenance of an ideal air speed in the collector lines for adult moths is required to avoid damage to the moths or accumulation of moths within the lines rather than in the collectors. The escape of adult moths from the egg-laying room to other parts of the facility is a constant challenge and, therefore, authorized access is restricted and the security against potential release from the facility is reviewed regularly. This issue could become more important if conventional strains and genetically engineered strains of pink bollworm were to be reared in the same building.

g. Maintenance of Desired Traits in Insects Mass-reared in Colonies within the Facility

The issue of isolation of each genetically engineered strain from other strains was discussed briefly in the previous section and is critical to maintaining a given strain. Appendix C discusses stability of genetically engineered traits in strains of fruit flies and the pink bollworm. This appendix also discusses the issue of remobilization of the genetic elements through excision (deletions), transposition, and horizontal gene transfer. Using a filter rearing system, any insects carrying deletions are mostly eliminated from the colony. The filter rearing system involves the maintenance of a mother colony that is checked within each new generation for unwanted individuals which are then removed. Eggs from this mother colony are harvested as required, and following three to four generations of amplification (increase through mass-rearing), the males are sterilized and released. However, in the filter release system, no insects that have been through mass-rearing are returned to the mother colony and, therefore, there is no accumulation of highly selected genotypes in the mother colony. The mother colony can be kept under more natural conditions, at reduced adult and larval densities, and with reduced selection pressure for genotypes that become adapted to mass-rearing conditions (Robinson and Hendrichs, 2005). Also, the use of the TSL strain of Medfly helps to control the genetic makeup of the colony by eliminating females from those flies that are not intended for continuing colony maintenance. Transpositions for these insects are extremely infrequent, with none detected in one study of the pink bollworm after 58 generations (Peloquin et al., 2000).

Horizontal gene transfer refers to movement of the genes to an individual of another species. Appendix D discusses the theoretical possibility of this happening—this is not expected to affect mass-rearing colonies within a secure facility.

Routine monitoring of brood stock for genetic composition and routine fitness testing will be necessary to ensure that the filter rearing system and other measures used to maintain the genetically engineered strain continue to provide the genotype required for effective use in SIT. Likewise, these and other monitoring tools and traps will be required for detection and identification in the event of any accidental release, and for use in monitoring the SIT releases.

4. Program Mitigations and Risk Reduction Strategies

APHIS fruit fly and pink bollworm control and eradication programs have established program mitigations and risk reduction strategies that have been expanded and refined to meet the changing risk potential and the challenges posed by global travel and trade. Most of this section describes those operating procedures that relate to the programs, in general. The

mitigations related specifically to genetically engineered insects are discussed in the previous section on logistical considerations and maintenance of genetic phenotypes, and also in appendix K, which describes procedures for application-specific evaluations of genetically engineered plant pests.

Fruit flies, in the family Tephritidae, are among the most destructive, feared, and well-publicized pests of fruits and vegetables around the world. Within this taxonomic family, the genera *Anastrepha*, *Bactrocera*, and *Ceratitis* pose the greatest risk to U.S. agriculture and are the focus of APHIS program mitigations and risk reduction strategies. Tephritid fruit flies spend their larval stages feeding and growing in over 400 host plants. Introduction of these pest species into the United States causes economic losses from destruction and spoiling of host commodities by larvae, costs associated with implementing control measures, and loss of market share due to restrictions on shipment of host commodities. The extensive damage and wide host range of tephritid fruit flies become obstacles to agricultural diversification and trade when these pest fruit fly species become established in production areas.

California and Florida are at highest risk from exotic fruit fly establishment. This conclusion is based on the historical record of frequent outbreaks and the costs to eradicate them, the high importation rate of fruit fly host material at the major ports of entry coinciding with the climatic conditions favorable to establishments of reproducing populations, public opposition to chemical control measures, and the availability of hosts. The market value of exotic fruit fly host commodities totaled about $7.2 billion in the United States in 2002, with approximately $5.1 billion of that grown in California and $1.8 billion in Florida.

APHIS responds to exotic fruit fly risks with an integrated system that incorporates surveillance activities, fruit fly control programs, prevention activities, and regulatory actions. This multitactical approach is the product of close collaboration and consultation between APHIS and its exotic fruit fly program cooperators and stakeholders.

States and other countries play a significant role in funding and implementing the fruit fly safeguarding system. APHIS has cooperative agreements with States to share resources and conduct detection programs, Medfly and Mexican fruit fly preventive releases, and control programs. APHIS also cooperates with other countries through international agreements to perform off-shore risk reduction activities to reduce the imminent threat of introduction or spread of Medfly and Mexican fruit fly from existing populations in Mexico and Central America.

APHIS is committed to continually reexamine fruit fly programs for the purpose of achieving maximum risk reduction. APHIS intends to improve efforts of early detection of fruit fly introductions, fruit fly prevention activities, and fruit fly control activities. APHIS convenes international teams of subject matter experts to review and make recommendations for these programs on a periodic basis. The fruit fly SIT PRPs were reviewed in 2003, and the fruit fly surveillance programs were reviewed in 2006. APHIS also developed an exotic fruit fly strategic plan in 2006 found at http://www.aphis.usda.gov/plant_health/plant_pest_info/fruit_flies/downloads/strategicplan06-19-06.pdf

The risk-based strategy outlined in the plan discusses three tenets for success:

1. Detection and PRPs using SIT are critical to stop small introductions from becoming established populations in high risk areas.
2. In order to reduce the likelihood of exotic fruit fly introductions into the United States, APHIS should participate in off-shore programs to reduce the threat at its source.
3. SIT is an essential operational tool for successful exclusion and eradication programs.

Fruit fly programs are burdened by increasing challenges to maintain risk reduction and mitigation activities, at an appropriate level, to reduce increasing risks posed by exotic fruit fly introductions. Challenges facing fruit fly programs include:

- There are numerous fruit fly species of economic importance, and they approach the United States via several high-risk pathways.
- A broad range of agricultural commodities are subject to fruit fly damage.
- Previously established populations of pest fruit fly species within the United States pose a constant threat to fly-free areas.
- The origins and nature of *Ceratitis*, *Bactrocera,* and *Anastrepha* species introductions to the United States are not fully understood.
- Lures and toxicants for detecion and control programs are not available for all species of concern. Existing and future lures and toxicants must comply with current environmental mandates.
- Aerial applications of bait sprays are unpopular with the urban public and restricted by the manufacturer.
- SIT is a species-specific population management tool that has been developed for a limited number of species.
- Exotic fruit fly species have worldwide distribution.
- Delineating high risk pathways for introduction of pest fruit fly species requires technology to differentiate species complexes and

identify potential source populations; this technology is not generally available.

- Expansion of international travel facilitates the movement of fruit fly host material, and effectively increases the introduction rate of exotic fruit flies to the United States.
- Fruit fly populations in Mexico and Central America are a significant threat to agriculture in the continental United States due to the large numbers of people migrating north from fruit fly infested areas.
- Foreign governments with endemic populations of fruit flies exotic to the United States do not necessarily concur with our need to mitigate risk to U.S. agriculture.
- Sterile fly production and distribution are resource-intensive processes.
- Efficient and effective SIT implementation requires continuous cooperation and consensus within APHIS and with outside entities.
- SIT is a species-specific population management tool that is dependent upon mass-production methodologies and facilities for each target fruit fly species; several years are required to implement SIT for a new species.
- New population suppression technologies, such as mass production and release of biological control agents, must be cost-effective and fully integrated with SIT and pesticides.

a. Fruit Fly Program Mitigations

APHIS employs a global approach to defend against pest threats and to lessen the economic impact posed by the new establishment or the spread of economically important exotic fruit fly species in the United States. The multifaceted approach includes the following components:

- Maintenance of an extensive bait and trap surveillance system coupled with the initiation of emergency response action plans, when appropriate.
- Application of sterile fruit fly PRPs over areas of historically high risk for exotic fruit fly introductions.
- Containment of any established fruit fly populations in the United States through regulatory and control activities.
- Facilitation of domestic production and interstate commerce of host commodities impacted by established fruit fly populations through the implementation or supervision of certification measures.
- Support, through direct participation, in the elimination or management of existing populations of economically important fruit flies in neighboring countries.

- Active encouragement and support of the development of fruit fly detection and control programs in the Caribbean Basin and Central America to act as an early warning system for the United States.
- Provision of technical assistance to all parts of the world to encourage fruit fly management at the source.
- Partnerships with the U.S. Department of Homeland Security by supporting the enforcement of agricultural quarantine requirements and the performance of agricultural quarantine inspections at points of entry into the United States.
- Solicitation of the assistance of world-wide subject matter experts in fruit fly surveillance, eradication, management, and SIT to review, on a periodic basis, our national fruit fly programs.

(1) Prevent Individual Exotic Fruit Fly Introductions from Becoming Established Populations

The establishment of new populations of exotic fruit flies in the United States would reduce yields of host commodities, create losses in domestic markets, and cause trade disruptions. APHIS' first risk mitigation priority is the prevention of the establishment of introduced populations of exotic fruit flies. This is accomplished by early detection and elimination of newly introduced populations of exotic fruit flies, in conjunction with the prevention of any exotic fruit fly entries into high risk areas from becoming established populations through the implementation of sterile fruit fly PRPs.

(a) Early Detection

Early detection offers the best chance to successfully eradicate newly introduced populations of exotic fruit flies. When fruit fly outbreaks are detected early, this gives program managers the ability to implement emergency response programs with minimum impact on the public and the environment. The duration of emergency response programs is reduced in both size and duration.

APHIS cooperates with various State and territorial departments of agriculture to implement the network of fruit fly surveillance programs. The current exotic fruit fly detection programs are risk-based. Focus is placed on risk areas in fruit fly-susceptible parts of the country, which includes surveillance traps being placed in 13 States and territories throughout the southern tier of the United States. There are a total of almost 160,000 traps arrayed from Hawaii to the U.S. Virgin Islands. Risk areas are identified in each State or territory based upon the examination of historical detections, socioeconomic population dynamics, and approach rates through points of entry. Most species of tephritid fruit flies

of the genera *Anastrepha*, *Bactrocera*, *and Ceratitis* are readily located by the detection program.

(b) Emergency Response

APHIS works with State or territorial departments of agriculture to put into place cooperative fruit fly action plans upon the detection of an exotic fruit fly in the United States. APHIS now utilizes the Incident Command System (ICS) as outlined in the government-wide National Response Plan and National Incident Management System during fruit fly outbreaks by setting up a Unified Command under ICS protocols.

The initial action of an emergency response to exotic fruit fly detection is the intensification of the trapping array surrounding the fruit fly detection site to delimit any suspected introduced fruit fly populations. Subsequent detections will trigger fruit fly control activities and, if appropriate, regulatory actions. SIT and pesticides are the primary control technologies used by APHIS to respond to exotic fruit fly incursions, coupled with localized fruit stripping. The environmental impacts of fruit fly control technologies have been thoroughly discussed in a final EIS published by APHIS in 2001 entitled "Fruit Fly Cooperative Control Program, Final Environmental Impact Statement—2001."

SIT has been developed for use in the United States for two fruit fly species, *Ceratitis capitata* (Medfly) and *Anastrepha ludens* (Mexican fruit fly). In an outbreak situation, an over-flooding rate of sterile male fruit flies is released in the areas of fruit fly detections for a period of at least two projected fruit fly life cycles to disrupt the reproduction cycle of any introduced population. In the States of California, Florida, and Texas, where SIT PRPs have been implemented, the infrastructure afforded by these programs is utilized as an eradication tool during fruit fly outbreaks. The use of SIT to control Medfly and Mexican fruit fly outbreaks is coupled with ground applications of pesticides.

The pesticide spinosad has replaced malathion as the pesticide of choice for use in bait spray fomulations during fruit fly outbreak campaigns. Bait spray formulations can be conducted with either ground or aerial applications. Aerial applications of pesticides can be utilized in crop production areas; however, these are reserved for limited use in urban environments, and only used under extreme outbreak situations, where other control technologies are not deemed to be adequate. Spinosad is a metabolite that results from the fermentation of a bacterium, and has been classified as organic. Malathion remains available for use to control fruit flies, and is reserved as an alternative to spinosad.

For those fruit flies where an effective male attractant has been found, (e.g., methyl eugenol for certain species of *Bactrocera*), the application of

pesticides in the control of fruit fly outbreaks can be minimized through the use of male annihilation technique. In male annihilation technique, a formulation of the attractant and a pesticide combined with an adhesive gelling agent can be selectively applied to telephone poles or tree trunks in a uniform density surrounding detections to attract and kill male fruit flies and disrupt the reproduction cycle of the introduced population. This control strategy has been demonstrated to be very effective during outbreaks of oriental fruit fly where the attractant, methyl eugenol, is mixed with the pesticide, naled, and a silicate adhesive gel; the formulation is then applied by spot spraying in treatment areas. Spinosad, as an alternative to naled, is currently being registered in a similar attractant/pesticide/gel adhesive combination.

(c) Preventive Release Programs (PRPs)

SIT is the biological control of pests using an area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species. There are three possible ways to use SIT for the control of tephritid fruit flies—in PRPs, in suppression programs, and in eradication programs. A fruit fly PRP involves the prophylactic use of SIT in an area where the risk of entry of a nonindigenous fruit fly into a fly-free area is high, that is, it is used to prevent any entries of the target fruit fly from becoming an established population.

The most frequent use of SIT to control fruit flies in the United States is the maintenance of two continual PRP programs, implemented in California and Florida. Both of these programs involve the release of sterile male Medflies weekly, on a year-round basis, over areas which have historically been subject to the entry and establishment of Medfly populations. Although these programs do not prevent new entries of Medlfy, they have been proven to greatly reduce the numbers of introductions and establishment of populations of Medfly in the coverage areas. In the case of Florida, there have been no detections of established Medfly populations in the PRP areas since the initiation of the Florida program in 1998.

APHIS maintains two sterile fruit fly production facilities which regularly produce sterile fruit flies for APHIS domestic and off-shore SIT programs. A large APHIS sterile Medfly production facility is located in Guatemala; it supports both the Moscamed Program and Medfly PRP programs in California and Florida. The other APHIS sterile fruit fly production facility produces sterile Mexican fruit flies in support of the Mexican fruit fly eradication campaign in the Lower Rio Grande Valley of Texas, and the suppression release program on the Mexican side of the Lower Rio Grande Valley. APHIS also supports, in a cooperative effort, a Medfly sterile production facility maintained by CDFA, located in Hawaii, which also provides sterile Medflies for the California sterile Medfly PRP.

The production and release of sterile fruit flies is closely monitored for sterile insect quality through an active quality control program of the produced sterile fruit flies. Internationally accepted quality control tests are conducted at both the production facility and the eclosion facility for each shipment of sterile fruit flies. The quality of mother stock colonies for the Medfly production strain is maintained through the administration of a filter rearing system.

Quality control for SIT used by APHIS is divided three categories:

- Production quality control, which monitors the inputs into the production system (such as diet ingredients, equipment, and so on).
- Process quality control, which measures how things are done (such as diet preparation, environmental conditions, and so on). (Note: the attention to quality control is demonstrated by the fact that the APHIS sterile Medfly production facility in Guatemala has recently received ISO 9000 certification. One of the requirements of this certification is to extensively document and follow all production processes.)
- Product quality control, which evaluates insects produced for effectiveness. APHIS utilizes tests for product quality control published by the joint Food and Agriculture Organization of the United Nations/International Atomic Energy Agency Pest Control found at http://www.iaea.org/programmes/nafa/d4/indx-tephritid.html

One of the concerns of strain management during mass-production is the rapid selection of individuals better adapted to laboratory rearing conditions resulting in the decline of genetic diversity. This raises concerns that laboratory strains may develop significantly differently than wild populations and possibly become noncompetitive. The maintenance of a filter rearing system will ensure a competitive strain in a production system. The concept known as a filter rearing system involves maintaining a small colony at a low density or under semi-natural conditions. This is designed to create low-selection pressure. Surplus insects from this low-density mother stock are fed into the high-density amplification chain of the production cycle in order to keep the production strain true. Before individual insects are fed back into the amplification chain, each one is examined and screened for undesirable traits. The concepts of strain management and sterile insect quality are explained in chapters 3.2 and 3.4 of "Sterile Insect Technique, Principles and Practice in Area-wide Integrated Pest Management" (IAEA, 2005).

Even with the great success of the use of SIT as a fruit fly control tool, the expansion of its use has several limitations including high cost, biological specificity, and production and eclosion infrastructure. The success of the

use of SIT in the control of fruit flies and the cost benefit, as compared to the cost of eradication campaigns when SIT was not available, is evident. The most severe limitation for SIT is the cost of resources to initiate and maintain a successful program. Even with the limited use of PRPs in historically high risk areas and during eradication campaigns. the portion of current allocations given to APHIS for fruit fly SIT to maintain the fruit fly exclusion and detection programs comprises nearly half of these allocations. Given the high cost of SIT and the proven effectiveness, the technological development of more efficient components of production, eclosion, and release operations, or, modifications to the strains of released fruit flies to improve competitiveness, longevity, or other biological aspects are desired by program managers.

The biological control aspect of SIT serves to disrupt the reproduction cycle of a single species of fruit fly; therefore, the development of SIT programs is obviously species-specific. After several years of research and methods development, APHIS has incorporated SIT technologies into full production and release programs for only two species of fruit flies, Medfly and Mexican fruit fly. It is within the vision of APHIS programs to expand SIT programs to other fruit fly species; APHIS is working toward that goal but is limited by the extensive time needed for strain development and for SIT production modifications to accommodate individual species needs.

The production, eclosion, and release of sterile fruit flies require the construction and maintenance of both production and eclosion facilities. In addition, these program activities require either access to or construction of a facility for aerial release. Independent of the need of the obvious resources to both construct and maintain a major facility, SIT facilities have some unique location restrictions due to biosecurity and operational logistic needs. APHIS is extremely concerned regarding accidental release of fertile fruit flies into areas susceptible for their establishment, and only allows fruit fly production facilities in susceptible areas where fruit fly species are established. Fruit fly production facilities also need to be located near transportation facilities capable of facilitating daily shipments of sterile pupae to eclosion/release areas with ease. Similarly, eclosion facilities need to have access to transportation systems which facilitate the daily receipt of sterile fruit fly pupae. Eclosion facilities also need to have immediate access to aerial release facilities to quickly transfer loaded release containers to aircraft for quick dispersion.

(2) Emerging Fruit Fly Threats

Although Medfly and Mexican fruit fly are currently the primary focus of APHIS' domestic and off-shore preventive and control activities, the threats posed by species of the genus *Bactrocera* have raised concerns due to a rise in the number of detections, the variety of species now detected, and the expansion of detections into new areas of the United States. By

far, the largest increased threat has been felt in the State of California where the highest number of detections occurs, and where the risk of establishment poses the highest economic impact.

Even though the most commonly detected exotic *Bactrocera* species is not known to currently infest California, and oriental fruit fly is easily and inexpensively controlled and eliminated with the use of male annihilation technique, there is some concern that some program pesticides may be carcinogenic and alternatives may have to be sought in the near future. Also, beyond oriental fruit fly, other *Bactrocera* species that are not as easily controlled with male annihilation technique now approach California from all directions of the world, as is evident by recent detections. The threat of establishment of an exotic species of *Bactrocera* is shown by the recent establishment and quick spread of the olive fruit fly (*Bactrocera oleae*) throughout California.

APHIS is in the process of implementing a cooperative *Bactrocera* initiative with CDFA. This initiative is envisioned as an integrated research and technical alliance for the advancement of mass-production principles and enhancement of detection, control, and risk management strategies for *Bactrocera* species. This new initiative will attempt to analyze the extent of the existing and emerging pest threat posed by invasive species of *Bactrocera* by identifying pest pathways, and enhancing detection and control strategies to address the threat. The exploration of new control strategies will include the development of SIT capabilities for species of *Bactrocera*, and the evaluation of new formulations and presentation of chemical control applications.

(3) Reduce Threat of Fruit Fly Introduction Through Off-shore Activities

The main source of any new exotic fruit fly entries that may lead to infestations in the United States is from off-shore populations found in infested countries. The pathways for these off-shore risks can be categorized into a "long-distance" pathway associated with the movement of infested fruit from countries distant from our borders, and the more immediate risk of natural spread over shared borders from fruit fly populations approaching the United States directly from Mexico or through Mexico from Central America. APHIS reduces these threats by various means, ranging from active participation in off-shore detection and control activities to capacity building efforts through technology exchanges.

(a) Medfly––Moscamed

The entry, establishment, and spread of Medfly throughout Central America, since 1955, has been a continual threat to the agricultural

economy of both Mexico and the United States by either natural spread or by the human-aided movement of infested host material. Since 1978, the United States has actively participated in a control program, known as the Moscamed Program, with Guatemala and Mexico, to combat the spread of Medfly into the fly-free areas of Guatemala and Mexico.

Maintaining a barrier near the Guatemala–Mexico international border to contain the spread of Medfly populations northward successfully keeps Medfly populations far from the United States–Mexico international border. The establishment of Medfly populations in northern Mexico, along the U.S. border, would severely strain our abilities to prevent Medfly from becoming established in the continental United States. In the event of this unfortunate occurrence, the result would be increased detections in the United States, increased use of pesticides in eradication campaigns, and the costly increase of Medfly SIT programs, especially along the lengthy United States–Mexico border.

Current control strategies used in the Moscamed Program include the aerial release of sterile Medflies, both the aerial and ground application of pesticides (spinosad), and fruit stripping. The Moscamed Program also employs an extensive surveillance program and maintains a regulatory program through the enforcement of fruit fly host quarantine at road stations.

(b) Mexican Fruit Fly—Mexico

APHIS partners with Mexico, in the use of SIT, to release sterile Mexican fruit flies in two areas of northern Mexico along the U.S. border. One area is south of California in the Tijuana area; the other is south of Texas, in the Lower Rio Grande Valley. The goal of these two release programs is to suppress any populations of Mexican fruit fly in those areas of Mexico and, therefore, reduce any introduction rate afforded by the natural spread of these populations into the United States.

The SIT program includes the weekly release of sterile Mexican fruit flies in both areas. Sterile Mexican fruit fly pupae, used for the sterile release in the Tijuana area, are produced in the Mexican sterile fruit fly production facility in Tapachula, Chiapas, Mexico. Sterile Mexican fruit fly pupae, for the release of sterile Mexican fruit fly on the Mexican side of the Lower Rio Grande Valley, are produced in the APHIS–Mexican fruit fly production facility in Mission, Texas.

(c) Enhanced Detection––Mexico

As an extension of the national detection program within the United States, APHIS cooperates with some of the northern Mexican States, which border the United States, to actively participate in an exotic

fruit fly detection program. This program proved its value in 2004, with the detection of Medfly in Tijuana, Baja California, Mexico. In cooperation with Mexico, APHIS played an active role in an emergency response to this outbreak which included the aerial spraying of spinosad, coupled with the release of sterile Medflies, in a successful eradication campaign. Molecular analysis of the captured adult Medflies targeted the origins of the outbreak to likely source populations in Central America, thus validating the concern of the fruit fly pest threats to Mexico and the United States emanating from that area of the world.

(d) Caribbean Basin

APHIS has identified a ready pathway for pests to move from Caribbean nations into the continental United States, using Florida as a gateway. The size and geographic location of Florida extends across several climatic zones, from a semi-tropical climate in the south to a near temperate climate in the north. The entire State of Florida is considered susceptible to fruit fly infestations, at least on a seasonal basis, with parts of the State being susceptible year-round. To mitigate this pest risk, afforded by the Caribbean pest pathway, APHIS encourages and provides technical assistance in the development of exotic fruit fly detection systems by Caribbean nations. APHIS is also exploring the use of biological control programs to suppress *Anastrepha* species currently infesting some island nations within the Caribbean Basin.

(e) Capacity Building

APHIS cooperates with international organizations and countries worldwide to reduce the threat posed by tephritid fruit flies. This is achieved through an information exchange offered by APHIS experts in fruit fly surveillance, regulatory activities, control actions, and by providing technical assistance through the transfer of technology. APHIS ascribes to fruit fly-free and low-prevalence area concepts for areas in foreign countries, which subsequently reduces the fruit fly pest risk to the United States.

(4) Mitigate Impact of Exotic Fruit Flies Established in Portions of the Continental United States, Hawaii, and U.S. Territories

Several species of exotic fruit flies have become established within the United States. These include—

- Mexican fruit fly (*Anastrepha ludens*) in the Lower Rio Grande Valley of Texas;
- oriental fruit fly (*Bactrocera dorsalis*), melonfly (*B. cucurbitae*), solanum fruit fly (*B. latifrons*), and Medfly (*Ceratitis capitata*) in Hawaii;
- Caribbean fruit fly (*Anastrepha suspensa*) in Florida;

- olive fruit fly (*Bactrocera oleae*) in California;
- Caribbean fruit fly (*Anastrepha suspensa*) and West Indian fruit fly (*A. obliqua*) in Puerto Rico and the U. S. Virgin Islands; and,
- melonfly (*Bactrocera cucurbitae*) in Guam and the Commonwealth of Northern Mariana Islands.

These economically important exotic species are contained and managed through APHIS and State and territorial fruit fly control programs both to prevent the spread of exotic fruit flies from infested areas within the United States, and also to facilitate the safe movement of host commodities produced within the infested areas into the commercial market.

(a) Mexican Fruit Fly—Lower Rio Grande Valley of Texas

APHIS, in cooperation with the Texas Department of Agriculture, has maintained a continuous blanket of sterile Mexican fruit flies over the Lower Rio Grande Valley of Texas since 1984. This release of sterile Mexican fruit flies was used to suppress wild populations of Mexican fruit flies in the Lower Rio Grande Valley of Texas, and to facilitate the movement of host commodities to noninfested parts of the United States.

Increased urbanization along the Lower Rio Grande Valley in Texas has resulted in more hosts in backyards that are in close proximity to commercial citrus groves. This has resulted in increased Mexican fruit fly populations that jeopardized the suppression program which facilitates the movement of host commodities out of infested areas. To address this increasing pest threat, in 2006, the overall goal of the Mexican fruit fly mitigation program in the Lower Rio Grande Valley was changed from a suppression program to an eradication program.

The eradication program includes fruit fly surveillance and regulatory and control activities. The control activities include the typical activities explained previously that are utilized in fruit fly eradication campaigns including the application of pesticides, fruit stripping, and the use of SIT. The SIT program in the Lower Rio Grande Valley was intensified to work toward achievement of this new goal. To support the eradication program, APHIS operates a sterile fruit fly production/eclosion facility in Mexico, and an additional separate eclosion facility in the Lower Rio Grande Valley.

(b) Domestic Fruit Fly Suppression and Certification Programs

APHIS works with CDFA to identify potential biological control agents for the suppression of olive fly in California. APHIS supports similar activities for the suppression of the West Indian fruit fly through the use of biological control agents in Puerto Rico. In Hawaii, APHIS provides

technical assistance through methods development and detection surveys in support of a USDA–ARS sponsored fruit fly suppression program. In

Florida, APHIS monitors the Florida Department of Agriculture and Consumer Services-sponsored Caribbean fruit fly management program to satisfy phytosanitary requirements in support of export markets.

(c) Regulatory Activities and Certification Programs

APHIS promulgates and enforces domestic quarantines to mitigate the risk of movement of infested host commodities from infested areas of Texas, Hawaii, Puerto Rico, the U.S. Virgin Islands, Guam, and the Commonwealth of the Northern Mariana Islands. In Hawaii, Puerto Rico, and the U.S. Virgin Islands, APHIS maintains preclearance operations of aircraft, cargo, and passenger baggage through an agricultural inspection system. Host commodities are subject to certification systems which can include treatments. Host commodities from infested areas of Texas are also subject to similar certification programs before movement is allowed outside of the quarantine area.

(5) Fruit Fly Risk Reduction Strategies

APHIS has developed several implementation strategies to both enhance current fruit fly programs and to ensure the successful continuation of fruit fly mitigation programs faced with an increasing threat from exotic fruit fly introductions into the United States.

(a) Enhance Early Detection, Emergency Response, and Prevention Capabilities

- Implement recommendations of the National Exotic Fruit Fly Surveillance Program review. Ensure national and international standards are followed in all U.S. States and territories.
- Complete implementation of the National Preventive Release Programs recommendations.
- Conduct periodic reviews (every 4 years) of detection, response, and preventive release programs to ensure cost-effective use of best technologies and methodologies.
- Develop improved strains of sterile fruit flies for use in current SIT programs.
- Develop alternative control technologies, such as SIT, for *Bactrocera* species.
- Provide stable and secure sources of sterile Medfly and Mexican fruit fly in order to ensure emergency preparedness.
- Update the National Exotic Fruit Fly Trapping Manual to integrate procedures for new trapping technologies.

- Develop molecular identification protocols for *Bactrocera* species to help identify source populations leading to the identification of introduction pathways.
- Design and construct a permanent building for the sterile fruit fly eclosion facility in Los Alamitos, California.

(b) Ensure Exotic Fruit Flies Do Not Move into the United States Across Shared Border with Mexico

- Stabilize U.S. Moscamed Program funding by minimizing reliance on emergency funding. Explore alternative sources to appropriated funding, both in the United States and from international donors.
- Form an international commission with Mexico/Secretariat of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA) to ensure long-term joint management of the Moscamed Program activities in southern Mexico.
- Continue to work closely, through the Moscamed Program, with the Guatemala Ministry of Agriculture to fortify field activities and continue the production of sterile flies in the APHIS sterile Medfly production facility in Guatemala.
- Conduct periodic reviews of strategies, tactics, technologies, and administration used by the Moscamed Program to ensure cost-efficient and effective operations. Enhance quality assurance and quality control processes and activities.
- Cooperate with Mexico to enhance the exotic fruit fly detection program throughout Mexico to assure effectiveness of the Moscamed Program and to serve as an early warning system for all exotic fruit flies.
- Develop strategies to collaborate with Mexico on its plan to establish northern Mexican States as free of Mexican fruit fly.
- Ensure that the Mexican fruit fly suppression program activities in Reynosa, Tamaulipas, Mexico, and Tijuana, Baja California, Mexico, maintain an adequate, ongoing sterile release program.

(c) Eradicate, Suppress, or Contain Established Populations of Exotic Fruit Flies within the United States

- Augment the current Texas–Mexico fruit fly SIT program and implement survey, regulatory, and control activities to eradicate Mexican fruit fly from Texas.
- Implement a filter rearing system for the sterile Mexican fruit fly production facility in Mission, Texas.
- Develop new and improved regulatory pre- and postharvest treatments for Mexican fruit fly.
- Conduct periodic reviews of strategies, tactics, technologies, and administration of the Texas–Mexican fruit fly eradication program

to ensure cost efficient and effective operations. Enhance quality assurance and quality control processes and activities.

b. Southwest Pink Bollworm Eradication Program Mitigations

The mitigations and risk reduction strategy for this program are patterned after the mitigation measures designed for the National Cooperative Boll Weevil Eradication Program. The primary difference is that pink bollworm eradication uses only minimal amounts of pesticide application and depends more heavily upon SIT, cultural control, Bt transgenic cotton, and mating disruption to achieve population reductions. The operational procedures and mitigation measures for this program were presented in previous EAs for the eradication program (USDA–APHIS, 2002a, 2001c). These operational procedures and mitigation measures are presented below to assist in understanding the program approaches to preclude undesirable impacts.

The operational procedures and mitigation measures described in this subsection have been adopted by, and are an integral part of, the Southwest Pink Bollworm Eradication Program. As with all procedures, these are subject to change as new issues and site-specific circumstances require adjustments to address specific environmental, human health, and nontarget wildlife risks.

Adherence to the above listed measures precludes or mitigates the most likely environmental impacts to a level where the potential impacts are negligible. Any reductions in pesticide applications or use of nonchemical methods (such as SIT) to replace those applications further reduce potential program impacts to environmental quality.

Table 4–1. Standard Operational Procedures for Southwest Pink Bollworm Eradication Program

A. All Methods of Control

1. All applicable Federal, State, and local environmental laws and regulations will be followed during pink bollworm control programs.

2. Sensitive areas (water bodies, parks, and occupied dwellings such as homes, schools, hurches, hospitals, and recreation areas) that may be adjacent to cotton fields will be identified. Some of the adjustments include, but are not limited to, the application of PB-Rope or PB Rope*L (pheromone only), or the release of sterile moths. If an insecticide application is required, wind speed, wind direction, and temperature will be monitored during the application to ensure sensitive areas are not adversely impacted.

3. Environmental monitoring of the program will be in accordance with the current environmental monitoring plans.

4. All cotton fields in each program increment will be trapped; however, only fields meeting the program criteria will be treated.

5. All program personnel will be instructed in the use of equipment, materials, and on operational procedures. Field supervisors will emphasize operational procedures and monitor the conduct of personnel.

B. Aerial Applications

1. All materials will be applied in strict accordance with EPA- and State-approved label instructions.

2. Aircraft, dispersal equipment, and pilots that do not meet all contract requirements will not be allowed to operate.

3. All USDA–APHIS, Plant Protection and Quarantine employees who plan, supervise, recommend, or perform pesticide treatments must be certified under the APHIS pesticide certification plan. They are also required to know and meet any additional requirements of the State where they perform duties involving pesticide use.

4. Unprotected workers will be advised of the respective reentry periods following treatment. If chlorpyrifos is used, unprotected workers will not reenter the field for 24 hours.

5. Two-way radios will be provided to personnel who direct or coordinate field operations. Radio communication will be available to provide close coordination of all application operations.

6. All APHIS field personnel will have baseline cholinesterase tests before the first application, and each spring and fall, thereafter. It is recommended that contract, State, and private personnel also participate in this testing program.

7. Only certified aerial applicators who have been familiarized with local conditions will be used by the program.

8. To minimize drift and volatilization, applications will not be made when any of the following conditions exist in the spray area:

- wind velocity exceeding 10 miles per hour (or less if required by State law);
- rainfall or imminent rainfall;
- foggy weather;
- air turbulence that could seriously affect the normal spray pattern; or,
- temperature inversions that could lead to offsite movement of spray.

9. Nozzle type and size, spray system pressure, and nozzle orientation will be specified in the program's aerial application contract, or as otherwise directed by program personnel.

C. Ground Applications

1. Mist Blowers
   - Operators will either be certified applicators or will be in constant radio contact with certified applicators.
   - Units will be operated from closed truck cabs, with operators using recirculated air.

2. High-clearance Machines
   - Operators will either be certified applicators or will be in constant radio contact with certified applicators.
   - Units will be operated from closed truck cabs, with operators using recirculated air.

Table 4–2. Program Mitigation Measures

All required State and local authorities will be notified upon initiation of the program. The notification will advise State and local authorities of the need for any assistance in identifying sensitive areas in the proposed treatment areas.

A. Protection of Workers

All program personnel will be instructed on emergency procedures to follow in the event of insecticide exposure. Equipment necessary for immediate washing procedures must be available for application personnel.

1. Aerial Applications

a. Pilots, loaders, and other personnel handling insecticides will be advised to wear safety equipment and protective clothing.

b. Program personnel observing applications of chlorpyrifos are required to wear protective clothing or remain inside a closed vehicle with recirculating air, depending on the circumstances of the application.

c. Application operations will be postponed in fields occupied by workers.

d. Flags, GPS equipment, or other markers will be used for pilot guidance at all times.

2. Ground Applications

a. Mist Blowers
   - Units will be operated from closed cabs with operators using recirculated air.
   - Operators will wear appropriate safety equipment when loading or servicing the unit and will be specially trained by program personnel.

b. High-clearance Machines
   - Operators must be certified applicators for chlorpyrifos applications, and they will exercise extreme caution when applying this material.
   - Operators will wear appropriate safety equipment and protective clothing when loading, servicing, and operating the unit.

3. Pesticide-handling Precautions

a. To the degree possible, insecticides will be delivered and stored in sealed bulk tanks and then pumped directly into the aircraft.

b. All insecticides will be stored in accordance with Federal, State, and local regulations and label instructions.

c. All mixing, loading, and unloading of insecticides will be in an area where an accidental spill will not contaminate a stream or other body of water.

d. In the event of an accidental spill, procedures set forth in "PPQ Guidelines for Managing and Monitoring Pesticide Spills" (USDA–APHIS–M390.1402, 1983) will be followed.

e. All insecticide drums must be triple-rinsed before disposal. Rinse solutions may be used to prepare spray tank mixes or may be stored for subsequent disposal, in accordance with label instructions. One of the following methods of drum disposal must be used:

- Require chemical companies, distributors, or suppliers to accept empty triple-rinsed drums.
- Transfer the empty triple-rinsed drums to State cooperators.
- Crush and/or puncture the empty triple-rinsed drums and dispose of as scrap metal.

B. Protection of the Public

1. Application aircraft shall avoid direct spraying of residences, garden plots, and adjacent crops at all times.

2. Program personnel shall immediately cease spraying operations if members of the public are observed within 100 feet of a cotton field being sprayed with chlorpyrifos.

3. Program personnel will establish a central telephone hot line (operational while the program is operational) for the public that can provide times and places of treatments, program information, and emergency referrals.

4. Program personnel will make available to the public, upon request, data from program environmental monitoring efforts.

5. Program personnel will publish public notices of the availability of the environmental assessment (EA) for this program in local newspapers; notices will be in both English and Spanish; copies of the EA will be provided to local libraries.

C. Protection of Bees

Before beginning treatment with chlorpyrifos, program personnel shall notify all registered apiarists in or near the treatment area of the date and the approximate time of chemical treatment.

D. Protection of Wildlife

All control operations will be conducted in a manner that avoids potential impact on endangered, threatened, and proposed species, and their critical habitats.

E. Additional Protective Measures

The following additional protective measures have been recommended to further reduce the potential for adverse environmental effects from this program.

1. Pesticide Applications

a. Program personnel overseeing applications of organophosphate and synthetic pyrethroid (chlorpyrifos and permethrin) pesticides are required to wear protective clothing or remain inside a closed vehicle with recirculating air, depending on the circumstances of the application.

b. Unprotected workers will be advised of the respective reentry periods following treatment.

c. Program personnel shall immediately cease spraying operations if members of the public are observed within 100 feet of a cotton field being treated with chlorpyrifos or permethrin.

d. Aerial applications will not be made to sensitive areas (residences, public buildings, water bodies, hospitals, primary and secondary schools, daycare centers, in-patient clinics, nursing homes, parks, churches); program treatments will be applied only to cotton fields.

e. Aerial applications will be made at a height of 5 to 12 feet above the cotton canopy, unless precluded by obstructions.

f. Program personnel will familiarize aerial applicators with applicable operational procedures, mitigation measures, and protection measures.

g. Before initiating operations, APHIS will obtain concurrence from the U.S. Department of the Interior's Fish and Wildlife Service on protection measures that are required for threatened and endangered species, or their critical habitats.

h. Program personnel will be present during all treatments near sensitive areas; they will use dye cards along field edges to detect off-site drift of pesticides.

i. The program will report any incident of pesticide poisoning to the local department of health; information about the validity and probable cause will be used to develop additional protective measures, as necessary.

2. Notification Procedures

a. Program personnel will provide advance notification, in writing or by telephone, of the approximate times and dates of treatments to area residents who reside within 3 miles of treatments and those who formally request special notification (having provided their name, address, and telephone number).

b. Program personnel will publish public notices of the availability of the EA for this program in local newspapers; copies of the EA will be provided to local libraries.

c. Growers participating in the program will be notified of treatment dates so that they may provide timely and appropriate notice of treatments and protective measures to persons in their employ or residing on properties that could be exposed to chemical pesticides.

d. Residents who are registered with the local State department of Agriculture, as having multiple chemical sensitivity, will be notified in writing or by telephone of the time of any program treatments to be made within 3 miles of their residence.

e. Before beginning treatment with chlorpyrifos or permethrin, program personnel shall notify all registered apiarists in or near the treatment area of the date and the approximate time of treatment.

Table 4–3. Production and Eclosion Facilities for Medfly and Mexican Fruit Fly.

Location	Type of Facility
California	
Los Alamitos	Eclosion facility
Florida	
Sarasota	Eclosion facility
Texas	
Harlingen	Eclosion facility
Mission	Production and eclosion facility
Guatemala	
El Pino	Production facility
Ixquisis	Eclosion facility
Peten	Eclosion facility
Retalhuleu	Eclosion facility
San Miguel Petapa	Production facility
Mexico	
Reynosa	Eclosion facility
Tijuana	Eclosion facility

5. Irreversible and Irretrievable Commitments of Resources

APHIS has been involved in the development of facilities and methodology for the use of SIT in control programs for more than a half a century. Considerable human, physical, and monetary resources have been applied to this development. The adaptation of genetically engineered strains to fit into the ongoing SIT programs is primarily an extension of the process. The decision to develop these strains involves a commitment of resources that can be used in the control programs; however, those developmental costs involve an irretrievable commitment of resources, of which some work has already been done. Application of the new strains to control programs will involve further commitments of irretrievable resources, in that the present and any future rearing facilities will need to be upgraded for the increased biosecurity and biosafety required for use of these genetically engineered strains. Although some parts of the present facilities are adequate for genetic engineering research and development purposes, APHIS has not yet committed to upgrade the entire production facilities for these strains.

a. Fruit Fly Exclusion and Detection Programs

APHIS has committed substantial resources toward SIT as a measure to mitigate the pest risk posed by tephritid fruit flies. APHIS spent approximately $32.9 million to support the production, eclosion, and distribution of sterile fruit flies between October 1, 2005, and September 30, 2006. APHIS maintains several facilities, both domestically and off-shore, for the production and eclosion of sterile fruit flies (Medfly or Mexican fruit fly). The production and eclosion facilities are listed in table 4–2.

APHIS has already invested approximately $250,000 toward the evaluation of strains of genetically engineered Mexican fruit flies for use in SIT production facilities in the facility in Mission, Texas. APHIS has committed resources to adapt the San Miguel Petapa production facility, in Guatemala, for the evaluation of genetically engineered Medflies.

The irreversible and irretrievable resources from ongoing PRPs and previous eradication efforts include various costs from chemical control, male fly annihilation, SIT, physical control, cultural control, and regulatory control measures. The impacts from these actions are described in the environmental consequences section, and most resources (other than the target insect) impacted by program actions recover within variable lengths of time, depending upon the method and affected resource.

b. Pink Bollworm Suppression and Eradication Programs

Likewise, APHIS has committed substantial resources towards SIT in the mitigation of potential damage posed by pink bollworm. APHIS has spent approximately $4,800,000 to support the production, eclosion, and distribution of sterile moths during fiscal year 2007. APHIS maintains a large facility in Phoenix, Arizona, for the production and eclosion of sterile moths for the ongoing suppression and eradication program efforts.

Over the last 6 years, APHIS has already invested approximately $870,000 toward the development and evaluation of strains of genetically engineered pink bollworm moths for use in the SIT production facilities at Phoenix, Arizona. APHIS has committed resources to adapt those facilities for the production and evaluation of genetically engineered moths.

The irreversible and irretrievable resources from present beltwide eradication and previous control efforts include various costs from chemical control, mating disruption, SIT, physical control, cultural control, use of transgenic cotton, and regulatory control measures. The impacts from these actions are described in the environmental consequences section. Most resources (other than the target insect) impacted by program actions recover within variable lengths of time, depending upon the method and affected resource.

6. Program Monitoring

APHIS and its State, county, international, and other cooperators monitor control program areas for fruit flies and pink bollworm to determine the environmental consequences and efficacy of program actions. The use of traps with various attractants and pheromones is a major part of ongoing program monitoring of invasive plant pests for all APHIS programs, where an infestation has been detected. This field monitoring of insect populations is used to assess the status of an infestation and to assess dispersion of sterilized insects that have been released to suppress pest

populations, as well as other program actions. The insect rearing facilities use quality control monitoring to ensure that desirable biological characteristics are maintained within the colonies. These facilities also use biosecurity monitoring to ensure that insect containment facilities preclude release to areas near the facility where suitable host plants may occur. Other than insect monitoring, some of the ongoing control and eradication programs currently use pesticides as part of their pest management efforts, which also involve surveillance, sampling, and monitoring activities. For further details on pesticide monitoring, there are several program monitoring plans that provide details about this aspect of large-scale APHIS programs (see appendix G, References: USDA–APHIS, 2007a; USDA–APHIS, 2001d), as well as NEPA documents (USDA–APHIS, 2002a; USDA–APHIS, 2001a; USDA–APHIS, 2001c; USDA–APHIS, 1991a).

In this EIS, the focus is on monitoring of invasive pest fruit flies and pink bollworm, monitoring of field-released genetically engineered fruit flies and pink bollworm that have fluorescent traits from marker genes, other pest suppression traits, and monitoring the rearing facilities that currently use SIT for those species. Some rearing facilities include irradiation equipment for the sterilization process. A brief section regarding radiation monitoring for personnel and the facilities is included in this chapter. Environmental monitoring is conducted in compliance with the following statutes or their implementing regulations:

- the National Environmental Policy Act (NEPA);
- the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); and,
- the Endangered Species Act (ESA).

In addition, environmental monitoring fulfills the intent of Executive Order 13112 that "directs Federal agencies to use their programs and authorities to prevent the spread or to control populations of alien species that cause economic or environmental harm" by providing the means by which successful control or eradication can be measured. For this EIS, NEPA-related monitoring is designed to assess the effectiveness and validity of the plant pest suppression or mitigation measures. Monitoring provides information to allow comparisons of the effects from using mitigations to the effects in the absence of mitigations.
Monitoring under FIFRA is sometimes required by EPA as a condition of registrations and experimental use permits for pesticide applications, particularly for pest resistance management for genetically engineered crops with pesticidal characteristics, such as the *Bt* insect toxin expressed in cotton or corn.

In compliance with ESA, monitoring is designed to assess the effectiveness of program protection measures for threatened and

endangered species or their habitats. The protection measures are ordinarily developed by APHIS and its cooperators, or through consultations with the U.S. Department of Interior's FWS and/or the U.S. Department of Commerce's NMFS. Because of the emergency nature of fruit fly control programs, program managers often need to consult by telephone with the FWS, NMFS, and local fish and game offices to confirm the presence or absence of threatened and endangered species, identify sensitive sites, and confirm the use of protective measures.

In fruit fly programs, which usually occur in suburban areas, the emphasis of the environmental monitoring is on the protection of human health. Monitoring with dye cards, water samples, and vegetation samples for insecticide residues is conducted to determine spray drift or misapplication of pesticides. Specific environmental components may be sampled in response to concerns about perceived impacts from program pesticide applications.

An APHIS environmental monitoring coordinator oversees the collection, packaging, and shipment of samples to the Analytical and Natural Products Chemistry Laboratory (ANPCL) in Gulfport, Mississippi, or to another private, accredited laboratory if the workload exceeds ANPCL's capacity. The results of the laboratory's residue analyses are then correlated with environmental conditions data recorded at the time of treatment and sampling, and are further analyzed by APHIS' environmental monitoring staff to determine whether there are any human or environmental risks related to the use of the pesticide. The data and analyses are reported at the end of the program, or intermittently during the program, as required.

APHIS recognizes that it cannot predict the exact locations, characteristics, or severity of future infestations of fruit flies and cannot be very specific in this discussion about the kinds and levels of monitoring that must be done for each program. Unlike monitoring for new introductions of exotic fruit fly species, the pink bollworm cooperative eradication program is more predictable because of the established presence of pink bollworm in certain States. APHIS has ongoing monitoring plans for pink bollworm that are revised each year (USDA–APHIS, 2007a). Specific monitoring plans are developed for each individual fruit fly control program, based upon the site-specific characteristics of each program activity as it arises with each new detection and infestation. The monitoring plans describe the purpose of the monitoring and the nature of the samples to be collected. The surveillance programs for these pests are described below.

a. Fruit Fly Surveillance Programs

Surveillance programs for tephritid fruit flies have been in operation in the continental United States for more than 75 years. Principle genera targeted in the program include *Anastrepha*, *Bactrocera*, and *Ceratitis.* Surveillance programs are primarily based upon a bait-and-trap system to capture adults. Traps are placed in host commodity trees to afford the most likelihood of capture. This survey is supplemented with fruit cutting to inspect eggs, larvae, and pupae during a delimitation survey after the initial capture of an adult in a detection trap.

The historic evolution of attractants has progressed from the use of kerosene in traps in the early part of the 20th century, to the current use of several classes of attractants including para-pheromones, pheromones, and two types of food-based attractants (protein baits or synthetic food lures).

Traps can be made from various materials including glass, waxed cardboard and plastic, and use different capture methods including liquid trapping agents, sticky material used to trap flies upon contact, and insecticides designed as knock-down agents once the fruit fly enters the trap.

The combination of trap and attractant chosen for a surveillance program is dependent upon the target species. Some baits are general attractants; some baits only attract certain taxa of fruit flies or even only one sex of certain taxa of fruit flies. Therefore, the fruit fly surveillance programs within the United States use several combinations of traps and attractants depending upon the type of surveillance program, the local conditions in which the program is being conducted, and the target species.

Fruit fly surveillance programs can be divided into three categories dependent upon the objective of the program including detection survey, delimiting survey, and monitoring survey. Detection survey is conducted in noninfested areas to determine if a species is present in the area or has newly entered an area. Delimiting survey is used to determine the boundaries of an infested or a suspected infested area. And, a monitoring survey is used to verify the characteristics of an existent fruit fly population in an infested area such as abundance, seasonal fluctuations, or relative host sequence.

(1) Detection Survey

Detection survey coupled with emergency response is applied as an exclusionary measure to minimize the risk of the introduction of exotic fruit flies into noninfested areas of the United States. APHIS cooperates domestically with State and territorial plant regulatory authorities to establish a bait-and-trap surveillance system for exotic fruit flies which

spans 13 States and territories along the southern tier of the United States, from Hawaii to the U.S. Virgin Islands. Over 160,000 fruit fly detection traps are deployed nationwide. Most of these traps are placed by government officials in host trees on residential private properties by permission of homeowners. Trap and lure combinations chosen for each of these State and territorial programs are selected based upon determination of the likelihood of the various target species being introduced into that risk area, and trapping arrays are determined by the characterization of risk areas. Risk areas are characterized through examination of historical detections, socioeconomic population dynamics, approach rates through points of entry, and area susceptibility through host availability and climatic conditions.

Due to the high risk economic consequences of the introduction of exotic fruit fly populations into the States of California and Florida and their extensive fruit fly detection program, each State has developed its own detection trapping guidelines. The California fruit fly surveillance program uses the CDFA's "Insect Trapping Guidelines" (Gilbert et al., 1984), and the Florida fruit fly surveillance program uses the "Florida Fruit Fly Detection Manual" (FDACS, 2004). For States and territories that do not have their own localized fruit fly detection guidelines, general guidelines has been developed for fruit fly surveillance as a cooperative effort between the appropriate State and territorial plant health authorities and APHIS, titled the "National Exotic Fruit Fly Trapping Protocol" (USDA–APHIS, 1991b). International fruit fly trapping guidelines published by the Insect Pest Control Section of the International Atomic Energy Agency, titled "Trapping Guidelines for Area-wide Fruit Fly Programmes" (IAEA, 2003), are also available for reference.

(2) Delimitation Survey

The capture of an exotic fruit fly under a detection survey will initiate an emergency response by both APHIS and the State official for the placement of additional traps in the area of the detection, followed by control and/or regulatory actions, if appropriate. A delimitation survey consists of the placement of an intensified trapping array in an 81-square mile area surrounding the detection site. The density of the traps is increased following bull's eye concept with the highest density of traps in the first square mile, surrounding and including the actual detection site, followed by a cascading array of decreasing densities of traps as you move out from the detection site in all directions. Any subsequent detection within the delimitation grid would initiate an overlapping grid, similar to pebbles thrown adjacent to each other in a still pond. Where delimitation grids overlap, a higher density of traps will be used. Delimitation trapping is also used in eradication programs to determine the efficacy of applied control measures used to eliminate fruit fly populations from an area.

(3) Monitoring Survey

Monitoring surveys measure the abundance of fruit fly populations in an infested area. APHIS and State officials use monitoring surveys to measure the efficacy of fruit fly suppression techniques to certify fruit for movement out of low prevalence areas. When combined with fruit fly control measures, such as the application of chemicals or the use of SIT, results from monitoring surveys can be used as a phytosanitary measure to allow fruit to be certified for movement outside of quarantine areas.

(4) Fruit Fly Surveillance under an SIT Program

Fruit fly surveillance under an SIT program is modified. Due to the volume of sterile flies captured in a surveillance program in an area where fruit fly SIT is being implemented, trap arrays can be modified by the decrease of density levels and the increased utilization of female attractants in traps. Identification programs also need to be adapted to discern wild from sterile fruit flies, using both primary and secondary methods. Sterile fruit flies are currently marked with powdered fluorescent dyes as pupae. Dye is then transferred to the adults during the eclosion process, which can normally be seen with the aid of a black light after captured in a trap; however, due to the volume of pupae that are processed, occasional specimens receive a lighter coating. Consequently secondary methods for discerning wild from sterile fruit flies are, therefore, needed occasionally when the applied dye is not easily detected. Currently, secondary determinations of sterility are conducted through the dissection and examination of internal organs for signs of sterility.

(5) Fruit Fly Surveillance Quality Assurance Systems

APHIS and State cooperators work in concert to establish and maintain the technical competency of their surveillance personnel. The quality assurance programs include the following components:

- All trap specialists receive training on procedures and proper use of equipment and materials with appropriate refresher training when needed.
- Field supervisors monitor the performance and conduct of trap specialists at regular intervals, including visiting trap sites, together with trap specialists and independently to assess the proper implementation of program procedures.
- Quality control marked flies are placed in traps to monitor trap specialist ability to recognize and submit target species for identification. Handling of the marked flies is monitored through the identification process to evaluate the entire system.

- Field work units are reviewed annually to ensure standard procedures are being implemented, including a local quality assurance program.

b. Pink Bollworm Surveillance Programs

Surveillance programs for pink bollworm have been in operation in the continental United States for almost 40 years. Surveillance programs are primarily based upon the use of pheromone to capture adults. Traps are placed around cotton fields to detect presence of infestations and relative density.

Detection Survey

Detection surveys for pink bollworm are conducted with delta traps baited with rubber septa impregnated with 4 milligrams (mg) of gossyplure and attached with brass fasteners to a wooden stake. Gossyplure is a pheromone used to attract pink bollworm. These traps are placed around the perimeter of cotton fields at planting, or shortly thereafter, at a rate of one trap per 10 acres, and are inspected weekly until defoliation and harvest or a killing freeze (Leggett et al., 1994). Both the traps and pheromone dispensers are replaced biweekly.

Special distribution surveys were conducted prior to the initiation of the present eradication program to delimit those areas where extant populations of pink bollworm exist. The surveys involve placement of traps from August to October at a density of 1 trap per 640 acres and weekly inspection for moths. An initial 2-year distribution survey was conducted in Arkansas, Louisiana, New Mexico, Oklahoma, and Texas in 2000 and 2001. The distribution survey found that pink bollworm populations were limited to west Texas and south-central New Mexico. This was confirmed through further trapping surveys in 2002 to 2004. Comparable trapping surveys have been conducted in Arizona and California as part of ongoing surveillance by the Arizona Cotton Research and Protection Council, the Imperial Valley Commissioner of Agriculture, and CDFAe. Their data indicate wide distributions of pink bollworm throughout Arizona and southern California.

In addition to trapping surveys, the eradication program employs visual inspection of cotton conducted by trained scouts. The scouts randomly select 20 cotton fields (30 percent Bt and 70 percent conventional) per work unit (12,000 to 15,000 acres) to inspect weekly for rosetted blooms beginning at the bloom stage. Weekly larval surveys in bolls are also conducted in the same fields by sampling 25 bolls per quadrant, starting at the boll formation (quarter size) stage until cut-out (El-Lissy et al., 2005b).

c. Monitoring of Exposure from Irradiation Equipment

All irradiation treatments are conducted in approved facilities in accordance with stringent safety guidelines (USDA, 1996). Each facility has monitors for detection of stray radiation. No problems with equipment design or shielding have been recorded with the operation of irradiation equipment under APHIS' programs and permits. Irradiation equipment at approved facilities is checked on a regular basis by the USDA Radiation Safety Staff, in accordance with standards set by the Nuclear Regulatory Commission. Workers involved in the treatment of insects are required to wear radiation badges when those irradiators are actively being used to sterilize insects, to ensure monitoring of any potential exposure.

7. APHIS Regulatory Oversight of Genetically Engineered Insects in Control Programs

a. Plant Protection Act

There are several U.S. laws that provide regulatory authority for transgenic insects, most of which fall under APHIS authority. The Federal Plant Protection Act of June 2000 (PPA) provides regulatory authority to detect, control, eradicate, suppress, prevent, or retard the spread of plant pests. Current Federal regulations governing the permitting of transgenic organisms, under PPA (7 U.S.C. 7701–7772), are described in 7 CFR Part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests." APHIS regulates exotic organisms, such as insects imported for use as biological control organisms as well as genetically engineered arthropods that are plant pests or indirectly affect plant pests (e.g., biological control organisms) under PPA.

Plant pests are defined, in CFR 340.1, as ". . .any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants." The four species (Medfly, Mexican fruit fly, oriental fruit fly, and pink bollworm) addressed in this EIS meet the definition of a plant pest. Regulated articles described in CFR 340.1 also include any product which contains an organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest. Examples of organisms which are or contain plant pests and exemptions are listed in CFR 340.2.

APHIS is obligated, by statute and regulation, to evaluate the potential impact to plants and the environment of transgenic organisms proposed for release into the environment that are or may become plant pests. This

evaluation process begins with a determination of jurisdiction: Does the proposed introduction involve a "regulated" article as defined in 7 CFR Part 330 or 340. A regulated article may be a plant pest listed in 7 CFR § 340.2 or a nonlisted organism engineered with sequences from an organism on the list. If it is determined that APHIS does not have authority to regulate the particular article, then the process is finished and the applicant is so informed.

b. National Environmental Policy Act

When it is determined that APHIS has jurisdiction, the evaluation proceeds to an assessment of the potential risks of the proposed introduction. In some circumstances, the organism or activity may already have been assessed and determined to be of no risk to plants or the environment. This may be the case for similar organisms previously permitted, or for certain activities with transgenic organisms categorically excluded by NEPA analysis listed in the NEPA Implementing Procedures for APHIS in 7 CFR Part 372. If the activity is not within these categories, an assessment is then conducted to examine the potential for additional risks associated with the introduction of the genetically engineered form relative to the non-genetically engineered form. When risks are identified, ways to manage or mitigate the risk may then be proposed.

If it is determined that the proposed introduction of the candidate organism represents a significant risk to agricultural crops or the environment and cannot be permitted, the applicant is so informed. When this is not the case, an EA may be prepared under NEPA and the Council on Environmental Quality (CEQ) guidelines, as described in 7 CFR Part 372. The EA describes the potential impacts of the introduction on the environment, and leads to either a finding of no significant impact (FONSI) or the preparation of an EIS. The availability of the EA and docket location are announced in the *Federal Register* and the public is given 30 days to comment on the EA. The docket is reviewed by APHIS and APHIS responds to the public comments. The information is used to inform the decisionmaker. Where appropriate, the EA is revised in response to public comment. If a FONSI can be reached, it is prepared and a permit can then be issued. When the EIS alternative is chosen, the decision to issue or deny a permit is not made until after the public comments are fully considered and the EIS is finalized.

c. Permit Requirements

This EIS addresses APHIS control programs on a programmatic basis rather then a site-specific basis. APHIS requires a permit for the importation, movement, or environmental release of genetically

engineered insects. A permit for the environmental release of genetically engineered insects will require a risk assessment and a formal analysis, as required under NEPA. Limited releases of genetically engineered insects for research purposes may not significantly affect the human environment and, therefore, may not require an EIS. In such cases, the preparation of an EA may be sufficient to address the agencies' NEPA obligations. Availability of the EA is published in the *Federal Register* with a public comment period, final deliberations, and then, if appropriate, an issuance of a FONSI and approval of the permit. For example, APHIS–Biotechnology Regulatory Services (BRS) has issued permits for caged and open-field releases of transgenic pink bollworm and predatory mites. An EA has been prepared for permits for environmental releases of genetically engineered pink bollworm (http://www.aphis.usda.gov/brs/aphisdocs/05_09801r_ea.pdf).

d. Containment and Confinement Guidelines

Appropriate containment or confinement of the transformed organism is required whether the organism is released, imported, or moved interstate. Arthropod and other invertebrate containment guidelines may be found at the following locations:

- NIH Guidelines: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html;
- Society of Tropical Medicine and Hygiene Arthropod Containment Guidelines: http://www.astmh.org/SIC/files/ACGv31.pdf.

- The APHIS, PPQ Containment Guidelines for Nonindigenous, Phytophagous Arthropods and their Parasitoids and Predators, Nonindigenous Snails, Plant Pathogenic Nematodes, and other organisms may be obtained by calling (301) 734–5304.

A confined field trial is a trial in which the candidate arthropod is prevented from becoming established and spreading. Confinement may be by physical barriers (such as screen cages), pesticides, cultural control, and biological measures, such as induced sterility or pheromone traps. Confined field trials can provide important information before unconfined release is requested and may be useful to observe changes in biology, ecology, and behavior of the transgenic form compared to the parental form.

e. Risk Assessment Considerations

When BRS receives an application, the agency evaluates its completeness for the purpose of doing a risk assessment. If APHIS finds the application deficient, the agency informs the applicant of the required additional

information and allots time for the information to be provided. The main purpose of the risk assessment is to determine if genetic alteration changes ecological or environmental properties of the organism. Such potential risks associated with the release of a transgenic arthropod, or other invertebrate, could include displacement of native populations, change in host or prey utilization, change in distribution, effects on endangered or threatened species, horizontal gene transfer, or the possibility of one of the characteristics of the transgenic arthropod increasing resistance to herbicides or pesticides.

The regulatory process concerning the introduction of plant pests involves the analysis of potential risk to the environment of a proposed introduction. The evaluation process must consider whether the genetic changes to the organism proposed to be released have altered the risks associated with the unmodified organism.

Information for risk assessments may include:
(http://www.aphis.usda.gov/biotechnology/arthropods.shtml)

(1) Purpose and Description of the Transformation

- Purpose and expression of inserted/altered genetic material?
    - Are there effects of the inserted material on other traits?
    - Does experience with similar constructs exist?
- Description of genetic engineering methods and genes involved including transduction viruses, transposons, symbionts?
- Phenotype, transgene, transposon stability over multiple generations?

(2) Ecological Consequences of the Transformation

- Horizontal movement of transgene, transposon, virus, symbiont to related species, parasites, predators?
- Possible result if introduced trait fails?
- Detection methods for presence or change in function of inserted/altered genes?
- For mobile DNA elements or genetic driving factors, such as transposable elements, viruses, symbionts (*Wolbachia*)—What is the known host range?
- Likelihood of dissociation of the drive mechanism from gene of interest?
- If natural selection is necessary to drive the trait in a population, what are selection criteria?

(3) Biology of the Host Organism

- What is the probability of establishment and movement of the genetically engineered arthropod? Are predictive models available?

- Effects on biology and life cycle? Are there associated fitness costs?
- Effects on invasive characteristics?
- Possibility of effects on threatened or endangered species?
- Effects on pesticide susceptibility?
- Effects on disease transmission, pest or parasitic abilities, host range, host utilization?

(4) Environmental Fitness Factors

- Net fitness supplied by the inserted transgene has been demonstrated in transgenic fish, and needs to be done with arthropods:
- Juvenile viability (probability of survival to sexual maturity)?
- Changes in adult viability (survival after sexual maturity)?
- Mating advantage, female fecundity (number of offspring)?
- Male fertility (male fertilization success)? Mating success?
- Transgene effects on age at sexual maturity?

(5) Monitoring and Mitigation

- Development, validation, and deployment of monitoring measures and models to track releases and potential unintended or adverse effects?
- Development of biological, chemical, and physical containments, limiting mechanisms, and other mitigation measures to reduce risks after environmental release?

(6) Baseline Data Needed for Risk Assessment (Parental or Wild-type)

- Taxonomy?
- Geographic occurrence or range including habitats and climates?
- Biology and life cycles in various habitats, climates and host range?
    - What is the ecology of the arthropod's occurrence?
    - Any dormant, hibernation, or estivation phases?
- Means of movement?
- Distribution of sexually compatible relatives?
- Invasiveness, associated threatened or endangered species, and sexually compatible wild relatives?
- Kinds of diseases vectored, pest, or parasite characteristics?
- Associated parasites, predators, pathogens, symbionts, and commensals?

(7) Baseline Data Needed for *Risk Assessment* (Gene Donor/Source or Source)

- Taxonomy and distribution?
- Biology?

- Characteristics including whether a disease organism or toxin is produced?
- Range of occurrence and function of genes of interest?
- Description of genetic construct, associated regulatory sequences, and marker genes?
    - Are there undetermined sequences present in the genetic material of interest?
    - Are sequences present that are not necessary for the intended effect?

(This page is intentionally left blank.)

Appendix A. Preparers

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
4700 River Road
Riverdale, MD 20737

David A. Bergsten
Biological Scientist
B.S. Environmental Science
M.S. Entomology
M.P.H. Disease Control
Ph.D. Toxicology

Background: Biological Scientist in Environmental Services with expertise in environmental toxicology, chemical fate, and pesticide research. More than 20 years experience with APHIS including environmental protection, field, and port inspection experience. Experience in preparing environmental documentation for other major APHIS programs, in compliance with Federal statutes.

- EIS Responsibility: Project manager for the draft EIS—wrote parts of the Executive Summary, chapter I, chapter II, chapter IV, some responses to comments in appendix E, and appendix K. Reviewed and contributed to other chapters and to the appendices. Responsible for coordination and team management on final documentation.

Wayne Burnett
Domestic Coordinator, Fruit Fly Exclusion and Detection Programs
B.A. Botany
B.A. Zoology

Background: National Fruit Fly Program Manager for Tephritid fruit fly detection, preventive, emergency response, and control programs, with over 27 years of experience in APHIS' plant health pest exclusion, detection, management, and eradication programs. Experience includes both field operations and policy development for plant health programs involving various plant pests.

- EIS Responsibility: Technical Expert—Prepared parts of Executive Summary, sections on fruit fly history in chapter I, sections on fruit fly monitoring and mitigations in chapter IV, and some responses to comments in appendix E.

Betsey L. Coakley
Writer/Editor
B.A. Sociology

Background: Over 17 years of service with APHIS, with administrative and clerical experience with Plant Protection and Quarantine, and Policy and Program Development. Currently serving as Writer/Editor with Environmental Services.

- EIS Responsibility: EIS Editor—desktop publishing of the EIS (including editing, format, and document security); supportive coordination and planning.

Tracy A. Horner
Ecologist
B.S. Biology
M.S. Entomology
Ph.D. Entomology

Background: Ecologist in Environmental Services. Eight years of service with APHIS. Experience in environmental compliance, especially those associated with the Endangered Species Act in the context of biological control and pest management. Prepares and provides assistance on environmental documents.

- EIS Responsibility: EIS Analyst—prepared the endangered species section of the EIS.

Elizabeth E. Nelson
Environment Protection Specialist
B.S. Biology
M.S. Healthcare Administration
M.B.A.

Background: Environmental Protection Specialist in Environmental Services. Eight years of service with APHIS. Experience in environmental compliance, especially those associated with the Endangered Species Act, in the context of trade agreements, pest management, and pesticide regulations. Prepares and provides assistance on environmental documents.

- EIS Responsibility: EIS Analyst—contributed to the preparation of the draft EIS. Wrote parts of chapter I and IV of the EIS.

Robyn I. Rose
Biologist

B.S. Agronomy
M.S. Entomology
Ph.D. Entomology

Background: Biologist with Plant Protection and Quarantine, Emergency and Domestic Programs. Previously a Biotechnologist with Biotechnology Regulatory Services and an Entomologist with U.S. EPA, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division. Extensive regulatory and risk assessement experience with genetically engineered crops and insects.

- EIS Responsibility: Resource person for permitting genetically engineered insects and provided comments on the EIS as needed. Presented at public scoping meetings and wrote section on APHIS regulatory oversight of genetically engineered insects.

Rhonda R. Solomon
Environment Protection Specialist

B.S. Biology
Juris Doctorate

Background: Environmental Protection Specialist in Environmental Services. Three years of service with APHIS. Experience in preparing environmental documentation for APHIS programs, ensuring compliance with Federal statutes especially those related to pest management.

- EIS Responsibility: EIS Analyst—contributed to the preparation of the draft EIS. Wrote parts of the affected environment section.

James E. Warren
Ecologist

B.S. Forest Management
M.S. Entomology
Ph.D. Environmental Toxicology

Background: Ecologist in Environmental Services with over 12 years of experience in environmental toxicology and risk assessment as well as environmental fate modeling of pesticides while working for the Federal government and the agrochemical industry.

- EIS Responsibility: EIS Analyst—Prepared host range descriptions for fruit fly species in chapter III.

Decision Support & Pest Management Laboratory
Center for Plant Health Science Technology
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
3645 E Wier Avenue
Phoenix, AZ 85040

Gregory S. Simmons
Supervisory Entomologist

B.S. Botany
M.S. Ecology and Evolutionary Biology
Ph.D. Entomology

Background: Supervisory Entomologist with the Center for Plant Health Science and Technology. Leads a team of scientists working on development of biological methods of pest control using beneficial insects, sterile insect release, and genetic control technology with genetically engineered insects. His focus over the last three years has been on the development of more than 20 strains of transgenic pink bollworm for use in genetic control programs.

- EIS Responsibility: Technical Expert— Organized and presented program information at public scoping meetings. Prepared parts of chapters I and IV.

Robert I. Rose, Ph.D.
Biotechnology Regulatory Consultant/Agent
8322 Sharon Drive; Frederick, MD 21704

B.Sc. Zoology/Chemistry
M.S. Insect Pathology
Ph.D. Insect Pathology/Biological Control

Background: Over 30 years private and corporate experience in the United States and several foreign countries in insect pathology, biological control, chemical control, risk assessment, and regulation of genetically engineered animals, plants, and biopesticides under FIFRA, TSCA, PPA, and AHPA (laws).

- EIS Responsibility: Provided several technical science sections of this EIS, plus two appendices (C and D), and some responses to comments in appendix E.

Appendix B. Cooperation, Review, and Consultation

William Grefenstette
National Coordinator
Boll Weevil Eradication Program
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 138
Riverdale, MD 20737–1236

Daniel Keaveny
Senior Economic Entomologist
California Department of Food and Agriculture
Pink Bollworm Program
5100 Douglas Avenue
Shafter, CA 93263–4036

Susan D. McCombs
Assistant Director of Fruit Fly Programs
Fruit Fly Exclusion and Detection Programs
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
300 Ala Moana Boulevard, Room 8–152
P.O. Box 50002
Honolulu, HI 96850

Jim Rains
Staff Environmental Scientist
California Department of Food and Agriculture
Plant Health and Pest Prevention Services
1220 N Street
Sacramento, CA 95814–5607

Leslie Rubin
Environmental Compliance Coordinator
Planning, Analysis & Regulatory Coordination
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 156
Riverdale, MD 20737–1236

Michael Stefan
Director
Fruit Fly Exclusion and Detection Programs
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 137
Riverdale, MD 20737–1236

Appendix C. Analysis of Repressible Lethal and Marker Genetic Engineering

Repressible Lethal and Marker Genetic Engineering with Analysis of Issues Pertaining to Transposon Mobility and Potentiation of Horizontal Transfer for Technology Under Development by APHIS

Review of and Fruit Fly and Pink Bollworm Transformation

Molecular Biology of Insect Transformation

Genetic transformation of insects usually means the stable integration of exogenous deoxyribonucleic acid (DNA) into the genome of the insect. This requires a method to get the DNA into the insect genome, and it also requires a method for identifying such events, that is, a genetic marker.

Transferring DNA into the Insect Genome

DNA can be physically placed in insect cells by methods such as microinjection; however, such DNA appears to be incorporated into the insect's genome at an extremely low (or zero) frequency. Therefore, genetic transformation of insects depends on the use of a method to increase the rate of incorporation into the genome. Such a method is the use of nonautonomous transposable elements. Autonomous transposable elements are genetic elements which have the ability to transpose (move) from one place to another in the host genome, and also to be incorporated into the host genome following microinjection, although, at a low frequency. A key part of this ability is the expression of a functional transposase enzyme, which is typically encoded by an autonomous transposon. This transposase gene acts on the ends of the transposable element in one or more essential steps of the transposition process.

Non-autonomous elements have been 'crippled' by the deletion of all or part of the gene encoding the transposase and, therefore, are incapable of transposing without external assistance, such as an appropriate transposase from a different source. In the present transformation systems, such assistance is typically provided by coinjection of an additional plasmid, which encodes the transposase gene. However, the injected elements do not include the ends of the transposable element and, therefore, cannot transpose. Other potential methods for providing a suitable transposase include coinjection of RNA encoding the transposase (Kapetanaki et al., 2002; Shinmyo et al., 2004), or transposase protein (Kaufman and Rio, 1991; Lampe et al., 1996). Another possibility is to inject a so-called jumpstarter strain which already contains a suitable transposase gene in its genome, this having been put there by a prior transformation (Cooley et al., 1988; Häcker et al., 2003; Robertson et al., 1988).

Since the initial development of a transformation system based on the use of the *P* element in *Drosophila melanogaster* flies (Fortini et al., 1992; Spradling and Rubin, 1982), several such transformation systems have been developed, based on different transposable elements, notably *piggyBac, Minos, Hermes*, and *mariner*/*Mos1*. These methods are now in widespread use in many labs around the world, and have been extensively reviewed (Handler and James, 2000;

Li et al., 2005; Wimmer, 2003). A wide phylogenetic range of insects has now been transformed by these methods, including several Lepidoptera, as well as Diptera, Coleoptera, and some non-insect species (e.g., Mediterranean fruit fly, *Ceratitis capitata* (Handler et al., 1998); oriental fruit fly, *Bactrocera dorsalis* (Handler and McCombs, 2000); vinegar fly, *Drosophila melanogaster* (Handler and Harrell, 1999); Caribbean fruit fly *Anastrepha suspensa* (Handler and Harrell, 2001); red flour beetle, *Tribolium castaneum* (Berghammer et al., 1999); silkworm, *Bombyx mori* (Tamura et al., 2000); pink bollworm *Pectinophora gossypiella* (Peloquin et al., 2000); yellow fever mosquito *Aedes aegypti* (Kokoza et al., 2001); malaria mosquitoes *Anopheles gambiae*, *An. albimanus* and *An. stephensi* (Grossman et al., 2001; Nolan et al., 2002; Perera et al., 2002); house fly, *Musca domestica* (Hediger et al., 2001); Australian sheep blowfly *Lucilia cuprina* (Scott et al., 2004); new world screwworm fly *Cochliomyia hominivorax* (Allen et al., 2004a; Allen et al., 2004b; and Allen, 2007); southern house mosquito *Culex quinquefasciatus* (Allen et al., 2001; and Allen and Christensen, 2004); and the planarian *Girardia tigrina*, which is not an insect. (Gonzalez-Estevez et al., 2003)].

"Plasmid backbone" sequences, antibiotic resistance genes, and bacterial origins of replication—these sequences and systems have not been incorporated into "Release of Insects carrying Dominant Lethal" (RIDL®) strains used to date by APHIS, Center for Plant Health Science and Technology (CPHST) and Plant Pest Quarantine research and development programs, nor is it anticipated that they will be in the future. However, because some other transgenic insects include these sequences, they are briefly discussed here.

Plasmids are circular DNA molecules capable of replication in suitable strains of bacteria, and are very widely used in standard procedures of molecular biology. They are routinely propagated and amplified in weakened laboratory strains of *Escherichia coli* (*E. coli*). When the plasmid DNA is purified from *E. coli* cultures grown for that purpose, essentially no bacterial protein or chromosomal genetic material remains associated with the plasmid.

Plasmids contain specific sequences which enable and direct their replication in suitable bacterial strains; for example, the specific weakened laboratory strains of *E. coli* were the immediate hosts for the plasmids carrying the cloned genes used to make the insect transforming constructs. Such sequences include a suitable origin of replication and usually an antibiotic-resistance gene. These sequences are collectively known as the plasmid backbone; however, the transformation systems used to transfer DNA from a plasmid to the insect genome do not incorporate the entire plasmid into the genome, but rather only the part between the ends of the transposon. Transposon vectors have been used which do or do not result in the incorporation of plasmid backbone sequences into the insect genome. For example, pBSII-ITR1.1k-ECFP does incorporate plasmid backbone sequences into the insect genome in the course of *piggyBac*-mediated transformation, and pXL-BacII-ECFP does not; both are described in Li et al. (2005). Plasmid backbone sequences are thought to be inert in the insect. Their inclusion potentially facilitates the replication of associated sequences if these have taken up by a suitable microbe. This is experimentally useful in some instances, such as in the "plasmid rescue" technique; however, it may be considered undesirable in strains intended for field release. Nonetheless, many engineered plant varieties include plasmid sequences and/or antibiotic resistance genes in the modified plant genomes.

Genetic Markers for Insect Transformation

The genetic transformation systems described above are relatively inefficient, so that only a low proportion, often less than 0.1%, of the candidate transgenics (the progeny of the individuals micro-injected with suitable DNA solution) actually have exogenous DNA inserted into their genome. An efficient marker system is, therefore, required with which to identify these rare transgenic individuals, both initially and during subsequent breeding experiments. The earliest transformations of pest insects used eye color markers, deriving from the widespread use of *white*$^{+}$ and *rosy*$^{+}$ as transformation markers in *Drosophila melanogaster* (Fortini et al., 1992; Klemenz et al., 1987). Such systems have been successfully used in a number of other insect species including Medfly, *Ceratitis capitata* (Loukeris et al., 1995), oriental fruit fly, *Bactrocera dorsalis* (Handler and McCombs, 2000) and the yellow fever mosquito, *Aedes aegypti* (Coates et al., 1998; Jasinskiene et al., 1998). However, such markers require the use of a mutant strain of insect which carries a recessive visible mutation, such as an eye color mutation, and a DNA sequence known to be able to complement this mutation, for example, a wild-type copy of the corresponding gene. Such strains and sequences are neither available for the pink bollworm nor for the majority of pest insects. In principle, it would be possible to use markers such as a chemical resistance gene including neomycin phosphotransferase (G418 resistance) or hygromycin B phosphotransferase (hygromycin resistance). These markers have been widely used in plants, and occasionally in *Drosophila*, but little, if any, in pest insects. Instead, the current markers of choice are fluorescent proteins of the green fluorescent protein (GFP) superfamily (Bevis and Glick, 2002; Matz et al., 1999; Shagin et al., 2004; Tsien, 1998). Most insects are not significantly fluorescent, or have at least some tissues or developmental stages which are not significantly fluorescent; therefore, expression of a suitable fluorescent protein allows the (fluorescent) transgenic individuals to be identified under ultraviolet (UV) light in a mixed population of wild-type and transgenic individuals.

Stability of the *piggyBac* Transposon in the Pink Bollworm and Fruit Flies

The original *piggyBac* transposon, from which current genetic transformation vectors are derived, is now known to be a member of a widespread and divergent family of transposable elements (Sarkar et al., 2003). Other than some other noctuid moths, the only species known to have elements closely related to those of the original element derived from the cabbage looper moth, *Trichoplusia ni,* are in the *Bactrocera dorsalis* species group (Handler and McCombs, 2000; Handler et al., 2004). It is highly unlikely that species which lack closely related elements will show transposase-mediated instability of transposase insertions; however, in a different family of transposons, it has been shown that distinct, although closely related, elements can cross-mobilize to some extent (Sundararajan et al., 1999). The lack of mobility of *piggyBac* elements, in the absence of exogenous transposase in pink bollworm (Thibault et al., 1999), indicates that non-autonomous *piggyBac* elements are immobile in pink bollworm unless artificially provided with *piggyBac* transposase. Similar data have been obtained for a range of other insect species (Grossman et al., 2000; Li et al., 2001; Lobo et al., 1999; Lobo et al., 2001; Shinmyo et al., 2004).

The enhanced green fluorescent protein (EFGP) positive lines were maintained as heterozygotes by serial backcrosses to the wild-type strain. At the time of backcross analysis, the lines had

been backcrossed for four generations. This would likely separate any transformed loci that were not tightly linked. Thus, the EGFP-positive parental insects used in the diagnostic backcrosses were expected to be heterozygous for a single copy of the gene. At the time of backcross analysis of the heterozygote lines, the first line produced 191 positive and 207 negative progeny, and the second line produced 555 positive and 616 negative progeny. These were not significantly different from the expected 1:1 ratio by X^2 statistical analysis. Therefore, a relatively close 1:1 ratio of EGFP versus wild-type supports the hypothesis that EGFP was transmitted as a singlelocus, dominant gene. This observation has also been confirmed by genomic Southern hybridization in the 58th generation that revealed only one 2.4-kb band for the insertion that was described for the original EGFP transgenic line (Peloquin et al. 2000; Park, unpublished data).

Another simultaneous transposon stability assessment conducted by Park was a survey of *piggyBac*-like elements (PLE) in various sources of pink bollworm. Surprisingly, multiple copies of a PLE that is distantly related to the *T. ni piggyBac* (56% similarity and 40% identity of the encoded transposase proteins) were found in the pink bollworm populations, including the mass-reared "C" stock of the APHIS, CPHST Pink Bollworm Rearing Facility, which is the strain that provided the genetic background for the EGFP line. This PLE shares little nucleotide sequence identity with the *T. ni piggyBac*, and would not be expected to cross-hybridize. This conclusion is consistent with the earlier Southern blot data indicating that there are no elements in pink bollworm closely related to the *T. ni piggyBac* element. As stated above, previous transposition assays indicated that mobilization of *T. ni piggyBac* in pink bollworm requires exogenous *T. ni piggyBac* transposase activity (Thibault et al., 1999). Therefore, these results indicate mobilization of a transgene based on a *T. ni piggyBac*-derived transposon vector by the transposase of an endogenous pink bollworm *piggyBac*-like element is highly unlikely.

Consequences of Remobilization of the *piggyBac* Element

Certain critics of the first transgenic pink bollworm environmental assessment (EA), (Confined Study of a Transgenic Pink Bollworm, *Pectinophora gossypiella* (Lepidopera: Gelechiidae), Environmental Assessment—October 2001) commented that the environmental use of a conventional non-autonomous *piggyBac* transposable element would pose a risk of horizontal gene transfer to other kinds of organisms. The exposure of a non-autonomous *piggyBac* element inserted into an insect (e.g., fruit fly or pink bollworm) genome to *piggyBac* transposase could have several possible effects on the non-autonomous element. These, in decreasing order of likelihood/frequency, are the following:

1. excision,
2. transposition within the genome of an individual, and
3. transposition into the genome of another individual of the same species or of a different species (horizontal gene transfer).

Excision

The least unlikely effect (in other words, the highest-frequency event should there be any transposase-mediated instability at all) would be excision of the transposable element. If this should happen at a significant frequency, it would potentially lead to loss of the element from the

released insects used for sterile insect technique (SIT) control. This would mean that some of the released insects would not have the intended transgenic genotype, and possibly not be transgenic. Using a filter rearing system, as described elsewhere in this environmental impact statement, insects carrying deletions should not accumulate; therefore, the proportion of released insects should be approximately equal to a small multiple of the excision rate. For genetic sterilization or a genetic marker, an excision rate of several percent is likely to be tolerable because radiation-sterilization presently used in SIT programs is typically also not completely 100% effective. The radiation dose used is based on a compromise between sterilization, which requires a relatively high dose, and minimizing the physiological damage to the insects, which increases with increasing radiation dose. The observed stability of *piggyBac* in the pink bollworm over 58 generations of the EGFP strain indicates that the rate of loss of transposable element function, by all methods combined (e.g., of spontaneous mutation, as well as transposase mediated excision), is so low that instability was not detected in the rearing facility (Peloquin et al., 2000), and in a field study of genetically modified pink bollworm (USDA–APHIS, 2005a) http://www.aphis.usda.gov/brs/biotech_ea_permits.html.

Transposition

Another possible effect on a transposable element exposed to a transposase specifically active for it is that it might transpose, in other words, move from one place in an insect's genome to another place in the genome of the same individual insect. Again, experiments with the original EGFP *piggyBac* element indicated this occurs at an extremely low rate, if at all, and no transposition was detected after 58 generations (Peloquin et al., 2000). The consequences of such an event, for the effectiveness and safety of the program, would be negligible. Transposition might also be associated with excision from the original locus. This would be the equivalent of loss of the element, which is discussed above. The element, in its new position, would probably impose a fitness penalty because the process of natural selection resulting from the proposed SIT usage eliminates the vast majority of mutational changes.

There is an extremely small, but possibly non-zero, chance that such changes result in species better adapted to their environment. Therefore, those few insects that might carry the change would not tend to spread through the habitat unless there was a change in the environment that favored them over their existing wild-type cohorts. Regardless, such a change would be of genetic material already present in the transgenic strain. This is the same process as insertional mutagenesis by natural transposons, which is a naturally occurring process. Artificial non-autonomous transposons of the type discussed here present no additional risks, in this regard, over the thousands of naturally occurring autonomous and non-autonomous transposable elements already present in the genomes of all insects and other animal and plant species.

Horizontal Gene Transfer

The most unlikely theoretical adverse effect of exposure of a transposon to a transposase capable of inciting movement is that it might transpose into the genome of another individual of another species. This theory is termed horizontal gene transfer. The term "horizontal" refers to movement of genes between different species, as distinguished from "vertical" transmission, which is the normal transmission of genetic information from parent to offspring. Phylogenetic

analysis indicates that the distribution and sequence of transposable elements is incongruent with phylogenetic trees constructed from morphological characteristics. However, sequences of genes, in some species, indicate that autonomous transposons, which carry their own transposases, have occasionally (over periods of millions of years) been able to move from one lineage to another. Autonomous transposons differ significantly from the non-autonomous transposons used as gene vectors in insect genetic transformation in that non-autonomous transposons do not encode their own transposase and are, therefore, incapable of driving their own transposition. Furthermore, the engineered transposons are inevitably much larger than native transposons, and transposition rates are known to decrease with increasing length (Berg and Spradling, 1991; Lampe et al., 1998).

Comparative analysis of full and partial genome sequences indicates that horizontal gene transfer is an extremely rare event in insects and other multicellular eukaryotes, far too rare a process in nature to be amenable to laboratory investigation. The main concern over risks associated with horizontal gene transfer relates mainly to the development of antibiotic-resistance in prokaryote bacteria arising from bacterial conjugation in which genetic material is transferred between bacteria through direct cell-to-cell contact. The genomes of insects and other eukaryote animals contain a very large number of transposable elements, comprising a significant percentage (e.g., 10 to 20%) of the total genome sequence. Despite this abundance of source material, horizontal gene transfer is extremely rare, even between closely related species, and even over geological timescales. For the *P* element of *Drosophila*, which may be a particularly mobile and invasive element, phylogenetic studies detected at least 11 events between 18 species of drosophilids over 3 million years (Silva and Kidwell, 2000). Sarkar et al. (2003) identified and analyzed *piggyBac*-related transposase genes over a very wide phylogenetic range and found only one clear-cut case of horizontal transfer which links the *piggyBac*-like sequences found in oriental fruit fly (Handler and McCombs, 2000) with the canonical form from *Trichoplusia ni* (Cary et al., 1989; Fraser et al., 1996). For an individual transposable element, from the many thousands in each insect genome, the phylogeny of the element is typically congruent with the phylogeny of the host over periods of millions of years, that is, vertically inherited through recent speciation events. In other words, the horizontal transfer rate is probably less than or part of the overall evolution rate of insect species.

The critical difference between autonomous and non-autonomous elements is the ability of the former to catalyze their own transposition and, thereby, spread through a single species. The best known example of such an invasion is the *P* element of *Drosophila melanogaster* (Engels, 1989; Engels, 1992). This self-mobilization ability would be essential to the element's ability to invade a new or different species. Non-autonomous elements cannot do this because the ability to transpose is removed due to lack of a transposase to cause movement. Analysis of *mariner* gene sequences in different insect species clearly indicated that the initial horizontal transfer event in each case was of an autonomous element, followed by neutral mutation, leading to the divergent population of elements now found in each species (Lampe et al., 2003). Given that there are far more non-autonomous *mariner* elements than autonomous ones, due to the accumulation of defective copies during the time the element is resident in a species, this strongly indicates that non-autonomous elements are incapable of invading a new species.

A non-autonomous element, like any other genetic mutation or element, would only spread through a large population (e.g., insect populations) if it conferred some kind of a phenotypic advantage that is selected for by its environment, although such elements alone are not associated with phenotypic characteristics. Such an advantage could, hypothetically, be provided by inclusion of an evolutionarily selective advantageous gene with the non-autonomous transposon. An equivalent issue has been raised for some transgenic crop plants, which variously include genes for insect tolerance or herbicide tolerance. However, no such genes are included in the repressible lethal RIDL® constructs. Similarly, uptake by microorganisms might be considered an issue if the transgenic insect carried an antibiotic resistance gene; nevertheless, they do not. This is an issue for some transgenic crop plants which carry a gene potentially encoding resistance to the antibiotic kanamycin and related compounds. No such genes are included in the RIDL® transgenic lines (see plasmid backbone sequences described in the section on Transferring DNA into the Insect Genome above).

Horizontal gene transfer flow, or movement, has arisen as one of the more significant theoretical concerns over development and deployment of transgenic or genetically modified insects for crop protection and human disease vector control. However, recombinant tools have been developed and used in genetically modified insect applications specifically to prevent transposon remobilization. Thus, current technology described in this appendix, which is the technology closest to development by APHIS, produces genetically modified insects with highly stable transgenes.

The theoretical assumption of risk by horizontal gene transfer is based on observations of gene transfer among prokaryote bacteria. These mechanisms are not present in higher multicellular eukaryote organisms. Exchange of genetic material between insects of different species, and between insects and other organisms, is biologically improbable. Insects exchange gametes internally and have complex mating behaviors and structures. Many higher organisms release genetic material into the surrounding environment, such as pollen or spores in air, or fish or mollusk sperm in water; however, insects are much more conservative in this respect and do not release their gametes promiscuously into the environment.

Post-integration Removal of *piggyBac* Ends

A method has been proposed and demonstrated, in *Drosophila*, for removing one end from a *piggyBac* element (Handler et al., 2004). Such a "one-ended" *piggyBac* would be more difficult to remobilise with exogenous *piggyBac* transposase because the missing end would also need to be supplied. However, this may not be impossible since an autonomous element could insert close to the one-ended element, thereby reconstituting a "three-ended" *piggyBac* element competent for transposition. As a consequence, use of this method would likely reduce the frequency of transposase-mediated events; however, it would not provide a mechanism to completely eliminate the possibility.

An alternative method has been developed which overcomes this potential limitation of the '3-end' stabilization approach described above (Dafa'alla et al., 2006). This method allows the removal of all transposon sequences after the initial transformation. This is illustrated schematically in figure 1, and in Dafa'alla et al., (2006). The other functional recombinant DNA

components of strains made using this system are unaffected. The only sequence difference between these strains and those made using conventional transposon-based methods is the absence in the former strains of the transposon sequences that are inevitably associated with the insertion in the latter strains. This transposon stabilization system may be particularly useful in those species where endogenous cross-reacting transposons are known or believed to be present in the recipient species. Amongst tephritids, this relates to a group of *Bactrocera,* related to *Bactrocera dorsalis*, oriental fruit fly (Handler and McCombs, 2000), however, not *Ceratitis* or *Anastrepha* species (e.g., *Ceratitis capitata*, *Anastrepha suspensa*), or other *Bactrocera* species, such as *Bactrocera oleae*. Among moths, an example is the cabbage looper, *Trichoplusia ni*, from which the original *piggyBac* clone was derived (Fraser et al., 1983), and which, presumably, contains potential sources of *piggyBac* transposase in its genome. This does not apply to pink bollworm. A survey of laboratory and natural populations of pink bollworm showed that none of these contained *piggyBac*, and there is strong evidence that the most closely related element in the pink bollworm genome is not capable of efficiently cross-mobilizing *piggyBac*. However, some researchers consider the field use of transgenic insects constructed using conventional non-autonomous transposon vectors to pose risks to the environment (Handler, 2004; Handler et al., 2004; Wimmer, 2003; Wimmer, 2005).

A Brief History of Genetic Transformation of the Pink Bollworm and Fruit Flies

Fruit Flies

For tephritid fruit flies, transformation has a longer history than for pink bollworm and has included use of a wider range of markers and non-autonomous transposon vectors. The first system for routine transformation of an insect was developed in the vinegar fly, *Drosophila melanogaster* (Fortini et al., 1992). This transformation used non-autonomous derivatives of the *P* element. The transposon, derived from the *P* element, had been identified and isolated from this species; however, for reasons that are still not entirely clear, the *P* element is inactive outside of a small group of species closely related to *Drosophila melanogaster*. Useful systems for genetic transformation of other species required the development of alternative transposon systems and suitable markers (for reviews see Atkinson et al., 2001; Handler, 2001; Handler, 2002; Handler and James, 2000; O'Brochta and Atkinson, 1996; Wimmer, 2003). The first such transformation of any insect, other than *Drosophila*, was of the Mediterranean fruit fly (Medfly), a tephritid fruit fly (Loukeris et al., 1995). This used *Minos*, a transposon originally isolated from *Drosophila hydei*. Subsequently, other transposons have been used for genetic transformation of tephritid fruit flies, including *piggyBac* (Handler et al., 1998) and *Hermes* (Handler, 2001). These are all members of the same class of transposons, and there is little practical difference between them in respect to their use as transformation vectors. Of the available transformation systems, *piggyBac* is currently the one most commonly used in tephritid insects.

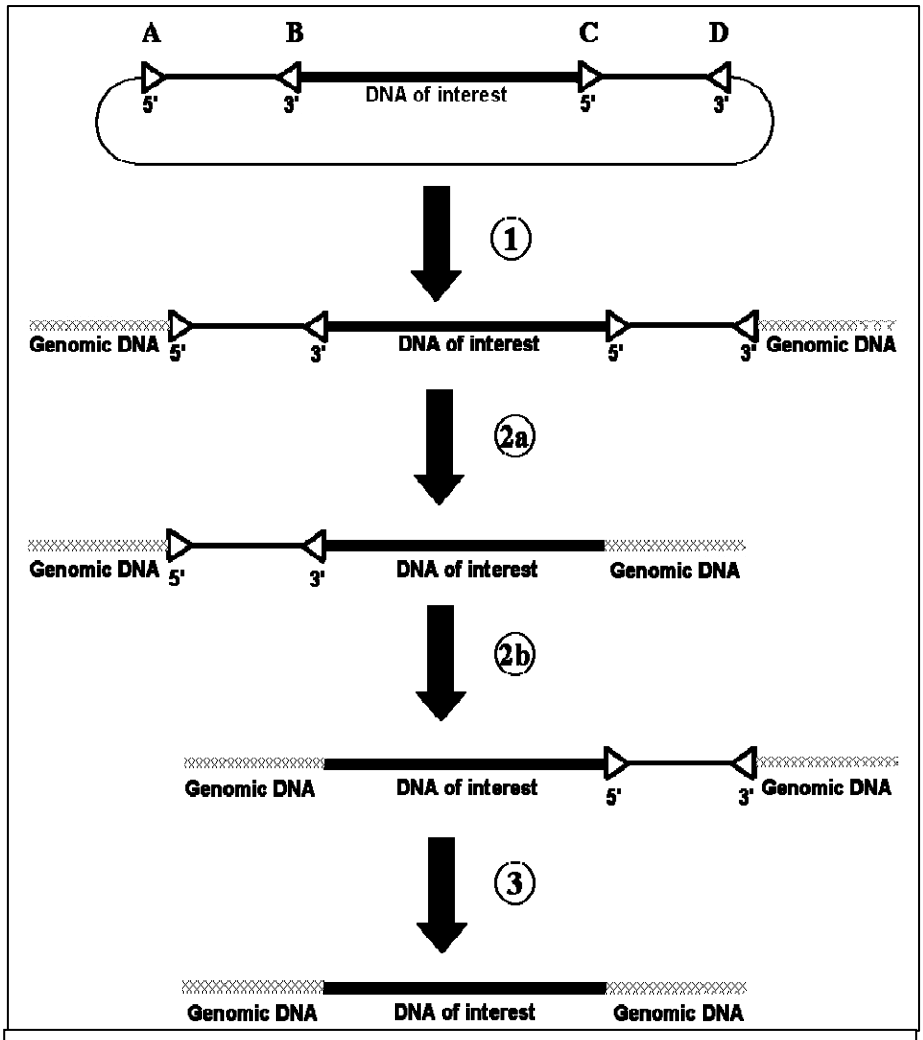

Figure 1: The process begins with a plasmid bearing a transposon with two repeated ends, within which is carried the DNA of interest. Triangles A-D represent the ends of the transposon; for example, the short inverted repeats at the ends of a class II element such as *piggyBac*. Transposition can occur between any two opposed triangles: in this case, A-B or C-D or A-D or C-B.

1. Introduce the plasmid into cells or embryos, by microinjection, with suitable transposase helper, e.g. helper plasmid or integrated transposase source ("jumpstarter"), and select transformants using suitable markers.
2. Expose to suitable source of active transposase, preferably jumpstarter. Select specific excision product, 2a or 2b, using suitable markers or molecular analysis.
3. Expose again to a suitable source of active transposase and select excision product using suitable markers or molecular analysis.

Although each logical step (insertion, each excision step, etc) is described separately, in practice exposure to transposase may induce several of these steps to occur within one generation, or without the intermediate being specifically identified. This approach leaves no transposon DNA whatsoever associated with the insertion. [Adapted from Dafa'alla et al., 2006.]

The initial transformation of Medfly used an eye color marker to identify the transgenics. In this system, the recipient strain carries (i.e., is homozygous for) a recessive visible marker (e.g., *white eye*), and the transformation marker is a sequence that can functionally complement this mutation. This type of marker is widely used in *Drosophila melanogaster*; for example, the well-known *rosy*$^{+}$ and mini-*white*$^{+}$ markers (Fortini et al., 1992; Klemenz et al., 1987). More recently, fluorescent proteins have become the markers of choice. One key advantage is that these are potentially detectable in a wild-type background and, therefore, do not require the prior identification of a suitable recessive mutation and complementing sequence. The first transformation of oriental fruit fly, *Bactrocera dorsalis,* used the same Medfly-derived *white* gene sequence to complement a white-eyed mutant of this species (Handler and McCombs, 2000). Both oriental fruit fly and Medfly were also subsequently transformed using fluorescent protein markers (reviewed by Handler, 2002). The first transformants of several other tephritid species were generated using fluorescent protein markers, for example, Caribbean fruit fly, *Anastrepha suspensa* (Handler and Harrell, 2001), olive fruit fly, *Bactrocera oleae* (Koukidou et al., 2006), and the Mexican fruit fly, *Anastrepha ludens* (Condon et al., 2007).

Autocidal strains of Medfly (Fu et al., 2007; Gong et al., 2005) and Mexican fruit fly (Condon, Alphey, and others, unpublished) have been constructed. Autocidal strains of Medfly and the Mexican fruit fly have also been constructed using dominant temperature sensitive (DTS) alleles of proteasome subunits (Xavier and Handler 2006, see http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=199795 and below).

Pink Bollworm

Genetic transformation of pink bollworm, *Pectinophora gossypiella*, was first achieved in the laboratory of Thomas Miller at University of California, Riverside (Peloquin et al., 2000). Genetic transformation of the silk worm, *Bombyx mori*, was described later in the same year (Tamura et al., 2000). These were the first two papers describing genetic transformation of Lepidoptera.

The transformation system used was based on a non-autonomous *piggyBac* transposable element, or transposon, co-injected with a non-integrating source of *piggyBac* transposase. A non-autonomous transposon is prevented from movement within or outside the genome of its host because it does not produce the transposase enzyme that is necessary for movement. Previous plasmid-based mobility assays had shown that the mobilization of the donor *piggyBac* transposon was induced in the presence of exogenous transposase produced without *piggyBac*, while no mobility was seen in the absence of exogenous transposase (Thibault et al., 1999). Both for the preliminary plasmid-to-plasmid transposition assays, and for the first genetic transformation of pink bollworm, the helper plasmid, contained a *piggyBac* transposase gene driven by the *Drosophila* hsp70 heat-shock promoter instead of the endogenous *piggyBac* promoter (Handler and Harrell, 1999). In construction of this helper plasmid, the endogenous *piggyBac* promoter was removed and replaced by the *Drosophila* hsp70 promoter. At the same time, one of the *piggyBac* inverted terminal repeats (ITRs) was removed; these are required for mobilization of wild-type *piggyBac.* This provided control and limitation of movement of the *piggyBac* transposon.

Based on the data from the plasmid-to-plasmid transposition assays above, together with the previous history of successful transformation of several insect species with *piggyBac*-based genetic transformation systems, it was considered that such a system could be a suitable for genetic transformation of pink bollworm; therefore, a *piggyBac*-based vector was constructed for transformation. Construction of the transformation vector resulted in deletion of approximately 1 kilo base ((kb), i.e., 1,000 nucleotide bases) within the original *piggyBac* transposase open reading frame, thereby rendering it non-autonomous. The complete PB{BmA3-EGFP} construct was approximately 2.6kb in length. The construct also contained a synthetic gene encoding a fluorescent marker. The well-established ability of the GFP and its derivatives to function as dominant, visible, non-destructive markers of insects (Brand, 1995), mammalian (e.g., Pines, 1995), and plant systems (Haseloff et al., 1997) were indicators of its potential use in pink bollworm. The gene encoding GFP (GFP gene) was cloned by Prasher (USDA–APHIS, Otis Air Force Base, Massachusetts), from the jellyfish, *Aequora victoria* (Cubitt et al., 1995; Heim et al., 1994; Heim and Tsien, 1996; Prasher, 1995; Prasher and Eckenrode, 1992). The best known derivative of GFP is a modified version with improved green fluorescence under blue light and with a reduced tendency to form insoluble aggregates of protein. EGFP is one improved derivative. The plasmid source of EGFP was purchased from Clontech, Inc.

Though transposon-based transformation systems have become well established for use in many insect species, they remain an inefficient system because only a low proportion of the candidate genetically modified insects generated actually carry the transgene. Therefore, a good marker system is required to enable these relatively rare transgenics to be identified from the many non-transformed individuals. By far, the most widely used marker system for pest insects is based on the expression of one or more fluorescent proteins of the GFP super family (Bevis and Glick, 2002; Matz et al., 1999; Shagin et al., 2004; Tsien, 1998). These proteins are intrinsically fluorescent, and cells or tissues that express these proteins at a suitable level can be detected by fluorescence microscopy. One such protein, EGFP, was used for the initial transformation of pink bollworm. All subsequent transformations of pink bollworm have also used fluorescent protein markers.

For the initial transformation of pink bollworm (Peloquin et al., 2000), the marker component was constructed by placing the coding sequence for EGFP between two other sequences, which were a promoter fragment from a silkworm gene and a polyadenylation signal from the SV40 virus. SV40 is the simian vacuolating virus 40. The *Bombyx mori* actin A3 (BmA3) promoter was cloned and modified by Steve Thibault, at the University of California–Riverside (UCR), from the embryos of the silkworm moth. In the silkworm moth, this promoter controls expression of a cytoplasmic actin gene. Cytoplasmic actin is a relatively abundant protein present in essentially every cell. It was, therefore, anticipated that this promoter could be used to express another protein, for example, GFP, at a reasonably high level in most or all cells of the silkworm moth and, by extension, in other moths including pink bollworm. Certain signals, for example, a polyadenylation signal, are required at the 3' end of a gene. The SV40 version has been shown to work in *Drosophila melanogaster* (for example in the widely used expression construct P{UAST} (Brand et al., 1994; Brand and Perrimon, 1993) and other species not closely related and was, therefore, thought likely to function as required in pink bollworm. This synthetic marker gene resulted in expression of EGFP in the transgenic pink bollworm.

Consequently, the transgenic individuals, at least as late larvae, are recognizably different from wild-type untransformed moths when viewed under a suitable fluorescence microscope.

Subsequent to the initial transformation described above, transformation of pink bollworm has been successfully performed at UCR, Oxitec Ltd., and the APHIS–CPHST, Decision Support and Pest Management Systems Laboratory (APHIS, CPHST, DSPMSL) in Phoenix, Arizona, using methods very similar to those described by Peloquin et al. (2000). For example, all subsequent transgenic pink bollworm have been constructed by microinjection into early embryos of a non-autonomous *piggyBac*-based system. All constructs have also carried a fluorescent marker (though the specific marker protein, the promoter used to drive its expression and other components of the synthetic marker gene, such as the 3' UTR) may also vary from those of the original PB(BmA3-EGFP) construct. Later constructs also contained additional functional components, notably a component designed to confer repressible dominant lethality.

Pink Bollworm Genetic Background

The transformed pink bollworm strains produced at UCR, Oxitec Ltd., or APHIS–CPHST–DSPMSL in Phoenix all originated from the mass-reared "C" stock of the Pink Bollworm Rearing Facility in Phoenix. The origin of this pink bollworm rearing facility stock is from commercial cotton fields located in the Colorado River Basin of California and Arizona. The pink bollworm strains maintained in the pink bollworm rearing facility have been in existence since at least 1970; however, the colonies are periodically out-crossed with endemic U.S. field populations of pink bollworm. The parental strain that was transformed was last out-crossed with wild-type pink bollworm in 1996.

In the molecular characterization of the first genetic transformation of pink bollworm (Peloquin et al., 2000), insertion of the *piggyBac* element into genomic DNA was detected by Southern blot analysis of one of the positive lines. The presence of at least two insertions was detected in this line with the probe recognizing two bands of approximately 1.9 kb and 2.3 kb, respectively. Individuals examined contained either one of the inserts or both. Based on inverse PCR and sequencing, the *piggyBac* integration appears to have been a singular event, which occurred in a transposase-dependent manner resulting in the expected TTAA target site duplication and with no plasmid sequences flanking the transposon ends. Immunoblot analysis, using a green fluorescent protein-specific antibody, was also used to differentiate expression of EGFP from auto fluorescence in wild-type insects, and to establish that the EGFP protein produced was the expected size. It also showed that no additional sequence was being translated into protein fused to the EGFP.

Phenotype of One Line Carrying PB(BmA3-EGFP), Compared to Wild-type

Of the transgenic pink bollworm strains produced by scientists at UCR (Peloquin et al., 2000), one strain (#35) was transferred to APHIS–CPHST–DSPMSL in Phoenix, Arizona, under USDA–APHIS permit No. 98–244–02m for movement of transformed insects between laboratories in Riverside, California, and Phoenix, Arizona. After 30 generations of rearing at the Phoenix Quarantine Facility, analysis by fluorescence microscopy found no evidence of change of expression of EGFP. Further studies found no differences in length of time spent in

larval instars, and the pupal stage in EGFP pink bollworm compared to non-transformed pink bollworm. However, the EGFP female moths had a number of minor defects or fitness costs, for example, reduced productivity in rearing (e.g., produced 19.8% fewer eggs than non-transformed APHIS strain pink bollworm (Miller et al., 2001)).

Autocidal or Lethal Genetic Systems

Radiation Sterilization

SIT is based on the large-scale rearing and release of sterile insects, which compete for mates with wild insects. (For a comprehensive discussion of SIT, see Dyck et al., 2005). Of paramount importance is that released insects need to be predominantly sterile for SIT to work. This sterility does not have to be 100% effective, but it does have to be reasonably close to 100%. The only method for sterilization currently in use is irradiation, in which gamma radiation from radioactive isotope sources (cobalt-60 [Cs-60 or ^{60}Co] or caesium-137 [Cs-137 or ^{137}Cs]) are used. This radiation ionizes atoms or molecules within cells. If enough of these ionizations occur, they can be destructive to biological organisms and can cause DNA damage. Ionizing radiation can cause mutations to future generations of the individual receiving the dose, which is the control concept for F_1 sterility. Irradiation injures the insect; therefore, the radiation dose used for sterilization is a compromise between a high dose, to get close to 100% sterility, and a low dose, to minimize the damage to the insect so it can compete for mates with wild-type male insects. Irradiation does not produce sterility in the classic sense, which is agametic sterility whereby individuals produce no functional gametes; instead, radiation induces dominant lethal mutations in the gametes, so that progeny derived from these gametes are not viable.

Dominance and Penetrance of Lethal Genetic Systems

Dominance is an important concept in classical genetics. Briefly, a fully dominant allele (or insertion of recombinant DNA) will show its full phenotypic consequence irrespective of whether the homologous chromosome has another copy of the same allele, or of any recessive allele. Conversely, the phenotypic consequence of a recessive allele will be masked if the homologous chromosome carries a dominant allele. Partially or incompletely dominant alleles show a stronger phenotype if homozygous rather than if heterozygous. Fluorescent marker genes sometimes express more fluorescence when homozygous then when heterozygous, to the extent that homozygotes show detectably brighter fluorescence than heterozygotes. In this case, the marker can be considered to be incompletely dominant. In other cases, no such difference between homozygous and heterozygous individuals can be discerned; therefore, the marker can be considered fully dominant, at least in respect of this means of assay.

Penetrance refers to the proportion of individuals of a certain genotype that show a phenotypic trait characteristic of that genotype. For example, if 5% of the individuals carrying a dominant lethal gene survive (where they would be expected to die), then the lethal gene is said to be have a penetrance of 95% (or 0.95). The penetrance of such a lethal phenotype is often higher when the inserted gene is present in two copies, for example, when homozygous (Fu et al., 2007).

Repressible *vs*. Inducible Lethal Systems

An autocidal system that was always active (on) would kill every individual that carried it; therefore, it would not be possible to rear a strain carrying such a system. Autocidal systems for SIT application need to be conditional so they are on under certain circumstances (conditions) and off under others. Those conditions that allow the strain to live, where the autocidal system is off, are called permissive; conditions where the system is on are called restrictive.

An engineered conditional lethal system might be repressible or inducible. In an inducible system, the effect is seen when a specific condition is applied, for example, heat treatment. In a repressible system, the effect is seen *unless* a specific condition is applied. Use of radiation is an inducible sterility system, in that the insects are fertile unless and until a suitable sterilizing dose of radiation is correctly applied. RIDL®, on the other hand, relies on a repressible lethal system. The system is repressed in the mass-rearing facility by applying an artificial condition, typically dietary tetracycline, but is not repressed and, therefore, active under other conditions, for example, on natural diet in the wild. This has the beneficial consequence that the RIDL® system is activated in escaped insects, or their progeny. This provides a fail-safe autocidal mitigation system to genetically modified insect SIT releases; this mitigation system is not readily available or effective in inducible systems.

Repressible Lethal Dominant Constructs

In principle, a genetics-based system could be used to remove the need for radiation as the sterilizing agent. Insects engineered to carry dominant lethal mutations in their gametes by genetics, rather than by irradiation, would provide the same effect as radiation sterilization (zygotic lethality) except without requiring irradiation. This approach is called "Release of Insects carrying a Dominant Lethal [gene or genetic system]" (RIDL®) (Alphey, 2002; Thomas et al., 2000). RIDL®, therefore, works in a way similar to radiation, especially for a non-sex-specific implementation of RIDL®. This would avoid the radiation damage to the insects' somatic and germ-line cells, the capital and recurrent costs of the irradiation process, and the need for a radioactive isotope source and its maintenance. One important variation of the basic system is the use of a female-specific lethal genetic system, which is somewhat different than the use of a system lethal to both sexes. Inherited radiation-induced lethals affect both sexes. This female-specific variant potentially allows genetic sexing, which is the removal of one sex, typically females, from the insect population during the mass-rearing process prior to release, resulting in release of males-only. This female-specific varient also could be used as a method of wild pest population control through the mating of released male insects with wild female insects, resulting in the subsequent death of female progeny through inheritance of the female-specific lethal genotype, and survival of males to pass this lethal trait on to other wild females. The use of repressible lethal systems is described, modeled, and/or discussed in a number of papers (Alphey, 2002; Alphey, 2007; Alphey and Andreasen, 2002; Alphey et al., 2007; Atkinson et al., 2007; Gould and Schliekelman, 2004; Heinrich and Scott, 2000; Horn and Wimmer, 2003; Phuc et al., 2007; Schliekelman and Gould, 2000b; Thomas et al., 2000; Wimmer, 2003). RIDL® is not the only potential way to eliminate the need for radiation; other autocidal systems have been described, as follows.

The autocidal system described by Fryxell and Miller (1995), and later modeled more extensively by Schliekelman and Gould (2000a), works differently than RIDL®. With this system, a gene is released to introgress into the wild population, which will kill them under some future triggering circumstance. The clearest example is a "diapause lethal" which kills the insects when they attempt to enter diapause at the onset of winter. Because of its cold sensitivity, the *Notch*^{cs} gene, used by Fryxell and Miller, would potentially have a similar effect, accumulating in the population during the summer due to mass-release of insects carrying it, and then killing all the insect progeny carrying it when the temperature drops. However, if the temperature frequently drops below the threshold temperature, this system becomes more like a RIDL® system, killing each individual insect that inherits the system. A potential difficulty with temperature as the lethal condition, particularly for field use, is the lack of control over this condition. For example, an unusually mild winter might render a cold-sensitive lethal ineffective.

Another autocidal system has been described by ARS researchers, Xavier and Handler (2006). As described in the following, these authors in a conference abstract posted on the Internet Web site: (http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=199795):

> "Proteasomes play a critical role in eukaryote development by regulating protein degradation. In *Drosophila*, mis-sense mutations in the 20S proteasome subunit lead to the production of dominant temperature-sensitive (DTS) "poison subunits" or antimorphs that disrupt proteasome function. DTS5 and DTS7 are two such mutations that result in late larval or pupal lethality at 29 ^{o}C. To study the potential of these genes to control the populations of tephritid fruit fly pests by conditional lethality, the *D. melanogaster* DTS5 mutation was genetically transformed into the Medfly, *Ceratitis capitata*, and the caribfly, *Anastrepha suspensa*. When reared at 30 ^{o}C transformed medflies homozygous for the transgene exhibited 90 to 95% late larval or pupal lethality, with lower lethality levels found in transformed caribflies. To enhance the temperature sensitive lethal effect we propose the use of native mutated proteasome genes in these species. The proteasome --2 subunit corresponding to DTS7 was isolated from an *A. suspensa* pupal cDNA library by gene amplification. Degenerate primers designed from the most conserved regions of insect DTS7 were used in combination with 5' and 3' adaptors, with subsequent isolation of DTS7 genomic DNA with gene specific primers. The *A. suspensa* DTS7 (AsDTS7) coding region contains 843 nts that potentially encode a 281 amino acid protein. Residues 40 to 224 comprise the proteasome beta domain conserved among eukaryotes, and the AsDTS7 amino acid sequence shares 85.7% identity to the *D. melanogaster* proteasome subunit. AsDTS7 transcript contains 1024 bp that is interrupted in the genome by 3 short introns ranging in size from 57-66 nts. Northern blot analysis indicates the presence of AsDTS7 transcript from embryonic through adult stages with quantitative variations during development, with an apparent maternal contribution to embryos. In vitro mutagenesis will be used to introduce the missense mutation in AsDTS7 that corresponds to the DTS7 mutation in *D. melanogaster*."

As with the system described by Fryxell and Miller (1995), this system is temperature-dependent; however, in the system of Xavier and Handler, increased rather than reduced temperature induces lethality.

Neuburger et al. (2006) describe apparently the same kind of temperature-sensitive lethal mutant system as that of Xavier and Handler with two dominant temperature-sensitive (DTS) lethal mutants of *Drosophila melanogaster*, which are *Pros26*^{1} and *Pros*β2^{1}, previously known as *DTS5* and *DTS7*. Heterozygotes for either mutant die as pupae when raised at 29 ^{o}C, but are normally viable and fertile at 25 ^{o}C. Previous studies have identified these as missense mutations in the genes encoding the β6 and β2 subunits of the 20S proteasome, respectively. To isolate additional proteasome-related mutants, a screen for dominant suppressors of *Pros26*^{1} was carried out, resulting in the identification of *Pros25*^{1SuDTS} (originally called *Su*(*DTS*)), a missense mutation in the gene encoding the 20S proteasome α2 subunit. *Pros25*^{1SuDTS} acts in a dominant manner to rescue both *Pros26*^{1} and *Pros* β2^{1} from their DTS lethal phenotypes. Using an in vivo protein degradation assay, it was shown that this suppression occurs by counteracting the dominant-negative effect of the DTS mutant on proteasome activity. *Pros25*^{1SuDTS} is a recessive polyphasic lethal at ambient temperatures.

Molecular Components of RIDL® Systems

The molecular constructs inserted into RIDL®, or other autocidal strains of pink bollworm or fruit flies, comprise several independent functional modules. These include the following:

1. a system for transferring DNA into the insect's genome,
2. a marker system for determining when this has been done and for monitoring the presence of the element in subsequent generations,
3. an autocidal or effector module, which confers repressible lethality or sterility.

Marker systems are discussed above, as are the transposon-based transformation systems used in insect genetic engineering. The molecular biology of the autocidal systems are presented in the following discussion.

Various methods for regulating gene expression are known that could be adapted to make a conditional autocidal system. One early proposal was to use temperature, specifically to use a cold-sensitive mutant version of the developmental signaling gene called *Notch*, which would kill individuals carrying this gene if they were exposed to low temperature (Fryxell and Miller, 1995). The system described by Xavier and Handler (above) is also temperature-dependent, though, in this case, it is high rather than low temperature that induces lethality. The basis of the conditionality of the RIDL® system, developed by Oxitec, is the 'tet-off' gene expression system (Gossen et al., 1994; Gossen and Bujard, 1992). In this system, gene expression is silenced in the presence of tetracycline, or tetracycline-like chemicals, which can, therefore, be used like an "antidote" to switch off the lethal system. The tet-off expression system is based on a synthetic fusion protein called "tTA" for tetracycline-repressible transactivator.

tTA as a Synthetic Transcription Factor

tTA is a synthetic transcription factor formed by fusing a sequence-specific DNA binding protein from a bacterial gene expression system (tetR) to a eukaryotic transcriptional enhancer from herpes simplex virus (VP16). This fusion protein has three key properties:

1. It binds to a short, specific, DNA sequence, known as the tet operator (tetO). The core 19bp (base pair) sequence of tetO to which tTA binds is 5'-TCCCTATCAGTGATAGAGA-3'.
2. When bound to tetO, tTA acts as a eukaryotic transcriptional activator, by virtue of the transcriptional activation domain from VP16. In effect, this means that tetO, in the presence of tTA protein, acts as a transcriptional enhancer, stimulating gene expression from nearby receptive promoters.
3. tTA, like tetR, binds tetracycline and suitable tetracycline-like chemicals with high affinity. The tetracycline bound form of tTA (or tetR) does not bind DNA. Therefore, in the presence of modest concentrations of a suitable tetracycline agent, tTA does not bind to DNA and, therefore, does not act as a transcriptional enhancer.

The net effect of these three properties is that tetO acts as a regulatable transcriptional enhancer in eukaryotes. tetO can be regulated in at least two ways:

1. Level of tTA: In the absence of tTA protein, tetO is inert.
2. Level of tetracycline: If tTA is present and tetracycline is absent, then tetO will act as a transcriptional enhancer. However, addition of tetracycline will prevent binding of tTA to tetO and, therefore, prevent it acting as an enhancer, even though tTA protein is present in the cell.

tTAV

The original tTA protein, described by Gossen and Bujard (1992), was not optimal for expression in insects or other eukaryotes. In particular, it included several potential cryptic splice sites; this means that a proportion of the primary transcripts may be spliced into mature mRNAs incapable of expressing functional tTA. To the extent that this happens, less tTA-encoding mRNA will be produced than anticipated or intended and, therefore, less of the effector molecule and potentially a lesser phenotypic expression. Subsequently, scientists have modified the sequence in various ways to improve expression or function of the protein. One such modified sequence was termed tTAV (Gong et al., 2005). To improve expression, the nucleotide sequence was modified primarily with silent substitutions to the nucleotide sequence, for example, to remove cryptic splice sites and/or adjust the codon usage. These modifications made only minor changes to the amino acid sequence encoded by the modified nucleotide sequence and are not predicted to significantly affect the properties of the encoded protein.

One- and Two-Component RIDL® Systems

In the original proof-of-principle implementation in Drosophila, the molecular configuration of the RIDL® systems comprised a two-component system (Thomas et al., 2000). A later implementation in the Medfly used a one-component system, in which tTA was placed under the

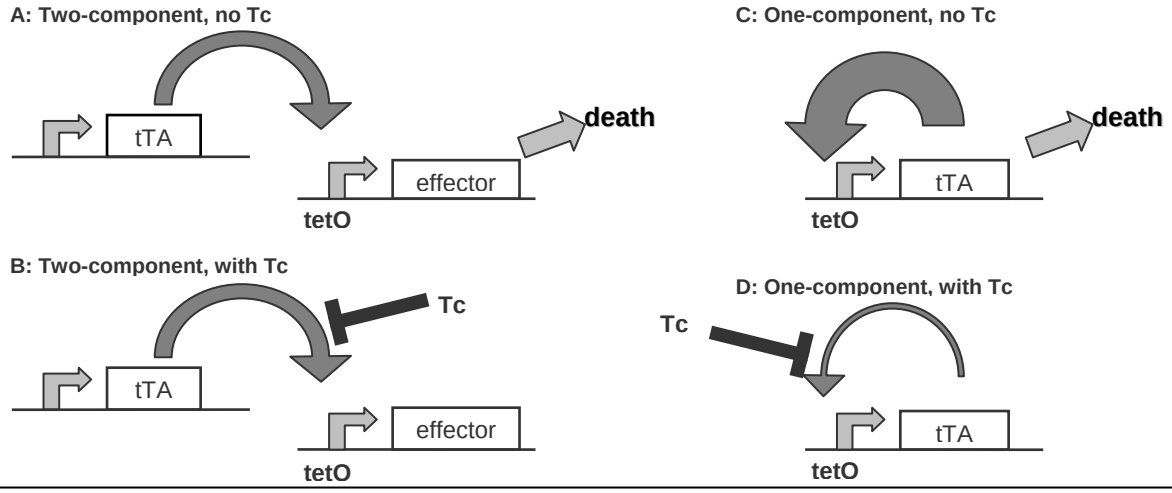

Figure 2: Tetracycline-repressible lethal systems.
A, B: Two-component system as previously published (Heinrich and Scott, 2000; Horn and Wimmer, 2003; Thomas et al., 2000). tTA (Gossen and Bujard, 1992) is placed under the control of a suitable promoter, e.g. constitutive, female-specific, embryo-specific, etc. A: In the absence of tetracycline (Tc) tTA binds tetO, drives expression of an effector leading, in the case of a lethal effector, to death. B: In the presence of tetracycline (Tc), tTA binds Tc; the Tc-bound form does not bind DNA, and therefore does not activate expression of the effector and the system is inactivated. C, D: is a simplified one-component system. C: In the absence of Tc, basal expression of tTA leads to the synthesis of more tTA, which accumulates to high level. This level can be regulated by modifying the stability and translational efficiency of the tTA mRNA. At the highest levels, expression is lethal, so tTA is both the driver and the effector. D: In the presence of Tc, tTA is inactivated by Tc and is therefore expressed only at basal levels. [adapted from figure 1 of (Gong et al., 2005)]

control of a minimal promoter linked to tetO (Gong et al., 2005). These two systems are illustrated in the following figure, adapted from figure 1 of Gong et al. (2005)]. Tc is tetracycline

Effector Molecules

The only requirement of an effector molecule is that it performs the desired effect (e.g., lethality or sterility) when induced and not when repressed or under the restrictive condition(s). In practice, this means that the effector molecule has to be selected in conjunction with the rest of the expression system. No expression system is perfectly regulated, that is, fully expressed under one condition and fully repressed under another; rather, there will be a relatively high level of expression under "induced" or "not repressed" conditions, and a relatively low level of

expression under non-induced or repressed conditions. The expression system must be designed so that the desired trait is expressed at the higher level of expression, but not under the lower level. For a lethal effector, it is important that the insects are not only viable at the lower level of expression, but also that their performance is not impaired—in other words, that expression under repressed conditions ("basal expression") is not only non-lethal, but also no more than minimally harmful. The actual repressed and unrepressed expression levels are determined by a combination of factors, which may include some or all of the following:

1. the activity of the promoter and enhancer elements in the construct, which may themselves be influenced by the site of insertion into the insect's genome,
2. the stability of the RNA(s) produced,
3. the efficiency of processing and translation of these RNAs for a protein effector, and
4. the stability of the effector molecule(s).

Pro-apoptotic Proteins

The cell is like a finely tuned machine with a multitude of proteins produced at the appropriate time and level for the cell to function correctly. Incorrect timing or level of production of any of a large number of endogenous or exogenous proteins may disrupt a cell's function, either killing it or changing its function in a way that might, in turn, disrupt or kill the function of the whole organism (for example, converting presumptive nerve cells into muscle cells). Therefore, it has not been found necessary to use effector molecules that might normally be considered toxins, for example, proteins such as ricin-A or diphtheria toxin (each of which have been described in the scientific literature as useful for cell ablation studies in *Drosophila* (Allen et al., 2002; Bellen et al., 1992; Han et al., 2000; Hidalgo et al., 1995; Moffat et al., 1992), but rather to use pro-apoptotic or cell signaling molecules, either naturally occurring ones (e.g., from *Drosophila*), or mutant versions thereof. Pro-apoptotic proteins are naturally occurring proteins involved in programmed cell death, a natural part of cell function and also an ancient defense against pathogens, in which cells may self-destruct to prevent pathogen replication. Two pro-apoptotic proteins that have been used in this context are *Hid* and *Reaper*, the products of the *Drosophila* genes, *head involution defective (hid)*, and *reaper* (*rpr*), respectively. These proteins are thought to bind to the anti-apoptotic protein IAP1 (in *Drosophila,* the product of the *Wrinkled* gene) and destabilize it (Hay and Guo, 2006; Yin and Thummel, 2004; Yokokura et al., 2004). *Hid* and *Reaper*, and other pro-apoptotic gene products from *Drosophila* and elsewhere, have been extensively used in *Drosophila* and other species to induce apoptosis; mutant versions of these proteins with higher activity have also been used, for example Hid^{Ala5} (Horn and Wimmer, 2003).

tTA As Effector Molecule

Another effector molecule that has been used is tTA. In the one-component "positive feedback" system, tTAV acts both as a repressible transcription factor and as the effector (Gong et al., 2005; Phuc et al., 2007). Low level expression of tTA has been widely used in gene expression studies and is thought to be innocuous; whereas, a high level expression of tTA is thought to be deleterious to cells, probably due to transcriptional "squelching" and/or interference with ubiquitin-dependent proteolysis (Berger et al., 1990; Damke et al., 1995; Gillespie et al., 1997;

Gong et al., 2005; Gossen and Bujard, 1992; Salghetti et al., 2001). tTA is a relatively unstable protein due to the presence of an ubiquitin degron. A degron is a specific sequence of amino acids in a protein that directs the starting place of degradation. It is not secreted or absorbed by cells in a functional form due to cell-autonomous effects on gene expression. Even if it were somehow taken up into another cell, modest amounts of the protein have no effect unless a specific tetO-based gene expression cassette is also present in the cell. A cassette is a pre-existing structure into which an insert can be moved.

RNA Effectors

Effector molecules do not have to be proteins. Some RNAs have biological activity, including ribozymes, anti-sense, and hairpin RNAs. Double-stranded RNA has been used to silence target genes in a sequence-specific manner in a wide range of eukaryotes including insects (Dietzl et al., 2007). A lethal RNA-based effector could be constructed by targeting an essential gene. It is also possible to modify the sexual phenotype of insects by silencing specific genes involved in sex determination (Dietzl et al., 2007; Fortier and Belote, 2000; Pane et al., 2002). Gene silencing by dsRNA is extremely sequence-specific requiring substantial sequence identity to function. Single and double-strand RNAs are produced by all eukaryotes and effector molecules of this design would have no effect on another species, such as a predator or parasite, by contact or ingestion.

Female-specific RIDL® Systems

In order to make the phenotype arising from expression of an effector molecule be female-specific, either the effector molecule needs to be expressed at a higher level in females in the whole insect or in one or more female tissues, than in males; or, the effector molecule needs to have a differential effect on females (or female cells) relative to males (or male cells). Each of these approaches has been demonstrated in *Drosophila* (Thomas et al., 2000); however, few candidate effector molecules may be expected to show differential effects on females vs. males. Female-specific expression might be achieved by use of a female-specific promoter to drive expression of tTA in a two-component expression system. In this case, tTA would only be expressed in females and males would not, therefore, express the effector at a high level whether provided with the repressor or not. Females, in contrast, would express the effector molecule unless provided with a repressor, such as dietary tetracycline. This approach was demonstrated in *Drosophila* in experiments in which tTA was placed under the transcriptional control of promoter or enhancer elements from yolk protein genes (Heinrich and Scott, 2000; Thomas et al., 2000).

Sex-specific alternative splicing has been used, as another approach, to achieve female-specific expression of an effector molecule. In this approach, the tTA open reading frame (ORF) was disrupted by inserting a sequence from *Cctra* (Fu et al., 2007). An ORF is the part of the gene that is used to start the production of some RNA from a gene made of DNA. *Cctra* is the Medfly homolog of the *Drosophila* gene *transformer* (Pane et al., 2002). In a class of transcripts produced only in females, this *Cctra* sequence is spliced out, leading to the production of an mRNA encoding tTA. The classes of mRNA, produced in males, all contain stop codons and/or frame shifts in the tTA open reading frame and so do not encode functional tTA; therefore,

functional tTA is produced only in females. This approach has several advantages. Female-specific promoters may be difficult to identify in a particular species, especially ones that express early in development. In contrast, the female-specific splicing systems are likely to function correctly from a very early developmental stage, and in most or all tissues. The system has only been demonstrated, so far, for *transformer* homologs; however, several other genes showing sex-specific splicing are known. One such gene is *doublesex*; like *transformer*, *doublesex* is involved in sex-determination. Homologs of *transformer* have so far only been identified in higher Diptera, though they may well exist in other taxa; however, *doublesex* is much more highly conserved with recognizable homologs in a wide range of insects (e.g., silkworm (Saccone et al., 2002; Suzuki et al., 2001), and even vertebrates. Sex-specific splicing of *doublesex* appears to be a fundamental regulatory mechanism for insect sex determination, therefore, suitable splicing systems should be obtainable for a wide range of species.

Stage of Expression of RIDL® System and Lethal Phase

Sterile-release programs, regardless of the sterilization method used, work by reducing the size and reproductive potential of the target population by killing progeny that would otherwise survive and reproduce. In this respect, mortality, at any developmental stage prior to reproductive maturity, is functionally equivalent; however, in some cases, it may be desirable to have the affected individuals die at a particular developmental stage. One reason might be to prevent crop damage by immature stages. Mortality very early in development, such as an embryonic stage, might be desirable for this purpose; however, in a density-dependent population with significant competition for resources at the larval stage, mortality at a post-competition stage, such as pupal, might be preferred (Atkinson et al., 2007; Phuc et al., 2007).

Radiation, as typically used for sterilization in SIT programs, induces random dominant lethal mutations in the gametes of irradiated individuals. These are inherited by their progeny which die, typically at an early embryonic stage of development. An alternative strategy, which has been used on at least a small scale for some Lepidoptera, is to use a lower dose of radiation with which the affected individuals remain partially fertile (Carpenter et al., 2005). Some of their progeny die at various stages of development, but others survive to adulthood; however, with an appropriate radiation dose, these F_1 progeny are essentially 100% sterile. This is, therefore, referred to as F_1 sterility. Because F_1 sterility depends on an unusual feature of the response of Lepidoptera to radiation, it may not be readily applicable to other taxa.

RIDL® systems could, in principle, be designed to induce mortality at any specified developmental stage; however, the state-of-the-art knowledge of insect molecular biology is not yet advanced enough to allow a great deal of sophistication or precision in this regard. The yolk protein-based, female-lethal systems first constructed in *Drosophila* (Heinrich and Scott, 2000; Thomas et al., 2000) presumably caused affected individuals to die at a late larval or pupal stage, as this is the time at which the yolk protein genes and promoters are thought to be active. It was subsequently shown that a similar system, replacing the yolk-protein promoter with an embryo-specific promoter from the *serendipityα* (*sryα*) gene of *Drosophila melanogaster*, could give embryonic lethality (Horn and Wimmer, 2003). Unfortunately, embryo-specific promoters are not yet known for most pest insects.

Present RIDL® systems lead to mortality in affected individuals at various developmental stages, depending on the design or genetic construction and the species concerned. The "positive feedback" systems of Gong et al. (2005) gave mortality typically at a late larval stage in the Medfly. Strains have been developed for mosquitoes with mortality predominantly at either an early pupal stage or an early larval instar (Phuc et al., 2007).

(This page is intentionally left blank.)

Appendix D. Risk Assessment Criteria and Analyses for Genetically Engineered Fruit Flies and Pink Bollworm

Risk Assessment Criteria and Analyses for Genetically Engineered Fruit Flies and Pink Bollworm

Much of appendix D is modified after the technical document on risk assessment of the use of genetically engineered arthropods in plant protection, prepared by a joint meeting of the United Nations Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA, 2006). This document will be referred to as the IAEA/FAO report throughout this appendix. (Note: The formal citation is included in the reference list.) The IAEA/FAO report addresses genetically engineered arthropods that affect both plants and human health; however, the emphasis of our discussion focuses primarily on plant pests, such as fruit flies and pink bollworm. Risk assessment for release of genetically engineered arthropods must be done on a case-by-case basis because there are different methods to produce genetically engineered arthropods, the constructs and their expressions vary greatly, and the interactions of the organism with the environment also vary greatly. Therefore, not all of the criteria in the IAEA/FAO report may be addressed in this risk assessment because they may not be applicable due to the immobilization of the transposon, the self-mitigating or self-restricting nature of the SIT function, and how they are used in APHIS cooperative SIT programs.

Identification of Risks Associated With A Genetically Engineered Insect Release

Risk Analysis

The IAEA/FAO report focuses on identifying potential hazards but does not address hazard assessment, exposure assessment, or risk assessment; therefore, hazards may occur with varying probabilities (e.g., improbable, frequent, highly probable), and the consequences of these hazards can vary in magnitude. Risk is the product of the probability of a hazard to occur times the magnitude of the consequences. Typically, risk analysis includes three stages: risk assessment, risk management, and risk communication. The risk assessment stage is traditionally divided into four stages:

- hazard identification;
- hazard assessment (describing the hazards);
- exposure assessment (e.g., assessing the probability, likelihood, exposure, or frequency of a hazard); and
- assessment of consequences (including assessing the magnitude of the economic, environmental, cultural, and social consequences).

The International Plant Pest Convention (IPPC) pest risk analysis procedures (IAEA/FAO Report p. 17) follow this same general procedures for risk analysis using the following stages:

- initiation (pest or pathway initiated);
- pest risk assessment (including pest categorization, estimating introduction and spread potential, probability of introduction and spread, and estimating consequences of introduction and spread); and
- pest risk management (including the determination of acceptability of risk and identification of risk management options).

Hazards (IAEA/FAO Report p. 17) may be associated with the genetically engineered arthropods, in general, with the genetic construct used, or with specific genes, promoters, or sequences in the constructs, or the gene product(s). Distinction should be made between hazards associated with the unmodified organism and those unique to the genetically engineered arthropod. In order to be as comprehensive as possible, potential hazards are listed without considering specific genetically engineered arthropods. These are shown in table D–1, which is not necessarily a complete list.

Table D–1. Potential Hazards Associated With the Release of Genetically Engineered Arthropods (IAEA/FAO Report p. 18).

Area of Interest	Source of Hazard	Hazard	Significance
Environment	Change to biology of organism	Change in host range	Increased disease transmission
		Change in environmental tolerances (temperature, humidity, etc.)	Potential spread to new areas
		Changes in other aspects of physiology (reproduction, pesticide resistance, susceptibility to post harvest treatment)	Compatibility with other pest management programs
		Change in reproductive behavior	
		Change in feeding behavior on normal hosts	Feeding more frequently may increase host exposure to pathogens
		Change in pathogenicity	Increase pathogenicity
		Change in timing or length of development	Disease transmission issues
	Effects on nontarget organisms	Change in suitability of LMO to parasites or predators	Reduction in natural enemies
		Adverse effects on other beneficials (e.g., pollinators)	Altered pollination
		Effects on symbionts, including gut symbionts, pathogens, etc.	Altered survivorship, fitness, etc.
		Adverse effects on soil species/ community (or aquatic env.) (e.g., accumulation in soil of genetic material or gene product)	Changes in soil productivity
	Stability of construct in organism	Recombination potential	Various effects on ecosystem
		Mobility of the gene	
		Transfer of transposable element by hybridization	
		Horizontal transfer to a related species	
		Horizontal transfer to other organisms including soil organisms or symbionts	
Human Health	Genetic modification	Change in pathogenicity of parasites	Could arise from horizontal transfer of gene from mosquito to parasite
		Change in host range of mosquito	Change in disease transmission potential
Cultural/ Social/ Economic	Genetically modified organism	Impact on ecosystem	Cultural and religious concerns; effects on tourism or other industries; loss of trade opportunities

G)

Table D–2 compares two scenarios, genetically engineered sterile Medflies containing a gene for a marker (e.g., GFP) and unrestricted release of genetically engineered mosquitoes transformed using transposable elements, representing two extremes of how genetically engineered arthropods may be released. The scenario for the fruit fly is one that has been transformed using a construct with a very low or negligible mobility, and in which fruit flies are sterile. Under these circumstances, the IAEA/FAO Report Working Group considered that the probability of

most hazards occurring would be very low. Likewise, the risk (i.e., hazard x probability x consequences) would be very low or negligible.

It is important that each new application for the development and release of a genetically engineered arthropod be considered on a case-by-case basis because generalized assumptions regarding hazard and risk can be irrelevant.

Table D–2. Case-specific Examples of Hazards. (FF denotes sterile Medfly containing the GFP marker gene. Mosquito denotes fertile mosquito containing a transposable element (IAEA/FAO Report p. 19)).

Hazard	Fruit Fly	Mosquito
Change in host range	0	+++
Effects on symbionts including gut symbionts, pathogens, etc.	0	+++
Adverse effects on soil species/community (or aquatic environment) e.g., accumulation in soil of genetic material or gene product)	+	+
Change in suitability of genetically modified organism to parasite or predators	+/0	+
Horizontal transfer to other organisms including soil organisms or symbionts	0	+
Horizontal transfer to a related species	0	++

("0" denotes a neutral change or an effect that is not predicted to cause any significant risks; "+" denotes a change that could result in low or moderate risk; "++" denotes a change that could result in medium to high risk.)

Development of Risk Assessment Protocols (IAEA/FAO Report p. 19):

Lack of relevant experience in releasing genetically engineered arthropods warrants a cautious, incremental approach to the development and implementation of the technology. Release of genetically engineered arthropods for pest management programs should occur only after stepwise evaluations of identified hazards and potential risks in field trials. Table 1 includes questions relevant to contained field trials and release programs that will not result in establishment of the genetically engineered arthropod in the environment. Tables 1 and 2 are based on considerations by Tiedje et al. (1989), but were expanded or revised to reflect issues relevant to arthropods. Possible consequences considered include effects on nontarget species and biodiversity, disruption of ecosystem functions, threatened and endangered species, and disruption of genomes of nontarget species. These tables reflect the present knowledge, and these issues may change as more experience is gained.

Horizontal gene transfer has principally been an issue among microorganisms, such as drug resistance among prokaryote bacteria, which is a human health concern.

Definitions (IAEA/FAO Report p. 21):

(a) A genetically engineered arthropod is an arthropod that possesses a novel combination of genetic material obtained through the use of modern biotechnology.
(b) The accessible environment consists of the region into which the organism will be released and the areas into which it could spread.
(c) A donor organism is one from which genetic material was obtained to create the genetically engineered organism.
(d) A recipient organism is the one into which the donor material has been introduced.

The IAEA/FAO (page 21) considered several potential purposes for release of genetically engineered arthropods, with different intrinsic risks and hazards associated with them:

(i) short-term presence in the environment with a low risk of establishment; for example, the autocidal control of a targeted pest population using the SIT. In that example, even if some small percentage of the released arthropods are not sterile or the released arthropods demonstrate F_1 sterility, the persistence of these organisms, or alleles, is unlikely due to loss of intrinsic fitness relative to naturally occurring organisms or alleles.
(ii) release of fertile genetically engineered or paragenetically engineered arthropods carrying a "suicide" trait or one of reduced fitness to that some intended purpose may be achieved by the released individuals or their progeny carrying self-limited suicide alleles, but not through their establishment in the environment.
(iii) releases expressly intended for establishment of an allele or an organism in the environment for control of a pest or disease over time. Retrieval of the released organisms is difficult, if not impossible.

Of these purposes for release of genetically engineered arthropods, the release of genetically engineered insects being considered by APHIS corresponds to case (i): short-term presence in the environment with a low risk of establishment; autocidal control in SIT was used as a specific example of this category.

Short-term Presence in the Environment With A Low Risk of Establishment

The following section addresses field use of genetically engineered arthropods for evaluation, involving short-term presence following each release, and low risk of establishment in the environment. The following is a list of questions, or risk assessment criteria, developed by IAEA/FAO that may need or may not need to be considered when conducting a risk assessment, depending on the case-by-case circumstances. Following the questions or criteria is a discussion in relation to fruit fly and pink bollworm risk assessment.

Attributes of the unmodified, recipient arthropod (IAEA/FAO Report p. 22):

(a) Is the arthropod subject to regulatory control?
(b) Taxonomy and distribution
- What is the origin and current distribution of the recipient species?
- What is its normal dispersal range?
- Is the recipient strain recognized as a specific biotype or strain? If so, what are the distinguishing characteristics?
  (1) What is the specific origin (point of collection) or acquisition location of the recipient strain?
  (2) Has the recipient and genetically engineered arthropod strain been identified by a qualified taxonomist and voucher specimens deposited in a permanent location to allow future morphological reconfirmation and isolation of DNA by conventional methods (e.g., after preservation at -80 °C or in 95% ethanol)? Where were the voucher specimens deposited?

Ecological Relationships And Roles of the Unmodified, Recipient Arthropod (IAEA/FAO Report p. 22):

(a) What is the recipient arthropod's trophic level (parasitoid, predator, parasite, plant feeder, or vector of animal or plant diseases) and host range?
(b) If the arthropod is a vector of plant, animal, or human disease(s), what are these?
(c) What other ecological relationships does the arthropod have?
(d) Is the arthropod involved in basic ecosystem functions and processes (e.g., decomposers, pollination)?
(e) What are the environmental limits to growth or reproduction (habitat, microhabitat)?
(f) How does the arthropod survive during periods of environmental stress?
(g) What is the potential for gene exchange with other populations of the same or related species (before modification)?
(h) What methods are available for detection of the arthropod, and what is their specificity, sensitivity, and reliability?

Attributes of the Genetic Alteration (IAEA/FAO Report p. 17):

(a) What is the intent of the genetic alteration (e.g., marker, altered function)?
(b) Have similar components of the present genetic material been evaluated in field tests, and if so, how do the present components differ?
(c) What is the nature and function of the genetic alteration?
- From what organism(s), are the transgene and molecules derived that were used to produce the genetically engineered organism? Describe any synthetic portions.
- What is the range of function of the components (e.g., effector expression or promoter function in other organisms)?
- Is the transgene donor (parental organism) pathogenic or subject to regulatory control?

(d) By what mechanism was the alteration made?
(e) What are the structures of the molecules used to alter the genome (primary sequences, maps and peptides)?
- Describe from what sources the above structural information was obtained and any additional confirmation.
- Are there undetermined sequences present in the inserted material or sequences not necessary for the intended effect?
- How many copies of the alteration(s) are present and what is known about each?
- Where is the alteration in the genome? (nuclear, mitochondrial, plasmid, symbiont, DNA sequence of the insertion site.)
- How stable is the genetic alteration?
- What is the mode of inheritance of the alteration(s), and how was this demonstrated—
  (1) in the laboratory?
  (2) in a contained environment similar to the release site?
- Is the copy number, sequence, and location of the insertion(s) stable, and how was this demonstrated—
  (1) in the laboratory?
  (2) in a contained environment similar to the release site?

Phenotype of the Modified Organisms Compared to the Unmodified Organism (IAEA/FAO Report p. 23)—

Organism, in this section, refers to the arthropod alone, or to the arthropod and symbiont in cases of paragenetically engineered arthropods. In the latter case, responses to the following questions should consider both organisms.

(a) Have strains similar to the present material been evaluated in field tests, and if so, how does the present strain differ?
(b) What function has been deliberately enhanced, introduced, or diminished?
(c) Have any phenotypic traits been modified unexpectedly by the introduced alteration?
(d) What is the host/prey range relative to the unmodified organism?
(e) Are there detectable changes in behavior (e.g., mating, dispersal)?
(f) Have changes in life table attributes occurred in the altered strain?
(g) What is the level and pattern (stage, tissue) of expression of the trait?
(h) Does the altered phenotype persist in any way in dead material?
(i) Has the alteration changed the organism's susceptibility to control by natural or artificial means?
(j) Have the environmental1imits to growth or reproduction (habitat, microhabitat) been altered as a result of the modification?
(k) Has the alteration affected the expression of an existing gene(s)?
- Is the alteration in a gene?
- What is its effect on that (or other) genes' function?

(l) What quality control measures are available to detect changes in the desired function of the material during production or after release?
(m) What detection methods are available to distinguish the modified from unmodified material and what is their specificity, sensitivity, and reliability?

Attributes of the Accessible Environment (IAEA/FAO Report p. 17)—

(a) Describe the accessible environment or dispersal range, given the field conditions. (Under the IPPC process, this is the "endangered area.")
(b) Are there artificial or natural agents that could move the genetically engineered or paragenetically engineered arthropod or genetic components from within the release environment? What are they?
(c) Are there alternative hosts or prey in the accessible environment?
(d) What relatives/related arthropods occur within the accessible environment or dispersal range?
(e) Are endangered or threatened species present that could be affected?
(f) How effective is the monitoring of the goals of the release? Monitoring for unintended consequences?
(g) How can unintended and undesired outcomes be reversed (bioremediation)?
- Who would finance and implement the process and how much would it cost?
- How has the feasibility of this bioremediation been verified?
- What are the social, economic, and environmental consequences if this remediation is required and conducted?

(h) What monitoring survey programs are in place to assess the characteristics of the modified arthropod population?

Risk Assessment Discussion Pertaining to Genetically Engineered Fruit Flies and the Pink Bollworm

Attributes of the Unmodified, Recipient Arthropod (IAEA/FAO Report p. 17):

(a) Is the arthropod subject to regulatory control?

Pink bollworm and the Medfly, oriental fruit fly, and Mexican fruit flies are invasive plant pests subject to regulatory control in the United States, which is fully described elsewhere in the EIS and following referenced environmental documents.

(b) Taxonomy and distribution

Besides information available in textbooks of economic entomology, such as Destructive and Useful Insects by Metcalf et al., (1962) regarding fruit flies and pink bollworm, APHIS has published extensive environmental documents concerning these important plant pests. The taxonomy and distribution are reviewed elsewhere in the EIS and in the following most recent APHIS environmental documents for species or families of interest:

Fruit Fly Control Programs—

- Mexican Fruit Fly Cooperative Eradication Program, Laredo, Texas, Environmental Assessment (USDA–APHIS, 2007b)
- Oriental Fruit Fly Cooperative Eradication Program, Rialto, San Bernadino County, California, Environmental Assessment (USDA–APHIS, 2006c)
- Mediterranean Fruit Fly Cooperative Eradication Program, Rancho Cucamonga, San Bernadino County, California, Environmental Assessment (USDA–APHIS, 2005c)
- Fruit Fly Cooperative Control Program, Final Environmental Impact Statement (USDA–APHIS, 2001a)

Pink Bollworm Eradication Program—

- El Paso/Trans Pecos Pink Bollworm Cooperative Eradication Program, Environmental Assessment (USDA–APHIS, 2001c)
- Southwest Pink Bollworm Eradication Program, Environmental Assessment, (USDA–APHIS, 2002a)
- Pink Bollworm Eradication Plan in the U.S. (Greffenstette et al., 2005)

Pink Bollworm Genetic Engineering—

- Confined Study of a Genetically Engineered Pink Bollworm, *Pectinophora gossypiella* (Lepidopera: Gelechiidae), Environmental Assessment (USDA–APHIS, 2001b)
- Field Study of Genetically Modified Pink Bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), (USDA–APHIS, 2005a)

Ecological Relationships and Roles of the Unmodified, Recipient Arthropod (IAEA/FAO Report p. 22)—

Refer to the above listed environmental assessments, other documents, and other sections of the EIS for detailed information concerning the ecological relationships and roles of the unmodified recipient arthropods, which are well known invasive plant pests of important crops grown in the United States and other countries. These references and resources address trophic levels, plant pest disease attributes, ecosystem functions, environmental limits, diapause, sexual compatibilities, and detection or surveillance methods.

Attributes of the Genetic Alteration (IAEA/FAO Report p. 22)—

The attributes of each genetic alteration must be considered on a case-by-case basis because of the great diversity of traits possible from genetic engineering, and the even greater diversity of organisms and their interactions with the environment.

Appendix C of the EIS, Repressible Lethal and Marker Genetic Engineering with Analysis of Issues Pertaining to Transposon Mobility and Potentiation of Horizontal Transfer for Technology Under Development, by APHIS, describes the biotechnology in depth, and the primary mitigation against risk for the relevant parameters under *Attributes of the Genetic Alteration*. Thus far, only fluorescent protein marker genes have been tested in the field under permit in pink bollworm, but have not been tested in the field with fruit flies. Field cage-contained studies have been conducted for pink bollworm RIDL® repressible lethal strains under permit. Stability of the fluorescent protein marker construct has been examined by APHIS, CPHST over many generations for pink bollworm, and laboratory stability studies have been done for some genetically engineered strains of fruit flies.

The proposed genetic engineering traits for both fruit flies and pink bollworm are for use in application of SIT to APHIS cooperative invasive plant pest control programs. The principles of SIT are described elsewhere in the EIS and in the environmental document references provided above. Since SIT is based on release of sterile insects to mate with wild-pest populations in the field, it is essentially a self-extinguishing or self-limiting pest control technology in which wild cohorts in the field do not inherit the sterility trait, except to a limited degree in F_1 sterility for pink bollworm, in which the sterility trait is inherited in the offspring, which are then sterile. Therefore, genetic engineering, when used as a component of SIT, is self-mitigating in respect to most of the possible and theoretical hazards and risk that may be associated with arthropod genetic engineering.

The genetic SIT system is repressed in the mass-rearing facility by applying an artificial condition (e.g., dietary tetracycline in the case of the RIDL® system of Gong et al., (2005) and Fu et al. (2007)), but is not repressed and, therefore, active under other conditions (e.g., on natural diet in the wild). This has the beneficial consequence that the system is activated in released or escaped insects, or their progeny. This provides a "fail-safe" type of "autocidal" or lethal mitigation system to genetically engineered insect SIT releases or inadvertent escapees.

The traits being considered for use in APHIS' cooperative pink bollworm and fruit fly invasive pest programs are the following: (1) development of genetically engineered fruit flies and pink

bollworm with marker gene traits for use in SIT programs that allows these insects to be distinguished by suitable optical systems from wild fruit flies and pink bollworm caught in insect monitoring traps; (2) development and use of genetically engineered male-only production for sterilization by irradiation in fruit fly control programs; (3) development and use of genetically engineered male-only fruit fly production with field release of males that produce only male offspring, which then pass on an inherited lethal trait that prevent female offspring from occurring; and (4) development and use of genetically engineered sterile insects without irradiation for the pink bollworm eradication program.

Fruit Fly Control Programs

The adverse environmental consequences, effects, or impacts of using genetically engineered fruit flies with a marker gene trait, such as a protein that fluoresces under specific wavelengths of light, would be no more significant than the continuation of the present SIT fruit fly programs, as described in the no action alternative of the EIS, because the mass-reared fruit flies would be sterilized by radiation and produce practically no offspring. Their release would also result in elimination of the fruit fly population in the area of release. The genetically marked fruit flies are easily distinguished by their fluorescence from wild fruit flies caught in insect traps baited with pheromones or other insect attractants used to monitor the dispersal of the SIT fruit flies and evaluate program effectiveness.

The adverse environmental consequences of using genetically engineered fruit flies that produce only males and no females in the mass-rearing process would also be of no more adverse environmental significance than the continuation of present SIT fruit fly programs because the mass-reared fruit flies would be sterilized by radiation and produce practically no offspring. Production of males-only is achieved by using an agent, such as tetracycline in the diet or a temperature threshold during rearing, which results in both males and females; however, when the agent is withdrawn or changed, only male fruit flies result. These male fly pupae would then be sterilized by radiation and used in SIT fruit fly control and eradication programs. It is also desirable that these male fruit flies have a genetically engineered marker gene trait to monitor their dispersal and the overall effectiveness of the program.

The adverse environmental consequences of mass-rearing genetically engineered male fruit flies that produce only male insects upon release and mating with wild fruit flies would also have no more adverse environmental impact than the continuation of the present SIT fruit fly programs, because the genetically engineered male fruit flies would produce only male offspring that carry the male-only trait and no females would be produced. The male offspring of these genetically engineered mass-reared and released fruit flies would inherit the trait for no female offspring. As a result, the wild population would soon collapse because of elimination of females, thus providing control and eradication of the pests. It is also desirable that these male fruit flies have a genetically engineered marker gene trait to monitor dispersal and program effectiveness.

Pink Bollworm Eradication Program—

The adverse environmental consequences, effects, or impacts of using genetically engineered pink bollworm with a marker gene trait, such as a protein that can be easily detected in tissues by

virtue of its fluorescence, would have no more adverse environmental impact than the continuation of the present pink bollworm eradication program, as described in alternative one, the no action alternative of the EIS, because the mass-reared bollworms would be sterilized by radiation and produce practically no offspring. They could be used with either a high dose of 20 kilorads (KR) radiation for conventional or immediate sterilization, or a lower dose of 7 to 10 KR for F_1 sterility in which their offspring inherit a high degree of sterility. A "rad" means radiation absorbed dose, a basic unit of absorbed dose of ionizing radiation representing an amount of energy absorbed per unit of absorbing material, such as body tissue. One rad is equal to an absorbed dose of 100 ergs/gram. An "erg" is a unit of work or energy, which is the work done or energy expended by a force of 1 dyne acting through a distance of 1 centimeter. In terms of the joule, one erg equals 0.0000001 joule. A "joule" is a standard international unit of energy and 1055 joules is equal to 1 BTU. The genetically marked bollworm moths are easily distinguished (by their fluorescence) from wild moths caught in insect traps used to their monitor dispersal and overall program effectiveness.

The adverse environmental consequences of using genetically engineered pink bollworm that are genetically sterile and do not require radiation sterilization would be no more significant than the continuation of the present pink bollworm eradication program because the mass-reared bollworms would be genetically sterilized and produce practically no offspring. This can be achieved by using an agent, such as tetracycline, in the diet that results in both fertile males and females; however, when the agent is withdrawn, the insects mass-reared for field release are reproductively sterile. The present state of the technology would result in mass-rearing and release of both sterile males and females; however, it would be more efficient and cost effective to improve the technology so that only sterile males are produced. It is also desirable that these pink bollworm moths carry a genetically engineered marker gene trait to monitor dispersal and program effectiveness.

Phenotype of the Modified Organisms Compared to the Unmodified Organism (IAEA/FAO Report p. 23)

The phenotype of the modified organisms compared to the unmodified organism in respect to the marker and repressible lethal genes of interest are described in appendix C of this EIS, Repressible Lethal and Marker Genetic Engineering with Analysis of Issues Pertaining to Transposon Mobility and Potentiation of Horizontal Transfer for Technology Under Development by APHIS. Many of the phenotypic characteristics considered to be hazard-related in the IAEA/FAO report, are contingent on or related to biological fitness factors or the presumption of some form of hypothetical horizontal gene transfer and genome incorporation of new genes occurring with unknown risks. Hazard from dead material persisting in the environment is highly unlikely because the dead material contains no known toxic compounds, and consists of ubiquitous proteins, nucleic acids, carbohydrates, naturally occurring minerals, fats, and other organic compounds. Horizontal gene transfer is addressed in this appendix, as well as in appendix C, and is highly improbable because of transposase removal. Biological fitness for genetically engineered fruit flies and pink bollworm in this EIS primarily relates to performance factors for use in APHIS' cooperative SIT control programs and does not relate to establishment, reproduction, and persistence in the environment because the insects are intended to be sterile and, therefore, unable to reproduce. Biological fitness would be 0% for male and

female sterile genetically engineered insects because neither gender would be able to produce offspring. This fitness would theoretically be 50% for the first generation of a 100% female-lethal system, in which all of the daughters die but the male offspring survive to reproduce only males. However, a female-lethal system would also soon lead to population collapse because these males produce no female offspring to bear young.

Repressible lethal fruit flies and pink bollworm have not been tested by APHIS long enough, under laboratory or field-cage conditions, to evaluate the applicable performance or fitness factors. However, because these performance factors are directly linked to the successful and environmentally safe use of genetically engineered insects to improve APHIS' SIT cooperative programs, they would be assessed in the process of evaluating the potential for each individual genetic construct or genetically engineered strain to improve APHIS' SIT cooperative programs. This testing would be done upon decision and funding, by APHIS, to proceed with the preferred alternative of the EIS to continue and expand research and development of repressible lethal and marker genetic engineering constructs for use in APHIS' SIT cooperative fruit fly and pink bollworm control programs.

A number of the biological characteristics of the genetically modified fruit flies and pink bollworm have not undergone testing by APHIS. The genetically modified fruit fly and pink bollworm biological characteristics and life table attributes that would be of importance, if the technology was not autocidal or self-mitigating, would be related to fitness factors, which are those aspects of the biology, physiology, or behavior of the genetically modified pink bollworm or fruit flies that would allow them to have a selective advantage in the environment over its wild-type cohort or sylvan strain. However, those fitness factors that pertain to establishment, persistence, and growth of genetically engineered animal populations in the environment when they can reproduce do not apply to conditional lethal autocidal fruit fly and pink bollworm strains that may be used in APHIS' cooperative SIT programs, in which the released insects die with no offspring. Any kind of a fitness advantage that might conceivably exist would have to compete for survival against overwhelming reproductive sterility, even if the penetrance of the sterility trait is less than 100%.

Muir and Howard (2001) described their research with fish that resulted in identification of six major fitness factors for establishment, persistence, and growth of genetically engineererd plant or animal populations in the environment. They are the following:

- Juvenile viability: the ability of a plant or animal to live long enough to reproduce.
- Age at sexual maturity: the age at which plants or animals begin to breed.
- Female fecundity: the ability to produce eggs in animals or seeds in plants.
- Male fertility: the ability of a male to fertilize eggs or seeds.
- Mating advantage: the ability to attract mates in animals or pollinators in plants.
- Adult viability: the number of breeding opportunities an animal or plant has during its lifetime.

According to Muir and Howard, there are two basic types of risks if a genetically engineered organism is released into the environment beyond human control. One is an invasion risk, where the new trait spreads through the population. The other risk is one in which the trait causes the population to become extinct and has been termed the "Trojan gene effect."

Muir and Howard estimated these components for wild-type and individuals using the fish, Japanese medaka (*Oryzias latipes*). They generalized their model's predictions using various combinations of fitness component values, in addition to experimentally derived estimates. Their model predicted that transgenes could spread in populations, despite high juvenile viability costs if transgenes also have sufficiently high positive effects on other fitness components. Sensitivity analyses indicated that transgene effects on age at sexual maturity should have the greatest impact on transgene frequency, followed by juvenile viability, mating advantage, female fecundity, and male fertility, with changes in adult viability resulting in the least impact.

There are several references on insect fitness factors; however, they mainly concern genetically engineered *Drosophila melanogaster*, culicine and anopheline mosquitoes, and the screwworm fly, *Cochliomyia hominivorax*. For anopheline mosquitoes, the intended effect has principally been for strains refractory to the malarial protozoan parasites, *Plasmodium falciparum* and/or *vivax*. With anophelines, the research objective has been to drive the gene into an existing wild population of the mosquitoes to prevent them from transmitting malaria to humans.

Irvin et al. (2004) examined the following fitness factors:

- Preimaginal development times—the mean number of days for eggs laid by F_0 females to hatch was determined and compared across mosquito lines,
- Adult longevity,
- Female fecundity,
- Partial life table construction,
- Offspring sex ratio, and
- Demographic growth parameters
    1. Net reproductive rates
    2. Mean generation time
    3. The intrinsic rate of natural increase, that is, the maximum exponential rate of increase by a population growing within defined physical conditions
    4. Doubling time in days is the time required by a population growing exponentially without limit to double in size when increasing at a given rate.

Irvin et al. (2004) examined the effects of these elements on the survivorship, longevity, fecundity, sex ratio, and sterility of transformed mosquitoes, and compared results to the nontransformed laboratory strain. The demographic parameters were significantly diminished in genetically engineered mosquitoes relative to the untransformed laboratory strain. Reduced fitness in genetically engineered mosquitoes has important implications for the development and utilization of this technology for control programs based on manipulative genetic modification.

Catteruccia et al. (2003) investigated factors influencing fitness in cage experiments with four lines of genetically engineered Anopheles stephensi. The results indicate direct costs of the introduced transgene in at least three out of the four lines, as well as an apparent cost of the inbreeding involved in making genetically engineered homozygotes. It has generally been assumed that genetically engineered organisms will have lower fitness than nongenetically engineered conspecifics in the absence of selection (Tiedje et al., 1989). However, Allen et al. (2004a) evaluated eight genetically engineered fluorescent protein marker gene strains of the

screwworm fly compared with the wild-type parental laboratory strain colony. Measurements of average weight of pupae, percentage of adults emerging from pupae, ratio of males to total emerged adults, and mating competitiveness were analyzed. None of the genetically engineered colonies exhibited significantly lower fitness characteristics than the control parental colony. The presence of the transgene used to produce the strains tested did not incur a measurable fitness cost to the colonies of laboratory-reared *C. hominivorax*.

In a similar article, Allen and Scholl (2005) compared the eight genetically engineered strains of screwworm to the wild-type parental laboratory strain in laboratory culture. Measurements of average fertility, fecundity, larval productivity, and longevity were analyzed. Two genetically engineered strains had significantly lower larval productivity than controls. Another strain produced significantly fewer eggs than controls. Overall strain characteristics, including measurements from egg, larva, pupa, and adult stages, were compared. The genetically engineered colonies did not consistently show significantly lower individual or aggregate strain quality characteristics than the control parental colony; hence, the presence of the transgene used to produce the strains tested did not incur a discrete cost to the colonies of laboratory-reared *C. hominivorax*.

The above two articles indicate genetically engineered insects do not necessarily incur biological fitness costs; however, these studies were laboratory studies where environmental selection factors were not present. Fitness equivalency or slight benefit under laboratory conditions does not mean that the same will occur under natural environmental conditions. Furthermore, the performance crteria for use in an APHIS' cooperative SIT program do not correlate in a positive fashion with environmental fitness factors pertaining to establishment, survival, and reproductive growth in the environment. An environmental fitness cost that would probably lead to extinction in the environment can be easily sustainable under environmentally isolated mass-rearing conditions, and of a particular benefit for use in SIT programs. For example, it is biologically improbable that the TSL sexing strain of the Medfly would establish and proliferate in the environment compared to its wild-type cohorts due to its fitness costs; however, it is sustainable and beneficial under the controlled conditions of the SIT program.

An article by Marrelli et al. (2007) discussed discussed the subject of genetically engineered malaria-resistant mosquitoes. They determined that there was a fitness advantage to the genetically engineered mosquitoes when feeding on *Plasmodium*-infected blood.

The above findings, together with previous work on *Drosophila melanogaster*, were reviewed by Marrelli et al. (2006). They concluded that genetically engineered insects, like naturally-occurring insertional mutants, would exhibit a spectrum of negative fitness effects. The magnitude of these effects would, on their own, be sufficient to prevent spread of the transgene through a wild population in most circumstances. In addition to the insertional effects, there would be an additional cost of the expression of the inserted genetically engineered sequence(s). This would normally have a somewhat negative effect on fitness; however, this would need to be assessed on a case-by-case basis: an insecticide resistance gene, for example, or a gene drive system, might behave very differently.

These articles are of relevance to genetically engineered fish and insect risk assessments when the genetically engineered traits of interest are—

- intended to be successfully inherited into a wild population,
- for use of genetically engineered strains that are intended to replace a wild population, or
- Genetically engineered strains that are intended for use independently of wild populations, such as pharmaceutical production or as a novel biological control agent added to an environment for control of a plant or animal pest when the biological control agent is expected to successfully reproduce, persist, and establish a population.

Fitness factors that may affect the survival and reproduction of sterile genetically engineered insects intended for use in SIT and not intended to become established and reproduce in the environment would be different than for genetically engineered insects intended to establish and persist. Because of this difference, they may be termed "performance" factors.

These performance factors are relevant to genetic engineering of fruit flies and pink bollworm for use in SIT because the insects must be fit enough to be amenable to mass-rearing conditions and handling conditions, and be able to mate successfully with wild-type pest populations of the same species. The SIT males must be able to live long enough and be sexually competitive with wild males to be able to ensure enough reproductive failure to significantly reduce the pest population. The following performance-fitness factors would be of importance for genetically engineered fruit flies and pink bollworm:

A. Suitability for mass-rearing under containment conditions:
   - Percent egg hatch compared to nonengineered mass-reared cohorts.
   - Time required to complete larval growth compared to nonengineered.
   - Percent survival of the larval growth phase compared to nonengineered
   - Time required for pupation.
   - Percent emergence from pupation.
   - Percent genetic sexing success for fruit flies.
   - Number of eggs produced per female compared to nonengineered.

B. Suitability for use in SIT program releases:
   - Longevity of engineered adult males compared to nonengineered cohorts or unmodified counterpart strains.
   - Size and weight of engineered males compared to nonengineered.
   - Competitive mating ability versus nonengineered cohorts and versus wild-type insects.
   - Percent sterility (or conversely percent surviving offspring) obtained from matings with nonengineered cohorts and wild-type insects.
   - Conventionally used insecticide susceptibility compared to nonengineered cohorts and wild-type insects.
   - Attractiveness to insect traps, as applicable, compared to nonengineered cohorts and wild-type insects.

C. Stability of the repressible lethal and marker constructs over multiple generations of mass-rearing:

- Phenotypic observations over multiple generations for changes in marker gene expression, and other changes in biology, under mass-rearing conditions compared to nonengineered cohorts.
- Competitive mating ability versus nonengineered cohorts and wild-type insects over time and multiple generations (periodic testing after implementation).
- Percent sterility (or conversely percent surviving offspring) obtained from matings with nonengineered cohorts and wild-type insects over time and multiple generations (periodic testing after implementation).

Genetically engineered repressible lethal fruit flies and pink bollworm have not been tested by APHIS long enough under laboratory or field cage conditions to evaluate the applicable performance or fitness factors listed directly above. However, since these performance factors are directly linked to the successful and environmentally safe use of genetically engineered insects to improve APHIS' SIT cooperative programs, they would be assessed in the process of evaluating the potential of each individual genetic construct or genetically engineered strain to improve APHIS' SIT cooperative programs. This testing would be increased upon decision and funding by APHIS to proceed with the preferred option of the EIS to continue and expand research and development of repressible lethal and marker genetic engineering constructs for use in APHIS' SIT cooperative fruit fly and pink bollworm control programs.

Fitness or performance testing was done in 2007 and in previous years under APHIS permits to compare APHIS mass-reared nonenginered pink bollworm to a pink bollworm strain genetically engineered to express the DsRed fluorescent protein marker. Results in 2007 showed that the DsRed strain of the pink bollworm was comparably fit to the APHIS mass-reared strain used for SIT. (See pages 54 to 57 of the section on the affected environment for more details on performance testing done in 2007.)

Attributes of the Accessible Environment (IAEA/FAO Report p. 24)

The attributes of the accessible environment have been described and discussed in other sections of this EIS and in the environmental assessments referenced for pink bollworm and fruit flies.

Horizontal Gene Transfer and Hazards of Transposable Elements Used

See appendix C, Repressible Lethal and Marker Genetic Engineering with Analysis of Issues Pertaining to Transposon Mobility and Potentiation of Horizontal Transfer for Technology Under Development by APHIS.

Horizontal gene transfer flow, or movement, has arisen as one of the more controversial and theoretical risk concerns over development and deployment of genetically engineered or genetically modified insects for crop protection and human disease vector control. However, recombinant mechanisms have been developed and used in genetically engineered insect applications, specifically to prevent transposon remobilization. The current technology is described in appendix C. It is the form of technology closest to development by APHIS, and it produces genetically engineered insects with highly stable transgenes through a process that prevents transposons from moving.

The concern over horizontal gene transfer-associated risks has arisen mainly due to antibiotic resistance development in prokaryote bacteria arising from bacterial conjugation, in which genetic material is transferred directly between bacteria through direct cell-to-cell contact. These mechanisms are not present in higher multicellular eukaryote organisms. Exchange of genetic material between insects of different species, and between insects and other organisms, is biologically improbable. Insects exchange gametes internally and have complex mating behaviors and structures. Many higher organisms release genetic material into the surrounding environment, such as pollen or spores in air, or fish or mollusk sperm in water; however, insects are much more conservative in this respect, and do not release their gametes freely into the environment.

Transposable elements (transposons or TEs), which are capable of transferring segments of DNA (or of being transferred) from one site to another within a genome, are already extremely abundant in eukaryotic genomes as the result of the long evolutionary process. Animals that eat plants and animals that eat other animals or microorganisms all consume abundant transposons in their daily diet without known adverse consequences.

The highly repetitive, largely noncoding sequence that is prevalent in eukaryotic genomes consists largely of TEs. TEs make up nearly half of the human genome (International Human Genome Sequencing Consortium 2001) and an estimated 50 to 80% of some grass genomes, such as that of maize (Meyers et al., 2001).

Eukaryotic TEs can be divided into two classes: class-1 elements (retrotransposons) transpose via an RNA intermediate, whereas class-2 elements (DNA transposons) do not (reviewed by Feschotte et al., 2002). There are numerous families of TEs within each class, based on features that include length and target site preference. TEs of both classes are further classified as autonomous or nonautonomous, based on whether or not they encode, within the element, the proteins or enzymes necessary for their own transposition.

The genome size of many plant species differs as a result of variable amounts of repetitive DNA. A significant portion of the maize genome is comprised of repetitive sequences (Hake and Walbot, 1980). Most of these sequences are retroelements, mobile DNA elements that transpose via RNA intermediates using reverse transcriptase (Bennetzen 2000). Retrovirus-like retrotransposons containing long terminal repeats (LTRs) have been found in many plant species, often at a very high abundance (Flavell et al., 1992; Voytas et al., 1992; SanMiguel et al., 1996; Kumar and Bennetzen, 1999). Non-LTR retroelements, such as long-interspersed nuclear elements (LINEs) and short-interspersed nuclear elements (SINEs), have also been identified in plants (Kumar and Bennetzen, 1999). Abundant LINE and SINE elements, however, have not been identified in maize, although they may make up a small percentage of other plant genomes (Leeton and Smyth, 1993; Yoshioka et al., 1993; The Arabidopsis Genome Initiative, 2000). Most plant genomes appear to contain a rich mixture of abundant LTR-containing retroelement families (SanMiguel et al., 1996; Kumar and Bennetzen, 1999). Five major classes, together, compose 25% of the maize genome (SanMiguel et al., 1996). Other plant retroelement families may be found in copy numbers varying from 5 to 50,000 (Bennetzen, 1996). In plant species with smaller genomes, such as Arabidopsis, retrotransposons, make up a very small percentage of the genome, perhaps <5% (The Arabidopsis Genome Initiative 2000).

Transposable elements in humans were discussed at length in the International Human Genome Sequencing Consortium (2001), summarized in the following discussion:

Most human-repeat sequence is derived from transposable elements. It is currently recognized that about 45% of the human genome as belonging to this class. Much of the remaining "unique" DNA must also be derived from ancient transposable element copies that have diverged too far to be recognized as such.

Classes of Transposable Elements

In mammals, almost all transposable elements fall into one of four types, of which three transpose through RNA intermediates and one transposes directly as DNA. These are long interspersed elements (LINEs), short interspersed elements (SINEs), LTR retrotransposons, and DNA transposons.

LINEs in humans, these transposons are about 6 kb long, harbour an internal polymerase II promoter, and encode two open reading frames (ORFs). Three distantly related LINE families are found in the human genome: LINE1, LINE2, and LINE3. Only LINE1 is still active.

SINEs are short (about 100 to 400 bp), harbour an internal polymerase III promoter, and encode no proteins. These non-autonomous transposons are thought to use the LINE machinery for transposition. Indeed, most SINEs "live" by sharing the 3' end with a resident LINE element.

LTR retroposons (retrotransposons) are flanked by long terminal direct repeats that contain all of the necessary transcriptional regulatory elements. Exogenous retroviruses may have arisen from endogenous retrotransposons by acquisition of a cellular envelope gene. Transposition occurs through the retroviral mechanism. Although a variety of LTR retrotransposons exist, only the vertebrate-specific endogenous retroviruses (ERVs) appear to have been active in the mammalian genome.

DNA transposons resemble bacterial transposons, having terminal inverted repeats and encoding a transposase that binds near the inverted repeats and mediates mobility through a "cut-and-paste" mechanism. The human genome contains at least seven major classes of DNA transposons, which can be subdivided into many families with independent origins.

Currently (2001), recognized SINEs, LINEs, LTR retroposons and DNA transposon copies comprise 13%, 20%, 8% and 3% of the sequence, respectively.
Comparison With Other Organisms

The human complement of transposable elements was compared with the genomes of yeast, *Saccharomyces cerevisiae*, the nematode, *Caenorhabditis elegans*, the vinegar fly, *Drosophila melanogaster*, and the mustard weed, *Arabidopsis thaliana*.

(1) The euchromatic portion of the human genome has a much higher density of transposable element copies than the euchromatic DNA of the other three organisms.

(2) The human genome is filled with copies of ancient transposons, whereas the transposons in the other genomes tend to be of more recent origin. The difference is most marked with the fly. The accumulation of old repeats is likely to be determined by the rate at which organisms do genomic deletion. Studies of pseudogenes have suggested that small deletions occur at a rate that is 75-fold higher in flies than in mammals.

(3) Whereas, in the human, two repeat families (LINE1 and Alu) account for 60% of all interspersed repeat sequence; the other organisms have no dominant families. Instead, the worm, fly, and mustard weed genomes all contain many transposon families, each consisting typically of hundreds to thousands of elements. These features of the human genome are probably general to all mammals. The relative lack of horizontally transmitted elements may have its origin in the well-developed immune system of mammals, as horizontal transfer requires infectious vectors, such as viruses, against which the immune system guards. (Source: International Human Genome Sequencing Consortium, 2001).

Quantification of Horizontal Gene Transfer

It would be difficult to quantify horizontal gene transfer empirically among different insect species in the laboratory. The following discussion is mainly theoretical; however, it presents a possible scenario encountered in an effort to prove or disprove horizontal gene transfer of a non-autonomous transposon under controlled laboratory conditions, not withstanding that field conditions may involve much different and far more complex environmental factors.

Theoretical Discussion On Difficulty of Empirically Proving Horizontal Gene Transfer

If a predatory and/or saprophytic (P, S) species consumes a genetically modified insect that is not severely decomposed, parts of that genetically modified insect will be present in the gut of the P, S for a period of time, until eliminated through the digestive and defecation process. If the inserted or altered nucleic acid sequences of interest are detected by PCR, this will only establish that material containing them has been consumed, and does not indicate horizontal gene transfer. The genes of interest would have to be assimilated into the genome of the P, S species, which would require rearing the P, S species to determine whether it shows the presence of the nucleic acid sequence(s) of interest in subsequent generations and, if so, in what tissues of any significance they are expressed, and with what phenotypic consequences, if any.

Relevant P, S species naturally consume other insects as all or part of their diet. A significant fraction of the genome of these insects, typically 10 to 20% or more, is comprised of non-autonomous transposable elements of various types, with a smaller, but large, number of individual autonomous elements. Therefore, if invasion of new species were feasible by this route, even through constant exposure for hundreds of years, these transposons would be completely homogenized between such species, which would all contain the complete repertoire of transposons of their prey. No such pattern of horizontal transfers has been recognized; even on the million-year timescales of known horizontal gene transfer of autonomous elements, no correlation with predator-prey or host-parasitoid relationships has been reported.

Many P, S species have no multigenerational rearing procedures published or available, which would contribute to the difficulty of establishing horizontal gene transfer in the laboratory. However, there are rearing procedures for some indicator species, such as lacewing flies (Chrysopidae) and lady beetles (Coccinellidae) that are commonly used for pesticide nontarget insect testing. These predatory insects are commonly sold for use in organic gardening, and are commercially available. Cockroaches (such as the German, *Blatella germanica*, or brown-banded, *Supella longipalpa*) could also serve as easily reared sentinel saprophytic species, and would probably be the most practical insects to use because they are easy to rear and capable of producing many generations over a relatively short time. Evaluations of insect predator diets, based on detecting nucleic acid sequences from prey within the predator gut using PCR, have resulted in short retention times, leading to the conclusion that nuclear materials are quickly digested by predators, as expected.

Continued ingestion of genetically modified insects by a P, S species might speculatively yield evidence of horizontal transfer in one P, S insect out of one billion (0.000000001), which would take many years of continuous exposure to a rapidly reproducing colony of P, S insects to detect, if it can occur at all. The duration of exposure and testing to establish this occurrence is likely to be at least 10 years, and more likely 20 or more years, and the resource costs would be enormous. Note that this rate—transfer into the germ-line of another species at a rate of one in one billion— is many orders of magnitude (factors of 10) higher than 1 would infer from phylogenetic data for short, autonomous, multicopy elements, and the single (or few) copy, non-autonomous, longer elements in question may be expected to transfer at a far lower rate than this, if at all. Even a rate of gene transfer occurrence for a single-copy non-autonomous element, so far above the maximum expected rate, would require continuous exposure of genetically modified insects to large numbers of P, S insects—and continuous monitoring, requiring the analysis of at least $3x10^9$ individual insects for a putative transfer event—and even low rates of occurrence might easily be confused with laboratory artifacts.

Acquisition of the gene of interest might provide selective advantage to the P, S insect, but will most likely provide a selective disadvantage as most mutational changes in nature do. Many mutations occur in nature; however, very few provide a selective advantage resulting in better adaptation to the environment of a species. A disadvantage is particularly likely for an autocidal element, which, by its nature and design, is intended to confer a significant disadvantage. The P, S insect bearing the gene of interest in its genome would then, probably, be selected against in the laboratory colony environment, further hampering detection.

Assuming, despite the improbability, that a subcolony of P, S insects with the gene of interest in its genome had been established in the laboratory, there is a low probability that they would survive under natural environmental conditions, due to adaptation to the laboratory-rearing environment. The probability that a given novel insertion of DNA sequence that does not itself encode a beneficial gene (e.g., insecticide resistance) confers a net fitness benefit is probably much less than one out of 1,000,000 (0.000001). However, unless the recipient P, S insects do indeed have equal or greater fitness (in the Darwinian sense) relative to their wild-type counterparts in the wild, the novel sequence would not be maintained in the population, even if it survived the significant possibility of stochastic loss from the population due to its low initial frequency (presumably a single individual). Assessing fitness in the laboratory is not an

adequate proxy for fitness in the wild. Practically all colony insects become adapted to the laboratory rearing conditions and laboratory diet over time and multiple generations. Testing, under natural environmental conditions, is very difficult and fraught with unexpected events and artifacts, resulting from lack of control over experimental conditions or environmental variables. This research could well take an additional 5 to 20 years.

The likelihood that a P, S insect bearing the gene of interest in its genome and being established in the environment has an adverse environmental impact might be one out of 1,000 (0.001). This may not be measurable until the P, S insect bearing the gene of interest is actually established in the environment, due to the unpredictable nature of environmental communities and associations. An additional human health or environmental adverse impact might be a further probability of 1 out of 1,000 (0.001) or possibly less.

All of these low probabilities would have to be compounded to gain an appreciation of the maximum likelihood of a negative consequence to the environment (i.e., 10^{-18}) or human health from horizontal gene transfer, based on extremely conservative estimates. This level of probability may be roughly equivalent to the naturally occurring selection process rate that produces environmental or public health detrimental species, and many orders of magnitude lower than the rate at which harmful exotic species are introduced. It may be difficult to distinguish the effects of horizontal gene transfer from natural selection over time, especially in a rapidly changing environment, as is the case due to adverse human population development impacts on the environment. Some horizontal gene flow may be a natural phenomenon that contributes to variability and environmental selection of new species. Models to predict the frequency and/or effects of horizontal gene transfer or flow will likely be unreliable due to the lack of valid data to use as input parameters.

It is virtually certain that rigorous research of this kind will not be conducted with genetically modified insects because of the cost, the prolonged time involved, the difficulty in conducting the research, the questionable relevance of the research, and the fact that there is little economic or commercial interest in investing much money for development of genetically modified insects, unlike genetically modified plants like corn, cotton, and soybeans, for which there are potentially large markets and profits.

(This page is intentionally left blank.)

Appendix E. Summary of Public Comments on the Draft Environmental Impact Statement

I. Introduction

The Animal and Plant Health Inspection Service (APHIS) thanks all who reviewed the draft environmental impact statement on the "Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs" (draft EIS) and provided their comments on this document. APHIS welcomes public involvement and considers public perspectives in its decision processes.

Copies of the draft EIS were submitted to the U.S. Environmental Protection Agency (EPA), Office of Federal Activities for their review and to announce availability of the draft document to the public. EPA prepared a notice of availability of the draft EIS which included provision for a public comment period from May 30 to July 14, 2008 (May 30, 2008, 73 FR 31115, Docket No. ER-FRL-6699-3). The seven public comments that were received on the draft EIS are available for review at the APHIS Reading Room and are reproduced in the third section of this appendix. Although some comments were submitted to APHIS outside of the formal comment period, their inclusion in this document furthers the intent of APHIS to seek as much public input as possible in our decisionmaking process.

Although this EIS focuses on the genetic engineering technology, there are a number of other technical issues related to the pest risks associated with these insect pests that were expressed in the comment letters submitted to APHIS. Those issues are addressed, along with the primary focus, to the extent that their potential environmental impact affects agency decisions to be made. Comments from individual respondents are addressed and summarized, as provided in 40 CFR §1503.4. The comment summaries are designed to concisely cover the issues and provide responses that clarify agency perspectives in the second section of this appendix. Respondents' complete addresses are provided in the Distribution List, appendix F.

II. Summarization of Comments and Responses

For the ease of presentation and thoroughness of coverage, the issues from the comment responses are generally discussed in the order of their submission to APHIS and in the order of their occurrence within the individual comments. However, some responses pertain to more than one comment letter, so the issues are identified and addressed by topic where most appropriate. The seven review comments, in order of their receipt by APHIS, were sent from: (1) Dr. Alan S. Robinson of the Entomology Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory, (2) Dr. Margaret Allen of the USDA/ARS Biological Control of Pests Research Unit, (3) Dr. Jorge Hendrichs of the Insect Pest Control Section of the FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, (4) Dr. Charles T. Allen of the Texas Boll Weevil Eradication Foundation, Inc., (5) Dr. E. Keith Menchey of the National Cotton Council of America, (6) Dr. Alfred M. Handler of the USDA/ARS Center for Medical, Agricultural, and Veterinary Entomology, and (7) U.S. EPA, Office of Federal Activities. Some public comments relate to specific parts of the text of the draft EIS, and the basic text changes were made to those sections accordingly. Other issues are addressed through text changes and formal responses in this section. General comments of support by the respondents are appreciated. Comments concerning an issue cited in more than one comment letter are addressed together in a single

response to minimize repetition. This is particularly true for issues raised by Dr. Jorge Hendrichs and Dr. Alfred Handler. The substantive issues related to specific concerns expressed by respondents are discussed by topic with a focus on the technical aspects.

The genetic engineering technology continues to develop and our understanding improves as more research is completed, so our responses are complete to the extent that our analysts were able to find and review documentation related to the topics of interest. In that this document is programmatic in nature, environmental reviews of individual actions are expected to tier to this document and proceed to address risk issues related to specific genetically engineered insect pests and specific traits before agency decisions are made to use those organisms in any sterile insect release programs. The ongoing effort to improve APHIS' sterile insect technique programs is important to the mitigation of the continuing pest risks to U.S. agriculture.

Issue 1: Tetracycline Safety and Disposal

Three comments concern issues related to the use of tetracycline in regards to:

1) Disposal of large quantities of Medfly larval and adult diet containing tetracycline.
2) Effect of the long-term addition of tetracycline on the bacterial environment of the Medfly.
3) Worker safety in relation to exposure to tetracycline over long periods.

The impact of mass-rearing and use of tetracycline is addressed within the draft EIS. Chlortetracycline has been used for many years in the diet of mass-reared pink bollworms in Phoenix, Arizona, which are radiation-sterilized and deployed in the area-wide APHIS' SIT cooperative State program for eradication of the pink bollworm. Therefore, diet disposal and worker safety issues have already been considered and addressed in actual practice. Regarding the disposal of diet containing tetracycline, it photo-degrades rapidly (Pouliquen et al., 2007; Kühne et al., 2000). In Sanderson et al. (2005), the dissipation times of parent compounds were monitored and half-lives of 1 to 4 days were recorded. This rapid degradation was an issue of early program concern for maintaining adequate persistence in the diet. Worker safety and toxicity of tetracycline has been comprehensively addressed in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in JECFA document FAS 41-JECFA 50/69. Tetracycline rarely causes allergic reactions, central nervous system side effects are rarely described, and irritation studies in rabbits described only mild effects which were completely reversible within 48 hours.

From the draft EIS (p. 102): "The potential impacts to the physical environment, human health and safety, and biological resources from the expansion of the existing program would not be much different from those effects anticipated for the no action alternative…" The disposal of rearing media and other waste products from increased production and general maintenance would not contribute significantly to overall impacts."

Neither tetracycline, chlortetracycline, nor other antibiotics are currently incorporated into mass-reared fruit fly diets to prevent bacterial contamination and growth. However, the draft EIS, (p. 109) states: " The quality control, biosafety, physical containment, and security measures used at the present fruit fly and pink bollworm mass-rearing facilities are not expected to pose any new or novel risks as a result of development and potential use of genetically engineered

fruit flies and pink bollworm. The mass-rearing mitigation measures will be reevaluated before mass production and field release commences."

On p. 113, the draft EIS states: "There are no foreseeable unavoidable environmental effects expected from this technology that would be different from those of the current SIT programs; however, if this technology led to significant expansion of APHIS' SIT programs to include other plant pests and much larger SIT program operations, the present unavoidable environmental effects that result from mass-rearing operations, such as the amount of waste produced, would increase accordingly. Prior to implementation of this technology, research testing and strain development will also involve unavoidable environmental effects that are comparable to those from the classical genetic selection methods currently used to develop new strains."

Issue 2: "Insecticidal" Protein or Toxic Effects from the Effector Protein on the Environment

It is somewhat of a misnomer to call RIDL® effectors "insecticidal proteins". Insecticides are chemicals, including proteins like *Bacillus thuringiensis* toxins, capable of killing an insect through external application or ingestion by the insect. The RIDL® effector proteins are not insecticides or toxins by the same consideration. They affect internal functions only in the cell or organism that carries and expresses the effector gene(s), and are not intended to act externally by animal contact or ingestion.

The comment that "This protein seems to be toxic for any insect species so far tested" would refer to tTA, which is used as both transactivator and effector in some RIDL® systems (Fu et al., 2007; Gong et al., 2005). This tTA can be expressed at significant levels in cells of any of a wide range of organisms, including insects without significant deleterious effects, and is used in lab studies to allow the tTA-dependent expression of any of numerous sequences placed under tRE control. Such expression is cell-autonomous, which shows that expression of tTA in one cell does not affect adjacent cells in the same animal. This lack of toxicity is one of the valuable aspects of tTA that has made it a valuable tool for basic biology. It has been used experimentally in various taxa, from microbes to plants to insects to animals, with no known deleterious effects, even from ubiquitous expression, except when expressed at exceptionally high levels. Evidence for stability of tTA is in Horn & Wimmer (2003): "The observed time lag between transient expression of *tTA* and detectable accumulation of hid^{Ala5} mRNA is typical for binary expression systems, and the strong delayed expression of hid^{Ala5} is likely due to stability of tTA."

The origin and characteristics of the tTA protein are described in appendix C. A sufficiently high level expression of this protein within a cell can adversely affect the cell, which has been shown in both fruit flies and pink bollworm, while moderate to low levels of expression are not harmful. This does not necessarily lead to a general conclusion that this protein has insecticidal toxicity because a sufficiently abnormal level of expression of practically any cell protein may result in cell malfunction, thus suggesting that any protein produced in sufficiently abnormal quantities could lead to symptoms of insecticidal toxicity. However, this does not mean that all proteins are "insecticides." The protein tTA is an innocuous protein with no known binding site or function in normal insect cells.

Protein tTA is only mildly deleterious to mammalian tissue culture cells (not tissues) when produced intracellularly at high levels. The evidence for this is that these cells tend to lose tTA expression over time. This suggests a degree a selection for lower tTA-expressing clones which infers a selective disadvantage for high tTA expressing cells and fitness cost or other biological disadvantage for high tTA producing cells. It is also known as a relatively unstable protein due to the presence of an ubiquitin degron, which is addressed in the draft EIS on page C–20. In transgenic mammals, a positive feedback system producing tTA analogous to some RIDL® systems (Fu et al., 2007; Gong et al., 2005), showed no detrimental effects (Shockett et al., 1995). Of importance to risk consideration is that intracellular expression would give a much higher exposure to functional tTA protein than other potential exposure routes, such as ingestion of tTA protein in the context of transgenic prey or food items. Therefore, experience of expressing tTA proteins in plants, mammals, insects, fungi, and other organisms strongly indicates lack of toxicity or environmental impact by ingestion or dermal contact.

Issue 3: Preference for Genetic Sexing Systems

One comment encourages use of transgenic sexing systems, referred to as temperature sensitive lethal (TSL) systems for SIT. However, currently available transgenic genetic sexing systems, which kill females, actually operate on the same molecular biochemistry as the RIDL® system. The comments mainly focuses on the use of the transgene autocidal system versus radiation for SIT programs and suggests that genetic sexing strains could further benefit SIT programs. However, filter-rearing systems, which are closely managed, are required for temperature sensitive lethal (TSL) sexing strains. These strains are currently the best sexing technology for the Medfly only, because reversion to wild-type insects commonly occurs. The wild-type insects have a large biological fitness advantage over the TSL strain and could take over the colony very rapidly because the TSL translocation strains (e.g. Vienna-7 or Vienna-8 for Medfly) have low fitness compared to wild-type Medflies. The fitness disadvantage is from aneuploidy in the progeny of a cross between any two individuals of the TSL strain, which results in the death of approximately 50 percent of such offspring and that constitutes a 50 percent fitness penalty. Aneuploidy is when there are additions or deletions of a small number of whole chromosomes, or large segments of chromosomes, from the normal diploid configuration. Wild-type or near-wild-type Medflies in the rearing facility can arise by recombination between the translocation chromosomes and they have approximately twice the fitness of the TSL-translocation strain.

Filter rearing systems may not be absolutely necessary for RIDL® strains, but would likely be employed, at least initially. No fitness cost, as described above, has been detected with RIDL® strains and there is no theoretical reason to expect such an effect. For this reason, it is unlikely that a hypothetical mutated RIDL® strain would have a significant large fitness advantage over the RIDL® strain. Also, there would be no equivalency for transgenic strains to the recombination-mediated breakdown of TSL translocation strains.

Issue 4: Comparing the Risks and Costs of Genetic Sterilization to Radiation Sterilization

This comment reflects the proponent's advocacy of the use of ionizing radiation in SIT programs. However, their comment overlooks the risk issues associated with powerful radiation sources that must be accommodated with safety measures, training, protective equipment and shielding, and unique supply and recycling, all of which add to program operating costs.

The adverse or debilitating effects of radiation alone on the TSL translocation Medfly strain after mass-rearing, handing, irradiation, and release from aircraft would be difficult to isolate in the multifaceted context of all the other unnatural treatment. The assertion that radiation has little or no negative impact on Medflies raises some doubts when the biologically disruptive effects of high doses of ionising radiation are irrefutable; therefore, it would be the degree or amount of radiation damage that is of concern. The cited paper from Todd Shelly et al. (2005) showed very poor performance of both non-irradiated and irradiated TSL males (equal numbers of TSL males gained only 20 to 25 percent of mates); irradiated males did worse, but not much worse compared to the poor performance of non-irradiated males. Other studies have shown that the microbial biofilm in the insect's gut is severely disrupted by radiation, with presumed adverse impact. Overall, irradiated, mass-reared, TSL translocation males are extremely short-lived (less than 3 days in the field, compared with 12 to 15 days for wild males), and less than fully competitive. Employing RIDL® strains and strategy would eliminate the use and effect of radiation and TSL translocation strains, but not the adversities of rearing, handling, and distribution, which would remain unchanged.

Issue 5: Potential Escape of Transgenic Insects into the Environment and Gene Transfer to Other Insects of the Same Species or Vertical Gene Transfer

Three concerns were directed at the containment of insects and genes as follows:
1) Escape from the facility of transgenic insects carrying fluorescent and insecticidal proteins;
2) Presence in the environment of large numbers transgenic insects expressing fluorescent and insecticidal proteins following release; and
3) Transfer of transgenes into the males of the natural insect population.

Escape of transgenic insects into the environment and gene transfer to other insects of the same species issues were addressed in the draft EIS, appendix D–8, which discussed the potential for the escape of insects from the rearing facility stating: "The genetic SIT system is repressed in the mass-rearing facility… but is not repressed and therefore active under other conditions...This has the beneficial consequence that the system is activated in released or escaped insects or their progeny. This provides a "fail-safe" type of "autocidal" or lethal mitigation system to the genetically engineered insect SIT releases or inadvertent escapees."

The issues of transgene persistence and presence in the environment have been addressed in the following sections of the draft EIS: pp. 104–105, D–9 for fruit flies, and D–10 for the pink bollworm: "The adverse environmental consequences of using genetically engineered fruit flies or pink bollworm… would be of no more significance than the continuation of the current SIT program."

Additionally, appendix D–11 states: "Those fitness factors that pertain to establishment, persistence, and growth in the environment…… do not apply to conditional lethal autocidal fruit fly or pink bollworm strains that may be used in APHIS cooperative SIT programs in which the released insects die with no offspring. Any kind of fitness advantage that might conceivably exist would have to compete for survival against overwhelming reproductive sterility, even if the penetrance of the sterility trait is less that 100%."

Issue 6: Effect of Environmental Conditions on Penetrance of RIDL®-induced Lethality

All biological gene systems are subject to mutation and variation, so 100 percent penetrance of a RIDL® system should not be assumed. Consequently, a low, but non-zero rate of survival is feasible. However, while the first paper describing a RIDL® system for Medfly (Gong et al., 2005) measured a non-zero survival rate for affected males (0.2-0.7 percent, depending on the line) and a later paper (Fu et al., 2007) describing a female-specific lethal system found no surviving females for each of two transgenic lines reared in the absence of tetracycline (LA3097B and LA3097C). This is already described in the draft EIS on page C–14. Therefore, the comment's premise that "the RIDL® system is not 100% lethal/sterile under lab conditions" would not be necessarily appropriate.

Page 54 of the draft EIS concludes that, even if penetrance is incomplete, there would be overwhelming selective pressure against reproductive sterility: "Any kind of fitness advantage that might conceivably exist would have to compete for survival against overwhelming reproductive sterility, even if the penetrance or successful expression of sterility trait is less than 100 percent."

The performance of any system intended for field use needs to tested in the field, and this would also relate to the penetrance of an autocidal system. Thus far, field performance of a genetic modification has been tested most comprehensively for the DsRed marker in the pink bollworm. These field experiments demonstrated that the performance of the marker in the field was as at least as good (brightness, persistence, penetrance) as was inferred from laboratory experiments. Cage-contained trials with autocidal strains have also found that the penetrance of the lethal trait is at least as high on cotton plants as on artificial diet in laboratory experiments.

Penetrance of the lethal trait of a RIDL® strain of Medfly has been shown to be the same on several different types of diet in different laboratories and environmental conditions. The concern that penetrance would need to be confirmed for new strains or genetic systems is first addressed in the Executive Summary of the draft EIS (p. viii) which states the following: "APHIS will be establishing standard operating procedures and mitigation measures for application of genetic engineering technology to SIT in specific control programs to ensure that potential applications are not compromised. This will require extensive monitoring of strain effectiveness, particularly for new strains that have not been used previously in SIT releases."

Page 56–57 of the draft EIS also addresses this comment with the following: "However, since these performance factors are directly linked to the successful and environmentally safe use of

genetically engineered insects to improve APHIS' SIT cooperative programs, their application would be assessed in the process of evaluating the potential for each individual genetic construct or genetically engineered strain to improve APHIS' SIT cooperative programs."

Issue 7: Effect of Survival in the Field of Some Transgenics Due to Incomplete Killing by the RIDL® Construct

If the autocidal construct is not 100 percent effective, then some transgenic insects may occur in the field. The survival of a small number of progeny poses neither a new environmental threat nor a threat to the SIT cooperative control or eradication programs because the currently used ionizing radiation is not 100 percent effective, so survival and reproduction of a very small proportion of the released insects is not unexpected. Furthermore, if the autocidal construct remains functional (loss of function of the construct through mutation is discussed above), then the overwhelming majority of insects that inherit the construct in the next (and following) generations will not survive and reproduce. This could be of benefit to the SIT program because if any RIDL® construct insects should have progeny, the progeny would likely carry the construct for sterility, which may then serve to further reduce its frequency of occurrence in any possible subsequent generations and result in eventual self-extinguishment of RIDL® from the genome of the pest in the field. (Also, see p. 54 of the draft EIS, as discussed in the response above.)

Short-term vs. Long-term Persistence

One comment questions the persistence determined in the draft EIS (p. D–4) in relation to an earlier FAO/IAEA Report: "Of these purposes for release of genetically engineered arthropods, the release of genetically engineered insects being considered by APHIS corresponds to case (i): short-term presence in the environment with a low risk of establishment; autocidal control in SIT was used as a specific example of this category." The comment states that any autocidal system that is not 100 percent lethal to both sexes should not be considered "short-term presence in the environment with a low risk of establishment." No autocidal system would, in all probability, be 100 percent lethal in actual practice. However, it may be close to 100 percent, but mutation would cost at least some small fraction of a percent. Therefore, this view would appear to be contrary to that expressed in the FAO/IAEA Report classification of autocidal control, which was placed in the "short-term" category. The comment also expressed the viewpoint that female-specific autocidal systems do not correspond to this category.

In the context of an ongoing release program, genetically engineered arthropods would be present in the environment for the duration of the release program, and this could be for many years. However, this is not the intended meaning of "short-term" or "long-term" persistence in the context of the FAO/IAEA Report. "Short-term" persistence refers to the one or few generations a transgene persists in a population even when it is periodically reintroduced, as with SIT, in contrast to "long-term," in which the transgene is introduced and then intended to be passed on from generation to generation without the continuous need for reintroduction. These are useful criteria because they pertain to a key difference between various proposed uses of recombinant DNA technology for reducing harm caused by pest and plant, animal, and human disease vector insects.

Short-term vs. Long-term Genetic Engineering Strategies

A major category of insect genetic engineering, is comprised of various "population replacement" long-term strategies. Population replacement has been proposed primarily for the control of insect-vectored diseases of humans such as malaria and dengue, but could potentially also be applied to the control of vector-borne diseases of plants or livestock. It postulates the introgression of one or more transgenes into a wild population by human interventions. These transgenes are designed to reduce the ability of an insect carrying them to transmit a specific disease. Introgression of such a transgene would, therefore, tend to reduce the vectorial capacity of the affected population and so reduce disease transmission. This strategy requires the transgene to spread, increase, or at least maintain, its frequency in the target population over many generations to a high allele frequency. Genetic systems to allow this, termed "gene drivers," have been discussed in the scientific literature, and a small number have been tested in small-scale laboratory experiments. However, the possibility of field use of such systems is generally considered to be many years in the future, and the theoretical environmental risks are very difficult to assess due to the "long-term" nature of a genome modification. Extrapolation from laboratory experiments to actual field circumstances with this kind of technology is also speculative and field-release experiments have prohibitive constraints. Such systems would manifest "long-term" presence in the environment with a significant probability of establishment, which is their intended purpose. In contrast, autocidal systems are self-limiting "short-term" because of the high fitness cost (death) associated with the transgenic construct. For this purpose, it does not matter whether the construct kills only one sex or only 99 percent of the individuals carrying it because any significant fitness cost will cause the transgene to be rapidly lost from the wild population. It requires repeated releases to be maintained, so it is "short-term." This means that the transgene would not spread significantly beyond the release area, nor will it persist for long in the environment if releases cease.

Issue 8: Mutation of Transgenes and Evolution of Resistance to the Effects of an Autocidal Construct

One comment suggests that resistance development is a theoretical potential detriment of using the RIDL® construct as a replacement for ionizing radiation. Resistance in agricultural pest control is commonly associated with repeated use of a single chemical class of pesticide with a specific mode of action and the resistance develops over time as susceptible individuals are eliminated from a breeding population, while more resistant individuals survive to pass on resistance phenotypes. However, as indicated above, resistance is neither a new issue nor is it a special issue for autocidal technologies. Considering the existing experience with pesticide resistance management strategies, especially in regard to genetically engineered crops that express highly selective insect toxins, the detection and management of incipient resistance can be accommodated.

For the majority of pest control interventions, there is some possibility of heritable resistance arising and spreading in pest populations. This resistance may be biochemical-based resistance or detoxification mechanisms, behavioural changes leading to avoidance of or reduced exposure to the control agent, or other possible mechanisms of resistance or avoidance. When two or more chemical pesticides with an identical mode of action are continuously used against a pest population, cross-resistance to this chemical class of pesticides may develop. This resistance

develops to a chemical with the same mode of action, even if that chemical has not been applied to control the pest population. However, certain pesticides have had little resistance develop over time against them, such as *Bacillus thuringinesis* (Bt) toxin producing cotton strains used to control the pink bollworm because the resistance mechanisms may have other biological fitness costs that impair the insect's reproduction or metabolism enough so that those fitness costs are equal to or greater than the effects of the pesticide on the pest population under existing conditions.

It is theoretically conceivable that resistance could arise in a wild population to a RIDL® SIT strategy, specifically a heritable resistance to or tolerance of the RIDL® effector. This could reduce the effectiveness of the RIDL® strain. Any resistance would be addressed by monitoring the population for such resistance, which can be achieved by simple adaptations of the monitoring already conducted by the fruit fly and pink bollworm cooperative SIT control programs. For example, monitoring of cotton bolls for larvae is already conducted for transgenic Bt toxin expressing cotton as part of pesticide resistance management requirements for EPA registration of the Bt cotton; refugia are also required. If resistance is suspected, monitoring would be conducted for RIDL® insects with a heritable fluorescent protein marker, then they would be reared to determine whether they die as expected, showing no resistance, or live.

Field cage mating competitiveness experiments are currently conducted as a quality assurance measure for sterile fruit flies in which sterile males are allowed to compete for mates with wild males for mating to wild females. A simple extension of this experiment, if resistance is suspected, would be to rear the progeny and determine whether the expected classes of progeny die, as expected. Such experiments could also be conducted on a larger-scale in the laboratory. In the unlikely event that resistance was detected, alternative strains could be used, possibly with stacked effectors to minimize the risk of subsequent resistance.

Radiation-based SIT is not immune to theoretical resistance development as a result of persistent use and selection. Since the early use of SIT, there have been concerns about resistance by assortive mating, whereby females acquire the ability to distinguish between wild and sterile males and preferentially mate with wild ones. This has rarely been observed in the field and remains a potential concern for any new radiation-based SIT programs.

While one comment suggests "there are other transgenic sterility systems which would be much less likely to be subject to resistance development, e.g. use of early acting endogenous apoptotic genes." This suggestion is theoretical considering it is very difficult to validate resistance development until after it occurs, usually in the field after many generations of selection in a genetically heterogeneous population. Theoretically, the two-component system to which the comment refers (described for *Drosophila* only, in Horn and Wimmer, 2003) could be prone to resistance because it has two components, rather than one, against which resistance mechanisms could evolve.

The comment does not acknowledge the benefits to pest resistance management that are inherent to the female-specific RIDL® systems (Alphey et al., 2007). The nature of this SIT system would tend to suppress any heritable resistance, which might also occur for other control tools, such as classes of pesticides used in conjunction with a RIDL® system in integrated pest

management (IPM) or for RIDL® itself. These potential benefits for resistance management would tend to compensate for theoretical resistances to control agents.

One comment suggests the possibility of resistance as a result of mutation of the RIDL® construct. Evolutionary genetics concerns change over extended time, multiple generations, and new environmental conditions. Mutations lead to changes, albeit with very low frequency, and the vast majority of mutations are deleterious to the organism. The affected individuals usually either die or do not reproduce to pass the new trait on to progeny. Under natural evolutionary circumstances, it is theoretical to estimate types of mutations, their persistence, and any biologically adaptive beneficial value under the conditions of what an organism's external environment may become.

Probability

The mutation rate presented in the comment by Dr. Handler may well be an overestimate of the practical or biologically relevant rate because the vast majority of released sterile males will die without progeny, in that females are outnumbered by males by at least 10:1 in most SIT programs, so most males fail to win a mate at all. Therefore, whether they carry a functional or defective RIDL® element becomes mainly irrelevant. However, such mutations may occur at some frequency. With mutation at the rate estimated by Dr. Handler, they might be expected to occur at a rate of up to one in 100,000 of Medfly developing in the wild. This is based on the following calculation using a 10^{-6} mutation rate based on estimates from *Drosophila*.

Filter rearing systems have already been devised and implemented, which successfully maintain the integrity of strains with non-wild-type genetics, such as TSL Medflies. Therefore, the frequency of mutant alleles (all mutations combined) in a release population should not be significantly higher than the mutation rate. However, a more conservative estimate of 10^{-5} could be considered rather than 10^{-6}. These non-wild-type released SIT males must then compete for mates with wild males. If it is artificially assumed that the released males do all of the mating, which is not what actually happens in the field, then 10^{-5} or one in 100,000 of the next generation would inherit a mutant dysfunctional copy of the autocidal construct, if they live. The actual number of these individuals depends more on the numerical size of the target population than on the number of males released because, in a situation like the Medfly California Preventative Release Program where the number of females in the target area is close to zero and may actually be zero much of the time, the number of such progeny will also be close to zero, even though 300,000,000 males are released per week.

Hazard

The overwhelming majority of such mutational changes to the autocidal construct would be loss-of-function mutations of one of the elements of the constructs, such as the marker or autocidal element. The hazards, or lack thereof, are explained in the following—

In the Mass-rearing Facility

Used for sexing, the strains would be homozygous and so have two copies of the female-lethal genetic element. Both copies would have to be defective for a female to survive. This suggests 10^{-10} females might survive because of this reason. This is far below the current rate with TSL, below the likely rate of female survival for other reasons, such as marginally incomplete penetrance (see above), and would have no significant negative impact on the SIT cooperative control or eradication program or the environment. Also, the draft EIS (p. 129) states that "to the extent that the genetically engineered strains require additional tests to ensure the strain maintains the desired fitness, genotype, and genetic marker, the rearing protocols and strain filters are even more comprehensive" [than the existing SIT protocols].

The maintenance of desired traits in the insects mass-reared in colonies within the mass-rearing facility is fully addressed in the draft EIS (p. 134) with the conclusion that insects carrying deletions are mostly eliminated from the colony when using a filter rearing system and that genetic changes for these insects are extremely infrequent.

In the Field

A small proportion, such as 10^{-5} to 10^{-6} based on the estimate above, of the RIDL® insects might survive in the field. This is much lower than the level of survival generally tolerated with radiation-based SIT. Because radiation doses are a compromise between using a higher dose for less fertility or more complete sterilization or a lower dose for less adverse impact on the health and performance of the irradiated insect, sterilization rates are not 100 percent for present radiation-based methods. Therefore a very low, but-non-zero survival rate would not adversely impact the program compared to the currently used radiation methods. This fact is acknowledged in the comment by the statement that "This is a valid point if all surviving insects are sterilized by irradiation and, as with TSL, there should be no substantial programatic or environmental effect if a few "non-lethals" get through." As a result, low-level mutations would have negligible or no environmental risk.

The draft EIS already concludes that the adverse environmental consequences of using genetically engineered fruit flies (p. 104) or pink bollworm (p. 105) would be no more significant than the continuation of the current SIT program. However, the benefits of increased effectiveness and efficiency of the control and eradication programs are expected.

One comment infers that many or most of the mutations that arise would confer resistance to the autocidal system, thereby reducing the population-suppressing effect of the vastly larger number of intact autocidal elements (e.g. "But if RIDL® were used with non-irradiated released males to directly control a field population, arguably many thousands of "mutated" fertile males could be released over time that are not autocidal, and these might proliferate easily in an otherwise suppressed population"). The basis for this assertion is not sufficiently explained. There are no known mutations of the published RIDL® constructs that would have this effect. The comment mentions one mutation, reverse tTA, that affects tTA function, but also notes that insects with this unusual complex mutation would die during rearing.

The suggested guarantee of 100 percent effectiveness and no possibility of resistance for the autocidal system sets a much higher standard than the requirements for any other control method. Other resistance issues are addressed in the draft EIS which discuss that genetic sterility would be part of a multifaceted integrated pest management, control, or eradication system including surveillance and monitoring traps baited with synthetic insect pheromones, kairomones, or allelochemicals; mating confusion technique with pheromones; male annihilation technique with insecticide treated attractant baits; chemical pesticides; biopesticides including *Bacillus thuringiensis* (Bt) toxin expressing cotton for the pink bollworm; and regulatory quarantines.

Issue 9: Horizontal Gene Transfer to Other Animal Species

Appendix C addresses the potential for horizontal gene transfer and the stability of the introduced genetic elements with the concomitant risk assessment in appendix D. In summary, appendix D (p. 8) concludes the following: "Since SIT is based on release of sterile insects to mate with wild pest populations in the field, it is essentially a self-extinguishing or self-limiting pest control technology in which wild cohorts in the field do not inherit the sterility trait, except to a limited degree in F_1 sterility for the pink bollworm, in which the sterility trait is inherited in the offspring, which are then sterile. Therefore, genetic engineering, when used as a component of SIT, is self-mitigating in respect to most of the possible and theoretical hazards and risk that could be associated with arthropod genetic engineering."

One comment letter appears to disagree with the statement that search for HGT "is very difficult to scientifically establish in the laboratory, may take millions of individual insects over innumerable generations, and may be difficult to differentiate from the normal evolutionary selection process that occurs over long periods of time"; and felt that "The arguments against the risk of HT, or the ability to test them, in appendix D are based upon incorporation of an autonomous element after feeding, but this is an unlikely mechanism for HT." However, the comment also acknowledges "That said, transposon-mediated HT is not expected for a stabilized vector, and if used, the dangers of HT may indeed be a moot point."

The issue of potential horizontal gene transfer is discussed in the draft EIS, primarily in C–6 to C–9 and D–15 to D–20. Stabilized vectors are discussed in C–8 to C–9.

Horizontal gene transfer (HGT) can be done by forced manipulation in the laboratory. Insect Transgenesis is itself an example; the *piggyBac* example cited by Dr. Handler is not a truly representative example. Because it used tissue-culture cells artificially infected by baculovirus, it is not a relevant example for field experiments or occurrences. An explanation of why HGT is very difficult, costly, and time consuming to study in the laboratory in realistic conditions is presented in the theoretical discussion on difficulty of empirically proving horizontal gene transfer in D–18 to D–20. Feeding is an obvious exposure route of predator or parasite species to DNA from transgenic insects, so the analysis in appendix D addressed that route. However, the discussion would not be specific to that route, especially in regard to consequences. Also, when discussed in the context of hypothetical laboratory studies, this is a route that one could test and, indeed, stimulate by artificially increasing the proportion of the insectivore's diet that consists of transgenic insect above that likely or feasible in the wild. For hypothetical infectious agents, laboratory detection of HGT becomes even more problematic in consideration of which

infectious agent or which exposure or infection route to use that provides results with relevance to the natural environment. The discussions in appendix C are not necessarily restricted to any particular exposure route. Therefore, the discussions of HGT in the draft EIS are not expressly limited to ingestion.

In a recent presentation, Atsushi Nakabachi (personal communication, 2008), showed evidence of a gene fragment that appears to be transferred from *Buchnera aphidicola* (Proteobacteria) into the pea aphid, *Acyrthosiphon pisum*, but with no apparent function. Genome fragments of *Wolbachia* (Proteobacteria) have been found in genomes of nematodes and arthropods. These genes are not expressed and do not confer novel functions in the host organisms.

Issue 10: Use of Marker Genes

Some of the problems associated with conventional marker dyes, either fed to the insect or topically applied, are the transiency and difficulty of positively identifying the dyed insects. Furthermore, the dyes are not heritable, therefore, viable F_1 progeny cannot be identified. These F_1 progeny occur at a low, but not-zero-rate in conventional radiation-based SIT programs, however, would occur at a high rate in a Lepidoptera F_1, inherited-sterility, SIT program, such as with pink bollworm. Lack of a way to identify the F_1 steriles and distinguish them from wild-type moths is one of the key barriers to the use of the F_1 method, so operational program have not used F_1 SIT due to monitoring problems.

A heritable marker could, in contrast to dyes, allow differentiation of released radiation sterilized males, F_1 sterile males in Lepidoptera inherited F_1 sterility, and heterozygous males in a female-specific RIDL® program from wild males which would not have the genetic marker. Therefore, genetic markers have value in nearly all SIT applications.

Marker genes, such as DsRed and GFP, were thoroughly analyzed in a previous EA and are not considered to have adverse environmental impact (USDA–APHIS, 2005a). Appendix V of this EA addressed all cnidarian (Phylum Cnidaria) fluorescent proteins, including DsRed, and refers to: "over 15,000 records of published documents and books demonstrating their widespread use as biological markers in organisms from all kingdoms…APHIS reports 58 approved permit requests for field testing organisms with GFP in the United States since 1997 (http://www.isb.vt.edu), and no adverse incidents or escapes have been reported." In summary of appendix V, the widespread use of cnidarian fluorescent protein markers has no reported or expected adverse effects on the human environment.

The comment advocates the use of neutral markers with negligible biological fitness costs in combination with radiation sterilization. This might be somewhat problematic for the following reasons. Radiation doses are a compromise between the dose needed to give 100 percent sterilization and the damage this radiation does to the insects. In a few cases, for example, the California Medfly Preventative Release Program, the radiation dose is high to minimize the number of viable progeny, even though this is known to decrease the effectiveness of the released insects. However, some other programs use lower doses resulting a few viable progeny from crosses between radiation treated insects and field insects. These progeny could inherit a genetic marker and, with very low levels of fertility following lower-dose irradiation, this could

result in the marker gene potentially entering the wild population. With a neutral marker used in conjunction with radiation sterilization, this could theoretically happen. Insects carrying the marker might then be incorrectly identified as steriles, which could lead to a monitoring problem in some SIT programs. Such genes with no biological benefit or penalty to an organism may

increase or decrease over time through genetic drift, although large changes in frequency due to drift are unlikely in a numerically large population.

However, in a RIDL® system, the marker is associated with a lethal gene and so has an extremely high biological fitness cost (death). Therefore, it would theoretically not introgress into a wild population with significant frequency. This is a biological containment aspect of the RIDL® system, which contains the marker as well as the genetic sterility mechanism.

However, this issue would not be an issue for the proposed use of a marker-only strain in the pink bollworm program because of the phenomenon of inherited F_1 sterility in Lepidoptera. So viable F_1, as they arise, will be sterile. They will also carry the fluorescent marker and so would be identified as steriles. With a marker, such as DsRed, the rate of F_1 progeny could be readily monitored in the field.

Issue 11: Presence of Subeconomic Populations of Pink Bollworm

Concern was expressed that the presence of pink bollworm at subeconomic levels in parts of Mexico, Texas, and New Mexico would preclude complete eradication under the present cooperative eradication program. Concern was also expressed about these populations posing an ongoing risk of reinfestation of those eradicated areas.

The commenter complimented APHIS on the program success at eradication so far. Grower and cooperator support in the eradication zones has been very high. The total of seven wild-type pink bollworm moths captured in pheromone traps in the eradication zone in west Texas and nine moths in adjacent Juarez in 2008 demonstrates a major pest population reduction. The distance between the eradication zone and the nearest locations of cotton and related host plants not subject to the eradication program provides a buffer zone, which precludes most potential for reinfestation. Tests of flight potential of pink bollworm were conducted recently (Wu et al., 2006). The authors of this study found that the daily flight distance for tethered 1-day old female moths averaged 41 kilometers (km), and for 1-day old male moths averaged 23 km. Although the buffer zone exceeds these distances, the moths can be transported via prevailing winds and movements of motor vehicles or infested cotton equipment and commodities. Most of the latter movement is precluded by program quarantine restrictions associated with the eradication programs for pink bollworm and the cotton boll weevil.

The commenter pointed out that there is a lack of grower interest in supporting a pink bollworm eradication program of the present magnitude at locations where the pest populations in cotton are presently below the economic threshold for control action. This is likely due to the high annual grower assessment costs for the present eradication effort. The suggestion was made for the eradication program in these locations to consider the use of more economical methods, including lower levels of irradiation, to obtain more competitive sterile moths (sterile F1

generation offspring) or the use of transgenic conditional lethal control systems and heritable marker genes. Although the program managers for the Southwest Pink Bollworm Eradication Program have not yet formally determined how they will handle the extant subeconomic populations of pink bollworm after the efforts in the eradication zones are completed, applications of these economical methods for eradication of low level populations will be considered along with other effective eradication techniques that meet program expectations, as well as receive grower support. It will be important to continue pheromone trap monitoring to detect reinfestation following the eradication program's successful regional control efforts, and to be able to reinstate and mobilize sterile insect release and other controls, as needed, to sustain pest population suppression. APHIS shares the concerns of the respondent over how best to complete eradication outside the present eradication zones and looks forward to working with growers and cooperators on this phase of the program when infestations in the eradication zones are extirpated.

Issue 12: Impact to Predators from Loss of Pink Bollworm as Prey

Concern was expressed over the potential implications to predators and parasites from loss of pink bollworm from their diet as a result of the completion of the eradication program.

The pink bollworm is a pest species first detected in the United States in 1917 (Scholl, 1919). Although it can survive in okra and various wild mallow species (family Malvaceae), it remains an insect pest dependent on cotton as its primary host plant. Based upon the presence of this pest in parts of the southwestern United States for close to a century, concern was expressed about the impact of eradication on the dependency of some insects, birds, bats, and rodents in the southwestern United States on the loss of availability of pink bollworm as part of their diet.

The pink bollworm spread from Texas through eastern New Mexico by 1926 and was recognized as a major economic pest of cotton in Arizona and southern California by 1965 (Burrows et al., 1982). Prior to its establishment in these areas, predators and omnivores did not have this insect available as part of their diets. The sterile insect technique program has kept the pink bollworm from infesting the San Joaquin Valley of California, but other locations have had ongoing infestations since 1965.

The eradication program depends upon a number of grower techniques implementing IPM. The populations of wild pink bollworm in many cotton-growing areas have decreased as a result of plantings prior to and in conjunction with the present eradication effort of transgenic cotton that expresses the BT toxin. Control by applications of chemical pesticides in the eradication program have been limited to cotton fields where monitoring indicates that at least 5 percent of the cotton is infested with pink bollworm larvae. Most eradication efforts to date have been successful with the use of sterile insect technique combined with pheromone trap monitoring and other IPM measures. The need for pesticide applications has been limited to those few fields lacking Bt cotton and having measurable pest infestations of at least 5 percent. This trigger for pesticide application in the program has only been met on a limited basis; therefore, the presence of pink bollworm as a major component of the diet of predators could only occur at these site-specific locations.

The ongoing Southwest Pink Bollworm Eradication Program has already eliminated economic infestations from west Texas and New Mexico, so further effects on predators will not occur there. No detectable impacts to predators or parasites have been observed from the continued monitoring of the eradication zone. However, potential impacts to predators in Arizona and southern California (other than the San Joaquin Valley) may be addressed by the following factors: (1) Cotton is a monoculture often treated with pesticides and is an inhospitable environment for predators. (2) The larval stage of the pink bollworm is predominantly within the cotton plant (boll) where it is protected from predators. (3) Ground hunters, such as ground beetles, spiders, and mice may eat some pupae, but cotton fields are not good ecological habitats for these predators or ominvores because these monoculture fields lack the environmental diversity needed by these generalist feeders. (4) Generalist predators or omnivores eat lots of other organisms and do not depend on the pink bollworm. (5) Bats may be the most significant predator of adult moths, but bats consume many other kinds of flying insects. (6) The pink bollworm is seasonal or temporal in occurrence, while predators are not. The survival of predators is dependent on a constant food supply, which the pink bollworm does not provide.

Pink bollworm may be eaten by predatory insects, birds, or mammals that venture into cotton fields in spite of pesticide use. In addition, pink bollworm may also serve as hosts for parasitic insects, nematodes, and various microorganisms (USDA–APHIS, 2005a). Natural enemies including predators and parasitoids have only been reported for the egg, larval, and pupal stages of the pink bollworm (Hagler and Naranjo, 1994; Naranjo and Hagler, 1998; Henneberry and Naranjo, 1998; UCR, 2008). There have been no reports of predators or parasitoids attacking pink bollworm adults (USDA–APHIS, 2005a). There have been a number of releases of parasitoids from foreign origins, but most were of limited success and their presence has not shown measurable ongoing benefit to the cotton crop in the United States or to the environment in general (UCR, 2008). The predation and parasitism of pink bollworm by native insects, birds, and mammals is not obligate or bollworm-specific, but opportunistic as these animals enter cotton fields seeking acceptable hosts. Based upon this, the impacts to native predators and parasitic animals from the ongoing eradication of pink bollworm are not expected to affect their survival or greatly increase their foraging efforts.

Issue 13: Potential Uses of Preventive-based Measures Through (a) Increased Controls On, and Inspection of Imported Agricultural Products, and (b) Improvement of Environmental Safeguards in Trade Agreements

Concern was expressed that explanations of potential uses of preventive-based measures needed to be expanded in the discussion of the No Action and Expansion of Existing Program alternatives sections such as (a) increased controls on, and inspection of, imported agricultural products and (b**)** improvement of environmental safeguards in trade agreements.

(a) APHIS uses a risk based strategy when implementing new or refining old pest control programs to face the challenges posed by the increased pest risk precipitated by the expansion of international travel and trade. Any increase to controls for the importation of agricultural products currently imported into the United States must be justified through the pest risk analysis process before implementation. This pest risk analysis process is also required before the design and implementation of any new phytosanitary program for an agricultural product not previously

imported into the United States. As a potential preventive measure, APHIS could continue to refine and strengthen this pest risk analysis process and quarantine parameters in general, to ensure proper risk mitigations are implemented to prevent pest introductions. An example of a recent significant refinement by APHIS to ensure that proper regulatory procedures were in place for pest exclusion was the successful completion and implementation of the revision of one of APHIS' implementing quarantine regulations for imported fruits and vegetables (7 CFR § 319.56) (USDA–APHIS, 2007e).

A specific potential activity to facilitate and further bolster the risk assessment process, and thereby increase controls of imported commodities by APHIS, could include the increased offshore information gathering efforts to obtain the most accurate information regarding the pest status of foreign countries. This would lead to more robust pest risk analyses and the subsequent implementation of the most appropriate phytosanitary control measures to reduce the pest risk posed by imported agricultural products.

Another potential activity would be for APHIS to strengthen the methods development of more efficient and more effective pest control technologies for application in improved pest population control measures at foreign production sites or in postharvest agricultural commodity treatments for commodities exported to the United States.

Examples of potential activities to enhance inspection controls at points of entry into the United States have been explained under "Exclusion Strategy in the Potential Risk Reduction Activities at a Glance" section of a previous EIS (USDA-APHIS, 2001a).

Most of these same potential activities are still applicable to enhance the inspection system for imported agricultural commodities. Since the completion of the 2001 EIS, the Department of Homeland Security has assumed the inspection duties for agricultural commodities at the points of entry and now works in partnership with APHIS in this exclusionary effort.

b) The United States is a ratified signatory to the International Plant Protection Convention (IPPC). APHIS has been delegated the task to act upon any responsibilities of the United States under the IPPC.

The IPPC official Web site home page states, "The IPPC is an international treaty to secure action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control. It is governed by the Commission on Phytosanitary Measures which adopts International Standards for Phytosanitary Measures" (IPPC, 2008). Through membership to this treaty, APHIS can and does contribute to the development of international and regional standards addressing the implementation of appropriate phytosanitary measures to prevent the spread of plant pests via international trade of plants and plant products. As a potential activity, APHIS could suggest a greater emphasis on environmental safeguards be included in any international standards before their adoption.

APHIS is tasked with developing and maintaining cooperative relationships with foreign governments in regard to phytosanitary activities surrounding the trade of agricultural products. These cooperative efforts are often outlined in bi-lateral agreements. As a potential activity,

APHIS could increase efforts to ensure that appropriate safeguards are included in the development of pest mitigation systems in foreign countries as provisions in bi-lateral agreements to prevent the spread of plant pests into the United States through trade of agricultural products.

Also as a cooperative effort, APHIS could work to include in provisions of bi-lateral agreements to provide technical assistance to foreign countries and to actively participate in the detection and control of plant pests in foreign countries. As a preventive measure, an increase in the active involvement of APHIS officials in the control of plant pests infesting other countries would help to limit one potential pathway of entry. This involvement could range from the establishment of pest surveillance systems, the development of clean stock programs for exported nursery stock, and active involvement in field control operations—all within the borders of the foreign country. These types of activities would decrease the approach rate of plant pests into the United States associated with imported agricultural products and, thereby, lower the pest risk posed by these products.

However, it should be noted that any of the above listed potentially new activities in section (a) or (b) above can only be implemented when weighed in balance with other program resource needs in consideration of budgetary constraints and government cost-cutting measures.

III. Comment Letters

All comment Letters submitted to APHIS are reproduced on the subsequent pages.

<A.S.Robinson@iaea.org> 05/26/2008 03:21 AM	To: cc: Subject:	<David.A.Bergsten@aphis.usda.gov> EIS on genetically modified insects

Dear David
Thank you very much for the draft EIS on the use of genetically modified fruit fly and pinkbollworm strains in control programmes. Together with the NAPPO standard it will provide a regulatory framework for the eventual use of these strains.

Best Wishes
alan
Alan Robinson
Entomology Unit
FAO/IAEA Agriculture and Biotechnology Laboratory
A-2444 Seibersdorf
Austria
tel +43-1-2600-28402
fax +43-1-26007-28274

"Allen, Meg"
<Meg.Allen@ARS.USDA.GOV>

05/28/2008 10:19 AM

To: <Gregory.S.Simmons@aphis.usda.gov>, <rirose1@juno.com>
cc:
Subject: RE: EIS for GE PBW and FF

My comments are attached.

WELL DONE!!!!!!!!!!!!!!!!!!!!!!!!!!
Meg

-----Original Message-----
From: Gregory.S.Simmons@aphis.usda.gov
[mailto:Gregory.S.Simmons@aphis.usda.gov]
Sent: Monday, May 12, 2008 12:55 PM
Subject: EIS for GE PBW and FF

Draft EIS statement comments.

Overall this document is exceptionally clear and well written. The authors should be very proud.

page 6, 3rd paragraph – the citation IPPC, 2007 is not in the reference appendix (G).
page 6, 4th paragraph – the species name for screwworm is *Cochliomyia hominivorax* Coquerel.

page 50 the figure must have color.

page 57, middle of page – appendix D info is in pages D-14 and D-15, not 15 & 16.

Appendix C. p. C-3 paragraph 1 includes a nearly exhaustive list of transgenic insects, primarily omitting those produced by M. Allen. (*Culex quinquefasciatus* and *Cochliomyia hominivorax*) The ladybird beetle and a sawfly have also been rendered genetically modified.

Page C-7 either spell eukaryote with a k or a c (eucaryote), not both.

Page C-8 1st line move comma to before would rather than after.

Page C-9 lines 1 and 2 are repeated as lines 3 & 4.

Page C-16 inapropriate citation. Not published, not peer reviewed. Substitute Neuburger et al, Genetics 173:1377-1387 (July 2006)?

Page D-2 2nd line from bottom “tramsfer” a misspelling of transfer.

I particularly like pages D-19-20. Well presented.

C:\Documents and Settings\Dr. Meg Allen\Desktop\Draft EIS statement comments.doc

<J.Hendrichs@iaea.org>

07/09/2008 08:40 AM

To: <david.a.bergsten@aphis.usda.gov>
cc: <R.Cardoso-Pereira@iaea.org>, <G.Franz@iaea.org>, <A.Jessup@iaea.org>
<Thomas.Andre@aphis.usda.gov>, <M.Vreysen@iaea.org>
Subject: Comments on Draft Environmental Impact Statement on the Use of Genetically

Dear Dr. Bergsten,

Attached please find our comments in relation to the APHIS/USDA Draft Environmental Impact Statement on the Use of Genetically Engineered Fruit Flies and Pink Bollworm.

Sincerely

Jorge Hendrichs

_____

Jorge Hendrichs, PhD
Head, Insect Pest Control Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
P.O. Box 100, A-1400 Vienna, AUSTRIA
E-MAIL: J. Hendrichs@iaea.org
FAX: (+43-1) 26007; TEL: (+43-1) 2600-21628
<http://www-naweb.iaea.org/nafa/index.html>

FAO/IAEA Comments on Draft Environmental Impact Statement on the Use of Genetically Engineered Fruit Flies and Pink Bollworm

Additional environmental concerns for SIT application using genetically modified fruit flies and pink bollworms, which have been designed to provide markers, sexing strains and molecular sterilization include:

1) Disposal of large quantities of Medfly larval and adult diet containing tetracycline.

2) Effect of the long term addition of tetracycline on the bacterial environment of the Medfly.

3) Worker safety in relation to exposure to tetracycline over long periods.

4) The introduction into the environment of insecticidal proteins, as proposed in the current RIDL system. The insecticide is produced in the transgenic female insects in the absence of tetracycline. This protein seems to be toxic for any insect species so far tested.

5) Escape from the facility of transgenic insects carrying fluorescent and insecticidal proteins.

6) Presence in the environment of large numbers transgenic insects expressing fluorescent and insecticidal proteins following release.

7) Transfer of transgenes into the males of the natural insect population.

It is very difficult in the document to find these specific concerns specifically dealt with although many of them are trivial and can probably be taken care of quite easily.

The protein kills the insect through reaching a toxic level late in the larval stage. A natural variant in a field female that is able to breakdown or sequester the protein would spread very rapidly through the population and render subsequent releases ineffective. This is a crucial difference from SIT (see below). However there are other transgenic sterility systems which would be much less likely to be subject to resistance development, e.g. use of early acting endogenous apoptotic genes.

According to the Draft Environmental Statement the use of genetically engineered fruit flies or pink bollworm has 3 potential benefits:

<u>1.) The use of a molecular marker (e.g. fluorescent proteins) to be able to distinguish released and wild insects</u>

To be acceptable in a practical application the marker has to be completely penetrant, stable, (even in dead trapped insects), have no effect on field competitiveness or rearing efficiency in the facility.

It is known that the expression of transgenic markers varies from strain to strain depending on the insertion site in the genome and a suitable strain would need to be identified.

Conclusion: Provided that the above criteria are met, a molecular marker would be a desirable improvement over current technology (i.e. the use of fluorescent powder). With current molecular markers (various forms of fluorescent proteins) no negative effect for the environment is to be expected when the released flies are sterilized using radiation (but see above).

(If a marker is used as component of a RIDL system however, it cannot be used to distinguish released males from wild males as there will be wild males in the population that have inherited the marker from released males.)

2.) To construct genetic sexing strains

It would be highly desirable to have a technique available that would allow a relatively easy transfer of a sexing mechanism to the various pest insect species. Such a mechanism must fulfil the following criteria in transgenic strains:

a) accuracy in sexing: laboratory experiments indicate this can be achieved;

b) stability during production: this will have to be maintained through the use of a filter rearing system, and this requires being able to identify the occurrence of transgene instability and changes in expression of the marker and the lethal factor; and

c) minimal effect on productivity and quality: in theory transgenic strains could be more productive than current sexing strains based on conventional genetics.
These criteria have to be evaluated for each individual strain.

Conclusion: With the currently available molecular sexing systems no negative effect for the environment is to be expected when the released flies are sterilized using radiation (but see above).

3.) Molecular lethality as an alternative to genetic sterility

The Draft Statement highlights on numerous occasions the advantages of transgene-based lethality over radiation-induced sterility. It is claimed that a significant improvement of the quality of the released insects is to be expected and it is argued that this outweighs any potential risk associated with the release of fertile transgenic insects. We disagree with this assessment:

a) Fruit flies: It was shown that the irradiation treatment, if applied properly, has either only a marginal or even no negative effect on the quality of the released insects (e.g. Shelly, T.E., Edu, J., and Pahio, E. 2005. Lack of an irradiation effect on the mating performance of mass-reared males of the Mediterranean fruit fly. Fla. Entomol. 88: 547-548). This is especially true compared to the negative impact resulting from the mass rearing and release procedure. Therefore, only marginal improvements can be expected as also the transgenic strains would have to be mass reared, packaged, aerially released, etc.

b) Pink bollworm: here the negative impact of irradiation is indeed much more significant, however only if an attempt is made to achieve 100% sterility of the released insects. If however

the “F1 sterility concept” is followed the radiation associated negative effects are minimized. Here again, however, a transgenic strain would also have to be mass reared, packaged, aerially released, etc.

Conclusion: The two arguments above show that the gain in efficiency by using a RIDL or a comparable system is much smaller than anticipated in the Draft Statement. On the other hand, this document overlooks a serious environmental issue that may arise if such technologies are used.

RIDL is in effect the introduction, albeit transitory, of a transgene into the environment as all the male progeny from the released fertile males will inherit the transgene and it will then be inherited by half of their progeny and so on. Lethality is restricted to the female offspring of the males carrying the RIDL construct. The transgene is maintained, at least for some time, in the target population through the male-lineage and will be continually increased in frequency by further releases. This will obviously also add the level of lethality in the population. However, following cessation of the releases, for whatever reason, the transgene will remain in the population and continue to kill females until eliminated by natural selection.

This scenario is completely different from SIT with radiation induced sterility, where following cessation of releases there is a rapid return to pre-release environmental conditions. Several days after stopping the releases the environment is free of any released flies and there is no change in the natural population.

This characteristic is not only important in a general regulatory context but could become an issue if one considers the potential of resistance developing against the lethality introduced by the RIDL system as currently foreseen. It is based on the lethality of a single, non-arthropod gene product being expressed in females, a sort of female specific insecticide. The dominant lethality of the RIDL system has only been shown in small scale tests with strains exhibiting much reduced genetic diversity. It cannot be excluded that a wild type target population, with its vastly larger genetic diversity, develops a resistance mechanism against this lethality. The ability of an insect to either metabolise or sequester the protein or to silence the transgene would lead to a rapid failure of control and permanent transgene contamination of the target population.

In contrast, radiation-induced sterility generates random and redundant dominant lethal mutations, i.e. every sperm carries a different set of multiple dominant lethal mutations. This makes the development of resistance virtually impossible. Secondly, the mode of action is completely different. Dominant lethality induced via radiation consists primarily of chromosome breaks followed by incorrect rejoining of the resulting fragments. This leads to death of the offspring due to genetic imbalance as opposed to the RIDL system, where the female offspring are killed by a toxin.

Overall Conclusion:

There would seem to be no unacceptable environmental risks associated with the use of transgenic insects to develop markers or sexing strains for conventional radiation-based SIT programmes. However, the Draft Statement overlooks a significant risk to the environment and

to the success of a control programme if transgene-based lethality systems using insecticidal gene-products are used.

TEXAS BOLL WEEVIL ERADICATION FOUNDATION, INC.

P.O. Box 5089 ♦ Abilene, Texas 79608-5089 ♦ Voice: (325) 672-2800 ♦ Fax: (325) 672-5034

July 11, 2008

Dr. David Bergsten
Biological Scientist
Policy and Program Development
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 49
Riverdale, MD 20737-1238

Dear Dr. Bergsten:

This letter is to provide comment on the May 2008 Draft Environmental Impact Statement, Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs. I will limit my comments to pink bollworm (PBW) since I work directly with the PBW eradication program in Texas and I have only limited knowledge of the work being done on fruit flies.

First, I would like to compliment the agency on its Draft Environmental Impact Statement. It is comprehensive in its treatment of PBW, the eradication program being conducted against it and the new technology in development.

The PBW eradication program has been successful. Grower support for the program has been high in the El Paso/Trans Pecos (EP/TP) zone. Growers have assessed themselves $20 per acre ($10 per acre for Bt cotton since 2001) for boll weevil and PBW eradication every year since 1999, and passed grower program retention referenda by votes of 80%, 89% and 95% in 1999, 2003 and 2005, respectively. To date this year, only seven wild type (native) PBW moths have been captured in the zone. No native PBW moths have been trapped in the adjacent South Central New Mexico program, and nine native moths have been captured in the adjacent Juarez, Chihuahua, Mexico program. Excellent progress is being made in Arizona, southern California and adjacent areas of northwest Mexico as well.

The eradication program in these areas has consisted of: 1) mapping of all cotton fields in the eradication zones, 2) pheromone trapping of cotton fields in the eradication zones, 3) use of Bt cotton, 4) use of hand applied and aerially applied pheromone mating disruption products, 5) limited use of insecticides (chlorpyrifos and pyrethroids), 6) cultural control via mandatory stalk destruction and 7) release of sterile PBW moths.

Concerns About Rearing DsRed Transgenic Moths

Rearing DsRed moths will require extra care to avoid escapes of transgenic moths that have not been irradiated or otherwise rendered sterile. Escapes of fertile DsRed moths in areas with remaining native PBW populations could compromise the marker system. In any operations in which DsRed moths are reared, rearing facility personnel would mitigate this risk through continual release of sterile insects.

Concerns Addressed by DsRed Transgenic Moths

The sterile PBW moths produced in the USDA-APHIS PBW Rearing Facility in Phoenix, AZ are dyed with calico red dye incorporated in the larval diet. This marking system allows field personnel to separate sterile moths from native moths. Ability of field personnel to correctly categorize moths is critical because actions taken in eradication are predicated largely on capture of native moths. Release rates of sterile insects and use of other population management technology are based on the number of native moths and the ratios of sterile moths to native moths captured in pheromone traps.

In 2007 and 2008, program personnel have caught moths that either have not shown the calico red marking or have shown it only very weakly. There are two possible errors that can occur when moths cannot be definitively separated into sterile or native categories. If sterile insects are misclassified as natives, unnecessary additional treatments (of various types) must be applied, adding to the cost of the program. If native insects are misclassified as sterile insects, active populations of native pink bollworms are not identified and do not receive appropriate treatment in a timely manner. In this case, native populations to become entrenched in fields and greatly increases program costs. Either misclassification is harmful to the program. The availability of transgenic DsRed moths provides two marker systems, virtually eliminating all sources of error except for human error. And, systems which could remove virtually all human error are being studied.

Pink Bollworm Issues in Texas Cotton Outside the EP/TP Zone

In recent years Texas growers have produced from 5.5 to 7 million acres of cotton each year. PBW trapping was conducted by the Texas Boll Weevil Eradication Foundation in cooperation with USDA-APHIS from 2000 to 2003 in the zones which had active boll weevil eradication programs during that time. PBW populations were confirmed in the following zones: Northern High Plains, Northern Rolling Plains, Northwest Plains, Permian Basin, Rolling Plains Central, Southern Blacklands, Southern High Plains/Caprock, Southern Rolling Plains and South Texas/Winter Garden. PBW presence was not confirmed in the Upper Coastal Bend zone (southwest of Houston). Three zones, Panhandle, Northern Blacklands and Lower Rio Grande Valley did not have active boll weevil eradication programs at the time and were not trapped for PBW. To briefly summarize this trapping data, PBW is present at sub-economic levels (or occasionally in economic levels in areas adjacent to the EP/TP zone) in practically all Texas cotton. Traps run by the Texas Boll Weevil Eradication Foundation in the Pecos Valley New Mexico zone confirmed presence of PBW in that area as well.

Due to the high cost of the PBW eradication program, there has not been grower interest in Texas, other than in the EP/TP zone, in developing PBW eradication programs. Neither has there been interest in PBW eradication in New Mexico outside of the South Central New Mexico zone and the southwestern counties adjacent to Arizona. As PBW is eliminated from the EP/TP, south central and southwestern New Mexico, Arizona, California and areas of Mexico adjacent to the U.S.; sub-economic PBW populations in the areas of Texas, New Mexico and Mexico which have not been eradicated will be a potential source of re-infestation for the eradicated zones. Cotton in the eradicated areas has a considerable degree of geographic isolation from infested cotton in the non-eradicated areas, but there is potential for native moths to move or be moved into the eradicated areas. Any potential re-infestation from non-eradicated areas can be managed through a monitoring and sterile moth release program as has been done successfully for years in the San Joaquin program in California. (The California program is grower funded with growers currently paying $2 per bale each year.) A San Joaquin style maintenance program will require the availability of a PBW rearing facility and on-call availability of sterile PBW moths for the foreseeable future to maintain the PBW free status of the eradicated areas.

At some time in the future, there may be a desire to eliminate the remaining PBW populations from New Mexico, Texas and parts of Mexico. Any such effort in Texas could take advantage of weather conditions and cultural practices that are less favorable to PBW populations. It would involve use Bt transgenic cotton. And, it would also require rearing and release of sterile PBW moths.

The current method of sterilization of PBW moths for use in eradication programs uses 20 kilorads (kR) of radiation to sterilize the moths. This sterilization procedure produces 100% sterilization but produces sterile moths with reduced survival and competitiveness compared with native moths. Currently active eradication programs compensate for the reduced competitiveness of these moths by inundation of the program area with large numbers of sterile moths. If available, other methods of sterilization, involving lower dose irradiation or sterile moths that are not irradiated, could potentially produce sterile moths whose survival and competitiveness were near equal to that of native moths. This would result in cost savings as fewer sterile insects would be needed. The ability to conduct PBW eradication in geographic areas in which the pest does not cause economic damage to the cotton crop will depend on whether inexpensive eradication technology is available.

There are two kinds of technologies which can be used to obtain more competitive sterile moths. Lower dose irradiation is one process that can be used. Moths irradiated with 10 kR of radiation can reproduce, but the offspring are sterile. In order for the 10 kR technology to be successful, program personnel must have a system to differentiate between the sterile F1 generation offspring of released 10 kR moths and native moths. This would require a heritable marker such as DsRed transgenic strain insects.

In addition, transgenic technologies can be used to produce competitive, effectively sterile moths without the use of radiation. Several constructs are in development. In one, survival of the insect is dependant on the presence of a dietary component which is present in the laboratory diet, but is not present in cotton. In another, mortality is triggered by low temperatures. The traits are dominant and express in the laboratory strains and in crosses of the laboratory strains with native insects. Moths carrying the weather mediated construct released early in the cotton season and allowed to reproduce spreading the gene through the population during the summer. Carriers of the gene could not survive cold temperatures in the fall. The adult transgenic moths are normal in their behavior and competitiveness. These insects could be reared with low risk in areas surrounded by large plantings of cotton because escaped laboratory strain moths pose no danger of initiating a persisting infestation. They are fully competitive so that fewer sterile moths could be used to obtain suppression/eradication. The ability to rear the population with reduced need to curtail escapes and the improved competitiveness of the sterile insects provide economic benefits. The heritable DsRed marker system would be very valuable if transgenic conditional lethal control/eradication systems are used in areawide programs. And, cultures with both a conditional lethal gene and the DsRed marker gene eliminate the risk of escaped, fertile, DsRed moths moving the DsRed gene into native populations and the cost of mitigating this gene movement into native populations.

Summary

Pink bollworms are present at sub-economic levels in millions of acres of Texas and New Mexico cotton. The sub-economic status of these populations makes eradication of the pest in the U.S. impractical and prohibitively expensive using the large program organizations and technology currently in use in the active PBW eradication programs. If complete elimination of PBW from the United States and northern Mexico is attempted, it will require additional technology to address low level PBW populations in large acreages of cotton in areas outside of the current active PBW eradication zones.

Texas Boll Weevil Eradication Foundation considers transgenic PBW technology very important if we are to achieve PBW eradication from the U.S. and northern Mexico, enhance long term program stability and reduce long term costs of program monitoring and maintenance.

I greatly appreciate the opportunity to provide comment on the Draft Environmental Impact Statement.

Sincerely,

Charles T. Allen, Ph.D.
Program Director, Extension Specialist and Professor

National Cotton Council OF AMERICA

1521 New Hampshire Avenue, NW, Washington, DC 20036
Telephone: 202-745-7805
Fax: 202-483-4040

July 11, 2008

David A. Bergsten, Ph.D.
Policy and Program Development
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 149, Room 3D-06D
Riverdale, MD 20737-1238

RE: Draft Environmental Impact Statement, May 2008. *Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs*.

Dear Dr. Bergsten:

The National Cotton Council (NCC) fully supports APHIS' preferred alternative of integrating genetically engineered insects into the sterile insect technique (SIT) component of its invasive plant pest control programs.

The NCC is the central organization of the U.S. cotton industry representing producers, ginners, oilseed crushers, merchants, cooperatives, warehousemen, and textile manufacturers in 17 states stretching from California to the Carolinas. The NCC represents producers who cultivate over 13 million acres of farmland. Annual cotton production of approximately 21 million 480-lb bales is valued at more than $5 billion at the farm gate.[1] While a majority of the industry is concentrated in the 17 cotton-producing states, the down-stream manufacturers of cotton apparel and home-furnishings are located in virtually every state. The industry and its suppliers, together with the cotton product manufacturers, account for more than 440,000 jobs in the U.S.[2] In addition to the cotton fiber, cottonseed products are used for livestock feed, and cottonseed oil is used for food products ranging from margarine to salad dressing. Taken collectively, the annual business revenue generated by cotton and its products in the U.S. economy is estimated to be in excess of $120 billion.[2]

The pink bollworm is a very destructive cotton insect pest. Pink bollworms damage squares and bolls, the damage to bolls being the most serious. Larvae burrow into bolls, through the lint, to feed on seeds. As the larva burrows within a boll, lint is cut and stained, resulting in severe quality loss. Under dry conditions, yield and quality losses are directly related to the percentage of bolls infested and the numbers of larvae/boll. With high humidity, it only takes one or two larvae to destroy an entire boll because damaged bolls are vulnerable to infection by boll rot fungi.

[1] Economic Services, NCC

[2] Retail Values of U.S. Agricultural Commodities, NCC

The NCC estimates pink bollworm costs western cotton producers more than $32 million annually for prevention, control, and yield losses. The pink bollworm's migratory nature and reproductive capability seriously challenge a cotton grower's control efforts. Fortunately, U.S. cotton producers now have access to the tools for eradicating the pink bollworm from this country. Absent this pest, cotton producers will see increased yields, reduced insecticide use, and improved profitability. The pink bollworm eradication plan promises to provide a permanent solution to the pink bollworm problem. The plan includes coordinated efforts by cotton producer communities and federal, state, regional, county, and local entities to combat and eliminate the pink bollworm from where it is inflicting damage — West Texas, New Mexico, Arizona, California, and northern Mexico. Effective control requires a proven area-wide management strategy similar to those employed in California's San Joaquin Valley pink bollworm control program and the highly successful National Boll Weevil Eradication Program. Pink bollworm technologies implemented in a managed system over a wide area include cultural practices, Bt cotton, pheromones applied for mating disruption, sterile insect release, and targeted use of insecticides if necessary.

Eradication, though, presents a more economically and environmentally viable solution than facing the pink bollworm on an annual basis. The National Cotton Council's Pink Bollworm Action Committee provides grower direction and the International Cotton Pest Work Committee provides coordination with Mexico. A bi-national organization will coordinate this project which is a cost-share effort combining significant grower contributions and federal assistance. USDA and state government and extension research specialists provide technical and operational assistance.

The sterile insect technique is an important component of the eradication strategy. SIT has been used for more than 30 years in the San Joaquin Valley to successfully prevent the pink bollworm from becoming established in that key cotton-producing area. The success of the technique lies in the regular release by air of large numbers of sterile moths into the wild. The males are irradiated at the Pink Bollworm Rearing Facility in Phoenix, Arizona which renders them sterile but otherwise leaves them reproductively active. Sterile male releases are initiated either when the pest insect population is at its seasonal lowest density or once the wild population of the pest has been reduced to relatively low numbers by other techniques. The large numbers of sterile males released out-compete wild males for females and, as no offspring result from these sterile matings, the insect population is reduced.

Genetically engineering will provide even greater possibilities for SIT programs. Of immediate interest is the utilization of a fluorescent protein marker gene which will enhance the ability to distinguish among sterile and native pink bollworms which will improve the surveillance and monitoring of the program. The release of sterile pink bollworm moths in an area is based on the number of native moths previously captured in pheromone traps for that area. It is, therefore, critical to be able to accurately distinguish between native moths and released sterile moths to ensure the appropriate number of steriles are released without releasing excess numbers that are costly to SIT programs. Future technologies could provide further improvements to this technique.

Thank you for the opportunity to comment on the draft EIS. This issue is important to the U.S. cotton industry and to the potential of SIT. We look forward to our continued efforts with APHIS in eradicating the pink bollworm.

Sincerely,

E. Keith Menchey

E. Keith Menchey

Manager, Science & Environmental Issues

Alfred M. Handler, Research Geneticist
USDA-ARS
Center for Medical, Agricultural, and Veterinary Entomology
1700 S.W. 23rd Drive
Gainesville, Florida 32608
e-mail: al.handler@ars.usda.gov
tel: 352-374-5793

Comments on:

Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs - Draft Environmental Impact Statement—May 2008

The following are comments on the draft EIS that relate to areas that fall within my expertise and I feel are relevant to the successful and safe initiation of a transgenic insect release program. Due to the large scope and length of the EIS I did not review the entire document in detail, and thus it is possible that some of these issues were addressed within the EIS, but overlooked.

One issue is that "negligible impact" is concluded for transgenic release due to lethality/sterility of the released organism, since this should result in the eventual elimination of transgenic insects from the environment. But the RIDL system is not 100% lethal/sterile under lab conditions, and this could change (for better or worse) under field conditions. An important question is whether sterility has been tested (in-lab) under simulated field conditions, and have the population effects of gene mutations/reversions been evaluated? Will the toxin accumulation and toxic effect remain the same under field conditons of elevated temperature, humidity, natural diet, etc. It cannot be assumed that there will be no effect, and this should be first tested under large scale in-lab rearing. If the lethal (sterile) effect is eliminated or diminished, then transgenics may persist in the environment and may proliferate in a generally suppressed population – and potentially be refractory to further RIDL control. While it is argued that RIDL provides a completely a "fail-safe" autocidal lethal mitigation for transgenics that remain in the environment, it is not apparent this will necessarily be the case if resistance to tTA or suppression of toxicity occurs.

In addition to the effects of environmental conditions, a very real concern is the possibility that spontaneous mutations/reversions will eliminate or suppress the lethality/sterility effect in the transgenics. In Drosophila these rates for a particular gene locus is estimated to occur at about 1 to 5 x 10^{-6} (see Ashburner, 1989 – Drosophila: A Laboratory Handbook for discussions). So if 3-4 billion flies are being reared per week (as in Guatemala currently), even at a mutation rate of 1 x 10^{-7}, one can expect 300 to 400 mutations per week to occur at any particular locus. Thus, mutations might be expected in the tTA or the TRE (tetO) promoter DNA that break the feedback loop stopping toxic tTA levels from occurring, or eliminate the toxic effect of tTA (or affect its site of action). A tTA mutation already exists (*reverse* tTA) that has the opposite effect of tTA - in that tetracycline causes increased, rather then suppressed, gene expression. For RIDL, such a mutation occurring in rearing would simply result in the mutated insect dying while on tet diet (assuming rtTA is also toxic), and these insects would not be released with no environmental effect. But if such a mutation occurred in the germline of released males, their progeny and descendants could survive in the absence of tetracycline. If surviving fluorescent-marked insects

became established, this would eliminate further use of that marker in released insects (and only a few easily distinguishable markers are currently available). The effect of mutations in the marker system should also be considered.

Since mutations are expected to arise relatively frequently under mass-rearing conditions, filtering systems are proposed to eliminate these modifications before the population effects are too great, and it is argued that filters would maintain the integrity of the transgenic strains being evaluated. This is a valid point if all surviving insects are sterilized by irradiation and, as with TSL, there should be no substantial programatic or environmental effect if a few “non-lethals” get through. But if RIDL were used with non-irradiated released males to directly control a field population, arguably many thousands of “mutated” fertile males could be released over time that are not autocidal, and these might proliferate easily in an otherwise suppressed population.

So this raises the risk of having "transgenics" that persist in the field – and this possibility must be evaluated for environmental impact. It is possible that some, if not many, non-lethal transgenics could eventually be controlled by the RIDL system itself (self-mitigation), but this will not necessarily work for all types of mutations. This includes mutations/modifiers that make the transgenic insect resistant to tTA overproduction by increased tTA metabolism or changes in its site of action (similar to the way insecticide resistance occurs). Potentially this could include mutations in ubiquitin or proteasome subunits involved in tTA degradation. These resistance mechanisms could make the transgenics refractory to the RIDL system resulting in re-population with transgenic flies that can't be controlled by RIDL – thus eliminating self-mitigation. Selection for resistance (especially for mass-reared organisms) is a particular weakness of any lethal system based on toxic product accumulation subject to metabolism, and experimental evaluation to assess the frequency of resistance and ways to ameliorate it is certainly critical. While this testing *en masse* may be impractical for some insects, relevant information could be gained from testing a model system such as Drosophila. The published report of RIDL in medfly indicated that progeny of surviving transgenics in small-scale rearing were subject to subsequent RIDL control, yet it is still highly important to understand the physiological and biochemical basis of their survival.

In the assessment and analysis of risks in Appendix D, there is reference to the FAO/IAEA report which assesses risk based on the period of time transgenics are expected to be in the field:

“Of these purposes for release of genetically engineered arthropods, the release of genetically engineered insects being considered by APHIS corresponds to case (i): short-term presence in the environment with a low risk of establishment; autocidal control in SIT was used as a specific example of this category.”

The RIDL system has been deemed to be short-term based on autocidal control and thus, having limited risk. However, RIDL transgenic-based sterility is not 100% based on the published data, and for the considerations of mutations/modifiers addressed above. If RIDL does not provide a complete “fail-safe” autocidal mitigation for transgenics remaining in the environment, this must be taken into consideration for further risk assessment. Where RIDL is used as a female-lethality system, having male transgenics persist in the field until the population collapses, short-term presence does not apply at all.

Another concern is the way in which potential horizontal transmission (HT) of the transgene has been evaluated, as in the following statement:

"Self-induced horizontal transfer by transposons is very difficult to scientifically establish in the laboratory, may take millions of individual insects over innumerable generations, and may be difficult to differentiate from the normal evolutionary selection process that occurs over long periods of time. (See appendix D for more information.)"

This is essentially a dismissal of the phenomena based on, what seems to me, to be a subjective if not an erroneous evaluation. This is actually contradicted by the demonstrated HT (established in the laboratory) that allowed the *piggyBac* transposon to be isolated originally – which ocurred by its transposition from the genome of the cabbage looper moth cell line into an infectious baculovirus (see Fraser et al., 1983). The arguments against the risk of HT, or the ability to test them, in Appendix D are based upon incorporation of an autonomous element after feeding, but this is an unlikely mechanism for HT. Most HT for an autonomous transposon is thought to be mediated by infectious or symbiotic organisms (as occurred for *piggyBac*), which is also noted in Appendix D:

"The relative lack of horizontally transmitted elements may have its origin in the well-developed immune system of mammals, as **horizontal transfer requires infectious vectors**, such as viruses, against which the immune system guards. (Source: International Human Genome Sequencing Consortium, 2001)."

So the extensive theoretical analysis given in Appendix D for HT mediated by feeding is not really relevant to the more likely mechanisms of HT (unless coupled with an infectious/symbiotic agent), and furthermore, it is based on assumptions that have a questionable scientific basis, especially that HT might occur at a frequency of 10^{-9} - what is the basis for the number? I have serious doubts as to whether the presented argument could stand up to scientific scrutiny. That said, transposon-mediated HT is not expected for a stabilized vector, and if used, the dangers of HT may indeed be a moot point.

Perhaps I missed this, but I do not recall seeing any discussion of potential tTA toxic effects in predatory organisms. Abundant tTA is certainly toxic to mammalian tissues when produced intracellularly, and while digested protein may not be problematic, the issue should be addressed and may need testing.

In summary, I believe there are risks associated with the field release of transgenic RIDL strains that have been overlooked or minimized, and should be addressed. Many of these issues require experimental testing, which could be accomplished initially in Drosophila (especially mutation rates), which would then provide insights as to whether the theoretical risks are real or not, and whether further tests in large-scale rearing would be warranted in the subject insects (and environmental influences would require such testing). Potentially RIDL strains could be put into mass-rearing with initial releases limited to irradiated males so that large-scale sampling could be done first to evaluate survival rates, and their biological basis, in the absence of tetracycline. If selection for survival due to resistance mechanisms, other mutations or environmental

pressures proved to be problematic, then consideration could be given to modifications in the system or alternatives. In general, experimental analysis necessary to understand the biological basis of tTA toxicity, potential resistance/suppression mechanisms, and means to ameliorate these possibilities seems to be seriously lacking.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
ENFORCEMENT AND
COMPLIANCE ASSURANCE

AUG 5 2008

David A. Bergsten
Biological Scientist
Policy and Program Development
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 49
Riverdale, MD 20737-1238

Dear Mr. Bergsten:

In accordance with our responsibilities under Section 309 of the Clean Air Act and the National Environmental Policy Act, the U.S. Environmental Protection Agency (EPA) has reviewed the Animal and Plant Health Inspection Service's (APHIS) draft environmental impact statement (DEIS), entitled the *Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs*.

APHIS is proposing further development of genetically engineered fruit fly species and pink bollworm for use in various applications of the sterile insect technique (SIT) to agency invasive plant pest control programs, and has prepared the DEIS to evaluate the environmental impacts associated with using genetically engineered insects in invasive plant pest control programs against fruit fly species of the family tephritidae and pink bollworm. This technology is under consideration for application to SIT used in preventive area-wide release programs, suppression programs, and emergency eradication programs. The DEIS states that there is an impending need for the development of more efficient, lower cost, and effective methods for control and eradication of pink bollworm and the invasive fruit fly species because of the continuing and increasing frequency of detection of invasive insects. Based on our review of the DEIS, EPA has identified a few issues that warrant further consideration.

Tephritid flies are not as well established in the U.S. or its territories; however, pink bollworm has been established in the southwestern U.S. for many years. Presumably, some insects, birds, bats and rodents have come to rely on this insect for at least part of their diet. We suggest, therefore, that the FEIS discuss the potential impact on predators if the pink bollworm is eradicated as a result of this project.

The DEIS analyzed three alternatives: 1) No Action; 2) Expansion of Existing Programs; and 3) Integration of Genetically Engineered Insects into Programs (Preferred Alternative).

We suggest that the discussion of the No Action and the Expansion of Existing Programs alternatives be expanded to address potential use of preventive-based measures, such as: 1) increased controls on, and inspection of, imported agricultural products; and 2) improvement of environmental safeguards in trade agreements.

In conclusion, EPA has no significant environmental concerns about this proposed action. Accordingly, we have rated the DEIS as "Lack of Objections (LO). (see enclosed "Summary of Rating Definitions").

We appreciate the opportunity to review this Draft EIS, and will continue to work with APHIS to resolve these issues in preparation of the Final EIS. If you have any questions, please contact me at (202) 564-2400, or have your staff contact Arthur Totten at (202) 564-7164.

Sincerely,

Susan E. Bromm

Susan E. Bromm
Acting Director
Office of Federal Activities

Enclosure

EPA's Criteria for Sec. 309 Review of Impact Statements

Rating Environmental Impacts:

LO--Lack of Objections

EC--Environmental Concerns--Impacts identified that should be avoided. Mitigation measures may be required.

EO--Environmental Objections--Significant impacts identified. Corrective measures may require substantial changes to the proposed action or consideration of another alternative, including any that was either previously unaddressed or eliminated from the study, or the no-action alternative). Reasons can include:

- o violation of a federal environmental standard;
- o violation of the federal agency's own environmental standard;
- o violation of an EPA policy declaration;
- o potential for significant environmental degradation; or,
- o precedent-setting for future actions that collectively could result in significant environmental impacts.

EU--Environmentally Unsatisfactory--Impacts identified are so severe that the action must not proceed as proposed. If these deficiencies are not corrected in the final EIS, EPA may refer the EIS to CEQ Reasons, in addition to impacts identified, can include:

- o substantial violation of a federal environmental standard;
- o severity, duration, or geographical extent of impacts that warrants special attention; or,
- o national importance, due to threat to national environmental resources or policies.

Rating Adequacy of the Impact Statement:

1 (Adequate)--No further information is required for review.

2 (Insufficient Information)--Either more information is needed for review, or other alternatives should be evaluated. The identified additional information or analysis should be included in the final EIS.

3 (Inadequate)--Seriously lacking in information or analysis to address potentially significant environmental impacts. The draft EIS does not meet NEPA and/or Section 309 requirements. If not revised or supplemented and provided again as a draft EIS for public comment, EPA may refer the EIS to CEQ.

Appendix F. Distribution List

Charles T. Allen, Ph.D., Program Director
Extension Specialist and Professor
Texas Boll Weevil Eradication
Foundation, Inc.
P.O. Box 5089
Abilene, TX 79608–5089

Margaret L. Allen
USDA–ARS
Biological Control of Pests Research Unit
59 Lee Road
P.O. Box 67
Stoneville, MS 38776

Dr. Luke Alphey
Chief Scientist
Oxitec Limited
71 Milton Park
Abingdon, Oxford OX14 4RX
United Kingdom

Earl Andress
USDA–APHIS–PPQ–PBWRF
3645 East Chipman Road
Phoenix, AZ 85040

Norman Barr
USDA–APHIS–PPQ
Moore Air Base
22675 Moorefield Rd.
Edinburg, TX 78541

Ted Batkin
Citrus Research Board
P.O. Box 230
Visalia, CA 93279

Mark Benedict
Center for Disease Control
4770 Buford Hwy.
MS F–42
Atlanta, GA 30341

Phil Berger
USDA–APHIS–PPQ–CPHST
1730 Varsity Dr. Suite 400
Raleigh, NC 27606

Ken Bloem
USDA–APHIS–PPQ–CPHST
1730 Varsity Drive Suite 400
Raleigh, NC 27606

Susan Carlson
USDA–OIG
1400 Independence Avenue, SW
Room 117–W
Washington, DC 20250

Jeff Conn
USDA–APHIS–PPQ
9 N. Grand Ave. Room 120
Nogales, AZ 85621

Hugh Conway
USDA–APHIS–PPQ–CPHST
Moore Air Base
22675 Moorefield Rd.
Edinburg, TX 78541

David Dean
FL Department of Agriculture
and Consumer Services
Division of Plant Industry/Entomology
923 10th Street E
Palmetto, FL 34221

Terri Dunahay
USDA–APHIS–BRS
4700 River Rd. Unit 146
Riverdale, MD 20737

Michael Euler
Texas Dept. of Agriculture
900–B E. Exp. 83
San Juan, TX 78589

Gerald Franz
Insect Pest Control Sub-Programme
Joint FAO/IAEA
Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency
Wagramerstrasse 5
P.O. Box 100
A–1400 Vienna, Austria

Richard Gaskalla
Division Director
Florida Department of Ag & Consumer Services
Division of Plant Industry
Doyle Conner Building
P.O. Box 147100
Gainesville, FL 32614–7100

Carolyn Gethers
USDA–APHIS–PPQ
4700 River Rd. Unit 156
Riverdale, MD 20737

Kevin Golden
Staff Attorney
Center for Food Safety
2601 Mission Street Suite 803
San Francisco, CA 94110

Michael Gottlieb
FNIH
45 Center Drive
Bethesda, MD 20892

Alfred M. Handler, Ph.D.
Research Geneticist
USDA–ARS
Center for Medical, Agricultural, and Veterinary Entomology
1700 S.W. 23rd Drive
Gainesville, FL 32608

Glen Hart
FDACS, DPI, Methods
1833 57th Street
Sarasota, FL 34243

Jorge Hendrichs
Insect Pest Control Sub-Programme
Joint FAO/IAEA
Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency
Wagramerstrasse 5
P.O. Box 100
A–1400 Vienna, Austria

Stephen James FNIH
45 Center Drive 3AN-44
Bethesda, MD 20892

Angus R. Kelly
Regulatory Affairs Representative
National Cotton Council of America
1521 New Hampshire Ave., NW
Washington, DC 20036

Ann Kramer
Head of Business Development
Oxitec Limited
71 Milton Park
Abingdon, Oxford OX14 4RX
United Kingdom

Jerry Levitt
USDA–APHIS–PPQ
3658 East Chipman Road
Phoenix, AZ 85040

David Madison
Arizona Department of Agriculture
1188 W. Adams
Phoenix, AZ 85007

Bob Maugan
USDA–ARS
2413 E Business 83
Weslaco, TX 78596

Andre McCarroll
USDA–APHIS–PPQ
9325 Bay Plaza Blvd. Suite 206
Tampa, FL 33619

Susan McCombs
USDA–APHIS–PPQ
4700 River Rd., Unit 137
Riverdale, MD 20737

E. Keith Menchey, Ph.D.
Manager, Science & Environment Issues
National Cotton Council
1521 New Hampshire Avenue, NW
Washington, DC 20036

Ernie Miller
USDA–APHIS–PPQ
3645 East Chipman Road
Phoenix, AZ 85040

Thomas A. Miller, Professor
University of California at Riverside
Entomology Department
Riverside, CA 92521

Soo Ok Miller
University of California at Riverside
Entomology Department
Riverside, CA 92521

George Nash
USDA–APHIS–PPQ
903 San Jacinto Suite 270
Austin, TX 78701

Don Parker, Ph.D.
Manager, Integrated Pest Management
National Cotton Council of America
1918 N. Parkway
Memphis, TN 38182

Paul Parker
USDA–APHIS–PPQ
Moore Air Base
22675 Moorefield Rd.
Edinburg, TX 78541

Mary Megan Quinlan
Regulatory Consultant
Suite 17
24–28 Saint Leonard's Road
Windsor, Berkshire SL4 3BB
United Kingdom

Sinead O'Connell
Oxitec Limited
71 Milton Park
Abington, Oxford OX14 4RX
United Kingdom

Jim Rains
California Department of Food
and Agriculture
1220 N Street
Sacramento, CA 95814

John Renshaw
USDA–APHIS–PPQ
1833 57th Street
Sarasota, FL 34243

Connie Riherd
FL Department of Agriculture and
Consumer Services
Division of Plant Industry
P.O. Box 147100
Gainesville, FL 32614

Jane Rissler
Deputy Director/Senior Scientist
Union of Concerned Scientists
Food and Environment Program
1707 H Street, NW Suite 600
Washington, DC 20006–3962

Alan S. Robinson
Insect Pest Control Sub-Programme
Joint FAO/IAEA
Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency
Wagramerstrasse 5
P.O. Box 100
A–14 Vienna, Austria

Robert I. Rose
Biotech Consultant
8322 Sharon drive
Frederick, MD 21704

Robyn Rose
USDA–APHIS–PPQ–PIM
4700 River Rd. Unit 150
Riverdale, MD 20737

James Rudig
California Dept. of Food and Agriculture
2895 N. Larkin Ave. Suite A
Fresno, CA 93727

Gary Russell
USDA–APHIS–PPQ
3658 E Chipman Road
Phoenix, AZ 85040

Barbara Sachau
15 Elm Street
Florham Park, NJ 07932

Josie Salinas
USDA–APHIS–CPHST
Moore Air Base
Edinburg, TX 78541

Jose Sanchez
Texas Dept. of Agriculture
900–B E. Exp. 83
San Juan, TX 78589

Ken Seeley
USDA–APHIS–PPD–ES
4700 River Rd. Unit 149
Riverdale, MD 20737

Harold T. Smith
1197 Kings Ranch Road
Bandera, TX 78003

Robert Staten
University of Arizona
Cotton/PBW Eradication Program
414 E Barbaritz Avenue
Gilbert, AZ 85234–4629

Don Thomas
USDA–ARS
2413 E. Highway 83
Weslaco, TX 78596

Don C. Vacek
USDA–APHIS–PPQ–CPHST–PDDML
Moore Air Base Bldg. 6414
22675 N. Moorefield Rd.
Edinburg, TX 78541

Bob Vlasik
USDA–APHIS–PPQ
320 N. Main Room 119
McAllen, TX 78501

John Worley
Moore Air Base Bldg. 6418
22675 N. Moorefield Rd.
Edinburg, TX 78541

Chris Wozniak
USDA–CSREES
1400 Independence Ave., SW
Washington, DC 20024

Helene Wright
USDA–APHIS–PPQ
650 Capitol Mall Suite 6–400
Sacramento, CA 95814

Appendix G. References Cited

Adair, D., Irwin, R., and Traynor, P.T., 2001. A practical guide to containment. Greenhouse research with transgenic plants and microbes. Information Systems for Biotechnology, Virginia Tech, Blacksburg, VA. 59 pp.

Adkisson, P., and Tumlinson, J., 2003. Edward F. Knipling, 1909–2000, A biographical memoir. Biographical Memoirs, Volume 83. National Academies Press, National Academy of Sciences. Washington, DC.

Allen, M., 2007. Transgenic screwworm applications and SIT. Internatinal Cong. Of Insect Biotech. And Industry Proc., Daegu, Republic of Korea. Entomol.Res. 37 (supplement 1):A54. [Online]. Available: http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115-214627. [2008, Sept. 9].

Allen, M., O'Kane, C., and Moffat, K., 2002. Cell ablation using wild-type and cold-sensitive ricin-A chain in *Drosophila* embryonic mesoderm. Genesis 34:132–134.

Allen, M.L., Berkebile, D.R., and Skoda, S.R., 2004. Postlarval fitness of genetically engineered strains of *Cochliomyia homonivorax* (Diptera: Calliphoridae). Journal of Economic Entomology 97(3):1181–1185.

Allen, M.L., and Christensen, B.M., 2004. Flight muscle-specific expression of act88F: GFP in transgenic *Culex quinquefasciatus* Say (Diptera Culicidae). Parasitol.Int 53(4):307–314.

Allen, M.L., Handler, A.M., Berkebile, D.R., and Skoda, S.R., 2004b. piggyBac transformation of the new world screwworm, *Cochliomyia hominivorax*, produces multiple distinct mutant strains. Med.Vet.Entomol. 18(1):1–9.

Allen, M.L., O'Brochta, D.A., Atkinson P.W., and Levesque, C.S., 2001. Stable, germ-line transformation of *Culex quinquefasciatus* (Diptera: Culicidae). J.Med.Entomol. 38(5):701–710.

Allen, M.L., and Scholl, P.J., 2005. Quality of genetically engineered laboratory strains of *Cochliomyia hominivorax* (Diptera: Calliphoridae). Journal of Economic Entomology 98(6):2301–2306.

Alphey, L., 2007. Engineering insects for the sterile insect technique, in area-wide control of insect pests: from research to field implementation, M. Vreysen, A. Robinson, and J. Hendrichs, eds. (Dordrecht, The Netherlands: Springer), pp. 51–61.

Alphey, L., 2002. Re-engineering the sterile insect technique. Insect Biochem. Mol. Biol. 32:1243–1247.

Alphey, L., and Andreasen, M.H., 2002. Dominant lethality and insect population control. Mol. Biochem. Parasitol, 121:173–178.

Alphey, L., Nimmo, D., O'Connell, S., and Alphey, N., 2007. Insect population suppression using engineered insects, *In:* Transgenesis and the management of vector-borne disease, S. Aksoy, ed. (Austin, Texas: Landes Bioscience).

Alphey, N., Coleman, P.G., Donnelly, C.A., and Alphey, L., 2007. Managing insecticide resistance by mass release of engineered insects. J.Econ.Entomol. 100:1642–1649.

Anonymous, 1976. Chronology of Mediterranean fruit fly 1975–76. Misc. fruit fly exclusion and detection programs files. USDA–APHIS, Riverdale, MD.

Anonymous, 1929. Med. fruit fly campaign. Misc. fruit fly exclusion and detection program files. USDA–APHIS–PPQ, Riverdale, MD.

APHIS—See U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

The Arabidopsis Genome Initiative, 2000. Analysis of the genome sequence of the flowering plant, *Arabidopsis thaliana*. Nature 408:796–815.

Atkinson, M., Su, Z., Alphey, N., Alphey, L., Coleman, P., and Lawrence, M., 2007. Analyzing the control of mosquito-borne diseases by a dominant lethal genetic system. Proc. Nat'l. Acad. Sci. (USA) 104:9540–9545.

Atkinson, P., Pinkerton, A., and O'Brochta, D., 2001. Genetic transformation systems in insects. Annual Rev. Entomol. 46:317–346.

Baumhover, A.H., 2002. A personal account of developing the sterile insect technique to eradicate the screwworm from Curacao, Florida and the southeastern United States. Florida Entomologist 85(4):666–673.

Bellen, H., D'Evelyn, D., Harvey, M., and Elledge, S., 1992. Isolation of temperature-sensitive diphtheria toxins in yeast and their effects on *Drosophila* cells. Development 114:787–796.

Benner, J., 2001. Frankenbugs in the wings wired. Available: http://www.wired.com/news/medtech/0,1286,48774,00.html. [October 10, 2007.]

Bennetzen, J.L., 2000. Comparative sequence analysis of plant nuclear genomes. Microcolinearity and its many exceptions. Plant Cell 12:1021–1030.

Berg, C., and Spradling, A., 1991. Studies on the rate and site-specificity of P element transposition. Genetics 127:515–524.

Berger, S.L., Cress, W.D., Cress, A., Triezenberg, S.J., and Guarente, L., 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell 61:1199–1208.

Berghammer, A.J., Klingler, M., and Wimmer, E.A., 1999. A universal marker for transgenic insects. Nature 402:370–371.

Berry, N.O., 1943. History of the Mexican fruit fly project, 1927 to 1943. Misc. fruit fly exclusion and detection programs files. Riverdale, MD. 1943.

Bevis, B., and Glick, B., 2002. Rapidly maturing variants of the *Discosoma* red fluorescent protein (DsRed). Nat. Biotechnol. 20:83–87.

Brand, A., 1995. GFP in *Drosophila*. Trends in Genetics 11:324–325.

Brand, A., Manoukian, A., and Perrimon, N., 1994. Ectopic expression in Drosophila. Meth Cell Biol. 44:635–654.

Brand, A., and Perrimon, N., 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118:401–415.

Briceno, D., Eberhard, W., and Shelly, T., 2007. Male courtship behavior in Ceratitis capitata (Diptera: Tephritidae) that have received aromatherapy with ginger oil. Florida Entomologist 90:175–179.

Burrows, T.M., Sevacherian, V., Browning, H., and Baritelle, J., 1982. The history and cost of the pink bollworm in the Imperial Valley. Bull. Entomol. Soc. Amer. 28:286–290.

Busch-Petersen, E., 1989. Genetic sex separation in sterile insect technique pest management programmes, with special reference to the Med-fly, Ceratitis capitata (Wied.). *In*: Fruit Flies of Economic Importance 87, April 1987. A.A. Balkema, ed., Commission of European Communities and International Organization for Biological Control. Rotterdam, Netherlands.

Calabrese, E.J., 1991. Multiple chemical interactions. Lewis Publishers, Chelsea, MI.

Calabrese, E.J., 1984. Ecogenetics. John Wiley and Sons, New York.

Calkins, C.O., and Parker, A.G., 2005. Sterile insect quality. *In*: Sterile insect technique. Principles and practice in area-wide integrated pest management, V.A. Dyck, J. Hendrichs, and A.S. Robinson, eds. (The Netherlands: Springer), pp. 269–296.

Carey, J.R., 1996. The future of the Mediterranean fruit fly *Ceratitis capitata* invasion of California: a predictive framework. Biological Conservation 78:35–50.

Carpenter, J.E., Bloem, S., and Marec, F., 2005. Inherited sterility in insects. *In:* Sterile insect technique. Principles and practice in area-wide integrated pest management, V.A. Dyck, J. Hendrichs, and A.S. Robinson, eds. (The Netherlands: Springer), pp. 115–146.

Cary, L., Goebel, M., Corsaro, B., Wang, H., Rosen, E., and Fraser, M., 1989. Transposon mutagenesis of baculoviruses: analysis of *Tricoplusia ni* transposon *IFP2* insertions within the FP-locus of nuclear polyhedrosis viruses. Virology 172:156–169.

Catteruccia, F., Charles, H., Godfray, J., Crisanti, A., 2003. Impact of genetic manipulation on the fitness of Anopheles stephensi mosquitoes. Science 299(5610):1225–1227.

Clark, R.A., Steck, G.J., Weems, Jr., H.W., 1996. Detection, quarantine, and eradication of exotic fruit flies in Florida. *In:* D.L. Rosen (ed.), Pest management in the subtropics: integrated pest management - a Florida perspective. Intercept Ltd. Andover UK, pp. 29–54.

Coates, C., Jasinskiene, N., Miyashiro, L., and James, A., 1998. Mariner transposition and transformation of the yellow fever mosquito, Aedes aegypti. Proc. Natl. Acad. Sci. USA 95:3748–3751.

Condon, K., Condon, G., Dafa'alla, T., Forrester, O., Phillips, C., Scaife, S., and Alphey, L., 2007. Germ-line transformation of the Mexican fruit fly. Insect Molecular Biology. 16(5):573–580.

Cooley, L., Kelley, R., and Spradling, A., 1988. Insertional mutagenesis of the *Drosophila* genome with single *P* elements. Science 239:1121–1128.

Cubitt, A., Heim, R., Adams, S., Boyd, A., Gross, L., and Tsien, R., 1995. Understanding, improving and using green fluorescent proteins. Trends Biochem. Sci. 20(11):448–455.

Cunningham, G.L., 1976. Minutes of Mediterranean fruit fly critique. Misc. fruit fly exclusion and detection program files. USDA–APHIS–PPQ, Riverdale, Maryland.

Dafa'alla, T.H., Condon, G.C., Condon, K.C., Phillips, C.E., Morrison, N.I., Jin, L., Epton, M.J., Fu, G., and Alphey, L., 2006. Transposon-free insertions for insect genetic engineering. Nature Biotechnology 24:820–821.

Damke, H., Gossen, M., Freundlieb, S., Bujard, H., and Schmid, S., 1995. Tightly regulated and inducible expression of dominant interfering dynamin mutant in stably transformed HeLa cells. Meth. Enzymol. 257:209–220.

Dietzl, G., Chen, D., Schnorrer, F., Su, K.-C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., and Scheiblauer, S., 2007. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. Nature 448:151–157.

Dowell, R.V., Siddiqui, I.A., Meyer, F., and Spaugy, E.L., 2000. Mediterranean fruit fly preventative release programme in southern California. *In*: Area-Wide Control of Fruit Flies and Other Insect Pests. Fifth International Symposium on Fruit Flies of Economic Importance, June, 1998, FAO/IAEA. Pulau Pinang: Penerbit Universiti Sains Malaysia.

Dutton, J.R., 1968. Chronology-Mexican fruit fly control program western region. Misc. fruit fly exclusion and detection program files. USDA–APHIS–PPQ, Riverdale, MD.

Dyck, V., Hendrichs, J., and Robinson, A., eds., 2005. Sterile insect technique: principles and practice in area-wide integrated pest management (Dordrecht: Springer).

El-Lissy, O., Staten, R.T., and Grefenstette, W., 2005a. Pink bollworm eradication plan in the U.S. USDA–APHIS–PPQ, Riverdale, MD and Phoenix, AZ. Available: http://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pest/index.shtml

El-Lissy, O., Staten, R.T., and Grefenstette, W., 2005b. Progress pink bollworm eradication in the U.S. and Mexico, 2005. USDA–APHIS–PPQ, Riverdale, MD and Phoenix, AZ.

Engels, W., 1992. The origin of *P* elements in *Drosophila melanogaster*. Bioessays *14*.

Engels, W., 1989. *P* elements in *Drosophila*, In Mobile DNA, D. Berg, and M. Howe, eds. Washington, DC: American Society of Microbiology., pp. 437–484.

ERS—See U.S. Department of Agriculture, Economic Research Service.

FAO—See Food and Agriculture Organization.

FDACS—See Florida Department of Agriculture and Consumer Services.

Feschotte, C., Jiang, N., and Wessler, S.R., 2002. Plant transposable elements: Where genetics meets genomics. Nat. Rev. Genet. 3:329–341.

Fisher, K., and Caceres, C., 2000. A filter rearing system for mass reared genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae). *In*: Area-Wide Control of Fruit Flies and Other Insect Pests. Fifth International Symposium on Fruit Flies of Economic Importance, June 1998, FAO/IAEA. Pulau Pinang: Penerbit Universiti Sains, Malaysia.

Flavell, A.J., 1992. Ty1-copia group retrotransposons and the evolution of retroelements in the eukaryotes. Genetica 86:203–214.

Florida Department of Agriculture and Consumer Services, Division of Plant Industry and USDA–APHIS, 2004. Florida Fruit Fly Detection Manual.

Food and Agriculture Organization, International Atomic Energy Agency, and U.S. Department of Agriculture, 2003. Insect pest control. FAO/IAEA/USDA manual for product quality control and shipping procedures for sterile mass-reared tephritid fruit flies. May 2003. Available: http://www.iaea.org/programmes/nafa/d4/indx-tephritid.html [August 22, 2007.]

Fortier, E., and Belote, J., 2000. Temperature-dependent gene silencing by an expressed inverted repeat in *Drosophila*. Genesis 26:240–244.

Fortini, M., Simon, M., and Rubin, G., 1992. Signalling by the sevenless protein tyrosine kinase is mimicked by Ras1 activation. Nature 355:559–561.

Franz, G., Rendon, P., Hendrichs, J., and Kerremans, P., 1996. Development and application of genetic sexing systems for the Mediterranean fruit fly based upon a temperature sensitive lethal. *In*: Fruit Fly Pests: World Assessment of Their Biology and Management. Delray Beach, Florida: St. Lucie P, 1996, pp. 185–191.

Fraser, M., Ciszczon, T., Elick, T., and Bauser, C., 1996. Precise excision of TTAA-specific lepidopteran transposons *piggyBac* (IFP2) and *tagalong* (TFP3) from the baculovirus genome in cell lines from two species of Lepidoptera. Insect Molecular Biology 5:141–151.

Fraser, M., Smith, G., and Summers, M., 1983. Acquisition of host-cell DNA-sequences by baculoviruses - relationship between host DNA insertions and FP mutants of *Autographica-californica* and *Galleria-mellonella* nuclear polyhedrosis viruses. J. Virol. 47:287–300.

Fryxell, K., and Miller, T., 1995. Autocidal biological control: a general strategy for insect control based on genetic transformation with a highly conserved gene. J. Econ. Entomol. 88:1221–1232.

Fu, G., Condon, K., Epton, M., Gong, P., Jin, L., Condon, G., Morrison, N., Dafa'alla, T., and Alphey, L., 2007. Female-specific insect lethality engineered using alternative splicing. Nature Biotechnology 25:353–357.

Gilbert, A.J., Bingham, R.R., Nicolas, M.A., and Clark, R.A., 1984. Insect Trapping Guide. California Department of Food and Agriculture, Pest Detection/Emergency Programs, Sacramento, CA: CDFA, 1984.

Gillespie, J.P., Kanost, M.R., and Trenczek, T., 1997. Biological mediators of insect immunity. Annual Rev. Entomol. 42:611–643.

Glick, P.A., 1967. Aerial dispersal of the pink bollworm in the United States and Mexico. U.S. Dept. Agric. Agric. Prod. Res. Rep. 96.

Gomulski, L.M., Torti, C., Murelli, V., Bonizzoni, M., Gasperi, G., and Malacrida., A.R., 2004. Medfly transposable elements: diversity, evolution, genomic impact and possible applications. Insect Biochemistry and Molecular Biology 34:139–148.

Gong, P., Epton, M., Fu, G., Scaife, S., Hiscox, A., Condon, K., Condon, G., Morrison, N., Kelly, D., and Dafa'alla, T., 2005. A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. Nat. Biotech. 23:453–456.

Gonzalez-Estevez, C., Momose, T., Gehring, W., and Salo, E., 2003. Transgenic planarian lines obtained by electroporation using transposon-derived vectors and an eye-specific GFP marker. Proc. Nat'l. Acad. Sci. (USA) 100:14046–14051.

Gossen, M., Bonin, A., Freundlieb, S., and Bujard, H., 1994. Inducible gene expression systems for higher eukaryotic cells. Curr. Opin. Biotechnol. 5:516–520.

Gossen, M., and Bujard, H., 1992. Tight control of gene expression in mammalian cells by tetracycline- responsive promoters. Proc. Natl. Acad. Sci. USA 89:5547–5551.

Gould, F., and Schliekelman, P., 2004. Population genetics of autocidal control and strain replacement. Annual Rev. Entomology 49:193–217.

Grefenstette, W., El-Lissy, O., and Staten, R.T., 2007. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Pink bollworm eradication plan in the U.S. Updated April, 2007. Riverdale, MD, Phoenix, AZ. Available: http://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/index.shtml. [July 16, 2007.]

Grossman, G., Rafferty, C., Fraser, M., and Benedict, M., 2002. The *piggyBac* element is capable of precise excision and transposition in cells and embryos of the mosquito, *Anopheles gambiae*. Insect Biochemistry and Molecular Biology 32:487.

Grossman, G., Rafferty, C., Clayton, J., Stevens, T., Mukabayire, O., and Benedict, M., 2001. Germline transformation of the malaria vector, *Anopheles gambiae*, with the piggyBac transposable element. Insect Molecular Biology 10:597–604.

Häcker, U., Nystedt, S., Barmchi, M., Horn, C., and Wimmer, E., 2003. *piggyBac*-based insertional mutagenesis in the presence of stably integrated *P* elements in *Drosophila*. Proc. Nat'l. Acad. Sci. (USA) 100:7720–7725.

Hagler, J.R., and Naranjo. S.E., 1994. Determining the frequency of heteropteran predation on sweet potato whitefly and pink bollworm using multiple ELISAs. Entomol.Exp.Appl. 72:59–66.

Hake, S. and Walbot, V., 1980. The genome of Zea mays, its organization and homology to related grasses. Chromosoma 79:251–270.

Han, D., Stein, D., and Stevens, L., 2000. Investigating the function of follicular subpopulations during *Drosophila* oogenesis through hormone-dependent enhancer-targeted cell ablation. Development 127:573–583.

Handler, A., 2004. Understanding and improving transgene stability and expression in insects for SIT and conditional lethal release programs. Insect Biochem. Mol. Biol. 34: 121–130.

Handler, A., 2002. Use of the *piggyBac* transposon for germ-line transformation of insects. Insect Biochem. Mol. Biol. 32:1211–1220.

Handler, A., 2001. A current perspective on insect gene transformation. Insect Biochem. Mol. Biol. 31:111–128.

Handler, A., and Harrell, R.A.I., 2001. Transformation of the Caribbean fruit fly, *Anastrepha suspensa*, with a *piggyBac* vector marked with polyubiquitin-regulated GFP. Insect Biochem. Mol. Biol. 31:199–205.

Handler, A., and Harrell, R.A.I., 1999. Germline transformation of *Drosophila melanogaster* with the *piggyBac* transposon vector. Insect Molecular Biology 8:449–457.

Handler, A., and James, A., 2000. Insect transgenesis: methods and applications (Boca Raton: CRC Press).

Handler, A., Zimowska, G., and Horn, C., 2004. Post-integration stabilization of a transposon vector by terminal sequence deletion in *Drosophila melanogaster*. Nat. Biotech. 22:1150–1154.

Handler, A.M., and McCombs, S.D., 2000. The *piggyBac* transposon mediates germ-line transformation in the Oriental fruit fly and closely related elements exist in its genome. Insect Molecular Biology 9:605–612.

Handler, A.M., McCombs, S.D., Fraser, M.J., and Saul, S.H., 1998. The lepidopteran transposon vector, piggyBac, mediates germ-line transformation in the Mediterranean fruit fly. Proc. Natl. Acad. Sci. (USA) 95:7520–7525.

Haseloff, J., Siemering, K., Prasher, D., and Hodge, S., 1997. Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. Proc. Nat. Acad. Sci. (USA) 94:2122–2127.

Hay, B.A., and Guo, M., 2006. Caspase-dependent cell death in *Drosophila*. Annual Review of Cell and Developmental Biology 22:623–650.

Hediger, M., Niessen, M., Wimmer, E., Dubendorfer, A., and Bopp, D., 2001. Genetic transformation of the housefly *Musca domestica* with the lepidopteran derived transposon *piggyBac*. Insect Molecular Biology 10:113–119.

Heim, R., Prasher, D., and Tsien, R., 1994. Wavelength mutations and posttranslational autoxidation of green fluorescent protein. Proc. Nat. Acad. Sci. (USA) 91:12501–12504.

Heim, R., and Tsien, R., 1996. Engineering green fluorescent protein for improved brightness, longer wavelengths, and fluorescence resonance energy transfer. Current Biology 6:178–182.

Heinrich, J., and Scott, M., 2000. A repressible female-specific lethal genetic system for making transgenic insect strains suitable for a sterile-release program. Proc. Nat'l. Acad. Sci. (USA) 97:8229–8232.

Hendrichs, J., Ortiz, G., Liedo, P., and Schwarz, 1983. Six years of successful Medfly program in Mexico and Guatemala. *In*: Fruit Flies of Economic Importance, November 1982. A.A. Balkema, ed., Commission of European Communites and International Organization for Biological Control. Rotterdam, Netherlands.

Henneberry, T.J., and Naranjo, S.E., 1998. Integrated management approaches for pink bollworm in the southwestern United States. Integrated Pest Management Rev. 3:31–52

Hidalgo, A., Urban, J., and Brand, A.H., 1995. Targeted ablation of glia disrupts axon tract formation in the *Drosophila* CNS. Development 121:3703–3712.

Horn, C., and Wimmer, E., 2003. A transgene-based, embryo-specific lethality system for insect pest management. Nat. Biotech. 21:64–70.

IAEA—See International Atomic Energy Agency

International Atomic Energy Agency, 2006. Status and risk assessment of the use of transgenic arthropods in plant protection. Proceedings of a technical meeting organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture of the International Plant Protection Convention held in Rome 8 to 12 April 2002. IAEA–TECDOC–1483. Available: http://www-pub.iaea.org/MTCD/publications/ResultsPage.asp

International Atomic Energy Agency, 2003. Trapping Guidelines for Area-Wide Fruit Fly Programmes. International Atomic Energy Agency. Available: http://www-pub.iaea.org/MTCD/publications/PDF/TG-FFP_web.pdf. [August 22, 2007.]

International Atomic Energy Agency, 1999. Thematic plan for fruit fly control using the sterile insect technique. IAEA, Vienna, Austria. Available: http://www.iaea.org/programmes/nafa/d4/public/d4_pbl_4_1.html

International Human Genome Sequencing Consortium, 2001. Initial sequencing and analysis of the human genome. Nature 409: 860–921.

International Plant Protection Convention, 2008. International Phytosanitary Portal. IPPC Home. [Online]. Available: https://www.ippc.int.IPP/En/default.jsp. [2008, Sept. 2].

International Plant Protection Converntion, 2007. Glossary of phytosanitary terms. International Standard for Phytosanitary Measures No. 5. Mar. 2007. Available: https://www.ippc.int/servlet/BinaryDownloaderServlet/184195_ISPM05_2007_E.doc?filename=1180102644600_ISPM_05_2007.doc&refID=184195. [August 17, 2007.]

International Plant Protection Convention, 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. Secretariat of the IPPC, FAO of the United Nations, Rome, Italy. Available: https://www.ippc.int/servlet/BinaryDownloaderServlet/76047_ISPM_3_E.pdf?filename=1146657660135_ISPM3.pdf&refID=76047. [August 17, 2007.]

IPPC—See International Plant Protection Convention

Irvin, N., Hoddle, M.S, O'Brochta, D.A., Carey, B. and Atkinson, P.W., 2004. Assessing fitness costs for genetically engineered *Aedes aegypti* expressing the GFP marker and transposase genes. PNAS 101(3):891–896.

Izekor, S. and Katayama, R.W., 2007. Okra Production Update for Small Acreage Growers. University of Arkansas at Pine Bluff, U.S. Department of Agriculture, and County Governments Cooperating.

Jasinskiene, N., Coates, C., Benedict, M., Cornel, A., Rafferty, C., James, A., and Collins, F., 1998. Stable transformation of the yellow fever mosquito, *Aedes aegypti*, with the Hermes element from the housefly. Proc. Natl. Acad. Sci. USA 95:3743–3747.

Kapetanaki, M., Loukeris, T., Livadars, I., and Savakis, C., 2002. High frequencies of *Minos* transposon mobilization are obtained in insects by using *in vitro* synthesized mRNA as a source of transposase. Nucl. Acids Res. 30:3333–3340.

Kaufman, P., and Rio, D., 1991. Germline transformation of *Drosophila melanogaster* by purified P element transposase. Nucl. Acids Res. 19:6336.

Klassen, W., and Curtis, C.F., 2005. History of the sterile insect technique. In Sterile Insect Technique. Principles and practice in area-wide integrated pest management, V.A. Dyck, J. Hendrichs, and A.S. Robinson, eds. (The Netherlands: Springer), pp. 16.

Klemenz, R., Weber, U., and Gehring, W., 1987. The white gene as a marker in a new P-element vector for gene transfer in Drosophila. Nucl. Acids Res. 15:3947–3959.

Knipling, E.F., 1985. Sterile insect technique as a screwworm control measure: the concept and its development. Entomological Society of America Misc. Publ. 62:4–7.

Kokoza, V., Ahmed, A., Wimmer, E., and Raikhel, A., 2001. Efficient transformation of the yellow fever mosquito *Aedes aegypti* using the *piggyBac* transposable element vector pBac[3xP3-EGFP afm]. Insect Biochem. Mol. Biol. 31:1137–1143.

Koukidou, M., Klinakis, A., Reboulakis, C., Zagoraiou, L., Tavernarakis, N., Livadaras, I., Economopoulos, A., and Savakis, C., 2006. Germ line transformation of the olive fly *Bactrocera oleae* using a versatile transgenesis marker. Insect Molecular Biol. 15:95–103.

Kűhne, M., Ihnen, D., Möller, G., and Agthe, O., 2000. Stability of tetracycline in water and liquid manure. J. Vet. Med. 47(6):379–384.

Kumar, A. and Bennetzen, J.L., 1999. Plant retrotransposons. Annual Rev. Genet. 33:479–532.

Lampe, D., Churchill, M., and Robertson, H., 1996. A purified mariner transposase is sufficient to mediate transposition in vitro. EMBO Journal 15:5470–5479.

Lampe, D., Grant, T., and Robertson, H., 1998. Factors affecting transposition of the *Himar1 mariner* transposon *in vitro*. Genetics 149:179–187.

Lampe, D., Witherspoon, D., Soto-Adames, F., and Robertson, H., 2003. Recent horizontal transfer of mellifera subfamily *mariner* transposons into insect lineages representing four different orders shows that selection acts only during horizontal transfer. Molecular Biol. and Evolution 20:554–562.

Leeton, P.R., and Smyth, D.R., 1993. An abundant LINE-like element amplified in the genome of *Lilium speciosum*. Mol. Gen. Genet. 237: 97–104.

Leggett, J.E., El-Lissy, O., and Antilla, L., 1994. Pink bollworm moth catches with perimeter and in-field gossyplure baited traps. Southwestern Entomologist 19(2):147–155.

Li, X., Harrell, R., Handler, A., Beam, T., Hennessy, K., and Fraser, M., 2005. *piggyBac* internal sequences are necessary for efficient transformation of target genomes. Insect Molecular Biol. 14:17–30.

Li, X., Heinrich, J., and Scott, M., 2001. *piggyBac*-mediated transposition in *Drosophila melanogaster*: an evaluation of the use of constitutive promoters to control transposase gene expression. Insect Molecular Biol. 10:447–455.

Lobo, N., Li, X., Hua-Van, A., and Fraser, M., 2001. Mobility of the *piggyBac* transposon in embryos of the vectors of Dengue fever (*Aedes albopictus*) and La Crosse encephalitis *(Ae. triseriatus*). Mol. Genet. Genomics 265:66–71.

Lobo, N., Li, X., and Fraser, M., 1999. Transposition of the *piggyBac* element in embryos of *Drosophila melanogaster, Aedes aegypti* and *Tricoplusia ni*. Mol. Gen. Genet. 261:803–810.

Loukeris, T.G., Livadaras, I., Arca, B., Zabalou, S., and Savakis, C., 1995. Gene transfer into the medfly, *Ceratitis capitata*, with a *Drosophila hydei* transposable element. Science 270:2002–2005.

Marrelli, M.T., Li, C,, Rasgon, J.L., Jacobs-Lorena, M., 2007. Transgenic malaria-resistant mosquitoes have a fitness advantage when feeding on Plasmodium-infected blood. Proc. Natl. Acad. Sci. 104(13):5580–5583.

Marrelli, M.T., Moreira, C., Kelly, D., Alphey, L., and Jacobs-Lorena, M., 2006. Mosquito transgenesis: what is the fitness cost? Trends Parasit. 22:197–202.

Matz, M., Fradkov, A., Labas, Y., Savitsky, A., Zaraisky, A., Markelov, M., and Lukyanov, S., 1999. Fluorescent proteins from nonbioluminescent Anthozoa species. Nat. Biotechnol. 17:969–973.

Metcalf, C.L., Flint, W.P., and Metcalf, R.L., 1962. Destructive and Useful Insects, McGraw-Hill Book Co. N.Y., San Francisco, Toronto, and London.

Meyers, B.C., Tingey, S.V., and Morgante, M., 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. Genome Res. 11:1660–1676.

Miller, E., Staten, R.T., Claus, J., Sledge, M., Peloquin, J., and Miller, T., 2001. A multiple generation life history study on rearing a genetically altered (EGFP) strain of pink bollworm (Lepidoptera: Gelechiidae). Paper presented at: Belt Wide Cotton Conference (Anaheim, CA).

Moffat, K., Gould, J., Smith, H., and O'Kane, C., 1992. Inducible cell ablation in *Drosophila* by cold-sensitive ricin A chain. Development 114:681–687.

Muir, W.M. and Howard, R.D., 2001. Fitness components and ecological risk of genetically engineered release: A model using Japanese medaka (*Oryzias latipes*). The American Naturalist 158(1). Available: http://www.journals.uchicago.edu/AN/journal/contents/v158n1.html

Nagel, P., and Peveling, R., 2005. Environment and the sterile insect technique. *In:* Sterile Insect Technique. V.A. Dyck, J. Hendrichs, A.S. Robinson [eds.]. IAEA, Springer, Dordrecht., pp. 499–524.

Nakabachi, A., 2008. Detection of genes laterally transferred from bacteria to aphid genome (personal communication to Robert Rose, August 18, 2008).

NAPPO—See North American Plant Protection Organization.

Naranjo, S.E., and Hagler, J.R., 1998. Characterizing and estimating the effect of heteropteran predation. Thomas Say Publications: Predatory Heteroptera. Pp. 173–195.

NASS—See U.S. Department of Agriculture, National Agricultural Statistics Service.

NCC—See National Cotton Council of America.

National Academy of Sciences, 1977. Drinking water and health, Vol. 1. National Academy of Sciences, Washington, DC.

National Cotton Council, 2001. Pink bollworm eradication. A window of opportunity. 6 pp. National Cotton Council of America, Memphis, TN. August 2001. Available: http://www.cotton.org/tech/pest/bollworm/index.cfm

National Institutes of Health, 2002. Guidelines for research involving recombinant DNA molecules. National Institutes of Health, Bethesda, MD. Available: http://www4.od.nih.gov/oba/rac/guidelines/guidelines,html

Neuberger, P.H., Saville, K.J., Zeng, J., Smyth, K-A., Belote, J.M., 2006. A genetic suppressor of two dominant temperature-sensitive lethal proteasome mutants of *Drosophila melanogaster* is itself a mutated proteasome subunit gene. Genetics 1731377–1387.

NIH—See National Institutes of Health.

Nilakhe, S.S., Worley, J.N., Garcia, R., and Davidson, J.L, 1991. Mexican fruit fly protocol helps export Texas citrus. Subtropical Plant Science 44:49–52.

Noble, L.W., 1969. Fifty years of research on the pink bollworm in the Unites States. U.S. Dept. Agric., Agric. Res. Serv. Handb. No. 357.

North American Plant Protection Organization, 2007. Guidelines for importation and confined release of transgenic arthropods in NAPPO member countries. RSPM No. 27. Secretariat of NAPPO, Ottawa, Ontario, Canada, 24 pp.

North American Plant Protection Organization, 2004. Guidelines for construction and operation of a containment facility for insects and mites used as biological control agents. RSPM No. 22. Secretariat of NAPPO, Ottawa, Ontario, Canada, 9 pp.

Nolan, T., Bower, T., Brown, A., Crisanti, A., and Catteruccia, F., 2002. *piggyBac*-mediated germline transformation of the malaria mosquito *Anopheles stephensi* using the red fluorescent protein dsRED as a selectable marker. J. Biol. Chem. 277:8759–8762.

NRCS—See U.S. Department of Agriculture, Natural Resources Conservation Service.

O'Brochta, D., and Atkinson, P.W., 1996. Transposable elements and gene transformation in non-drosophilid insects. Insect Biochem. Mol. Biol. 26:739–753.

Pane, A., Salvemini, M., Delli Bovi, P., Polito, C., and Saccone, G., 2002. The *transformer* gene in *Ceratitis capitata* provides a genetic basis for selecting and remembering the sexual fate. Development 129:3715–3725.

Parker, A.G., 2005. Mass-Rearing for Sterile Insect Release. *In:* Sterile Insect Technique. Principles and practice in area-wide integrated pest management, V.A. Dyck, J. Hendrichs, and A.S. Robinson, eds. (The Netherlands: Springer), pp. 209–232.

Peloquin, J.J., Thibault, S.T., Staten, R., and Miller, T.A., 2000. Germ-line transformation of pink bollworm (Lepidoptera: Gelechiidae) mediated by the *piggyBac* transposable element. Insect Mol. Biol. 9, 323–333.

Perera, O., Harrell, R., and Handler, A., 2002. Germ-line transformation of the South American malaria vector, *Anopheles albimanus*, with a piggyBac-EGFP tranposon vector is routine and highly efficient. Insect Molecular Biology 11:291–297.

Phuc, H., Andreasen, M., Burton, R., Vass, C., Epton, M., Pape, G., Fu, G., Condon, K., Scaife, S., and Donnelly, C., 2007. Late-acting dominant lethal genetic systems and mosquito control. BMC Biol. 5:11.

Pines, J., 1995. GFP in mammalian cells. Trends in Genetics 11:326–327.

Pouliquen, H., Delépée, R., Larhantec-Verdier, M., Morvan, M-L., and Le Bris, H., 2007. Comparative hydrolysis and photolysis of four antibacterial agents (oxytetracycline oxolinic acid, flumequine and florfenicol) in deionized water, freshwater and seawater under abiotic conditions. Aquaculture 262(1):23–28.

Prasher, D., 1995. Using GFP to see the light. Trends in Genetics 11:320–323.

Prasher, D., and Eckenrode, V., 1992. Primary structure of the *Aequorea victoria* green fluorescent protein. Gene 111:229–233.

Rendon, P., Franz, G., and Wood, R.J., 1996. Assessment of irradiation doses for TSL (thermal sensitive lethal) strain Vienna 42. *In:* Fruit Fly Pests: World Assessment of Their Biology and Mangement. Delray Beach, Florida: St. Lucie P., pp. 193–196.

Rhodes, R.H., 1976. The Medfly Containment in Central America. Citrograph (Mar. 1976):153+.

Richards, H.A., Han, C.-T., Hopkins, R.G., Failla, M.L., Ward, W.W., and Stewart, N., Jr., 2003. Safety assessment of recombinant green fluorescent protein orally administered to weaned rats. Journal of Nutrition, 133:1909–1912.

Robertson, H., Preston, C., Phillis, R., Johnson-Schlitz, D., Benz, W., and Engels, W., 1988. A stable genomic source of *P* element transposase in *Drosophila* melanogaster. Genetics 118:461–470.

Robinson, A.S., and Hendrichs, J., 2005. Prospects for the future development and application of the sterile insect technique. *In*: Dyck, V.A., Hendrichs, J., and Robinson, A.S., Eds., Area-wide integrated pest management. Springer, Dordrecht, The Netherlands, pp. 727–760.

Saccone, G., Pane, A., and Polito, C., 2002. Sex determination in flies, fruitflies and butterflies. Genetica 116:15–23.

Salghetti, S., Caudy, A., Chenoweth, J., and Tansey, W., 2001. Regulation of transcriptional activation domain function by ubiquitin. Science 293:1651–1653.

Sanderson, H., Ingerslev, F., Brain, R.A., Halling-Sørenson, B., Bestari, J.K., Wilson, C.J., Johnson, D.J., and Solomon, K.R., 2005. Dissipation of oxytetracycline, chlortetracycline, tetracycline and doxycycline using HPLC-UV and LC/MS/MS under aquatic semi-field microcosm conditions. Chemosphere 60:5. pp. 619–629.

SanMiguel, P., Tikhonov, A., Jin, Y.K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A., Springer, P.S., Edwards, K.J., Lee, M., Avramova, Z., and Bennetzen, J.L., 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science 274:765–768.

Sarkar, A., Sim, C., Hong, Y., Hogan, J., Fraser, M., Robertson, H., and Collins, F., 2003. Molecular evolutionary analysis of the widespread *piggyBac* transposon family and related "domesticated" sequences. Mol. Gen.Genomics 270:173–180.

Saul, S.H., 1984. Genetic sexing in the Mediterranean fruit fly, Ceratitis capitata (Wiedemann)(Diptera: Tephritidae): conditional lethal translocations that preferentially eliminate females. Annals of the Entomological Society of America 77:280–283.

Scholl, E.E., 1919. Report of pink bollworm of cotton (*Pectinophora gossypiella* Saunders), Texas Dept. of Agric. Bull. 65, 4 pp.

Schliekelman, P., and Gould, F., 2000a. Pest control by the introduction of a conditional lethal trait on multiple loci: potential, limitations, and optimal strategies. J. Econ. Entomol. 93:1543–1565.

Schliekelman, P., and Gould, F., 2000b. Pest control by the release of insects carrying a female-killing allele on multiple loci. J. Econ. Entomol. 93:1566–1579.

Scott, M., Heinrich, J., and Li, X., 2004. Progress towards the development of a transgenic strain of the Australian sheep blowfly (*Lucilia cuprina*) suitable for a male-only sterile release program. Insect Biochem. Mol. Biol. 34:185–192.

Sequeira, R., Millar, L., and Bartels, D., 2001. Identification of susceptible areas for the establishment of Anastrepha spp. fruit flies in the United States and analysis of selected pathways. USDA–APHIS–CPHST–PERAL Report, 47 pp.

Shagin, D.A., Barsova, E.V., Yanushevich, Y.G., Fradkov, A.F., Lukyanov, K.A., Labas, Y.A., Ugalde, J.A., Meyer, A., Nunes, J.M., and Widder, E.A., 2004. GFP-like proteins as ubiquitous Metazoan superfamily: evolution of functional features and structural complexity. Mol. Biol. Evol. 21:841–850.

Shaw, J.G., and Sanchez-Riviello, M., 1965. Effectiveness of tepa-sterilized Mexican fruit flies released in a mango grove. Journal of Economic Entomology 58:26–28.

Shaw, J.G., and Sanchez-Riviello, M., 1962. Investigations on the use of chemical products as sexual sterilants for the fruit-fly. Ciencia 22:17–20.

Shaw, J.G., Patton, W.P., Sanchez-Riviello, M., Spishakoff, L.M., and Reed, B.C., 1966. Mexican fruit fly control. California Citrograph 51:210–214.

Shelly, T.E., Edu, J., and Pahio, E., 2005. Lack of an irradiaton effect on the mating performance of mass-reared males of the Mediterranean fruit fly. Fl. Entomol. 88:547–548.

Shelly, T.E., Steiner, E., Bosco, V., and McInnis, D., 2006. Additional tests on the efficacy of ginger root oil in enhancing the mating competitveness of sterile males of the Mediterranean fruit fly, Ceratitis capitata (Diptera: Tephritidae). Proceedings of the Hawaiian Entomolocial Society 38:41–47.

Shinmyo, Y., Mito, T., Matsushita, T., Sarashina, I., Miyawaki, K., Ohuchi, H., and Noji, S., 2004. *piggyBac*-mediated somatic transformation of the two-spotted cricket, *Gryllus bimaculatus*. Development, growth and differentiation 46:343–349.

Shockett, P., Difilippantonio, M., Hellman, N., and Schatz, D., 1995. A modified tetracycline-regulated system provides autoregulatory, inducible gene expression in cultured cells and transgenic mice. Proc. Nat'l. Acad. Sci. (USA) 92, 6522–6526.

Silva, J., and Kidwell, M., 2000. Horizontal transfer and selection in the evolution of P elements. Molecular Biol. and Evolution 17:1542–1557.

Spradling, A., and Rubin, G., 1982. Transposition of cloned P elements into Drosophila germ line chromosomes. Science 218:341–347.

Staten, R.T., Rosander, R.W., and Keaveny, D.F., 1993. Genetic control of cotton insects, pp. 269–283. *In:* Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques. (Proc. Symp. Vienna, 1992), International Atomic Energy Agency, Vienna.

Steiner, L.F., and Christenson, L.D., 1956. Potential usefulness of the sterile fly release method in fruit fly eradication programs. Annual Meeting, 1956, Hawaiian Academy of Science.

Steiner, L.F., Mitchell, W.C., and Baumhover, A.H., 1962. Progress of fruit-fly control by irradiation sterilization in Hawaii and the Marianas Islands." International Journal of Applied Radiation and Isotopes 13:427–434.

Sundararajan, P., Atkinson, P., and O'Brochta, D., 1999. Transposable element interactions in insects: cross-mobilization of *hobo* and *Hermes*. Insect Molecular Biol. 8:359–368.

Suzuki, M., Ohbayashi, F., Mita, K., and Shimada, T., 2001. The mechanism of sex-specific splicing at the *doublesex* gene is different between *Drosophila melanogaster* and *Bombyx mori*. Insect Biochem. Mol. Biol. *31*:1201–1211.

Tamura, T., Thibert, C., Royer, C., Kanda, T., Eappen, A., Kamba, M., Komoto, N., Thomas, J.-L., Mauchamp, B., and Chavancy, G., 2000. Germline transformation of the silkworm *Bombyx mori* L. using a *piggyBac* transposon-derived vector. Nat. Biotech. 18:81–84.

Thibault, S.T., Luu, H.T., Vann, N., and Miller, T.A., 1999. Precise excision and transposition of piggyBac in pink bollworm embryos. Insect Molecular Biology 8:119–123.

Thomas, D., Donnelly, C.A., Wood, R.J., and Alphey, L.S., 2000. Insect population control using a dominant, repressible, lethal genetic system. Science 287:2474–2476.

Thomas, M.C., Heppner, J.B., Woodruff, R.E., Weems, H.V., Steck, G.J., and Fasulo, T.R., 2006. Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Insecta: Diptera: Tephritidae. University of Florida IFAS Extension, 15 pp.

Tiedje, J.M., Colwell, R.K., Grossman, Y.L., Hodson, R.E., Lenski, R.E., Mack, R.M., and Regal, P.J., 1989. The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70:298–315.

Tsien, R.Y., 1998. The green fluorescent protein. Annual Rev. Biochem. 67:509–544.

UCR—See University of California at Riverside

USDA–APHIS—See U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

USDA–ERS—See U.S. Department of Agriculture, Economic Research Service.

USDA–NASS—See U.S. Department of Agriculture, National Agricultural Statistics Service.

USDA–NRCS—See U.S. Department of Agriculture, Natural Resource Conservation Service.

U.S. Department of Agriculture, 2003. USDA security policies and procedures for laboratories and technical facilities (excluding Biosafety Level (BSL)-3 facilities). . DM9610-002. USDA-Agricultural Research Service, Washington, DC.

U.S. Department of Agriculture, 2002. USDA security policies and procedures for Biosafety Level-3 facilities. DM9610-001. USDA, Agricultural Research Service, Washington, DC.

U.S. Department of Agriculture, 1996. Safety and health Manual. Chapter 3, section 1. Radiation safety programs. USDA, Office of Human Resources Management, Safety and Health Management Division, September 1996.

U.S. Department of Agriculture, 1956. The Mediterranean fruit fly. USDA, Washington, D.C.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2007a. Environmental monitoring plan for applications of chlorpyrifos and permethrin, pink bollworm eradication program, Arizona, New Mexico, and Texas, 2007. USDA–APHIS, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2007b. Mexican fruit fly cooperative eradication program, Laredo, Texas, environmental assessment. USDA–APHIS, Riverdale, MD. Available: http://www.aphis.usda.gov/plant_health/ea/index.shtml

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2007c. Exotic fruit fly infestations and eradications in the continental United States, 39 pp.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2007d. Identification of areas susceptible to the establishment of fifty-three *Bactrocera* spp. (Diptera: Tephritidae: Dacinae) in the United States, 42 pp.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2007e. Revision of fruits and vegetables import regulations—final rule. Docket No. APHIS-2005–0106. July 18, 2007. USDA–APHIS, Plant Protection and Quarantine, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2006a. Exotic fruit fly strategic plan FY 2006–2010. USDA–APHIS, Fruit fly exclusion and detection programs, Riverdale, MD. Available: http://www.aphis.usda.gov/plant_health/plant_pest_info/fruit_flies/index.shtml. [June 19, 2006.]

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2006b. Risk assessment to determine placement of a new fruit fly production facility, 15 pp.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2006c. Oriental fruit fly cooperative eradication program, Rialto, San Bernadino County, California, environmental assessment. USDA–APHIS, Riverdale, MD. Available: http://www.aphis.usda.gov/plant_health/ea/index.shtml

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2005a. Field study of genetically modified pink bollworm, *Pectinophora gossypiela* (Lepidoptera: Gelechiidae) environmental assessment. USDA–APHIS, Riverdale, MD. Available: http://www.aphis.usda.gov/brs/biotech_ea_permits.html

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2005b. Mediterranean fruit fly cooperative eradication program, Rancho Cucamonga, San Bernadino County, California, environmental assessment. USDA–APHIS, Riverdale, MD. Available: http://www.aphis.usda.gov/plant_health/ea/index.shtml

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2005c. Plant Protection and Quarantine treatment manual, updated September 21, 2005. USDA–APHIS, Frederick, MD. Available: http://www,aphis.usda.gov/import_export/plants/manuals/ports/treatment.shtml

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2004. National pink bollworm eradication program, biological assessment, July 2004. USDA–APHIS, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2003a. Spinosad bait spray applications nontarget risk assessment, October 2003. USDA–APHIS, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2003b. Mediterranean fruit fly action plan. USDA–APHIS, Riverdale, MD, 84 pp.

U.S. Department of Agriculture, 2003c. USDA security policies and procedures for laboratories and technical facilities (excluding Biosafety Level (BSL)-3 facilities). DM9610-002. USDA, Agricultural Research Service, Washington, DC.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2002a. Southwest pink bollworm eradication program environmental assessment, April 2002. USDA–APHIS, Riverdale, MD. Available: http://www.aphis.usda.gov/plant_health/ea/index.shtml

U.S. Department of Agriculture, 2002b. USDA security policies and procedures for Biosafety Level-3 facilities. DM9610–001. USDA, Agricultural Research Service, Washington, DC.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2001a. Fruit fly cooperative control program final environmental impact statement—2001. USDA–APHIS, Riverdale, MD. Available: http://www.aphis.usda.gov/plant_health/ea/index.shtml

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2001b. Confined study of a transgenic pink bollworm, *Pectinophora gossypiela* (Lepidoptera: Gelechiidae). USDA–APHIS, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2001c. El Paso/Trans Pecos pink bollworm eradication program environmental assessment, April 2001. USDA–APHIS, Riverdale, MD. Available: http://www.aphis.usda.gov/plant_health/ea/index.shtml

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2001d. Environmental monitoring plan, Mexican fruit fly regulatory program, 2001. USDA–APHIS, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 2001e. Identification of susceptible areas for the establishment of *Anastrepha* spp. fruit flies in the United States and analysis of selected pathways, 47 pp.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 1997. Irradiation for phytosanitary regulatory treatment. Environmental assessment, October 1997. USDA–APHIS, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 1995. APHIS history. USDA–APHIS, Riverdale, MD. Available: http://permanent.access.gpo.gov/lps3025/history.html. [July 16, 2007.]

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 1993. Biological assessment, Mediterranean fruit fly cooperative eradication program. USDA–APHIS, Hyattsville, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 1991a. National boll weevil cooperative control program, final environmental impact statement—1991. USDA–APHIS, Hyattsville, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 1991b. National exotic fruit fly trapping protocol, 1991. USDA–APHIS, Riverdale, MD.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, 1989. Oriental fruit fly action plan. USDA–APHIS, Hyattsville, MD, 56 pp.

U.S. Department of Agriculture, Californina Department of Food and Agriculture, and Santa Clara and Alameda County Departments of Agriculture, 1981. Joint cooperative, Mediterranean fruit fly eradication project. Los Gatos, CA: Medfly Project, 1981.

U.S. Department of Agriculture, Economic Research Service, 2007. Cotton backgrounder. Washington, DC. March 2007.

U.S. Department of Agriculture, National Agricultural Statistics Service, 2006. USDA–NASS Agricultural Statistics 2006: Cotton, tobacco, sugar crops, and honey. U.S. Government Printing Office.

U.S. Department of Agriculture, Natural Resource Conservation Service, 2006. Land resource regions and major land resource areas of the United States, the Caribbean, and the Pacific Basin. United States Department of Agriculture Handbook 296, 682 pp.

University of California at Riverside, 2008. Pink bollworm, *Pectinophora gossypiella* (Saunders)—gelechiidae. [Online]. Available: http://faculty.ucr.edu/~legneref/biotact/ch-86.htm (2008, Aug. 26).

Venette, R.C., Naranjo, S.E., and Hutchison, W.D., 2000. Implications of larval mortality at low temperatures and high soil moistures for establishment of pink bollworm (Lepidoptera: Gelechiidae) in Southestern United States Cotton. Population Ecology, 29(5):1019–1026.

Venette, R.C., and Hutchison, W.D., 1999. Assessing the risk of establishment by pink bollworm (Lepidoptera: Gelechiidae) in the Southeastern United States. Environ. Entomol. 28(3):445–455.

Vera, M.T., Rodriquez, R., Segura, D.F., Cladera, J.L., and Sutherst, R.W., 2002. Potential geographical distribution of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), with emphasis on Argentina and Australia. Environ. Entomol. 31(6):1009–1022.

Voytas, D.F., Cummings, M.P., Koniczny, A., Ausubel, F.M., and Rodermel, S.R., 1992. Copia-like retrotransposons are ubiquitous among plants. Proc. Natl. Acad. Sci. 89:7124–7128.

Wimmer, E., 2005. Eco-friendly insect management. Nat. Biotech. 23:432–433.

Wimmer, E., 2003. Applications of insect transgenesis. Nat. Rev. Genet. 4:225–232.

Wu, H., Wu, K., Wang, D., and Guo, Y., 2006. Flight potential of pink bollworm, *Pectinophora gossypiela* Saunders (Lepidoptera: Gelechiidae). Environ. Entomol. 35(4):887–893.

Xavier, H., and Handler, A., 2006. Use of misscense proteaome subunits for conditional lethality in the tephritid fruit flies, *Anastrepa suspensa* and *Ceratitis capitata*. Proceedings of a symposium on fruit flies of economic importance, biologically-based technologies for management of crop insect pests in local and area-wide programs. USDA Insect Behavior and Biocontrol Research Unit. Available: http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=199795

Yin, V.P., and Thummel, C.S., 2004. A balance between the *diap1* death inhibitor and *reaper* and *hid* death inducers controls steroid-triggered cell death in *Drosophila*. Proc. Nat'l. Acad. Sci. USA 101:8022–8027.

Yokokura, T., Dresnek, D., Huseinovic, N., Lisi, S., Abdelwahid, E., Bangs, P., and White, K., 2004. Dissection of DIAP1 functional domains via a mutant replacement strategy. J. Biol. Chem. 279:52603–52612.

Yoshioka, Y., Matsumoto, S., Kjima, S., Ohshima, K., Okada, N., and Machida, Y., 1993. Molecular characterization of a short interspersed repetitive element from tobacco that exhibits sequences homology to specific tRNAs. Proc. Natl. Acad. Sci. 90:6562–6566.

(This page is intentionally left blank.)

Appendix H. Federal Register Notices

DATES: Tuesday, January 23, 2007, from 8 a.m. to 5 p.m., and Wednesday, January 24, 2007, from 8 a.m. to 12 noon.

ADDRESSES: The Committee meeting will be held at the Holiday Inn Central, 1501 Rhode Island Avenue, NW., Washington, DC.

FOR FURTHER INFORMATION CONTACT: Andrew Hatch, Designated Federal Official, USDA, AMS, Fruit and Vegetable Programs. Telephone: (202) 690–0182. Facsimile: (202) 720–0016. E-mail: *andrew.hatch@usda.gov.*

SUPPLEMENTARY INFORMATION: Pursuant to the Federal Advisory Committee Act (FACA) (5 U.S.C. App. II), the Secretary of Agriculture established the Committee in August 2001 to examine the full spectrum of issues faced by the fruit and vegetable industry and to provide suggestions and ideas to the Secretary on how USDA can tailor its programs to meet the fruit and vegetable industry's needs. The Committee was re-chartered in July 2003 and again in June 2005 with new members appointed by USDA from industry nominations.

AMS Deputy Administrator for Fruit and Vegetable Programs, Robert C. Keeney, serves as the Committee's Executive Secretary. Representatives from USDA mission areas and other government agencies affecting the fruit and vegetable industry will be called upon to participate in the Committee's meetings as determined by the Committee Chairperson. AMS is giving notice of the Committee meeting to the public so that they may attend and present their recommendations. Reference the date and address section of this announcement for the time and place of the meeting.

Topics of discussion at the advisory committee meeting will include: Invasive pests and disease initiatives; an update on U.S. produce industry labor and immigration issues; Perishable Agricultural Commodities Act (PACA) program budget and fees; and food safety initiatives.

Those parties that would like to speak at the meeting should register on or before January 15, 2007. To register as a speaker, please e-mail your name, affiliation, business address, e-mail address, and phone number to Mr. Andrew Hatch at: *andrew.hatch@usda.gov* or facsimile to (202) 720–0016. Speakers who have registered in advance will be given priority. Groups and individuals may submit comments for the Committee's consideration to the same e-mail address. The meeting will be recorded, and information about obtaining a transcript will be provided at the meeting.

The Secretary of Agriculture selected a diverse group of members representing a broad spectrum of persons interested in providing suggestions and ideas on how USDA can tailor its programs to meet the fruit and vegetable industry's needs. Equal opportunity practices were considered in all appointments to the Committee in accordance with USDA policies.

If you require special accommodations, such as a sign language interpreter, please use either contact name listed above.

Dated: December 13, 2006.

Lloyd Day,

Administrator, Agricultural Marketing Service.

[FR Doc. E6–21567 Filed 12–18–06; 8:45 am]

BILLING CODE 3410–02–P

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. APHIS–2006–0166]

Environmental Impact Statement; Genetically Engineered Fruit Fly and Pink Bollworm

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice of intent to prepare an environmental impact statement and proposed scope of study.

SUMMARY: We are advising the public that the Animal and Plant Health Inspection Service intends to prepare an environmental impact statement relative to the proposed use of genetically engineered fruit flies and pink bollworm in certain plant pest control programs. This notice identifies potential issues and alternatives that will be studied in the environmental impact statement, requests public comment to further delineate the scope of the issues and alternatives, and provides notice of public meetings.

DATES: We will consider all comments that we receive on or before February 20, 2007. We will also consider comments made at public meetings to be held in Washington, DC, on January 17, 2007; in Ontario, CA, on January 23, 2007; in Tempe, AZ, on January 25, 2007; in Weslaco, TX, on January 30, 2007; and in Tampa, FL, on February 1, 2007. Each meeting will be held from 9 a.m. to 12 p.m., local time.

ADDRESSES: You may submit comments by either of the following methods: Federal eRulemaking Portal: Go to *http://www.regulations.gov*, select ''Animal and Plant Health Inspection Service'' from the agency drop-down menu, then click ''Submit.'' In the Docket ID column, select APHIS–2006–0166 to submit or view public comments and to view supporting and related materials available electronically. Information on using Regulations.gov, including instructions for accessing documents, submitting comments, and viewing the docket after the close of the comment period, is available through the site's ''User Tips'' link.

Postal Mail/Commercial Delivery: Please send four copies of your comment (an original and three copies) to Docket No. APHIS–2006–0166, Regulatory Analysis and Development, PPD, APHIS, Station 3A–03.8, 4700 River Road Unit 118, Riverdale, MD 20737–1238. Please state that your comment refers to Docket No. APHIS–2006–0166.

Public Meetings: For the locations of the public meetings regarding this notice, see the Supplementary Information section of this notice.

Reading Room: You may read any comments that we receive in our reading room. The reading room is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW., Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 690–2817 before coming.

Other Information: Additional information about APHIS and its programs is available on the Internet at *http://www.aphis.usda.gov*.

FOR FURTHER INFORMATION CONTACT: Dr. David A. Bergsten, Biological Scientist, Environmental Services, PPD, APHIS, 4700 River Road Unit 149, Riverdale, MD 20737–1238; (301) 734–4883.

SUPPLEMENTARY INFORMATION:

Background

The Animal and Plant Health Inspection Service (APHIS) is considering using genetically engineered fruit flies (Diptera: Tephritidae) and pink bollworm (*Pectinophora gossypiella*) in our ongoing plant pest control programs for fruit flies and pink bollworm. Currently, these programs use a sterile insect technique that involves mass-rearing plant pests in a special facility, sterilizing the insects by irradiation, and releasing the insects to mate with wild plant pests. The release of sterile insects reduces the pest population through

associated decreases in the potential reproduction rate. Genetically engineered fruit flies and pink bollworm could augment the sterile insect technique by producing only male insects, insects with a genetic identification marker, insects that compete more effectively for mates, and/or insects that produce no viable offspring.

Under the provisions of the National Environmental Policy Act of 1969, as amended (42 U.S.C. 4321 *et seq.*), agencies must examine the potential environmental effects of proposed Federal actions and alternatives. We intend to prepare an environmental impact statement (EIS) relative to the proposed use of genetically engineered fruit flies and pink bollworm in the plant pest control programs for fruit flies and pink bollworm. The EIS will examine the range of potential effects that the proposed applications could pose to the human environment.

This notice identifies potential issues and alternatives that we will study in the EIS and requests public comment to further delineate the issues and the scope of the alternatives.

We have identified three broad alternatives for study in the EIS.

Take no action. This alternative contemplates no change to the plant pest control programs that use sterile insect technique. It represents a baseline against which proposed revisions may be compared.

Expansion of existing plant pest control programs. This alternative contemplates improving the current plant pest control programs by expanding rearing operations, irradiation treatment capacity, classical genetic selection methods for separation of insect sexes, and the plant pest species used in these programs.

Integrate genetically engineered insects into existing plant pest control programs. This alternative contemplates integrating genetically engineered fruit flies and pink bollworm into the current plant pest control programs.

We welcome comments on these alternatives and on other issues or alternatives that should be examined in the EIS. In addition, we invite responses to the following questions:

Are there any new or greater risks or apparent benefits associated with the strategy of using genetic engineering instead of classical genetic techniques to develop new insect strains to improve ongoing APHIS plant pest control programs? If so, please explain.

The proposed EIS focuses on the development and use of genetic engineering to improve specific APHIS plant pest control programs. Are there any unique risks that APHIS should consider in detail for genetic engineering of pink bollworm and fruit fly species?

What are the potential risks of non-target effects associated with this technology?

All comments will be considered fully in developing a final scope of study. When the draft EIS is completed, a notice announcing its availability and an invitation to comment on it will be published in the **Federal Register.**

Public Meetings

We are advising the public that we are hosting five public meetings on this notice of intent to prepare an EIS. The public meetings will be held as follows:

Wednesday, January 17, 2007, in the USDA Jamie L. Whitten Building, Room 107–A, 1400 Independence Avenue SW., Washington, DC.

Tuesday, January 23, 2007, in the Marriott Hotel, 2200 East Holt Boulevard, Ontario, CA.

Thursday, January 25, 2007, in the Holiday Inn, 915 East Apache Boulevard, Tempe, AZ.

Tuesday, January 30, 2007, in the Kika de la Garza Subtropical Agricultural Research Center, 2413 East Highway 83, Bldg. 213, Bill Wilson Conference Room, Weslaco, TX.

Thursday, February 1, 2007, in the Embassy Suites Hotel Tampa-Airport/Westshore, 555 North Westshore Boulevard, Tampa, FL.

All of the public meetings will be held from 9 a.m. to noon, local time.

A representative of the Animal and Plant Health Inspection Service will preside at the public meetings. Any interested person may appear and be heard in person, by attorney, or by other representative. Written statements may be submitted and will be made part of the meeting record.

Registration for each meeting will take place 30 minutes prior to the scheduled start of the meeting. Persons who wish to speak at a meeting will be asked to sign in with their name and organization to establish a record for the meeting. We ask that anyone who reads a statement provide two copies to the presiding officer at the meeting.

The presiding officer may limit the time for each presentation so that all interested persons appearing at each meeting have an opportunity to participate. Each meeting may be terminated at any time if all persons desiring to speak and that are present in the meeting room have been heard.

Done in Washington, DC, this 13th day of December 2006.

Kevin Shea,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. E6–21612 Filed 12–18–06; 8:45 am]

BILLING CODE 3410–34–P

DEPARTMENT OF AGRICULTURE

Commodity Credit Corporation

Amendment 2 of the Cotton Storage Agreement

AGENCY: Commodity Credit Corporation, USDA.

ACTION: Notice.

SUMMARY: This notice announces Amendment 2 to the Commodity Credit Corporation's (CCC's) Cotton Storage Agreement. This amendment alters the agreement that regulates the storage of CCC interest and commercial cotton in warehouses throughout the United States.

DATES: *Effective Date:* December 19, 2006.

FOR FURTHER INFORMATION CONTACT: Timothy R. Murray, Cotton Program Manager, Warehouse and Inventory Division, Farm Service Agency, USDA, STOP 0553, 1400 Independence Avenue, SW., Washington, DC 20250–0553. Telephone: (202) 720–6125. E-mail: *tim.murray@usda.gov.* Persons with disabilities who require alternative means for communication (Braille, large print, audiotape, etc.) should contact the USDA Target Center at (202) 720–2600 (voice and TDD).

SUPPLEMENTARY INFORMATION: The final rule published in the **Federal Register** on August 30, 2006 (71 FR 51422) amended the regulations at 7 CFR 1423.11 regarding delivery and shipping standards for CCC-approved cotton warehouses. Amendment 2 to the CCC Cotton Storage Agreement updates Part III, S., Delivery and Shipping Standard, to reflect the changes in 7 CFR 1423.11. The new Section S redefines the minimum weekly delivery and shipping standard to 4.5 percent of the CSA-approved storage capacity or the maximum number of bales on hand at any time during the crop year. A new mandatory reporting requirement is also included. This provision applies to all cotton shipped from the warehouse. Questions regarding Amendment 2, or any other aspects of the CCC Cotton Storage Agreement, should be addressed to Paul Rodriguez at the Kansas City Commodity Office (816) 929–6662 or e-mail *Paul.Rodriguez@kcc.usda.gov.*

EIS No. 20080118, ERP No. F–FAA–K51043–CA, Horizon Air Service to Mammoth Yosemite Airport Project, Proposed Operations Specifications Amendment to Provide Scheduled Air Service, Town of Mammoth Lakes, Mono County, CA.

Summary: No formal comment letter was sent to the preparing agency.

EIS No. 20080121, ERP No. F–FHW–F40818–00, Interstate I–94, I–43, I–894, and WI–119 (Airport Spur) I–94/USH 41 Interchange to Howard Avenue, To Address Freeway System's Deteriorated Conditions, Funding and U.S. Army COE Section 404 Permit, Kenosha, Racine, and Milwaukee Counties, WI and Lake County, IL.

Summary: EPA has environmental concerns about the proposed project regarding compensatory wetland mitigation sites, mobile source air toxics, and air quality mitigation efforts. EPA recommends the use of clean diesel strategies during construction.

EIS No. 20080123, ERP No. F–NPS–F65066–MN, Pipestone National Monument General Management Plan, Implementation, Pipestone County, MN.

Summary: EPA continues to express environmental concerns with off-site impacts from land use and development surrounding the site and the need for additional noise mitigation measures.

EIS No. 20080138, ERP No. F–NOA–G64007–00, Reef Fish Amendment 30A: Greater Amberjack—Revise Rebuilding Plan, Accountability Measures: Gray Triggerfish—Establish Rebuilding Plan, End Overfishing, Accountability Measures, Regional Management, Management Thresholds and Benchmarks, Gulf of Mexico.

Summary: No formal comment letter was sent to the preparing agency.

EIS No. 20080149, ERP No. F–SFW–K99037–AZ, Horseshoe and Bartlett Reservoirs Project, To Store and Release Water, Issuance of an Incidental Take Permit for Operation, Located Northeast of Phoenix, Maricopa and Yavapai Counties, AZ.

Summary: No formal comment letter was sent to the preparing agency.

Dated: May 27, 2008.

Ken Mittelholtz,

Environmental Protection Specialist, Office of Federal Activities.

[FR Doc. E8–12095 Filed 5–29–08; 8:45 am]

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

[ER–FRL–6699–3]

Environmental Impact Statements; Notice of Availability

Responsible Agency: Office of Federal Activities, General Information (202) 564–7167 or *http://www.epa.gov/compliance/nepa/.*

Weekly receipt of Environmental Impact Statements.

Filed 05/19/2008 Through 05/23/2008.

Pursuant to 40 CFR 1506.9.

EIS No. 20080204, Draft EIS, APH, 00, Programmatic—Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs, Implementation, Comment Period Ends: 07/14/2008, Contact: David A. Bergsten 301–734–6103.

EIS No. 20080205, Revised Final EIS, FHW, TX, Grand Parkway (State Highway 99) Selected the Preferred Alternative Corridor for Segment F–2 from SH 249 to IH 45, Right-of-Way Permit and U.S. Army COE Section 404 Permit, Harris County, TX, Wait Period Ends: 07/10/2008, Contact: Randy Paulk 512–536–5961.

EIS No. 20080206, Draft EIS, COE, CA, Middle Harbor Redevelopment Project, Proposal to Increase Container Terminal Efficiency to Accommodate a Portion of the Predicted Future Containerized Cargo, Section 10 and 404 Permits, Port of Long Beach, Los Angeles County, CA, Comment Period Ends: 07/11/2008, Contact: Antal Szijj 805–585–2147.

EIS No. 20080207, Final EIS, USN, FL, Shock Trail of the MESA VERDE (LPD 19), San Antonio (LPD 17) Class Ship designated as the Shock Ship for Proposed Shock Trail, Possible Offshore Locations are Naval Station Norfolk, VA; Naval Station Mayport, FL; and Naval Air Station Pensacola, FL, Wait Period Ends: 06/30/2008, Contact: Donald Shaver 703–412–7521.

EIS No. 20080208, Final EIS, NRC, OK, Sequoyah Fuels Corporation Site, Proposed Reclamation Activities for the 243-hectare (600 acre) Site, (NUREG–1888) in Gore, OK, Wait Period Ends: 06/30/2008, Contact: Allen H. Fetter 301–415–8556.

EIS No. 20080209, Draft EIS, AFS, WY, Inyan Kara Analysis Area Vegetation Management, Proposes to Implement Best Management Livestock Grazing Practices and Activities Associated with Adaptive Management and Monitoring Strategies, Douglas Ranger District, Medicine Bow Routt National Forest and Thunder Basin National Grassland, Niobrara and Weston Counties, WY, Comment Period Ends: 07/14/2008, Contact: Ernie Gipson 307–358–4960.

EIS No. 20080210, Draft Supplement, FHW, IN, US 31 Improvement Project (I–465 to IN 38), between I–465 North Leg and IN–38, Updated Information, NPDES Permit and U.S. Army Section 10 and 404 Permits, Hamilton County, IN, Comment Period Ends: 07/25/2008, Contact: Larry Heil 317–226–7480.

EIS No. 20080211, Final EIS, FRA, CA, Bay Area to Central Valley High-Speed Train (HST) Project, Provide a Reliable High-Speed Electrified Train System to Link Bay Area Cities to the Central Valley, Sacramento, and South California, Wait Period Ends: 06/30/2008, Contact: David Valenstein 202–493–6368.

EIS No. 20080212, Final EIS, BIA, WA, Cowlitz Indian Tribe Trust Acquisition and Casino Project, Take 151.87 Acres into Federal Trust and Issuing of Reservation Proclamation, and Approving the Gaming Development and Management Contract, Clack County, WA, Wait Period Ends: 06/30/2008, Contact: B.J. Howerton 503–231–6749.

EIS No. 20080213, Final EIS, COE, NC, PCS Phosphate Mine Continuation, New Information on Additional Alternative ''L'' and ''M'', Proposes to Expand its Existing Open Pit Phosphate Mining Operation into a 3,412 Acre Tract, Pamlico River and South Creek, near Aurora, Beaufort County, NC, Wait Period Ends: 06/30/2008, Contact: Tom Walker 828–271–7980 Ext 222.

EIS No. 20080214, Final EIS, AFS, ID, Yakus Creek Project, Proposes Timber Harvest, Watershed Improvement, and Access Management Activities, Lochsa Ranger District, Clearwater National Forest, Idaho County, ID, Wait Period Ends: 06/30/2008, Contact: Craig Trulock 208–926–4274.

Amended Notices

EIS No. 20080167, Draft EIS, COE, CO, Northern Integrated Supply Project, Construction and Operation of a Regional Water Supply to Serve the Current and Future Water Needs of 12 Towns and Water District, Approval of Section 404 Permit Application, Northern Colorado Water Conservancy District, Larimer and Weld Counties, CO, Comment Period Ends: 07/30/2008, Contact: Chandler J. Peter 303–979–4120. Revision of FR Notice Published: Extending the Comment Period from 06/30/2008 to 07/30/2008.

Dated: May 27, 2008.

Ken Mittelholtz,

Environmental Protection Specialist, Office of Federal Activities.

[FR Doc. E8–12096 Filed 5–29–08; 8:45 am]

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

[EPA–HQ–ORD–2007–0484; FRL–8574–1]

Board of Scientific Counselors, National Center for Environmental Research (NCER) Standing Subcommittee Meeting—2008

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of Meeting.

SUMMARY: Pursuant to the Federal Advisory Committee Act, Public Law 92–463, the Environmental Protection Agency, Office of Research and Development (ORD), gives notice of a meeting of the Board of Scientific Counselors (BOSC) National Center for Environmental Research (NCER) Standing Subcommittee.

DATES: The meeting (a teleconference call) will be held on Tuesday, June 24, 2008, from 1 p.m. to 3 p.m. All times noted are eastern time. The meeting may adjourn early if all business is finished. Requests for the draft agenda or for making oral presentations at the conference call will be accepted up to 1 business day before the meeting.

ADDRESSES: Participation in the meeting will be by teleconference only—meeting rooms will not be used. Members of the public may obtain the call-in number and access code for the call from Susan Peterson, whose contact information is listed under the **FOR FURTHER INFORMATION CONTACT** section of this notice. Submit your comments, identified by Docket ID No. EPA–HQ–ORD–2007–0484, by one of the following methods:

- *http://www.regulations.gov:* Follow the on-line instructions for submitting comments.
- *E-mail:* Send comments by electronic mail (e-mail) to: *ORD.Docket@epa.gov,* Attention Docket ID No. EPA–HQ–ORD–2007–0484.
- *Fax:* Fax comments to: (202) 566–0224, Attention Docket ID No. EPA–HQ–ORD–2007–0484.
- *Mail:* Send comments by mail to: Board of Scientific Counselors, National Center for Environmental Research (NCER) Standing Subcommittee—2007 Docket, Mailcode: 28221T, 1200 Pennsylvania Ave., NW., Washington, DC 20460, Attention Docket ID No. EPA–HQ–ORD–2007–0484.
- *Hand Delivery or Courier.* Deliver comments to: EPA Docket Center (EPA/DC), Room B102, EPA West Building, 1301 Constitution Avenue, NW., Washington, DC, Attention Docket ID No. EPA–HQ–ORD–2007–0484. Note: this is not a mailing address. Such deliveries are only accepted during the docket's normal hours of operation, and special arrangements should be made for deliveries of boxed information.

Instructions: Direct your comments to Docket ID No. EPA–HQ–ORD–2007–0484. EPA's policy is that all comments received will be included in the public docket without change and may be made available online at *http://www.regulations.gov,* including any personal information provided, unless the comment includes information claimed to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. Do not submit information that you consider to be CBI or otherwise protected through *http://www.regulations.gov* or e-mail. The *http://www.regulations.gov* Web site is an ''anonymous access'' system, which means EPA will not know your identity or contact information unless you provide it in the body of your comment. If you send an e-mail comment directly to EPA without going through *http://www.regulations.gov,* your e-mail address will be automatically captured and included as part of the comment that is placed in the public docket and made available on the Internet. If you submit an electronic comment, EPA recommends that you include your name and other contact information in the body of your comment and with any disk or CD–ROM you submit. If EPA cannot read your comment due to technical difficulties and cannot contact you for clarification, EPA may not be able to consider your comment. Electronic files should avoid the use of special characters, any form of encryption, and be free of any defects or viruses. For additional information about EPA's public docket visit the EPA Docket Center homepage at *http://www.epa.gov/epahome/dockets.htm.*

Docket: All documents in the docket are listed in the *http://www.regulations.gov* index. Although listed in the index, some information is not publicly available, e.g., CBI or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, will be publicly available only in hard copy. Publicly available docket materials are available either electronically in *http://www.regulations.gov* or in hard copy at the Board of Scientific Counselors, National Center for Environmental Research (NCER) Standing Subcommittee—2008 Docket, EPA/DC, EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number for the ORD Docket is (202) 566–1752.

FOR FURTHER INFORMATION CONTACT: The Designated Federal Officer via mail at: Susan Peterson, Mail Code 8104–R, Office of Science Policy, Office of Research and Development, Environmental Protection Agency, 1200 Pennsylvania Avenue, NW., Washington, DC 20460; via phone/voice mail at: (202) 564–1077; via fax at: (202) 565–2911; or via e-mail at: *peterson.susan@epa.gov.*

SUPPLEMENTARY INFORMATION:

General Information

Participation in the meeting will be by teleconference only—meeting rooms will not be used. Members of the public who wish to obtain the call-in number and access code to participate in the conference call may contact Susan Peterson, the Designated Federal Officer, via any of the contact methods listed in the **FOR FURTHER INFORMATION CONTACT**section above, by 4 working days prior to the conference call.

The purpose of the meeting is to discuss the subcommittee's draft letter report. Proposed agenda items for the conference call include, but are not limited to: Clarification for NCER of two of the recommendations from the final letter report and discussion of NCER's next charge question(s). The conference call is open to the public.

Information on Services for Individuals With Disabilities: For information on access or services for individuals with disabilities, please contact Susan Peterson at (202) 564–1077 or *peterson.susan@epa.gov.* To request accommodation of a disability, please contact Susan Peterson, preferably at least 10 days prior to the meeting, to give EPA as much time as possible to process your request.

Dated: May 22, 2008.

Maryellen Radzikowski,

Acting Director, Office of Science Policy.

[FR Doc. E8–12093 Filed 5–29–08; 8:45 am]

BILLING CODE 6560–50–P

(This page is intentionally left blank.)

Appendix I. Acronyms and Glossary

A

Application Rate The amount of pesticide product per unit area.

Aromatherapy The use of vapors from various substances to improve the health and vitality of an organism or a population (e.g., use of aromas from ginger oil to improve overall vigor of Medfly populations in rearing facilities).

Attractant, Insect A natural or synthesized substance that lures insects by stimulating their sense of smell; sex, food, or oviposition attractants are used in traps or bait formulations.

Autocidal A genetic mechanism in which some change of an element of the affected insect's environment triggers an expression of death of one gender (females) and/or of sterility of one gender (males) leading to no offspring. SIT, incorporating the use of dominant lethal genetic systems like RIDL®, is an example of an application of autocidal techniques.

B

Biodiversity The number and variety of different organisms in the ecological complexes in which those organisms occur naturally, the relative abundance and frequency of biological organisms within ecosystems.

Biological Control The reduction of pest populations by means of living organisms introduced or supplemented by humans; utilizes competitors, parasites, predators, or sterile insects to reduce pest populations (also called biocontrol).

Biosecurity The management conditions present in a rearing or eclosion facility to ensure that access is restricted to authorized personnel and supplies, and to ensure that adequate controls are in place to verify that releases and movement from the facilities are handled appropriately by properly trained and authorized staff.

Biosafety The containment conditions in a rearing or eclosion facility to ensure safe handling of potentially damaging pest life stages, diet, and facility equipment.

Biotechnological Control The use of genetic engineering or other forms of biotechnology to directly or indirectly control a pest; may involve genetic engineering of host plants, biocontrol agents, or the pest itself to achieve control.

C

CFR — Code of Federal Regulations (U.S.).

Chemical Sensitivity — An adverse reaction(s) of a person or organism to ambient levels of toxic substance(s) contained in environmental media such as air, food, soil, and water.

Community — An assemblage of populations of plants, animals, bacteria, and fungi that live in an environment and interact with one another, forming a distinctive living system with its own composition, structure, environmental relationships, development, and function; an association of interacting populations, usually defined by the nature of their interaction or the place in which they live.

Concentration — The ratio of the mass or volume of a solute to the mass or volume of the solution or solvent; the amount of active ingredient or herbicide equivalent in a quantity of diluent (e.g., expressed as lb/gal, ml/liter, etc.), or an amount of a substance in a specified amount of medium (e.g., air and water).

Confined — Specific physical, chemical, biological, and other conditions within a field test or other environmental release of genetically engineered organisms that are intended to minimize, restrict, and prevent their establishment, spread into, and interaction with the environment, as well as for any of their progeny.

Construct — An engineered piece of DNA designed to be transferred into a cell or tissue.

Containment — The use of physical, chemical, and operational controls, or a combination thereof, within an enclosed building with walls, a floor, and a ceiling or in an area within such a building, to restrict contact of an organism with humans and the environment (NAPPO, 2007).

Control — Action or treatment to reduce a pest population; also, an untreated test group.

Critical Habitat — Habitat designated as critical to the survival of an endangered or threatened species, and listed in 50 CFR 17 or 226.

Cumulative Chemical Risk — The sum of all potential adverse effects from all exposures to a specific chemical or related chemicals with the same mechanism of toxic action.

Cumulative Effects or Impacts Those effects or impacts that result from incremental impact of a program action when added to other past, present, and reasonably foreseeable future actions.

D

Deoxyribo-nucleic Acid (DNA) The molecule in which the genetic information for most living cells is encoded; viruses also contain DNA.

DNA See Deoxyribonucleic acid.

Dominant Lethal Genetic System A system where insects are genetically engineered to carry heritable traits that are expressed under certain conditions as either mortality of one gender (females) or sterility of one gender (males) in either the homozygous or heterozygous states, in which one allele is dominant. A genetic trait that, if present in the genome of the individual, is expressed and, therefore, prevents the individual from having any descendents (e.g., RIDL®).

Dose A given quantity of material that is taken into the body; dosage is usually expressed in amount of substance per unit of animal body weight often in milligrams of substance per kilogram (mg/kg) of animal body weight, or other appropriate units, such as parts per million; to radiology, the quantity of energy or radiation absorbed; see Concentration.

Drift The airborne movement of a pesticide away from the targeted site of an application.

Dyes Temporary visual markers used to identify those sterile mass-reared insects that are subjected to irradiation prior to SIT. Theses dyes are primarily fluorescent. Their visual persistence on the marked insect and their application to individual insects has been inconsistent such that more effective program alternatives (genetic markers) to these dyes are being developed.

E

Eclosion The emergence of an adult insect from a pupal case (e.g., adult fruit fly emergence from pupae), or the emergence of an insect larva from an egg.

Ecoregion A geographic area that is relatively homogeneous with respect to ecological systems.

EGFP See Enhanced Green Fluorescent Protein

Enhanced Green Fluorescent Protein	Enhanced green fluorescent protein, a marker for positive lines of pink bollworm.
EIS	See Environmental Impact Statement
Endangered Species	A plant or animal species identified by the Secretary of Commerce or the Secretary of the Interior in accordance with the 1973 Endangered Species Act, as amended, that is in danger of extinction throughout all or a significant portion of its range.
Environment	The sum of all external conditions affecting the life, development, and survival of an organism; all the organic and inorganic features that surround and affect a particular organism or group of organisms.
Environmental Assessment	A concise public document which provides sufficient evidence and analysis for determining whether to prepare an environmental impact statement (EIS) or a finding of no significant impact (FONSI). It aids in compliance with the National Environmental Policy Act (NEPA) when program impacts are not likely to be significant or require the preparation of an EIS.
Environmental Impact Statement	A document prepared by a Federal agency in which anticipated environmental effects of alternative planned courses of action are evaluated; a detailed written statement as required by section 102(2)(C) of the National Environmental Policy Act (NEPA).
EPA	U.S. Environmental Protection Agency
Eradication	The complete elimination of a pest species; for some agricultural pests, this may mean the reduction of the pest populations to nondetectable levels.
Exposure	The condition of being subjected to contact with a substance that may have a harmful effect.
Excision	Removal of the transposable element or a functional portion of it, such as the ability to produce transposase that results in its inability to either move or transport other genetic material to other parts of the organism's cellular genome. Also, the loss of genes that potentially leads to loss of the intended genotype for insects used in SIT. This infrequent event would be eliminated from the population through maintenance of the filter rearing system described in this EIS.

F

FAO — Food and Agriculture Organization of the United Nations.

F_1 Sterility — Establishment of a partially sterile first generation (F_1) of Lepidoptera insects based upon exposure of the parent generation to a reduced dose of gamma radiation compared to the high dose used to produce complete sterilization. This does not completely sterilize the adults, but results in progeny in the next generation (F_1) that are sterile, thus propagating the sterility effect.

Filter — The use of a separation system to remove insects with deletions (excisions) and with other with undesirable qualities from the insect colony. This may be a function of the mother colony that is carefully maintained, monitored, and selected for optimum performance characteristics. The mother colony becomes the source for scale-up colony mass-rearing.

Finding of No Significant Impact (FONSI) — A document prepared by a Federal agency that presents the reasons why a proposed action would not have a significant impact on the environment and thus would not require preparation of an environmental impact statement (EIS). A FONSI is based on the results of an environmental assessment (EA).

Fitness — The extent to which an organism is adapted to or able to survive and reproduce in a particular environment for which the organism is selectively adapted.

Fitness Factor — An adaptive biological characteristic or characteristics of certain individuals in a population that contribute/s to increased ability to survive and reproduce in a particular environment. Organisms with some biologically beneficial or adaptive factors can become established and increase the frequency of these factors by interbreeding with populations lacking the factors.

FONSI — See Finding of No Significant Impact.

FWS — Fish and Wildlife Service; an agency of the U.S. Department of the Interior.

G

Gene — Part of a chromosome that controls expressions of certain biological characteristics of an organism; a portion of DNA that directs the synthesis of a protein.

Genetic Engineering	The process in which one or more genes and other genetic elements from one or more organism(s) are inserted into the genome of a second organism using recombinant DNA techniques.
Genetic Marker	A gene that produces a protein or enzyme, which is a reliable indicator that a particular organism possesses a specific trait of interest.
Genetically Engineered	Modified in genotype and phenotype using recombinant DNA techniques.
Genome	All of the hereditary material in a cell.
Genotype	The total genetic makeup (all characteristics) that an individual receives from its parents.
GFP	See Green Fluorescent Protein
Green Fluorescent Protein	A marker for insects that is selective due to the lack of significant natural fluorescence in most wild-type insects.

H

Habitat	The place occupied by wildlife or plant species; the environmental niche with all its attributes occupied by an organism or group of organisms; the total environment occupied.
Hazard	The intrinsic ability of a stressor to cause adverse effects to man or the environment under a particular set of circumstances.
Hazard Quotient (HQ)	The ratio of the exposure to a chemical or mixture by a specific route and duration divided by the estimated exposure level (regulatory risk value) at which an adverse health effect is likely to occur for that chemical or mixture. The HQ is used to quantify the level of concern for risk from exposures in a given exposure scenario or a set of exposure scenarios.
Horizontal Gene Transfer	The transfer of genetic material from one organism (the donor) to another organism (the recipient) that is not sexually compatible with the donor; frequently occurs in prokaryotes (bacteria), but infrequently occurs in eukaryotes (higher organisms). This event occurs with considerably lower frequency than random mutations that are normally associated with genetic changes.
Host	Any plant or animal fed upon by a pest or a parasite.

HQ See Hazard Quotient.

Hypersensitivity Abnormal or excessive reactivity (often of a pronounced allergenic nature) to a substance, sometimes attributed to low-levels of exposure to pesticides.

I

IAEA International Atomic Energy Agency.

Infestation The presence of a living pest in a plant or plant product; the presence of an undesirable organism in any product.

Insecticide A pesticide compound specifically intended and designed to kill or control the growth stages of insects.

Integrated Pest Management (IPM) The selection, integration, and implementation of pest control actions on the basis of predicted economic, ecological, and sociological consequences; the process of integrating and applying practical methods of prevention and control to keep pest situations from reaching damaging threshold levels, while minimizing potentially harmful effects of pest control measures on humans, nontarget species, and the environment.

Introduction The entry of a pest resulting in its establishment; usually refers to the pest's introduction into the country and the local establishment of a pest population.

IPM See Integrated Pest Management.

IPPC International Plant Protection Convention.

Irradiated Treated with any type of ionizing radiation (e.g. gamma radiation).

M

Male Annihilation A control method that reduces fruit fly populations by employing male attractants with insecticide baits and mass trapping to lure and kill the male fruit flies before they have a chance to mate.

Marker Gene A gene that produces a protein or enzyme, which is a reliable indicator that a particular organism possesses a specific trait of interest.

Media Specific environments (e.g., air, water, soil) that are the subject of regulatory concern and activities.

Mitigate To lessen the effect; to make less harsh or less harmful.

Monitoring The act of measuring environmental conditions through periodic time or continuous surveillance or testing to determine the level of compliance with statutory requirements and/or pollutant levels in various media, humans, animals, or other living things; also the act of measuring operational components or results to verify the efficacy of treatments (e.g. checking the presence of pests in surveillance traps).

Mother Stock Colony A colony of insects kept under more natural conditions (reduced adult and larval densities and reduced selection pressure) than the mass-reared colonies. This colony is designed to maintain the genetic diversity of the colony and prevent accumulation of genotypes that are highly selectively adapted to mass-rearing. The mother colony is used to filter out undesirable characteristics and provide insects to scale-up for mass production.

N

NEPA The National Environmental Policy Act of 1969 and subsequent amendments.

Nontarget Organisms Those organisms (species) that are present in the environment and that are not the focus of control efforts.

O

Organism Any living thing.

Outbreak A recently detected pest population involving a sudden significant increase of an established pest population in an area.

P

PARC See Plastic Adult Rearing Container.

Persistence The quality of a pesticide or other compound to persist as a residue, usually with some associated biological acivity; persistence is related to persistence is related to volatility, chemical stability, and biodegradation.

Pest An insect, rodent, nematode, fungus, weed, or other form of terrestrial or aquatic plant or animal life, or virus, bacterial, or microorganism that is injurious to human, plant, or animal health or the environment.

Pesticide Any substance or mixture of substances intended and designed to kill insects, rodents, fungi, weeds, or other forms of plant or animal life that are considered to be pests.

Phenotype The appearance or other biological characteristics of an organism, resulting from the interaction of its genetic constitution (genome) with the environment.

Pheromone A chemical substance released by an animal or synthetic analog that causes an attraction response, usually of a sexual nature, in other individuals of its species. Pheromones are frequently used as attractants for trapping and surveillance of insect populations.

Physical Control Physical actions (e.g., fruit stripping or host destruction) taken to control a pest.

piggyBac The most commonly used insect transposable element or transposon, which is a transposase-induced enzyme transformation system intended to place DNA from one species or synthetic DNA into another insect's genome. This system has been developed for lines of fruit flies and pink bollworm.

Plastic Adult Rearing Container (PARC) A system of containers designed to rear and collect emerging adult Mexican fruit flies and Medflies. This system has been largely replaced by Worley Eclosion towers, which have resulted in increased efficiency.

Population A potentially interbreeding group of organisms of a single species, occupying a particular space; generically, the number of humans or other living creatures in a designated geographic or environmental area.

Potentiation The interaction of two or more substances in which one or more enhances or synergizes the biological activity, effects, or toxicity of another. The potentiating agent generally is not as toxic as the substance being potentiated.

Preventive Release Program (PRP) An ongoing program to release sterile fruit flies throughout the active growing season at locations where the risk of entry of nonindigenous fruit flies into fly-free areas is high. This prophylactic use of the sterile insect technique serves to prevent any entries in these high risk areas from becoming established.

Programmatic Documentation that covers broad usage of new and existing methods in programs covering large portions of the United States (e.g. EIS analysis of control and eradication efforts for the fruit fly and pink bollworm programs throughout the United States). This can be contrasted with site-specific actions that are not programmatic.

PRP See Preventive Release Program.

R

Rearing The ongoing production of insects in all life stages for maintenance of the colony and for use in sterile insect control and preventive release programs.

Reasonable Alternative Alternatives to the proposed or preferred alternative that are practical or feasible from the technical, economic, and common sense standpoints.

Recombinant DNA Technology Modern techniques in molecular biology for cutting apart and splicing together different pieces of DNA. When segments of foreign DNA are transferred into another cell or organism, the substance or biological attributes for which they code may be produced or expressed.

Recombination The reciprocal exchange of portions of two homologous chromosomes (usually equivalent) during gamete formation. The process by which progeny derive a combination of genes different from that of either parent.

Regulatory Control A combination of control methods including quarantines and certification treatments; regulatory controls may include chemical and/or nonchemical treatment methods that are applied to specific crops within quarantined areas.

Repressible Lethal System A genetically engineered system in which insects are genetically engineered to carry heritable traits that are expressed in the absence of a repressor agent, such as tetracycline in the RIDL® system. In the absence of the repressor, these heritable traits (e.g., a lethal condition for females or a reproductively sterile condition for males) are expressed.

Resistance The ability of a population of organisms or biological system to absorb a usually adverse impact without significant change from normal fluctuations; for plants and animals, the ability to withstand adverse environmental conditions and/or exposure to toxic chemicals or disease.

Ribonucleic Acid (RNA) A nucleic acid composed of a long, often single-stranded chain of chemical building blocks called 'nucleotides.' RNA is similar to DNA, but contains ribose rather than deoxyribose. RNA has multiple functions in the process of translating information stored in genes (DNA) into proteins.

RIDL® Release of Insects carrying a Dominant Lethal [gene or genetic system]; a system in which insects are engineered to carry dominant lethal mutations in their gametes to induce sterility by genetics rather than achieving sterility by exposure to radiation.

Risk The probability that a substance or organism will produce harm under specified conditions.

RNA See Ribonucleic Acid

S

Scope The span of issues to be addressed for a proposed action and its alternatives.

Scoping A process for determining the span of issues to be addressed and for identifying the significant issues related to a proposed action.

SIT See—Sterile Insect Technique

Socioeco-nomics Sociological and economic factors considered together.

Species A group of closely related, morphologically similar individuals which typically interbreed; a reproductively isolated aggregate of interbreeding populations of organisms.

Spot Treatment A pesticide application to a small, discrete, or otherwise restricted area of a larger area or of the whole unit.

Sterile Insect Technique (SIT) A method of pest control using area-wide inundative release of reproductively sterile insects to reduce reproduction in a field population of the same species.

Strain A group of organisms of the same species having distinctive characteristics but not usually considered a separate breed or variety (e.g., TSL strain of Medfly).

Suppression The reduction of a pest population to below some predetermined economic threshold level.

Susceptibility The capacity to be adversely affected by exposure to pesticides, other substances, or factors.

Synergism The interaction of two or more substances which results in an effect that is greater than the sum of independent effects; the interaction of elements that, when combined, produce a total effect that is greater than the sum of the individual contributions.

T

Target The plants, animals, structures, areas, or pests to be treated by a pesticide application or biocontrol agent release.

Temperature Sensitive Lethal (TSL) A strain of Medfly that can effectively be restricted to production of only male flies by certain temperature exposures that eliminate female flies during the egg stage. The efficient separation of Medfly genders by temperature control and use of an effective filter system ensures maintenance of integrity of the colony (Fisher and Caceres, 2000).

Threatened Species Any species listed in the *Federal Register* that is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range.

Toxic Poisonous to living organisms.

Toxicity The capacity or property of a substance to cause any adverse effects, based on scientifically verifiable data from organism exposure tests; capacity of a chemical to induce an adverse effect.

Trait A characteristic of an organism, such as eye color (as compared to phenotype, which is the description of the trait, such as blue eye color).

Transcription The synthesis of an RNA copy from a sequence of DNA (a gene); the first step in gene expression in which a DNA sequence is copied by an RNA polymerase to produce a complementary RNA. In short, the process by which a messenger RNA is created from the nucleotide sequence of a gene (DNA).

Transformation The genetic alteration of a cell resulting from the introduction, uptake into the cell's genome, and expression of foreign genetic material, in which the introduced DNA is intended to alter the phenotype of the recipient organism.

Transgene A foreign gene that is inserted into the genome of a cell via recombinant DNA techniques.

Translation	The process by which the sequence of nucleotides in a messenger RNA directs the sequence of amino acids in a new protein during protein synthesis at a ribosome in the cytoplasm.
Transposable Elements (Transposons)	Sequences of DNA that can move to different positions within the genome of a single cell through a process called transposition. In the process, these elements can cause mutations and change the amount of DNA in the genome. They have the ability to be incorporated into the host genome following egg microinjection.
Transposase	An enzyme encoded by a transposon that catalyses the movement of DNA sequences to a different locations in the cell genome.
Transposon	See Transposable Elements.
Transposition	The movement of a transposable element from one location in the genome to another place in the genome following exposure to a transposase usually made by that transposon and specifically active for its movement. This infrequent event could potentially interfere with expression of the intended genotype for insects used in SIT; however, mother colony screens are expected to detect this.
Uncertainty	May be due to missing information, or gaps, in scientific theory; whenever uncertainty is encountered, a decision, based upon scientific knowledge, probability, and policy, must be made; the term "scientific judgment" is used to distinguish this decision from policy decisions made in risk management.
USDA	United States Department of Agriculture.
Vector	A DNA molecule, containing regulatory sequences and coding sequences, into which a fragment of foreign DNA is inserted with the intention of transfer to another organism. The inserted fragment is referred to as the 'insert.' The vector is often a plasmid, used by researchers to carry new genes into cells; also an agent known to carry pathogens or diseases.
Wild-type	Feral, sylvan, wild-pest, or nonengineered; applies to fruit fly or pink bollworm pest populations rather than to sterile insect populations used in release programs.
Worley Eclosion Towers (WET)	Devices designed to improve efficiency, produce less waste, and reduce expenses for accomplishing emergence and collection of adult Mexican fruit flies and Medflies over those incurred with previous rearing systems, such as PARC system.

(This page is intentionally left blank.)

Appendix J. Index

Appendix K. Procedures for Application-specific Evaluations of Genetically Engineered Plant Pests Used in Sterile Insect Technique Release Programs

This EIS has covered in detail the potential issues of greatest concern when developing and releasing genetically engineered insects for sterile insect technique release programs. The sterile insects subject to genetic or gamma radiation sterilization have the mechanism for biological containment designed intrinsically, which mitigates the majority of plant pest risk issues. SIT offers not only an immediate biological containment of any genetically engineered insect, but serves the purpose of eradication, control, or mitigation of wild-type pests of the target species in the field, including any of those few released program insects that might be fertile**.** Addressing the other impacts can be largely accomplished by determining and fulfilling the nine general procedural steps below:

1. Determination of potential adverse impacts to plants and the environment from proposed biologically or physically unconfined or uncontained release of transgenic organisms that are or may become plant pests (compare pest risks for the genetically engineered organism to the same plant pest organism which is not engineered). When applicable, such as for genetically engineered traits that are intended to be spread throughout a wild-insect population, this may include preparation of a pest risk assessment, NEPA documentation, and any associated ESA compliance.

2. Determine the logistics for mass-rearing of the organism and maintenance of transgenic traits for their intended uses to improve SIT programs. Address any unique containment issues for rearing facilities and costs related to colony establishment and maintenance.

3. Determination of the program benefits of the mass-reared genetically engineered arthropod strains for use in SIT over the non-engineered strains

4. Determine the stability of the genetic construct in the organism. Will it continue to be expressed in the phenotype in subsequent generations?

5. Identify the environmental fitness factors of the genetically engineered organisms relative to the non-engineered organism. When used under the circumstances of a SIT eradication and control program, these fitness factors become performance factors as described in Appendix D of this EIS. These performance factors are relevant to genetic engineering of fruit flies and the pink bollworm for use in SIT because the insects must be fit enough to be amenable to mass-rearing and handling conditions and be able to mate successfully with wild-type pest populations of the same species. SIT males must be able to live long enough and be sexually competitive with wild males to ensure enough reproductive failure to substantially and effectively reduce or locally eradicate the wild-type pest population.

6. Identify the available means to successfully monitor and mitigate the genetically engineered organism, if the biological containment provided by either genetic or gamma radiation sterilization provides insufficient mitigation and some substantive risks still exist.

7. Identify the quality control methods for packaging and transport to ensure secure movement of the proper insects for release.

8. Prepare appropriate documentation to address guidelines in the International Standards related to ISPM No. 3 (IPPC, 2005), RSPM No. 22 (NAPPO, 2004), and RSPM No. 27 (NAPPO, 2007). The use of SIT with its intrinsic biological containment mitigation will typically be subject to the case-by-case guidelines.

9. Submit permit requests addressing all importation, interstate movement, and environmental releases associated with site-specific or larger scale usage of genetically engineered organism, if the organism is still classified as posing potential plant pest risks after being reproductively sterilized.

Appendix L. Federally Listed Endangered, Threatened, and Proposed Species in Potential Program Areas

Appendix L. Federally Listed and Proposed Species in Fruit Fly Preventative Release Program Areas in Florida, Texas, and California and Pink Bollworm Eradication Areas in New Mexico, Arizona, Texas, and California.

Common Name	Scientific Name	Status	Critical Habitat	County, State
Plants				
San Diego thornmint	*Acanthominta ilicifolia*	T	No	San Diego, CA
Munz's onion	*Allium munzii*	E	Yes	Riverside, CA
South Texas ambrosia	*Ambrosia cheiranthifolia*	E	No	Cameron, TX
San Diego ambrosia	*Ambrosia pumila*	E	No	Riverside, San Diego, CA
Crenulate lead-plant	*Amorpha crenulata*	E	No	Miami-Dade, FL
Kearney's blue-star	*Amsonia kearneyana*	E	No	Pima, AZ
Tobusch fishhook cactus	*Ancistrocactus tobuschii*	E	No	Val Verde, TX
Del Mar Manzanita	*Arctostaphylos glandulosa* ssp. *crassifolia*	E	No	San Diego, CA
Marsh sandwort	*Arenaria paludicola*	E	No	Los Angeles, San Bernardino, CA
Bear Valley sandwort	*Arenaria ursine*	T	No	San Bernardino, CA
Four-petal pawpaw	*Asimina tetramera*	E	No	Palm Beach, FL
Cushenberry milk-vetch	*Astragalus albens*	E	Yes	San Bernardino, CA
Braunton's milk-vetch	*Astragalus brauntonii*	E	Proposed	Los Angeles, Orange, CA
Holmgren milk-vetch	*Astragalus holmgrenorium*	E	Proposed	Mohave, AZ
Lane Mountain milk-vetch	*Astragalus jaegerianus*	E	Yes	San Bernardino, CA
Coachella Valley milk-vetch	*Astragalus lentiginosus* var. *coachellae*	E	Yes	Riverside, CA
Peirson's milk-vetch	*Astragalus magdalenae* var. *peirsonii*	T	Yes	San Diego, Imperial, CA
Ventura marsh milk-vetch	*Astragalus pycnostachyus* var. *lanosissimus*	E	Yes	Los Angeles, Orange, CA
Coastal dunes milk-vetch	*Astragalus tener* var. *titi*	E	No	Los Angeles, San Diego, CA
Triple-ribbed milk-vetch	*Astragalus tricarinatus*	E	No	San Bernardino, Riverside, CA
Star cactus	*Astrophytum asterias*	E	No	Cameron, Hidalgo, Starr, TX
San Jacinto Valley crownscale	*Atriplex coronata* var. *notatior*	E	Yes	Riverside, CA
Texas ayenia	*Ayenia limitaris*	E	No	Cameron, Hidalgo, Willacy, TX
Encinitas baccharis	*Bacharis vanessae*	T	No	San Diego, CA
Nevin's barberry	*Berberis nevinii*	E	No	Los Angeles, San Bernardino, Riverside, San Diego, CA
Florida bonamia	*Bonamia grandiflora*	T	No	Charlotte, Hardee, Polk, FL
Thread-leaved brodiaea	*Brodiaea filifolia*	T	Yes	Los Angeles, Orange, San Bernardino, Riverside, San Diego, CA
Ash-gray paintbrush	*Castilleja cinerea*	T	No	San Bernardino, CA
San Clemente Island Indian paintbrush	*Castilleja grisea*	E	No	Los Angeles, CA
Vail Lake ceanothus	*Ceanothus ophiochilus*	T	No	Riverside, CA
Catalina Island mountain-mahogany	*Cercocarpus traskiae*	E	No	Los Angeles, CA
Deltoid spurge	*Chamaesyce deltoidea* ssp. *deltoidea*	E	No	Miami-Dade, Monroe, FL
Garber's spurge	*Chamaesyce garberi*	T	No	Miami-Dade, FL

Pygmy fringe-tree	*Chionanthus pygmaeus*	E	No	Polk, FL
Orcutt's spineflower	*Chorizanthe orcuttiana*	E	No	San Diego, CA
Florida golden aster	*Chrysopsis floridana*	E	No	Hardee, Hillsborough, Pinellas, FL
Florida perforate cladonia	*Cladonia perforate*	E	No	Manatee, Palm Beach, Polk, FL
Pigeon wings	*Clitoria fragrans*	T	No	Polk, FL
Short-leaved rosemary	*Conradina brevifolia*	E	No	Polk, FL
Salt marsh bird's beak	*Cordylanthus maritimus* ssp. *maritimus*	E	No	Los Angeles, Orange, San Diego, CA
Nellie cory cactus	*Coryphantha minima*	E	No	Brewster, TX
Bunched cory cactus	*Coryphantha ramillosa*	T	No	Brewster, Terrell, TX
Cochise pincushion cactus	*Coryphantha robbinsorum*	T	No	Cochise, AZ
Pima pineapple cactus	*Coryphantha scheeri* var. *robustispina*	E	No	Pima, AZ
Sneed pincushion cactus	*Coryphantha sneedii* var. *sneedii*	E	No	Dona Ana, NM; El Paso, TX
Avon Park harebells	*Crotalaria avonensis*	E	No	Polk, FL
Terlingua Creek cat's-eye	*Cryptantha crassipes*	E	No	Brewster, TX
Okeechobee gourd	*Cucurbita okeechobeensis* ssp. *Okeechobeensis*	E	No	Palm Beach, FL
Jones cycladenia	*Cycladenia jonesii* (*humilis*)	T	No	Mohave, AZ
Beautiful pawpaw	*Deeringothamnus pulchellus*	E	No	Charlotte, FL
Otay tarplant	*Deinandra* (*Hemizonia*) *conjugens*	T	Yes	San Diego, CA
San Clemente Island larkspur	*Delphinium variegatum* ssp. *kinkiense*	E	No	Los Angeles, CA
Slender-horned spineflower	*Dodecahema leptoceras*	E	Yes	Los Angeles, San Bernardino, Riverside, CA
Conejo dudleya	*Dudleya abramsii* ssp. *parva*	T	No	Los Angeles, CA
Marcescent dudleya	*Dudleya cymosa* ssp. *marcescens*	T	No	Los Angeles, CA
Santa Monica Mountains dudleya	*Dudleya cymosa* ssp. *ovatifolia*	T	No	Los Angeles, Orange, CA
Laguna Beach liveforever	*Dudleya stolonifera*	T	No	Orange, CA
Verity's dudleya	*Dudleya verityi*	T	No	Los Angeles, CA
Nichol's Turk's head cactus	*Echinocactus horizontalonius* var. *nicholii*	E	No	Pima, AZ
Chisos Mountain hedgehog cactus	*Echinocereus chisoensis* var. *chisoensis*	T	No	Brewster, TX
Davis' green pitaya	*Echinocereus viridiflorus* var. *davisii*	E	No	Brewster, TX
Lloyd's Mariposa cactus	*Echinomastus maripoensis*	T	No	Presidio, Brewster, TX
Santa Ana River woolly-star	*Eriastrum densifolium* ssp. *sanctorum*	E	No	Orange, San Bernardino, Riverside, CA
Parish's daisy	*Erigeron parishii*	T	Yes	San Bernardino, Riverside, CA
Southern mountain wild buckwheat	*Eriogonum kennedyi* var. *austromontanum*	T	No	San Bernardino, CA
Gypsum wild-buckwheat	*Eriogonum gypsophilum*	T	Yes	Culberson, TX
Scrub buckwheat	*Eriogonum longifolium* var. *gnaphalifolium*	T	No	Polk, FL
Cushenbury buckwheat	*Eriogonum ovalifolium* var. *vineum*	E	Yes	San Bernardino, CA
San Diego button-celery	*Eryngium aristulatum* var. *parishii*	E	No	Riverside, San Diego, CA

Johnston's frankenia	*Frankenia johnstonii*	E, Proposed for delisting	No	Starr, TX
Mexican flannelbush	*Fremontodendron mexicanum*	E	No	San Diego, CA
Small's milkpea	*Galactia smallii*	E	No	Miami-Dade, FL
Johnson's seagrass	*Halophila johnsonii*	T	Yes	Broward, Miami-Dade, Palm Beach, FL
Todsen's pennyroyal	*Hedeoma todsenii*	E	Yes	Sierra, NM
Island rush-rose	*Helianthemum greenei*	T	No	Los Angeles, CA
Pecos sunflower	*Helianthus paradoxus*	T	No	Reeves, Pecos, TX
Highlands scrub hypericum	*Hypericum cumulicola*	E	No	Polk, FL
Beach jacquemontia	*Jacquemontia reclinata*	E	No	Broward, Miami-Dade, Palm Beach, FL
San Bernardino Mountains bladderpod	*Lesquerella kingii* ssp. *bernardina*	E	Yes	San Bernardino, CA
Zapata bladderpod	*Lesquerella thamnophila*	E	Yes	Starr, TX
Scrub blazingstar	*Liatris ohlingerae*	E	No	Polk, FL
Huachaca water-umbel	*Lilaeopsis schaffneriana* var. *recurva*	E	Yes	Cochise, Pima,AZ
San Clemente Island woodland-star	*Lithophragma maximum*	E	No	Los Angeles, CA
San Clemente Island broom	*Lotus dendroideus* var. *traskiae*	E	No	Los Angeles, CA
Scrub lupine	*Lupinus aridorum*	E	No	Miami-Dade, FL
San Clemente Island bush-mallow	*Malacothamnus clementinus*	E	No	Los Angeles, CA
Walker's manioc	*Manihot walkerae*	E	No	Hidalgo, Starr, TX
Willowy monardella	*Monardella linoides* ssp. *viminea*	E	No	San Diego, CA
Spreading navarretia	*Naverretia fossalis*	T	Proposed	Los Angeles, Riverside, San Diego, CA
Britton's beargrass	*Nolina brittoniana*	E	No	Polk, FL
California orcutt grass	*Orcuttia californica*	E	No	Los Angeles, Riverside, San Diego, CA
Cushenbury oxytheca	*Oxytheca parishii* var. *goodmaniana*	E	Yes	San Bernardino, CA
Papery whitlow-wort	*Paronychia chartacea*	T	No	Polk, FL
Siler pincushion cactus	*Pediocactus sileri*	T	No	Mohave, AZ
Lyon's pentachaeta	*Pentachaeta lyonii*	E	Proposed	Los Angeles, CA
Key tree cactus	*Pilosocereus robinii*	E	No	Monroe, FL
San Bernardino bluegrass	*Poa atropurpurea*	E	No	San Bernardino, San Diego, CA
San Diego Mesa mint	*Pogogyne abramsii*	E	No	San Diego, CA
Otay mesa mint	*Pogogyne nudiuscula*	E	No	San Diego, CA
Lewton's polygala	*Polygala lewtonii*	E	No	Polk, FL
Tiny polygala	*Polygala smallii*	E	No	Broward, Miami-Dade, Palm Beach, FL
Wireweed	*Polygonella basiramia*	E	No	Polk, FL
Sandlace	*Polygonella myriophylla*	E	No	DeSoto, Polk, FL
Little Aguja pondweed	*Potamogeton clystocarpus*	E	No	Jeff Davis, TX
Scrub plum	*Prunus geniculata*	E	No	Polk, FL
Arizona Cliff-rose	*Purshia* (=*Cowania*) *subintegra*	E	No	Graham, Mohave, Maricopa, AZ
Hinckley oak	*Quercus hinckleyi*	T	No	Presidio, Brewster, TX
Gambel's watercress	*Rorippa gambellii*	E	No	Los Angeles, Orange, San Bernardino, San Diego, CA

Santa Cruz Island rock-cress	*Sibara filifola*	E	No	Los Angeles, CA
Pedate checker-mallow	*Sidalcea pedata*	E	No	San Bernardino, CA
Texas snowbells	*Styrax texanus*	E	No	Val Verde, TX
Canelo Hills ladies'-tresses	*Spiranthes delitescens*	E	No	Cochise, AZ
California taraxacum	*Taraxacum californicum*	E	No	San Bernardino, CA
Slender-petaled mustard	*Thelypodium stenopetalum*	E	No	San Bernardino, CA
Ashy dogweed	*Thymophylla tephroleuca*	E	No	Starr, TX
Hidden Lake bluecurls	*Trichostema austromontanum* ssp. *Compactum*	T	No	Riverside, CA
Big-leaved crown beard	*Verbesina dissita*	T	No	Orange, CA
Wide-leaf warea	*Warea amplexifolia*	E	No	Polk, FL
Carter's mustard	*Warea carteri*	E	No	Polk, FL
Florida ziziphus	*Ziziphus celata*	E	No	Polk, FL

Birds

Cape Sable seaside sparrow	*Ammodramus maritimus mirabilis*	E	Yes	Miami-Dade, Monroe, FL
Florida grasshopper sparrow	*Ammodramus savannarumafloridanus*	E	No	Polk, FL
San Clemente sage sparrow	*Amphispiza belli clementeae*	T	No	Los Angeles, CA
Florida scrub jay	*Aphelocoma coeruluscens*	T	No	Charlotte, DeSoto, Hardee, Hillsborough, Manatee, Palm Beach, Pinellas, Polk, Sarasota, FL
Marbled murrelet	*Brachyramphus marmoratus*	T	Yes	Los Angeles, CA
Western snowy plover	*Charadrius alexandrinus nivosus*	T	Yes	Los Angeles, Orange, CA
Piping plover	*Charadrius melodus*	E,T	Yes	Cameron, Willacy, TX; Hillsborough, Manatee, Monroe, Pinellas, FL
Masked bobwhite	*Colinus virginianus ridgwayi*	E	No	Pima, AZ
Southwestern willow flycatcher	*Empidonax traillii extimus*	E	Yes	Brewster, El Paso, Culberson, Hudspeth, Jeff Davis, Presidio, TX; Dona Ana, Luna, Sierra, NM; Cochise, Graham, La Paz, Pima, Maricopa, Mohave, Yuma, AZ; Los Angeles, Orange, San Bernardino, Riverside, San Diego, Imperial, CA
Northern aplomado falcon	*Falco femoralis septentrionalis*	E	No	Brewster, Cameron, El Paso, Hidalgo, Hudspeth, Jeff Davis, Presidio, Reeves, Willacy, TX; Dona Ana, Luna, Sierra, NM; Cochise, AZ
Whooping crane	*Grus Americana*	E, EXPN	Yes	Brewster, TX
California condor	*Gymnogyps californianus*	E, EXPN	Yes	Mohave, AZ; Los Angeles, San Bernardino, CA
San Clemente loggerhead shrike	*Lanius ludovicianus mearnsi*	E	No	Los Angeles, CA
Wood stork	*Mycteria americana*	E	No	Broward, Charlotte, DeSoto, Hardee, Hillsborough, Manatee, Miami-Dade, Monroe, Palm Beach, Pinellas, Polk, Sarasota, FL

Brown pelican	*Pelecanus occidentalis*	E	No	Cameron, Val Verde, Willacy, TX; Cochise, Graham, La Paz, Pima, Maricopa, Mohave, Yuma, AZ; Los Angeles, Orange, San Bernardino, Riverside, San Diego, Imperial, CA
Short-tailed albatross	*Phoebastria albatrus*	E	No	Los Angeles, Orange, San Diego, CA
Red-cockaded woodpecker	*Picoides borealis*	E	No	Charlotte, Hillsborough, Manatee, Monroe, Palm Beach, Pinellas, Polk, FL
Coastal California gnatcatcher	*Polioptila californica californica*	T	Yes	Los Angeles, Orange, San Bernardino, Riverside, San Diego, CA
Audobon's crested caracara	*Polyborus plancu audobonii*	T	No	Charlotte, DeSoto, Hardee, Manatee, Monroe, Palm Beach, Polk, FL
Light-footed clapper rail	*Rallus longirostris levipes*	E	No	Los Angeles, Orange, San Bernardino, CA
Yuma clapper rail	*Rallus longirostris yumanensis*	E	No	La Paz, Maricopa, Mohave, Yuma, AZ; Riverside, Imperial, San Bernardino, CA
Everglade snail kite	*Rostrhamus socialilis plumbeus*	E	Yes	Broward, Miami-Dade, Monroe, Palm Beach, Polk, FL
Least tern	*Sterna antillarum*	E	No	El Paso, Jeff Davis, Starr, Val Verde, TX; Dona Ana, NM
California least tern	*Sterna antillarum browni*	E	No	Los Angeles, Orange, Riverside, San Diego, Imperial, CA
Roseate tern	*Sterna dougallii dougallii*	T	No	Miami-Dade, Monroe, FL
Mexican spotted owl	*Strix occidentalis lucida*	T	Yes	El Paso, Hudspeth, Culberson, Jeff Davis, TX; Dona Ana, Sierra, NM; Cochise, Graham, Pima, Maricopa, Mohave, AZ
Black-capped vireo	*Vireo atricapilla*	E	No	Brewster, Crockett, Jeff Davis, Pecos, Terrell, Val Verde, TX
Least Bell's vireo	*Vireo bellii pusillus*	E	Yes	Los Angeles, Orange, San Bernardino, Riverside, San Diego, Imperial, CA
Mammals				
Sonoran pronghorn	*Antilocapra americana sonoriensis*	E	No	Maricopa, Pima, Yuma, AZ
Gray wolf	*Canis lupus*	E, T, EXPN	No	Luna, NM; Graham, AZ
San Bernardino kangaroo rat	*Dipodomys merriami parvus*	E	Yes, Proposed	Los Angeles, San Bernardino, Riverside, CA
Stephens' kangaroo rat	*Dipodomys stephensi*	E	No	San Bernardino, Riverside, San Diego, CA
Southern sea otter	*Enhydra lutris nereis*	T, EXPN	No	Los Angeles, Orange, San Diego, CA
Gulf Coast jaguarundi	*Herpailurus yagouaroundi cacmitli*	E	No	Cameron, Hidalgo, Starr, Willacy, TX
Ocelot	*Leopardus pardalis*	E	No	Cameron, Hidalgo, Starr, Willacy, TX; Cochise, Pima, AZ
Lesser long-nosed bat	*Leptonycteris curasoae yerbabuenae*	E	No	Cochise, Graham, Maricopa, Pima, AZ
Mexican long-nosed bat	*Leptonycteris nivalis*	E	No	Brewster, Presidio, TX
Hualapai Mexican vole	*Microtus mexicanus hualapaiensis*	E	No	Mohave, AZ

Black-footed ferret	*Mustela nigripes*	E, EXPN	No	Sierra, NM
Key Largo woodrat	*Neotoma floridana smalli*	E	No	Monroe, FL
Key deer	*Odocoileus virginianus clavium*	E	No	Monroe, FL
Peninsular bighorn sheep	*Ovis Canadensis*	E	Yes	Riverside, San Diego, Imperial, CA
Jaguar	*Panthera onca*	E	No	Cochise, Pima, AZ; Riverside, Imperial, CA
Pacific pocket mouse	*Perognathus longimembris pacificus*	E	No	Los Angeles, Orange, San Diego, CA
Key Largo cotton mouse	*Peromyscus gossypinus allapaticola*	E	No	Monroe, FL
Puma	*Puma concolor*	T (S/A)	No	Broward, Charlotte, Hardee, Miami-Dade, Monroe, Palm Beach, Polk, FL
Florida panther	*Puma concolor coryi*	E	No	Broward, Charlotte, Miami-Dade, Monroe, Palm Beach, FL
Rice rat	*Oryzomys palustris natator*	E	CH	Monroe, FL
Lower Keys marsh rabbit	*Sylvilagus palustris hefneri*	E	No	Monroe, FL
Mount Graham red squirrel	*Tamiasciurus hudsonicus grahamensis*	E	Yes	Graham, AZ
West Indian manatee	*Trichechus manatus latirostris*	E	CH	Broward, Charlotte, Hillsborough, Manatee, Miami-Dade, Monroe, Palm Beach, Pinellas, Sarasota, FL
Santa Catalina Island fox	*Urocyon littoralis catalinae*	E	Yes	Los Angeles, CA
San Joaquin kit fox	*Vulpes macrotis mutica*	E	No	Los Angeles, CA
Reptiles				
American alligator	*Alligator mississippiensis*	T (S/A)	No	Cameron, Willacy, TX: Broward, Charlotte, DeSoto, Hardee, Miami-Dade, Monroe, Palm Beach, Polk, Sarasota, FL
Loggerhead sea turtle	*Caretta caretta*	T	No	Cameron, Willacy, TX; Broward, Charlotte, Hillsborough, Manatee, Miami-Dade, Monroe, Palm Beach, Pinellas, Sarasota, FL
Green sea turtle	*Chelonia mydas*	E,T	Yes	Cameron, Willacy, TX; Broward, Charlotte, Hillsborough, Manatee, Miami-Dade, Monroe, Palm Beach, Pinellas, Sarasota, FL
American crocodile	*Crocodylus acutus*	E	CH	Broward, Miami-Dade, Monroe, FL
New Mexico ridge-nosed rattlesnake	*Crotralus willardi obscurus*	T	Yes	Cochise, AZ
Leatherback sea turtle	*Dermochelys coriacea*	E	Yes	Cameron, Willacy, TX; Broward, Hillsborough, Manatee, Miami-Dade, Monroe, Palm Beach, Pinellas, FL
Eastern indigo snake	*Dymarchon corais couperi*	T	No	Broward, Charlotte, DeSoto, Hardee, Hillsborough, Manatee, Miami-Dade, Monroe, Palm Beach, Pinellas, Polk, Sarasota, FL
Hawksbill sea turtle	*Eretmochelys imbricata*	E	Yes	Cameron, Willacy, TX; Broward, Miami-Dade, Monroe, Palm Beach, FL

Bluetail mole skink	*Eumeces egregious lividus*	T	No	Polk, FL
Blunt-nosed leopard lizard	*Gambelia silus*	E	No	Los Angeles, CA
Desert tortoise	*Gopherus agassizii*	T	Yes	Mohave, AZ; Los Angeles, San Bernardino, Riverside, Imperial, CA
Kemp's ridley sea turtle	*Lepidochelys kempii*	E	No	Cameron, Willacy, TX; Hillsborough, Manatee, Pinellas, FL
Sand skink	*Neoseps reynoldsi*	T	No	Polk, FL
Coachella Valley fringe-toed lizard	*Uma inornata*	T	Yes	Los Angeles, CA
Island night lizard	*Xantusia riversiana*	T	No	Los Angeles,
Amphibians				
Sonora tiger salamander	*Ambystoma tigrinum stebbinsi*	E	Yes	Cochise, AZ
Desert slender salamander	*Batrachoseps aridus*	E	No	Riverside, CA
Arroyo toad	*Bufo microscaphus californicus*	E	Yes	Los Angeles, Orange, San Bernardino, Riverside, San Diego, CA
California red-legged frog	*Rana aurora draytoni*	T	Yes	Los Angeles, Orange, San Bernardino, Riverside, San Diego, CA
Chiricahua leopard frog	*Rana chiricahuensis*	T	No	Luna, Sierra, NM; Cochise, Graham, Pima, AZ
Mountain yellow-legged frog, southern CA DPS	*Rana mucosa*	E		Los Angeles, San Bernardino, Riverside, CA
Fish				
Gulf sturgeon	*Acipenser oxyrhynchus desotoi*	T	Yes	Hillsborough, Manatee, Pinellas, FL
Santa Ana sucker	*Catostomus santaanae*	T	Yes	Los Angeles, Orange, San Bernardino, Riverside, CA
Beautiful shiner	*Cyprinella Formosa*	T	Yes	Luna, NM; Cochise, AZ
Leon Springs pupfish	*Cyprinodon bovinus*	E	Yes	Pecos, TX
Comanche Springs pupfish	*Cyprinodon elegans*	E	No	Jeff Davis, Reeves, TX
Desert pupfish	*Cyprinodon macularius*	E	Yes	Graham, La Paz, Maricopa, Pima, AZ; Riverside, San Diego, Imperial, CA
Devils River minnow	*Dionda diaboli*	T	No	Val Verde, TX
Tidewater goby	*Eucyclogobius newberryi*	E	Yes	Los Angeles, Orange, San Diego, CA
Big Bend gambusia	*Gambusia gaigei*	E	No	Brewster, TX
Pecos gambusia	*Gambusia nobilis*	E	No	Jeff Davis, Pecos, Reeves, TX
Unarmored threespine stickleback	*Gasterosteus aculeatus williamsoni*	E	No	Los Angeles, San Bernardino, San Diego, CA
Mohave tui chub	*Gila bicolor mohavensis*	E	No	San Bernardino, CA
Humpback chub	*Gila cypha*	E	Yes	Mohave, AZ
Bonytail chub	*Gila elegans*	E	Yes	La Paz, Mohave, AZ: San Bernardino, Riverside, Imperial, CA
Gila chub	*Gila intermedia*	E	Yes	Cochise, Graham, Maricopa, Pima, AZ
Yaqui chub	*Gila purpurea*	E	Yes	Cochise, AZ
Virgin River chub	*Gila seminude*	E	Yes	Mohave, AZ
Rio Grande silvery minnow	*Hybognathus amarus*	E	No	Dona Ana, Sierra, NM

Yaqui catfish	*Ictalurus pricei*	T	Yes	Cochise, AZ
Spikedace	*Meda fulgida*	T	Yes	Graham, AZ
Apache trout	*Oncorhynchus apache*	T	No	Graham, AZ
Gila trout	*Oncorhynchus gilae*	T	No	Sierra, NM
Southern California steelhead	*Oncorhynchus mykiss*	E	Proposed	Los Angeles, Orange, San Diego, CA
Woundfin	*Plagopterus argentissimus*	E, EXPN	Yes	Mohave, AZ
Gila topminnow	*Poeciliopsis occidentalis*	E	No	Graham, La Paz, Maricopa, Pima, AZ
Colorado pikeminnow	*Ptychocheilus lucius*	E	Yes	San Bernardino, Riverside, Imperial, CA
Loach minnow	*Tiaroga cobitis*	T	Yes	Graham, AZ
Razorback sucker	*Xyrauchen texanus*	E	Yes	Graham, La Paz, Maricopa, Mohave, Yuma, AZ; San Bernardino, Riverside, Imperial, CA
Snails				
Pecos assiminea snail	*Assiminea pecos*	E	Yes	Pecos, Reeves, TX
Stock Island tree snail	*Orthalicus reses*	T	No	Monroe, FL
Arthropods				
Vernal pool fairy shrimp	*Branchinecta lynchii*	T	Yes	Riverside, CA
San Diego fairy shrimp	*Branchinecta sandiegonensis*	E	No	Orange, San Diego, CA
El Segundo blue butterfly	*Euphilotes battoides allyni*	E	No	Los Angeles, CA
Quino checkerspot butterfly	*Euphydryas editha quino*	E	Yes	Los Angeles, Orange, San Bernardino, Riverside, San Diego, CA
Palos Verdes blue butterfly	*Glaucopsyche lygdamus palosverdensis*	E	Yes	Los Angeles, CA
Schaus swallowtail butterfly	*Heraclides aristodemus ponceanus*	E	No	Miami-Dade, Monroe, FL
Laguna Mountains skipper	*Pyrgus ruralis lagunae*	E	Proposed	San Diego, CA
Delhi Sands flower-loving fly	*Rhaphiomidas terminatus abdominalis*	E	No	San Bernardino, Riverside, CA
Riverside fairy shrimp	*Streptocephalus wootoni*	E	Yes	Los Angeles, Orange, Riverside, San Diego, CA

(This page is intentionally left blank.)