Systems Approaches for Managing the Risk of Citrus Fruit in Texas during a Mexican Fruit Fly Outbreak

Photo courtesy of Texas A&M Kingsville Citrus Center

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EXECUTIVE SUMMARY

Fruit flies of the family Tephritidae are important pests of fruits and vegetables worldwide and their presence in areas where the pest is not established represents a significant threat to commercial agriculture and trade. Where the threat of introducing fruit flies in host materials exists, local and national governments utilize a number of methods to reduce the risk associated with movement from potentially infested areas that range from outright prohibition of the commodity to approval based on verification of approved quarantine treatments designed to eliminate the risk. Single quarantine treatments have been shown to be effective when research can be done to design and rigorously verify the efficacy of the treatments. More recently international phytosanitary agreements have embraced the concept of "systems approaches" as an alternative means of allowing movement of commodities while reducing the risk of fruit fly introductions to acceptable levels.

Areas previously considered "fruit fly free" are impacted by the discovery of a small number of flies exceeding a predetermined "trigger" which results in an emergency quarantine. This situation is usually temporary and methods for their eventual "eradication" are often routine and very efficacious. We propose to use systems approaches to mitigate fruit fly risks in cases where "low prevalence" of flies and other independent measures, such as regulatory trapping and certified pre-harvest foliar bait spray treatments, can be applied and verified.

Previously we developed systems approaches utilizing areas of low prevalence and poor host status as independent measures against Mediterranean fruit fly on tomatoes (the tomato systems approach), and areas of low prevalence and a less-than-probit 9 fumigation as independent measures against oriental fruit fly on sweet cherries (the cherry systems approach). Here we address the specific case of the movement of citrus from the Rio Grande Valley in Texas, where the Mexican fruit fly, *Anastrepha ludens* (Mexfly) is a recurring quarantine issue. In these cases the previously fruit-fly-free areas temporarily become areas of low prevalence, and additional independent mitigation measures (approved bait sprays and limited distribution) are combined to mitigate the risk associated with fruit for shipping. Quantitative assessments based on empirical information data, and expert opinion indicates that this systems approach reduces the level of risk to an acceptable level (i.e., negligible).

In this document we present systems approaches for Texas fruit of any member in the genus *Citrus* regulated by quarantine for Mexfly. The three major components (independent measures) proposed for these systems approaches are: 1.) Area of low pest prevalence (ALPP), 2.) Pre-harvest foliar bait spray treatments, and 3.) Limited distribution of shipped fruits.

Qualitative and quantitative results indicate that systems approaches as described in this document will provide acceptable pest risk reductions for Mexflies in quarantined Texas citrus fruit as follows: **Fruit from quarantined areas that were under routine SIT** can ship from anywhere in the quarantined area after at least 30 d of regulatory trapping, 30 d of certified sprays until the end of harvest, and with a shipping restriction (Option 1); **Fruit from quarantined areas not under routine SIT** can ship from a grove *more than 250 m from the center of the infestation* with regulatory trapping for at least 30 d, 30 d of certified sprays prior to harvest, and a shipping distribution restriction (Option2), and fruit from a grove *within 250 m of the center of the infestation* can be shipped if harvest is delayed 60 d with weekly certified sprays until the end of harvest, and a shipping distribution restriction (Option 3). In this case, the pest risk is negligible for all quarantines that begin in October, even without a distribution requirement. With the shipping restriction in place, the pest risk is low for quarantines that begin in November and December (harvest dates into February).

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1. INTRODUCTION

1.1. Fruit fly risks

Fruit flies of the family Tephritidae are important pests of fruits and vegetables worldwide and their presence in areas where the pest is not established represents a significant threat to commercial agriculture and trade. Fruit flies represent a particular risk because 1) eggs are laid inside the fruit, so immature fruit flies often develop undetected, at least during the early stages of an invasion, and 2) fruit flies lay multiple eggs inside fruit, resulting in a "clumped" distribution within a consignment or smuggled fruit that poses a higher risk. One infested fruit may harbor a potential mating pair of adults.

Where the potential risk of fruit fly introduction and spread exists, local, regional and national governments utilize a number of methods to reduce the risk associated with host movement ranging from outright prohibition of the commodity from potentially infested areas to approval based on quarantine treatments designed to eliminate the risk. Single quarantine treatments have been shown to be effective but can damage the commodity and/or result in reduced shelf life for some commodities. More recently, regulatory officials have embraced the use of "systems approaches" as an alternative means to allow movement of commodities in lieu of using a single or combination treatment while effectively mitigating the risk posed by fruit flies to acceptable levels where quarantine treatments may not exist, may cause unacceptable damage, or are not practical (ISPM 14. 2002 & ISPM 35. 2012).

1.2. Systems approaches to manage risk

Movement of commodities where fruit flies are present has historically been subject to approval by the importing localities and/or (in some cases) bilateral agreements between importing and exporting entities. These agreements are normally based on commodity pest risk assessments determining whether pests present in the commodity could enter and become established in incoming commodity shipments. Sometimes these assessments have resulted in functional "trade barriers" that are not always based on scientific "risk assessments", such as the likelihood that a mating pair of insects could survive any treatment and become established. Mitigation of risk associated with new pest introduction has historically employed single quarantine treatments such as fumigation, heat, cold treatments, irradiation, etc. that are meant to alleviate/reduce risk to a low (near zero) level (Sharp and Hallman 1994, Paull and Armstrong 1994). The standard measure of efficacy has been 99.9968 percent (probit 9) (Baker 1939) especially for fruit flies. While this standard measurement of efficacy has largely been effective in risk mitigation, it is based on the premise that significantly large population pressures of the pest exist where the crop is grown. In practice the high populations that might be associated with the "generally infested" condition are rare in U.S. commercial production areas due to pest management procedures put into practice by growers. Additionally, some commodities can be damaged by single quarantine treatments (e.g. heat, cold, fumigation, irradiation) resulting in quality and shelf life problems. Intra- and Interstate quarantines such as those which might occur as a result of a new detection of fruit flies or other pest could limit host movement outside of the quarantine areas unless suitable risk mitigation measures are undertaken and approved.

Over the last 20 years scientifically-based concepts have been internationally adopted to provide a more biologically-based framework to assess and mitigate risk. These concepts include "probability of a mating pair" (Landolt et al. 1984), "maximum pest limits" (Baker et al. 1990), "pest free areas and areas of low prevalence" (Riherd et al. 1994), "host status and resistance"

(Greany 1989 & 1994, Liquido et al. 1995) and “systems approaches” (Jang and Moffitt 1994, Jang 1996). Recent reviews (Follett and Neven 2006; Aluja and Mangan 2008) discuss in more detail these and other concepts related to quarantine entomology and fruit fly biology. Recently, regional standards such as the North American Plant Protection Organization, (NAPPO 1994, 2002, 2003 & 2008) and international standards from the FAO’s International Plant Protection Convention (https://www.ippc.int/en/core-activities/standards-setting/ispms/) have been developed with the overall goal of harmonizing methods for dealing with risk associated with the threat of establishment of invasive species. The concept of the “systems approach” (Moffitt 1990, Vail et al. 1993, Jang et al. 2006) was developed largely to support biologically-based risk assessments and mitigations that could occur in a broader based “system” of activities that cumulatively meet quarantine requirements of the importing country when they are backed up by strong scientific data (or in some cases - expert opinion). While not new, systems approaches are now internationally recognized by member parties of the World Trade Organization (WTO), and the IPPC providing a framework for harmonizing risk assessment and mitigation, and a forum for oversight when disagreements exist.

1.3. Need for systems approaches for Texas citrus

Most of the outbreaks of exotic fruit flies in California and Florida occur in urban areas where there are a limited amount of commercial host crops present. Many of the outbreaks of Mexican fruit fly, *Anastrepha ludens* (Mexfly), in Texas occur in commercial citrus production areas, or one to two hundred yards from commercial production in residential sites. As a result, it is common that sizeable areas of commercial citrus production fall within a core area (one sq. mile or more) when quarantine actions are triggered due to the presence of an infestation (Attachment 9.4).

Under the current federal and state approved quarantines regulations citrus can be harvested and shipped from outside the 1 sq. mile core area after application of approved bait spray treatment (see CFR 2014a). Citrus from the core areas can only be shipped under approved probit 9 treatments using methyl bromide (MB) fumigation or cold treatment. In Texas, the capacity to treat citrus with MB is very limited and it cannot be used on organically-marketed citrus. Facilities for cold treatment are likewise not available. Therefore other options are needed to provide relief for growers in core areas (typically a square mile) within a quarantined area. The proposed systems approach is intended to achieve this while adequately addressing the fruit fly risk.

2. SELECTION OF THE SYSTEMS APPROACH

In this document we present systems approaches for Texas fruit of any member in the genus *Citrus* regulated by quarantine for Mexfly. The three major components (independent measures) proposed for systems approaches are: 1.) Area of low pest prevalence (ALPP), 2.) Certified pre-harvest foliar bait spray treatments, and 3.) Limited distribution of shipped fruits. For purposes of this assessment, major components and independent measures are synonymous (FAO/IAEA 2011).

Under new IPPC guidelines for fruit flies, with appropriate trapping and verification, areas recently invaded could be considered ALPPs. The USDA uses ALPP as an independent measure in systems approaches concerned with the importation of fruit fly host material. Examples include the importation of pitahaya, papaya and tomato from Mediterranean fruit fly (Medfly;

Ceratitis capitata)-infested countries (FAVIR 2015). The specified level of low pest prevalence used for the same pest can differ for each systems approach which in part is determined by the effectiveness of the other independent measures (ISPM 22. 2005, FAO/IAEA 2011).

U.S. Federal and State authorities declare emergency quarantines when new detections of exotic fruit flies are found at levels exceeding a predetermined “trigger”. The quarantine area encompasses approximately 81 sq. miles around a detection site (Attachment 9.4). The size of the area is adjusted if fruit flies are found at multiple sites. Robust eradication actions are conducted within the core 1 sq. mile around each find. These actions include application of sterile insects (SIT) [if available], pre-harvest foliar bait sprays, soil drenches, fruit stripping, and quarantine controls to further reduce the probability that the infestation will spread outside the quarantine area. This declaration of quarantine changes the designation of an area from “fruit fly free” to one that is considered “generally” infested. In reality, the new designation as a generally infested area may be too severe given the historical success of many fruit fly eradication programs.

Pre-harvest foliar bait spray treatment was assessed as a second major component based on its successful use for decades in non-core areas for eradication of fruit fly outbreaks. This includes pre-harvest foliar bait sprays for Mexfly in Texas citrus in non-core areas. This treatment is also justified by research discussed below. We are proposing a pre-harvest certified spray program beginning at least 30 days prior to harvest and continuing through end of harvest using certified applicators in impacted areas.

Lastly, we propose limiting the distribution of shipped fruit to more temperate U.S. states as a third major component. We will discuss the risk if distribution of this fruit is not allowed to AL, AZ, CA, FL, GA, HI, LA, MS, NM, SC, American Samoa, Guam, Northern Mariana Islands, Puerto Rico, and the Virgin Islands of the United States. Boxes or other containers should be marked to indicate this restriction. For locally distributed fruit, we propose additional trapping and labeling (as above) to mitigate risk.

3. MAJOR COMPONENTS OR INDEPENDENT MEASURES OF THE SYSTEMS APPROACHES FOR CITRUS FROM CORE AREAS

In this section evidence is presented concerning the prevalence of Mexfly during an outbreak under robust emergency response measures, certified bait sprays and limited distribution within the U.S. These factors are major components in potential systems approaches for movement of commercial citrus fruit from core areas within Mexfly quarantine area.

3.1 Area of low pest prevalence

3.1.1. General surveillance and preventive measures for Mexfly

USDA and Texas Departments of Agriculture (TDA) employ a “grid’ of traps baited with food-based attractants in urban and agricultural areas targeting early detection of Mexfly. The normal trapping grid for detection surveys is five traps / sq. mile. The program uses McPhail or Multilure traps baited with protein (Torula yeast/Borax, Nu-lure in water or the 2 component lure) both before and after a detection.

Mexflies are detected on a recurring basis in the Lower Rio Grande Valley (LRGV), Texas, resulting in implementation of emergency detection and/or eradication procedures depending on whether or not trapping triggers are exceeded (USDA APHIS, 2014).

To mitigate repeated occurrences of Mexfly detections, USDA and TDA employ a preventive release program (PRP) of sterile flies over 90 percent of the area where commercial citrus occurs at a rate of 250 to 500 male sterile flies weekly. Sterile insect technique (SIT) reduces the occurrences of fruit fly outbreaks in Texas and other states considered to be high risk locations (Dowell et al. 1999, Thomas et al. 1999).

Intensive **delimitation trapping** is triggered by the capture of a single fly. Trapping is increased in the core area (typically 1 square mile or possibly greater if multiple cores are present) to 80 or more traps (a 16-fold increase) within 24 hours of an outbreak and serviced daily for at least the first week. Trap densities in the remainder of the delimitation area are increased from the core outward within 72 hours of a find. Optimally, delimitation traps are placed over an 81-square-mile area in an 80-40-20-10-5 array (Fig. 1 and Table 1). Thus, the number of traps increases after a detection from 405 up to as many as 1,120 traps depending upon the presence of Mexfly hosts in the area. The density of delimitation traps within a PRP area increases to 20 traps per square mile within a 9 square mile grid around the find (Fig. 2 and Table 2). These increased trapping arrays are meant to increase the likelihood of detecting additional Mexflies anywhere within the quarantine area. Apart from information on prevalence of flies in the trapped area, trapping results also show the effectiveness of treatments and can be used to verify an ALPP based on FAO IPPC guidelines. Given that the normal grid of five traps/sq. mile remains unchanged outside the quarantine area suggests intuitively that areas within it are more "secure", further justifying the designation of ALPP (ISPM 30. 2008). Delimitation trapping is conducted within the quarantine area for at least three life cycles based on day-degree calculations (Attachment 9.4).

DELIMITATION TRAPPING ARRAYS

5	5	5	5	5	5	5	5	5
5	10	10	10	10	10	10	10	5
5	10	20	20	20	20	20	10	5
5	10	20	40	40	40	20	10	5
5	10	20	40	80	40	20	10	5
5	10	20	40	40	40	20	10	5
5	10	20	20	20	20	20	10	5
5	10	10	10	10	10	10	10	5
5	5	5	5	5	5	5	5	5

Fig. 1 Delimitation Trapping Array (Areas outside of Mexfly PRP / other species of *Anastrepha* apart from *A. ludens*

Buffer	Total Mi^2	Traps/Mi^2	Total Traps
C (Core Area)	1	80	80
1st (1 mi^2 buffer)	8	40	320
2nd (1 mi^2 buffer)	16	20	320
3rd (1 mi^2 buffer)	24	10	240
4th (1 mi^2 buffer)	32	5	160
Totals	81	13.8 avg	1120

Table 1 Delimitation Trapping Array (Areas outside of Mexfly PRP / Other species of *Anastrepha* apart from *A. ludens*

20	20	20
20	20	20
20	20	20

Fig. 2 Delimitation Trapping Array within a Mexfly PRP Release Area

Buffer	Total Mi^2	Traps/Mi^2	Total Traps
C (Core Area)	1	20	20
1st (1 mi^2 buffer)	8	20	160
Totals	**9**	**20 avg**	**180**

Table 2 Delimitation Trapping Array within Mexfly PRP Release Area

Larval surveys are conducted near sites where adult captures occur. At least 100 fruit selected at random from host plants in the immediate area of an adult capture are cut open and closely examined by experienced personnel for signs of oviposition ("stings") or exit holes left by larvae. Fruit can be either from the tree or ground. If multiple flies are captured in close proximity, fruit cutting may be extended to all properties within a 200-meter radius of the finds (Attachment 9.4).

Bait spray treatments also are applied to hosts on the property where the find occurred. The first treatment of ground bait spray generally is completed within the first week within a radius of 200-500 meters around fly captures.

Quarantine activities are triggered whenever the detection of a single mated female Mexfly, immature stages (larvae or pupae) or capture of five adults occurs within a 3-mile radius during 1 life cycle of the fly based on day-degree calculations. The area under quarantine will include that area which is within 4.5 miles in all directions from each find site. Regulatory actions are taken to limit the movement of host fruits from this area by placing hold orders on properties where finds occurred. The quarantine will remain in effect until the declaration of eradication. This will consist of certified foliar bait sprays applied for two life cycles of the fly past the date of the last fly find and post-treatment monitoring for one additional life cycle (Attachment 9.4).

Sterile Mexflies are released over all or most of the 81 sq. mile outbreak area based on availability of sterile insects. Outbreak sites are given priority for release of sterile insects. In the core and first one mile buffer, the sterile flies are released at a rate of about 500 males per acre per week. Bait sprays and increased release of sterile flies will continue for two life cycles after the date of the last adult or larval detection (Attachment 9.4).

The effectiveness of the detection trapping helps ensure the Mexfly outbreak is found early and intensive eradication activities ensure the introduced population remains small and quickly eliminated. For these reasons, ALPP is considered as an effective major component of systems approaches for Texas citrus.

3.1.2. Integrated pest management in commercial citrus production in support of ALPP

In 2013-14, the production value of commercial citrus in Texas totaled $72 million (NASS, 2014). The majority of citrus grown in the U.S. comes from California, Florida and Texas. Pre- and post-harvest management practices are routinely applied by growers with guidance of farm advisors and cooperative extension agents.

When invasive fruit flies are not present, pesticides used for their control are not applied. However, the presence of other citrus pests, such as mites, mealybugs, scales, psyllids, whiteflies, thrips, etc., requires pesticide–based management strategies applied to commercially-grown citrus in most cases (Anciso et al. 2002). Effective pest control normally follows integrated pest management practices in accordance with state and federal regulatory guidelines. Many of the pesticides approved for use to control other pests also have activity against fruit flies to some extent and will reduce fruit fly populations when applied thereby contributing to ALPP (Attachment 9.3). An exception to this would be groves using organically-approved IPM practices or methods where few pesticides, if any, are applied.

USDA-APHIS-CPHST personnel have noted the effects of insecticide sprays aimed at control of the Asian citrus psyllid (ACP) in recently carried out studies to assess the effects of insecticide sprays on Mexfly trapping (Attachment 9.2). In their trapping study, captures of Mexfly were significantly reduced after application of zeta-cypermethrin for control of ACP. Currently there are two area-wide applications of insecticide for ACP: One occurring in

November and a second in late January - early February (Texas Citrus Mutual 2015). During 2014, a third area-wide application was made combining a miticide, pesticide and fungicide (Setamou 2015).

Although specific efficacy data against fruit flies on formulations approved for other pests (scales and other surface pests) is scarce, the fact that the same or similar active ingredients or formulations (e.g. malathion, lambda-cyhalothrin, spinosad) approved for fruit fly control are used suggests that application of such chemicals will adversely affect any fruit flies that might be present at the time of treatment. A list of the pesticides approved for insect control on citrus in TX are included in Attachment 9.3 as an example of the types of chemicals already in use that may kill fruit flies or prevent their establishment. However, specific formulations, rates and timing of application need to be tested to determine to what degree they kill fruit flies.

3.2 Effectiveness of the Pre-harvest foliar bait spray treatment

Under declaration of a fruit fly quarantine, regulated crops which are located within a quarantined area but outside the infested core area may receive regular treatments with either malathion or spinosad bait spray as an alternative to post-harvest quarantine treatments. The bait spray treatments take place at 6-10 d intervals at least 30 d prior to harvest and continue on a weekly basis throughout the harvest period. Currently, movement of host fruit within the core area(s) is only allowed with an approved post-harvest treatment.

Published reports support the efficacy of bait sprays for tephritid fruit flies including Mexfly to be 99 percent effective (Mangan, R 2014: Mangan et al 2006; Attachment 9.1).

Under ALPP, chemical treatments in the core area as stated above would be effective as an independent measure if applied preemptively under regulatory supervision coupled with delimitation trapping at least 30 days prior to harvest.

3.3 Effectiveness of the limited U.S. distribution of harvested fruit

The potential risk of fruit shipments from the core area to more temperate areas come from two possible scenarios. One is that the fruit within the shipment is infested and Mexfly become established in the more temperate U.S. states and directly or indirectly cause economic damage. The second is that a portion of the fruit shipped to the more temperate states may be moved to more susceptible areas by the traveling public or that some of the boxes or other containers shipped north are reshipped south to high risk states such as California and Florida.

Shipments of citrus from the core area will be prohibited distribution to AL, AZ, CA, FL, GA, HI, LA, MS, NM, SC, American Samoa, Guam, Northern Mariana Islands, Puerto Rico, and the Virgin Islands of the United States and the shipping boxes or other containers would be marked to indicate this restriction. APHIS has not commonly allowed tropical fruit fly host material into the continental United States using limited distribution and boxes or other containers markings as a major mitigation, but has allowed commodities under other systems approaches. For example melons and watermelons from Ecuador imported under a systems approach for the South American melon fly, *Anastrepha grandis,* have limited distribution within the U.S. (CFR 2014b). To date, no interceptions have been found in commercial shipments nor have any detections of this pest occurred in the U.S.

Since 1929, there have been over 150 reported outbreaks of tropical fruit flies in the Continental United States representing 10 different species not counting Mexfly outbreaks in

TX. Apart from Hawaii, all fruit fly outbreaks in the U.S. have occurred in three States (CA, FL and TX) and Puerto Rico.

In Texas, harvest can start as early as mid-October and peak commercial harvest runs from approximately December 1 through April 1 annually (Nash 2013). Since the Mexfly free area has been established in Texas, the majority of outbreaks (>15) occurring there were detected in either the winter or the spring (USDA APHIS 2014). Thus, any Mexfly (larvae, pupae or adults) arriving in the temperate States to the north via this pathway will arrive at a time when unfavorable temperatures occur and suitable host material is lacking. With no outbreaks reported from all known pathways into these northern temperate States during the summer or fall, then the risk from TX citrus is negligible.

Reshipment of citrus under a limited permit in boxes or other containers marked/stamped is unlikely to move to prohibited states for the following reasons:

- A large volume of TX citrus can already be shipped directly to all of the southern states,
- Distribution is illegal and subject to fine,
- AZ, CA and FL maintain inspection stations for commercial vehicles transporting agricultural commodities, and
- Grapefruit makes up the majority of citrus shipped from Texas and it is not commonly carried south by the traveling public via plane or cars.

An assessment of the effectiveness of this mitigation was conducted for Mexican Hass avocados (Firko and Podleckis, 2001). The findings show that during the first two seasons only 0.12 and 0.36 percent of the containers shipped to the allowed states were detected in other U.S. States. These boxes were moved either inadvertently or intentionally smuggled. After more stringent compliance requirements became effective, only 0.004 and 0.006 percent of containers were detected in other U.S. states during third and fourth seasons respectively.

Unlike Mexican avocados which were prohibited into those fruit fly susceptible states, 90 percent or more of the TX citrus will not be restricted movement. Only the citrus fruits from the core area of the outbreaks would not be allowed movement to those susceptible areas. Therefore, reshipping of whole or partial shipments back into Texas that were sent north should be extremely rare.

Firko and Podleckis (2001) did not directly assess the risk of the traveling public moving small lots of Hass avocados to susceptible area in the southern states. About 68 percent of the citrus shipped from the lower Rio Grande Valley is grapefruit (TVCC, 2013). With no hard data available, we assumed that smaller fruits that are easier to eat, such as apples, plums, bananas, grapes and cherries, are far more likely to be carried by travelers within the Continental United States than larger fruits, such as sweet melons and grapefruit. In this case, we know that some TX citrus shipped to temperate States to the north will be moved south by the traveling public, mostly by plane or car, but the amount moved is highly likely to be very small.

4. DESCRIPTION OF PROPOSED SYSTEMS APPROACHES

4.1. Systems Approaches for Managing the Risk of Citrus Fruit in Texas during a Mexican Fruit Fly Outbreak

General surveillance, delimitation trapping and eradication activities can and often do precede the declaration of a quarantine. The proposed systems approaches take effect once a quarantine action is triggered by detection of:

1) Five adult Mexfly within three miles (4.8 km) of each other and within a time period equal to one life cycle of the fly; or,
2) One mated female (known or suspected to have been mated to a wild male). A single mated female captured during and within an existing Mexfly sterile fruit fly release program area, in the absence of evidence to the contrary, is treated as if it has mated with a sterile male, and therefore is not an quarantine trigger in and of itself; or,
3) One or multiple eggs, larvae or pupae.

Currently fruit can only move from the core area with the MB fumigation (including the 600 fruit cutting), cold treatment or from the non-core area with the pre-harvest certified sprays beginning at least 30 days prior to harvest and continuing through the end of harvest. Note that there are only 5 MB chambers in the valley and no cold treatment facilities for postharvest treatment.

Table 3. Current protocols for moving citrus fruit out of a Mexfly quarantine area

Options	**Mitigation Method**	**Eligible Quarantine Area**	**Eligible Distribution**
A	MB fumigation (Post-harvest)	Whole quarantine area	All of the U.S.
B	Processing the fruit (Post-harvest)	Whole quarantine area	All of the U.S.
C	Certified pre-harvest foliar bait spray 30 days before harvest to harvest end	Quarantine area except designated **core areas**	All of the U.S.

4.2. Proposed mitigations to allow regulated citrus to move from a core area(s) (1 sq. mi or greater) without fumigation

As stated in the Introduction, the purpose of the proposed systems approaches is to provide industry with some options that relieves this regulatory burden while adequately addressing the fruit fly risk.

The proposed mitigations include three main components as discussed in the document

1) Areas of low pest prevalence (ALPP)
2) Certified pre-harvest foliar bait sprays
3) Limited U.S. distribution

The three main components of these systems approaches will vary slightly depending on the specific scenarios as summarized in Table 4 below. Although we believe that these three scenarios will cover the majority of the issues surrounding the movement of citrus from these quarantine core areas, other unanticipated situations may occur. If necessary, a local risk assessment will determine the applicability of the current mitigations to the specific situation.

4.2.1. Fruit from quarantined areas which have been under routine SIT

Option 1. All fruit in the quarantined area is eligible for movement under these systems approaches, including fruit from groves within the core area.

Requirements:

a. The particular core area grove in the quarantine area is under at least 30 days of regulatory trapping beginning 30 days prior to harvest and continuing through end of harvest
b. No flies have been detected within the grove
c. The particular grove is under pre-harvest certified sprays beginning 30 days prior to harvest and continuing through end of harvest.

4.2.2. Fruit from quarantined areas that have not been under routine SIT

Option 2. Under this systems approach, fruit is eligible to move if it comes from a grove greater than 250 meters from a Mexfly detection.

Requirements:

a. The grove is under pre-harvest certified sprays beginning within 72 hrs. of first detection continuing on a weekly basis through end of harvest
b. Minimum 30 days of regulatory bait sprays prior to harvest.
c. The particular core area grove in the quarantine area is under at least 30 days of regulatory trapping beginning 30 days prior to harvest and continuing through end of harvest
d. No flies found within the grove
e. Fruit from groves within 250 meters of a detection is NOT eligible for movement.

Option 3. Under this systems approach, fruit under quarantine is eligible to move if it comes from a grove within 250 meters of a Mexfly detection.

Requirements:

a. The grove is under pre-harvest certified sprays beginning within 72 hrs. of first detection continuing on a weekly basis through end of harvest
b. Minimum 60 days of certified bait sprays after the establishment of the quarantine prior to harvest and continuing through end of harvest
c. The particular grove in the quarantine area is under at least 60 days of regulatory trapping beginning 60 days prior to harvest and continuing through end of harvest
d. No flies found within the grove

Table 4. Proposed systems approach options allowing interstate movement of citrus fruit from a **core** area under Mexfly quarantine.

Options	Under SIT before detection of first Mexfly	Other Conditions	Eligible Area In Core
1	Yes	a. The particular core area grove in the quarantine area is under at least 30 days of regulatory trapping beginning 30 days prior to harvest and continuing through end of harvest b. No flies have been detected within the grove c. The particular grove is under pre-harvest certified sprays beginning 30 days prior to harvest and continuing on a weekly basis through end of harvest.	Whole core
2	No	a. The grove is under pre-harvest certified sprays beginning within 72 hrs. of first detection continuing through end of harvest b. Minimum 30 days of regulatory bait sprays prior to harvest. c. The particular core area grove in the quarantine area is under at least 30 days of regulatory trapping beginning 30 days prior to harvest and continuing through end of harvest d. No flies found within the grove e. Fruit from groves within 250 meters of a detection are **NOT** eligible for movement.	Core except within 250 meters from fly finds
3	No	a. The grove is under pre-harvest certified sprays beginning within 72 hrs. of first detection continuing through end of harvest b. Minimum 60 days of certified bait sprays after the establishment of the quarantine prior to harvest and continuing on a weekly basis through end of harvest c. The particular grove in the quarantine area is under at least 60 days of regulatory trapping beginning 60 days prior to harvest and continuing through end of harvest d. No flies found within the grove	Within 250 meters from fly finds

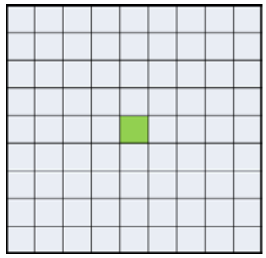

Fig. 3 A typical quarantine area (shown in gray) consisting of 81 square miles. The "core area " (in green) represents 1 square mile area around a fly find.

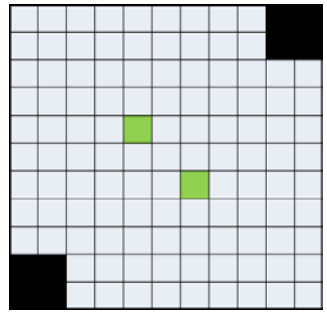

Fig. 4 A quarantine area (shown in gray) can have multiple "core areas" (shown in green). When this occurs, the quarantine boundary expands outward 4.5 miles around each fly find. The black areas fall outside the quarantine boundary. In some cases, the "core areas" may be adjacent to one another.

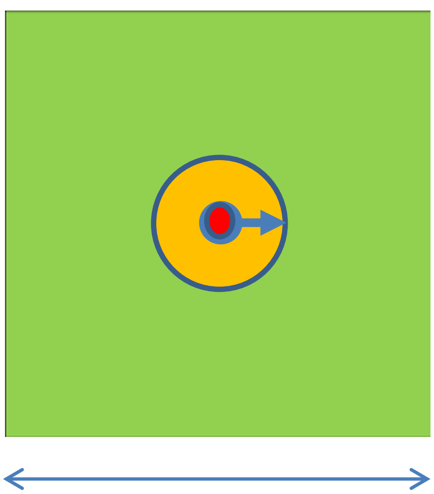

Fig. 5 The green and orange areas combined represent a hypothetical "core area" (typically 1 square mile) around a fly find. The fly find is represented by the small red circle at the center, and the arrow is a radius of 250 meters from the fly find.

Option 1 (with SIT PRP), fruit can move from the core area shown in green and orange after a minimum of 30 days of regulatory trapping at weekly intervals, a minimum of 30 days of certified pre-harvest foliar bait sprays at weekly intervals through the end of harvest, and no flies or larvae found within a grove.

Option 2 (no SIT), fruit can move from the area shown in green after a **minimum of 30 days** of regulatory trapping at weekly intervals, a **minimum of 30 days** of certified pre-harvest foliar bait sprays at weekly intervals through the end of harvest, and **no flies or larvae** found within a grove. **Fruit harvested in groves within the orange area (250 meter radius from a find) would be excluded**.

Option 3 (no SIT), fruit can move from the orange area within a 250 meter radius from a fly find after a **minimum of 60 days** of regulatory trapping at weekly intervals, a **minimum of 60 days** of certified foliar bait sprays at weekly intervals through the end of harvest , and no flies or larvae found with a grove.

<u>4.2.3. Additional Requirements or conditions for all three options in core area systems approaches</u>

1. APHIS/TDA Regulatory trapping establishment in the groves – 1 trap per 25 acres with at least 1 trap per grove. Note: regulatory trapping refers to the specific requirement of placing an additional program approved trap(s) in each grove as part of the systems approaches.
2. If a portion of a grove falls within an area not eligible for systems approaches, the entire grove is ineligible.
3. A compliance agreement will be required and will stipulate the systems approach requirements.
4. A local risk evaluation by PPQ and State program personnel will be required prior to initiation of a compliance agreement.
5. Limited fruit cutting will be carried out periodically in high risk residential sites next to grove(s).
6. When a new Mexfly is detected within the core area, groves shipping under systems approaches must increase trapping from 1 trap per 25 acres to 3 traps per 25 acres. **Note:** The capture of additional flies within a core area do not reset the clock in terms of the number of days of trapping or bait spray treatments required prior to movement.
7. If additional flies (5 or more individuals) or immature stages are detected within a core, systems approaches will be suspended for the remainder of the season.
8. Certified pre-harvest foliar bait sprays are under the same requirements now used for citrus grown outside the core area and the applicator must be under a compliance agreement.
9. Fruit found to be eligible for interstate movement under systems approaches must be accompanied by a PPQ530 Limited Permit.
10. The boxes or other containers in which the fruit is packaged, and any shipping documents accompanying the boxes or other containers must be clearly marked with the statement "**Limited Permit: USDA-APHIS-PPQ, Not for distribution in** AL, AZ, CA, FL, GA, HI, LA, MS, NM, SC, American Samoa, Guam, Northern Mariana Islands, Puerto Rico, and the Virgin Islands of the United States"
11. Only fruit that meets all of these requirements for interstate distribution may be packed in boxes or other containers that are marked with this statement.

5. Summary of Risk

5.1. Qualitative Risk Description

As stated in Section 2, **Area of Low Pest Prevalence**, **Certified Field Treatment,** and **Limited Distribution** are the major components (independent measures) of proposed systems approaches.

<u>5.1.1. Low Pest Prevalence</u>

The first major component of systems approaches is that the quarantine area is an ALPP. When eradication is triggered, various measures covered in Section 3.1.1 and Attachment 9.4 ensure that the area is an ALPP. In the LRGV about 90 percent of the area with commercial citrus is under a Mexfly PRP program. Within 24 hours after a Mexfly adult is captured, trapping in the core area is increased from five traps/ sq. mile to a minimum of 80 traps and serviced daily for the first week. Fruit cutting occurs around each adult capture.

Under systems approaches, the program groves require additional measures to maintain ALPP:

- Trapping within the grove
- At least 30 day delay before harvest
- Additional fruit cutting
- Other safeguards as needed

5.1.2. Certified pre-harvest foliar bait spray treatment

Certified pre-harvest foliar bait sprays are the second main component for systems approaches. The grove would be required to have pre-harvest certified foliar bait sprays beginning 30 days prior to harvest and continuing on a weekly basis through end of harvest using certified applicators**.** The certified applicators must use an approved malathion or spinosad bait spray. These certified foliar bait spray programs have been used successfully during outbreaks of various exotic fruit flies in non-core areas for decades. Section 3.2 gave the details on the effectiveness of the approved pesticides. With the degree of low prevalence in the grove area and this approved treatment, none or only a few fruits would be infested from the groves' harvest.

5.1.3. Limited Distribution

The third major component is limited distribution of fruit to States not susceptible to fruit fly establishment. For reasons stated in Section 3.3 above, the likelihood of inadvertent or deliberate movement of citrus fruit to States susceptible to fruit fly establishment or back into Texas is deemed to be very low.

If a few infested fruits finally arrive into a susceptible area, Mexfly larvae within these fruits must overcome several serious impediments before an outbreak can occur. These impediments include:

- Fruit needs to arrive to susceptible areas with favorable climatic conditions and ample host material suitable for oviposition. Since the fruit will be shipped during the winter and early spring, climatic conditions will be unfavorable in those States where movement is allowed. Furthermore, there will be an absence of host fruit suitable for oviposition.
- Finding a suitable mate that is sexually mature, successfully copulate and find a suitable host fruit in which to oviposit.
- A suitable niche where pupa(e) survival can occur. Garbage cans generally would not favor survival in northern States.
- Insecticides used for other pests that may also kill immature stages or adults.
- Presence of predators that can eat larva, pupa or adult fruit flies that includes ants, birds and spiders.

The risk of allowing program fruit under systems approaches to be distributed within TX is minimal because:

- Large expanses of TX outside the LRGV are free of any hosts with the exception of the "coastal bend" where winter temperatures are unfavorable for tropical fruit flies.
- Fruit moving out of areas under systems approaches will have been treated under compliance agreement.

- Since 90 percent of the commercial citrus in the LRGV is under a PRP program, this also serves to reduce the risk to other parts of TX.
- The only recorded outbreak in TX outside of the LRGV occurred in Laredo, TX when a single mated female was captured on March 06, 2007. This triggered an eradication response (USDA APHIS 2014)
- Most Texas citrus will be eligible to move to other parts of Texas from non-core areas.

5.1.4. Colonization Potential

Miller et al. (1996) estimated the probability of an *Anastrepha*-infested lot of fruits causing an outbreak. Using available evidence (examples, foreign interception records and outbreak records), and expert judgment, they assessed that it takes 62,170 (mean value) of infested lots (mostly carried by the traveling public) destined to a favorable state to cause one outbreak of an *Anastrepha* fruit fly. The states considered favorable for colonization for this study were AZ, CA, and FL. TX was not considered because at that time, the LRGV was not a Mexfly-free area. It was under a Federal/State pest management program for Mexfly. Although the preferred host material is commonly intercepted from Mexico and Central America, only a fraction of the *Anastrepha* interceptions are Mexfly. All of the *Anastrepha* outbreaks during the study period were Mexfly which is expected since Mexfly is generally assumed to have the greatest or almost the greatest colonization potential of the *Anastrepha* fruit flies (Miller et al. 1996). Given the above and with a large amount of uncertainty we can estimates that it would take 1,000's (but not 10s of 1,000's) of infested lots on average arriving in a susceptible State to cause one outbreak.

Given the effectiveness of the three major components of systems approaches and the rather low colonization potential of Mexfly, the risk of this program causing an outbreak is extremely low.

5.2. Quantitative Risk Analysis Summary

One important factor we need to highlight is that in the presence of host material, Mexfly are unlikely to spread very far across the environment (Aluja et al. 2001, Bressan and da Costa Teles 1991, Hernandez et al. 2007, Plummer et al. 1941 and Thomas and Loera-Gallardo 1998). Flies from outbreaks in groves may not move very far at all, and any flies from outside a grove may not move very far into any grove they encounter. Likewise, Bateman (1972) wrote that when ample host fruit are available, "Adults tend to remain in such areas…." Dispersive movement, in contrast, occurs when individuals have either not located suitable hosts or left when the supply declined. The low likelihood of dispersal largely limits the risk posed to those fruit within about 250 meters of the center of the incipient population. We also note, however, that this behavior enhances the chances of successful mating under SIT because it tends to concentrate available wild males into a smaller area (also see below). With a very small number of mature females and males present (see above), dispersal would likely *reduce* the overall chance of reproduction and establishment, since the likelihood of flies going to the same sector—and being in proximity to each other for mating—becomes smaller and smaller as the flies get farther and farther away from the center of the outbreak. Accordingly, the simulation results below indicate that the risk is usually greatest for fruit in the inner-most areas.

We based our initial population estimate on the numbers of flies that might emerge from infested smuggled mangoes from Mexico, which represents a likely scenario and also one which creates a larger population size than would happen if single mated females were dispersing long distances from Mexico. We assumed people would carry and discard one to six fruit, with empirical estimates of number of eggs and survival probabilities at each stage up to adult emergence (Appendix 8.1 Quantitative assessment). For areas under SIT, we based the estimate on the emerging generation (i.e., Gen0 or adults emerging from smuggled-in fruit), since subsequent generations are highly unlikely to develop. That gave a mean initial population size of about 20 adult flies for generation 0 (initial) with a maximum size of 53. In areas not under SIT we based the estimate on numbers for generation 1, which had a mean size of about 53, but a maximum of about 540 flies.

For most situations below we made the following simplifying and conservative (i.e., risk *over*-estimating) assumptions:

- In the option for areas under routine SIT, we ignored natural mortality in wild adult Mexflies. We still accounted for the likelihood of eggs to develop into viable larvae.
- At a drop rate of only 300 flies per acre, a square mile contains 192,000 sterile male Mexflies. To allow for some low probability of mating, we restricted competition from sterile males to the immediate area around the incipient population (e.g., inner 50 m radius; see below). At distances of 150 m or more from center we further restricted the area considered, by assuming all dispersing adult flies (both wild males and females) would spread to the same small area (50 or 25 percent of the total).
- Based on available data we specified that certified sprays would cause 99 percent mortality in Mexfly (1 percent survival). Actual mortality rates may be greater considering that mortality is high following one treatment but foliar bait sprays are applied weekly over generations.
- In non-SIT options, we allowed unhindered fly reproduction and oviposition for two weeks before the start of either spray operations or SIT after official detection, to account for logistics (e.g., trap servicing, official identification, and notification).
- We assumed each clutch of eggs went into a different fruit. Fruit with multiple clutches might increase the chance of having a mating pair, but that would likely decrease the overall risk via this pathway because of the low likelihood of a box being misdirected. Furthermore, fruits with multiple stings are more likely to prematurely drop from the tree. Our assumption maximizes the number of boxes with fruit infested with a potential mating pair.

5.2.1. Fruit from quarantined areas that were under routine SIT

Option 1. This option requires at least 30 d of regulatory trapping and 30 d of certified sprays until the end of harvest. Because SIT has been going on in the area even before the quarantine began, the model results indicate very few mated females are likely to occur (not shown). In addition, once certified sprays start, they quickly mitigate against any adult Mexfly that do occur.

The risk is negligible for all fruit from groves that are more than 150 meters from a Mexfly detection, even without a restricted shipping distribution (Table 5). With the restriction in place, the risk is negligible for all fruit within the quarantine area.

Doubling the estimated initial population size significantly affected the quantitative results but did not change the qualitative conclusion. For example, mean years to the first misdirected box for fruit within 150 meters of center increased almost seven-fold, to 0.0001 from 0.000015, but mean years to first misdirected box remained very large, at 10,000 years (not shown).

5.2.2. Fruit from quarantined areas not under routine SIT

Option 2. Under this systems approach, fruit is eligible to move if it comes from a grove greater than 250 meters from a Mexfly detection. The requirements include regulatory trapping for 30 d and at least 30 d of certified sprays prior to harvest.

Because SIT is not ongoing in these areas, the new Mexfly population can reproduce unhindered until detection. Consequently some fruit near the epicenter of the outbreak are highly likely to be infested, which results in a greater likelihood of infested boxes being misdirected to suitable places for Mexfly establishment. Fruit from areas more than 250 meters from a detection, however, have negligible risk (see Table 6).

In this option, for fruit outside 250 meters of the center, doubling the estimated initial population size did almost double the probability that a misdirected box with a potential mating pair could occur, but the overall risk remained very low: mean years to the event dropped only to about 4,550 years.

Table 5. Simulation results for Mexican fruit fly infestation in the scenario described in Option 1

Distance from center	Probability of mating pair in fruit	Expected years to first potential mating pair (no.)		Probability of misdirected box with mating pair	Expected years to first misdirected box with mating pair (no.)	
		Mean	5th pctile		Mean	5th pctile
0-50m	0.0209	48	3	0.00007	14,286	733
50-150m	0.0091	110	6	0.000035	28,571	1,466
0-150m	0.01896	53	3	0.000015	66,667	3,420
150m+	0.00003	33,333	1,710	<0.000005	200,001	10,258

Table 6. Simulation results for Mexican fruit fly infestation in the scenario described in Option 2

Distance from center	Probability of mating pair in box	Years to first mating pair (no.)		Probability of misdirected box with mating pair	Expected years to first misdirected box with mating pair (no.)	
		Mean	5th pctile		Mean	5th pctile
0-100m[a]	1.000	1	1	0.504	2	1
101-200m[a]	0.984	1	1	0.153	7	1
0-200m[a]	1.000	1	1	0.575	2	1
200m+	0.00512	195	10	0.00013	7,692	395

[a] Fruit near the epicenter of the outbreak are highly likely to be infested; therefore they are excluded under this option.

Option 3. Under this systems approach, fruit is eligible to move if it comes from a grove within 250 meters of a Mexfly detection. The requirements are to delay harvest for 60 d and use weekly certified sprays until the end of harvest. We list potential quarantine dates and associated harvest times in Table 5 for easy reference.

Waiting 60 d for harvest accomplishes two things: letting sprays decimate the incipient population, and allowing any developing Mexfly to leave infested fruit to pupate (which would likely be culled during harvesting or processing). Harvesting typically begins in October (e.g., Wagner and Sauls, N.D.), and 40 to 50 percent could be harvested before January (Setamou 2015 pers. comm.).

Because of the degree day requirements of the Mexfly (Thomas, 2012), fruit harvested the week of 23 December or earlier poses a negligible risk (Table 8). Thus, the risk is negligible under this option for any October quarantines, even without the shipping distribution measure.

For quarantines beginning in November and December, the risk is greater because with cooler weather larvae are more likely to be present in the fruit at harvest. For these quarantines, however, the shipping distribution requirement reduces the risk to very low levels for harvest dates into February (Table 8).

We did not need to model dates beyond those shown in the table because the number of days to pupation peaked for harvests in the last week of January/first week of February. Consequently, the risk would decrease in this option as harvests move beyond the week of February 10.

As before, doubling the estimated initial population size did not significantly change the risk estimates associated with any harvest date. For example, the mean years to first misdirected box for a harvest date of January 13 dropped by only 6 percent, from 926 to 870 years.

In addition, we verified in a separate simulation that with the shipping distribution requirement, the risk is very low in this option regardless of whether or not we accounted for the likelihood of larvae to be in fruit at harvest (see Table 7). We ignored that calculation and just estimated reproduction and oviposition under the certified spray program. We found no differences between harvest dates, and a mean probability of a misdirected box equal to 0.025 percent overall (or about 500 years to the first misdirected box with a mating pair).

Table 7. Quarantine dates by week and the corresponding harvest times by week after 60 d-delay under Option 3.

Quarantine start week	**Harvest week with 60-d delay**
17-October	16-December
24-October	23- December
31-October	30- December
7-November	6-January
14-November	13- January
21-November	20- January
28-November	27- January
5_December	3-February
12-December	10-February
19-December	17-February

Table 8. Simulation results for Mexican fruit fly infestation in the scenario described in Option 3

Harvest date (week)	**Probability of mating pair in box**	**Years to first mating pair (no.)**		**Probability of misdirected box with mating pair**	**Years to first misdirected box with mating pair (no.)**	
		Mean	5th pctile		Mean	5th pctile
16-Dec	0.0	200,001	10,258	0.0	200,003	10,258
23-Dec	0.000745	1,342	69	0.0	200,003	10,258
30-Dec	0.088	11	1	0.000255	3,922	202
6-Jan	0.361	3	1	0.000985	1,015	53
13-Jan	0.431	2	1	0.00110	909	47
20-Jan	0.626	2	1	0.00171	587	31

Harvest date (week)	Probability of mating pair in box	Years to first mating pair (no.)		Probability of misdirected box with mating pair	Years to first misdirected box with mating pair (no.)	
		Mean	5th pctile		Mean	5th pctile
27-Jan	0.768	1	1	0.00219	458	24
3-Feb	0.757	1	1	0.00196	512	27
10-Feb	0.683	1	1	0.00189	529	28
17-Feb	0.635	1	1	0.00164	610	32

6. CONCLUSIONS

Qualitative and quantitative results above indicate that the following systems approaches provide acceptable pest risk reductions for Mexflies in quarantined Texas citrus fruit for different situations.

Fruit from quarantined areas that were under routine SIT can ship from anywhere in the quarantined area after at least 30 d of regulatory trapping, 30 d of certified sprays until the end of harvest, and with a shipping restriction (Option 1). Model results indicated that fruit outside 150 m from the center of the infestation poses a negligible risk even without the shipping restriction.

Fruit from quarantined areas not under routine SIT can ship if it comes from a grove more than 250 m from the center of the infestation, with regulatory trapping for at least 30 d, 30 d of certified sprays prior to harvest, and a shipping distribution restriction (Option2).

If the fruit comes from a grove within 250 m of the center of the infestation, it can be shipped if harvest is delayed 60 d with weekly certified sprays until the end of harvest, and a shipping distribution restriction (Option 3). In this case, the pest risk is negligible for all quarantines that begin in October, even without a distribution requirement. With the shipping restriction in place, the pest risk is low for quarantines that begin in November and December (harvest dates into February).

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9. APPENDICES

Appendix 9.1. 2013 Trip Report - Citrus Production Sites in Texas

During April 2013, Eric Jang, Barney Caton, Ed Miller and Pat Gomes visited citrus production sites in TX. We visited various groves, packing houses and met with the following PPQ program personnel as follows:

Stuart Kuehn - State Plant Health Director – Austin
George Nash - Assistant SPHD & Mexfly Program Coordinator - Austin
Hugh Conway - Entomologist – CPHST Laboratory at Mission
Robert Vlasik - Officer-In-Charge (OIC) - McAllen Work Unit
Vela Chapman - Plant Health Survey Specialist (PHSS) – McAllen Work Unit
Velma Saenz - PHSS – McAllen Work Unit
Guadalupe (Lupita) Gracia - OIC Harlingen Work Unit

The main objective of the site visit was to see a cross-section of the fresh Citrus production industry in TX.

During the trip we visited five packinghouses and two groves from in the LRGV, TX. The two groves were next to packing houses. We also visited a large flea market where a lot of the vendors sell fresh fruits. These businesses are regulated during Mexfly quarantines. We visited two citrus groves which appeared fairly free of all pests. The fallen citrus fruit was present on the ground in the groves indicating that sanitation following harvest could pose a risk. Concerning Texas production we were informed or observed the following:

1. GENERAL OBSERVATIONS

- Mexfly generally are detected during the months of February, March and April, with peak detections normally occurring in March and April.
- About 50 percent of the harvested citrus in the Lower Rio Grande Valley is sold as fresh fruit and about 50 percent is processed (for juice, oil and cattle food). A small amount is discarded because of post-harvest diseases of fruits.
- They currently ship citrus fruit from Texas counties officially recognized Mexican fruit fly pest free areas: Willacy County declared pest free since 2008; Cameron County since 2010; and, Hidalgo County since 2012.
- The irrigation systems are generally flood irrigation.
- Several of the insecticides used in the field are for non-fruit fly pests (thrips, scales, mealybugs, psyllids, mites, etc.) and routine sprays for these other pests also cause a certain degree of mortality for fruit flies.
- No shipments of Citrus have been rejected for presence of fruit flies by officials in the States of AZ or CA since 2005.

- They currently export to Japan. Japanese officials are known to rigorously inspect commodities; however, no shipments of citrus have been rejected due to the presence of Mexfly larvae in fruit.

- From Oct 1, 2012 to April 1, 2013, they exported 162,148 boxes or other containers (6,485,920 lbs.) of citrus mostly to Germany, China, Japan and the Netherlands. This included at least 17 shipments (22,051 boxes or other containers) of grapefruit to Japan.

- Around every adult Mexfly capture, program personnel cut at least 100 fruits (if the fruits are available) up to 200 plus fruit. In the 2012 season, large numbers of adult Mexfly were captured and over 1200 fruits were cut with 8 larval sites found. In sour orange (dooryard) 1 or 2 larvae are found per fruit and in grapefruit (grove) up to 20 larvae are found per fruit.

- There is limited capacity for conducting methyl bromide fumigations (only 5 chambers in the LRGV) and no cold treatment facilities in the LRGV.

- The general process of fresh fruit in the packing houses including de-greening (early season only), a chlorine wash (with an organic substitute in South Tex Organics), culls removed two or more times during the process, grading and sizing, washing and brushing, drying and waxing (not in the Triple "J" Organic Packinghouse). It appeared that all of the packers produce a high quality product.

- A total of 27,300 ac. of citrus is grown in the LRGV

2. PACKING HOUSE SPECIFICS - for places visited

- Paramount – Contact was Robert Martin
  They process citrus from about 16,000 acres (approximately 70 percent of the total commercial citrus acreage in the LRGV). About 80 percent of what they process is grapefruit and the remainder is sweet oranges. The packing house visited was entirely enclosed and capable of excluding adult Mexfly.

- South Tex Organics – Contact was Dennis Holbrook
  They process citrus from about 475 ac. The packing house was completely open and would be very hard to enclose it to exclude adult Mexfly.

- Edinburg Citrus Association – Contact was Jeff Husfeld
  They have about 110 members. The packing house visited was entirely enclosed and capable of excluding adult Mexfly.

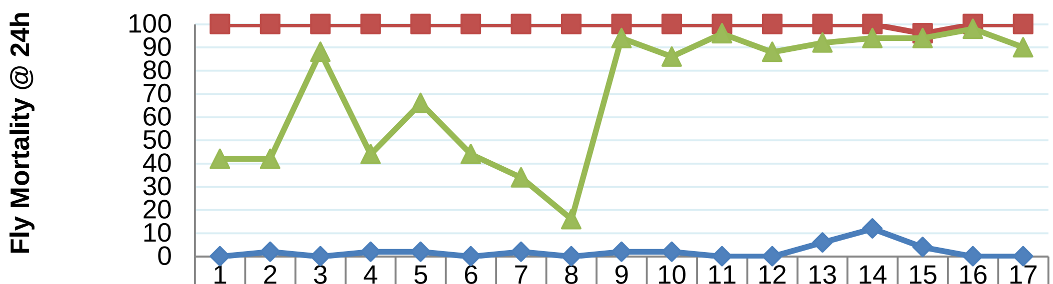
- Lone Star Citrus Growers – Contact was Jud Flowers
  They process citrus from about 6,000 ac. (20 percent or more of the commercial citrus acreage present in the LRGV). The packing house visited was entirely enclosed and capable of excluding adult Mexfly.

- Triple "J" Organic – Contact was J. J. Luzano

They process citrus from about 95 acres. The packing house was completely open and would be very hard to enclose it to exclude adult Mexfly. The packing house was small and much of the fruit was packed by hand.

Appendix 9.2. Validation bioassays of Spinosad GF-120 and Malathion-Nulure, October 2012 - Hugh Conway CPHST Mission Lab

Bioassays to test the length of effectiveness of Malathion Nulure 12 oz. /acre (1.2 oz. Malathion to 10.8 oz. Nulure) and Spinosad **GF-120** NF **Naturalyte**® 48 oz. / acre (40 to 60: GF-120 to water) in the citrus orchard were conducted after a field application on 21 October 2012. Three sample branches 8-12 inches long containing spray residue were collected daily from citrus trees treated with Malathion Nulure and Spinosad. Three plastic observation cages were set up. Each cage contained a supplemental source of food and water. Cages for observing effect of treatments received three field collected branches with residue that were slid through a ¼ inch centered drilled hole in the lid of a sealed 7 oz plastic cup containing water and placed into the observation cage. The control cage was set up with no leaves. Each cage with field collected leaves contained samples branches from only Malathion Nulure or Spinosad. Each cage received 50 sterile Mexican fruit flies. Mortality of *Anastrepha ludens* was observed and recorded by treatment set at 24, 48, and 72 hour periods after the initial placement of leaves with residue into the cages and values graphed across a seventeen day test period.



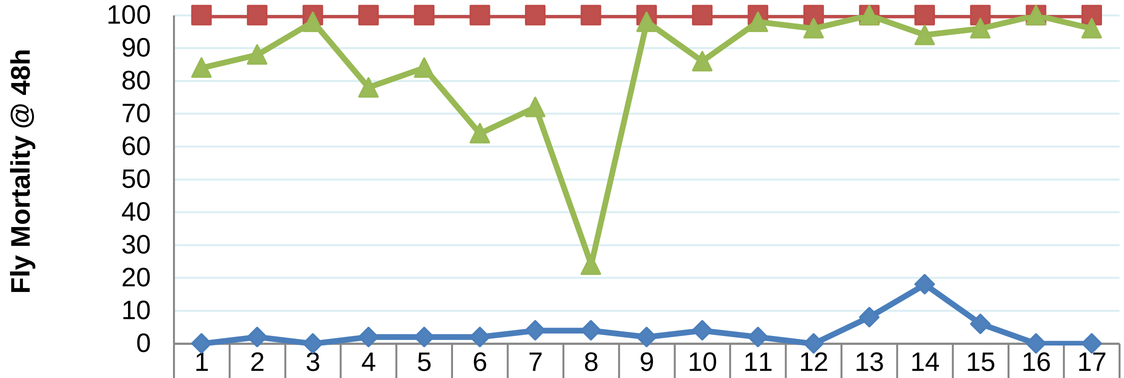
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Control	0	2	0	2	2	0	2	0	2	2	0	0	6	12	4	0	0
Malathion	100	100	100	100	100	100	100	100	100	100	100	100	100	100	96	100	100
Spinosad	42	42	88	44	66	44	34	16	94	86	96	88	92	94	94	98	90

Fig. 1. Daily percent Mortality of Mexican fruit flies after 24 hours in cage from field collected leaves with residual chemical spray from single spray on 21 October 2012

At 24 hours, Malathion spray resulted in nearly 100 percent mortality while mortality from Spinosad dropped to less than 20 percent at day eight (Fig.1). On the afternoon of day eight, growers applied a psyllid control spray (Mustang) that increased Spinosad effectiveness as seen from day nine onward.

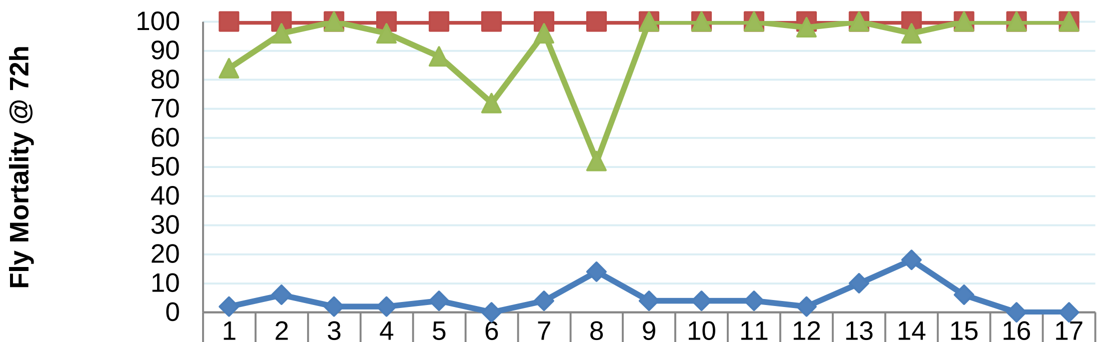
At 48 hours, Malathion spray caused 100 percent mortality to Mexican fruit flies. Fly mortality from Spinosad dropped day three to eight from 90 to 52 percent. The psyllid spray late on day eight resulted in increased mortality in the Spinosad treated fields (Fig. 2).

At 72 hours, fly mortality from Spinosad was down to 50 percent at day eight, but increased from day nine onward probably due to psyllid spray in the orchard (Fig. 3).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Control	0	2	0	2	2	2	4	4	2	4	2	0	8	18	6	0	0
Malathion	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Spinosad	84	88	98	78	84	64	72	24	98	86	98	96	100	94	96	100	96

Fig. 2. Daily percent Mortality of Mexican fruit flies after 48 hours in cage from field collected leaves with residual chemical spray from single spray on 21 October 2012



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Control	2	6	2	2	4	0	4	14	4	4	4	2	10	18	6	0	0
Malathion	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Spinosad	84	96	100	96	88	72	96	52	100	100	100	98	100	96	100	100	100

Fig. 3. Daily percent Mortality of Mexican fruit flies after 72 hours in cage from field collected leaves with residual chemical spray from single spray on 21 October 2012

Malathion produced ~100 fruit fly mortality after 24 hours in the observation cage across the 17 day period. Fly mortality increased from Spinosad with time but dropped at 72 hours post spray on day eight to 52 percent. There was a large increase in fly Mortality in the Spinosad observation cages after day nine possibly due to a psyllid spray (Mustang) that the growers applied to the test field on the afternoon of day eight.

Conclusion: Based on this test, the current chemical control methods used in the Lower Rio Grande Valley are effective in controlling Mexican fruit flies when applied as per label. For additional information contact Hugh Conway hugh.e.conway@aphis.usda.gov (956) 205-7644

Appendix 9.3. Validation bioassays of Spinosad and Malathion-Nulure, October 2014
Hugh Conway, CPHST Mission Lab

Malathion was affective across the 15 day testing period in killing Mexican fruit flies. Spinosad was affected by rain on the day 5 and 6 drastically dropping mortality. On 25 Oct 14, the field was sprayed with Imidacloprid & Envidor causing increased mortality for four day post psyllid spray.

Methods: Bioassays to test the length of effectiveness of Malathion Nulure 12 oz. /acre (1.2 oz. Malathion to 10.8 oz. Nulure) and Spinosad **GF-120** NF **Naturalyte**® 48 oz. / acre (40 to 60: GF-120 to water) in the citrus orchard were conducted after a field application on 16 October 2014. Three sample branches 8-12 inches long containing spray residue were collected daily from citrus trees treated with Malathion Nulure and Spinosad GF 120. Three plastic observation cages were set up with supplemental source of food and water. Cages for observing effect of treatments received three field collected branches with residue that were slid through a ¼ inch centered drilled hole in the lid of a sealed 7 oz plastic cup containing water and placed into the observation cage. The control cage was set up with no leaves. Each cage with field collected leaves contained samples branches collected from only Malathion Nulure or Spinosad spray fields. Each cage received 50 sterile Mexican fruit flies. Mortality of *Anastrepha ludens* was observed and recorded by treatment set at 24, 48, and 72 hour periods after the initial placement of leaves with residue into the cages and values graphed across a fifteen day test period.

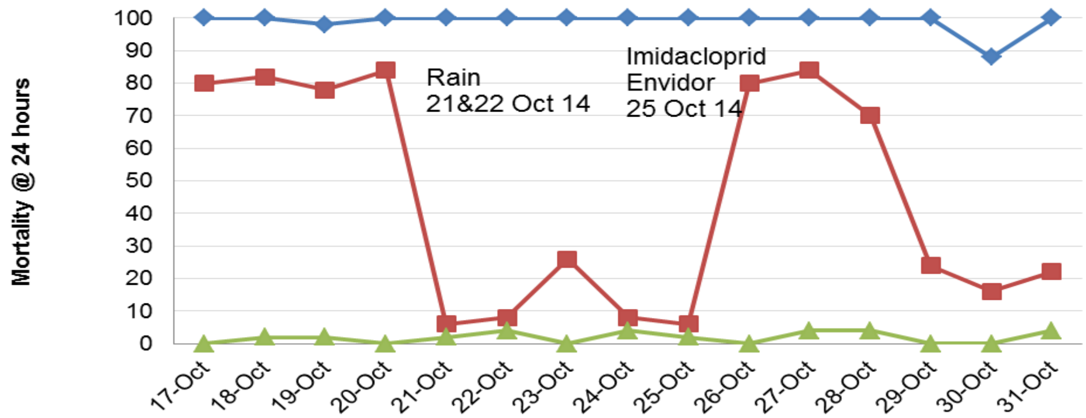
Results:

At 24 hours, Malathion spray resulted in nearly 100 percent mortality while mortality from Spinosad dropped to less than 10 percent on 21 Oct after a rain event (Fig.1). On the afternoon of 25 Oct, growers applied a psyllid control spray (Imidacloprid & Envidor) that increased Spinosads effectiveness across four days.

At 48 hours, Malathion spray caused 100 percent mortality to Mexican fruit flies. Fly mortality from Spinosad decreased on 21 Oct from 100 to 36 percent. The psyllid spray on 25 Oct resulted in increased mortality in the Spinosad treated fields for four days (Fig. 2).

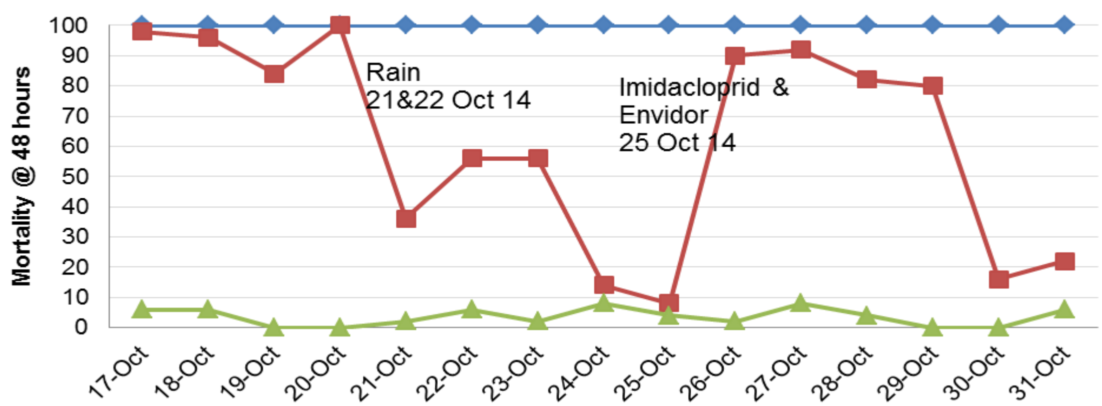
Fly mortality from Malathion was 100 percent at 72 hours across the entire test period.

At 72 hours, fly mortality from Spinosad decreased from 100 to ~70 percent on 21 Oct then dropped as low as 8 percent by 25 Oct which was similar to the control. On 25 Oct, an afternoon spray of Imidaclorprid & Envidor caused significantly higher mortality at 72 hours over 90 percent for the next four days before dropping to ~20 percent for the last two days of the test (Fig. 3).



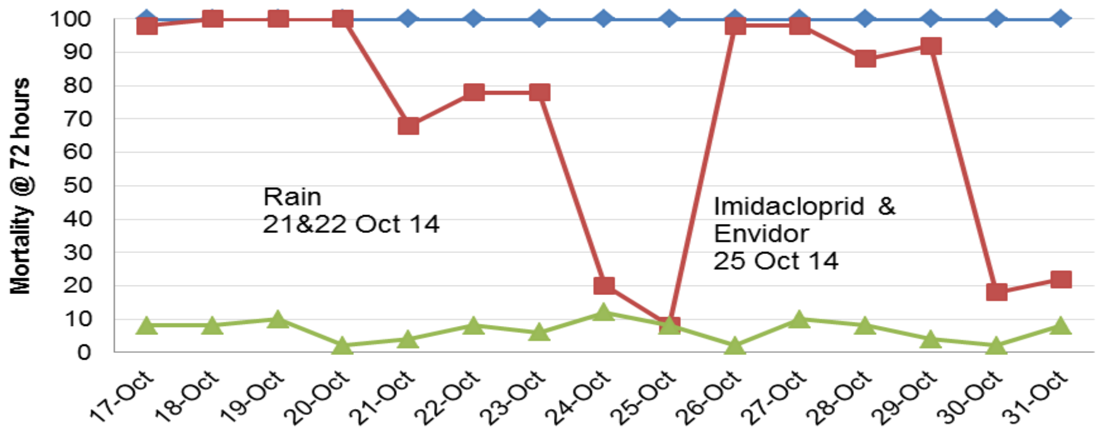
	17-Oct	18-Oct	19-Oct	20-Oct	21-Oct	22-Oct	23-Oct	24-Oct	25-Oct	26-Oct	27-Oct	28-Oct	29-Oct	30-Oct	31-Oct
Malathion	100	100	98	100	100	100	100	100	100	100	100	100	100	88	100
spinosad	80	82	78	84	6	8	26	8	6	80	84	70	24	16	22
Control	0	2	2	0	2	4	0	4	2	0	4	4	0	0	4

Fig. 1 Daily percent Mortality of Mexican fruit flies after 24 hours in cage from field collected leaves with residual chemical spray from single sprays on 16 October 2014



	17-Oct	18-Oct	19-Oct	20-Oct	21-Oct	22-Oct	23-Oct	24-Oct	25-Oct	26-Oct	27-Oct	28-Oct	29-Oct	30-Oct	31-Oct
24h	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
48h	98	96	84	100	36	56	56	14	8	90	92	82	80	16	22
72h	6	6	0	0	2	6	2	8	4	2	8	4	0	0	6

Fig. 2 Daily percent Mortality of Mexican fruit flies after 48 hours in cage from field collected leaves with residual chemical spray from single sprays on 16 October 2014



	17-Oct	18-Oct	19-Oct	20-Oct	21-Oct	22-Oct	23-Oct	24-Oct	25-Oct	26-Oct	27-Oct	28-Oct	29-Oct	30-Oct	31-Oct
24h	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
48h	98	100	100	100	68	78	78	20	8	98	98	88	92	18	22
72h	8	8	10	2	4	8	6	12	8	2	10	8	4	2	8

Fig. 3 Daily percent Mortality of Mexican fruit flies after 72 hours in cage from field collected leaves with residual chemical spray from single sprays on 16 October 2014

Discussion: Malathion produced ~100 fruit fly mortality after 24 hours in the observation cage across the 15 day period. Fly mortality increased from Spinosad with time (24h, 48h, and 72h) but dropped at 72 hours post spray on 21 Oct to 68 percent because of rain wash off. There was a large increase in fly Mortality in the Spinosad observation cages after 25 Oct possibly due to a psyllid spray (Imidacloprid and Envidor) that the growers applied to the test field. Increased fly mortality from psyllid spray lasted for four days before dropping in the Spinosad treated fields.

Imidacloprid is a neonicotinoid systemic insecticide that acts as a neurotoxin. Envidor is a miticide. This combination provided four days of additional Mexican fruit fly control in this test.

Conclusion: Based on this test, the current chemical control method of Malathion used in the Lower Rio Grande Valley is effective in controlling Mexican fruit flies when applied as per label. Spinosad provides good control but should be reapplied after any rain event because of wash off from rain. Spinosad at 72hours provided above 68 percent mortality for seven days even after two rain events on day 5 and 6. After fly mortality dropped below 10 percent in Spinosad spray field on 25 October, a psyllid spray of Imidacloprid & Envidor provided increased Mexican fruit fly control 88-98 percent across four days.

For additional information contact Hugh Conway hugh.e.conway@aphis.usda.gov (956) 205-7644

Appendix 9.4. List of Pesticides Used for Citrus Rust Mite and Psyllid Control In Texas
Source: Table modified from 2011-2012 EFFICACY AGAINST SPECIFIC CITRUS PESTS AND DISEASES” published by theTexas A&M Kingsville Citrus Center

Pesticide Brand Name	Active Ingredient (Irac-Moa)	Rate/Acre	REI (Hr)	Phi (D)	Maximum Rate/Yr Amount (#)	Citrus Rust Mites	Citrus Psyllid
ADMIRE PRO *	Imidacloprid (4)	7 -14 oz	12	0	14 oz		++
ACTARA	Thiamethoxam (4)	3 – 5.5 oz	12	0	11 oz		++
AGRI-FLEX	Abamectin/Thiamethoxam (6, 4)	5.5 – 8.5 oz	12	7	17 oz (3)	++	++
AGRIMEK 0.15EC**	Abamectin (6)	5 - 20 oz	12	7	40 oz (3)	++	+
BAYTHROID XL	Beta-Cyfluthrin (3)	1.6 – 6.4 oz	12	0	6.4 oz (4)		++
CARZOL SP	Formetanate hydrochloride (1B)	2 – 4 oz	216	30	1.25 lbs	++	
COMITE EC	Propargite (12 C)	2 - 3 pt	480	21	80 oz (2)	+	
DANITOL 2.4 EC	Fenpropathrin (2)	16 - 21 oz	24	1	42-2/3 oz	+	++
DELEGATE	Spinoteram (5)	3 - 6 oz	4	1	12 oz. (3)		++
DICOFOL 4E (KELTHANE)	Dicofol (UN)	1 – 2 pt	12	7	6 pints	++	
DIMETHOATE 4E	Dimethoate (1B)	½ - 2 pt	48	15-45	(2)		++
ENVIDOR 2 SC	Spirodiclofen (23)	13 - 20 oz	12	7	20 oz. (1)	++	
ESTEEM 0.86 EC	Pyriproyifen (7C)	10 - 16 oz	12	1	26 oz		+
IMIDAN	Phosmet (1B)	1 - 2 lb	24	7	4 oz (2)		++
LEVERAGE 360	B-Cyfluthrin /Imidacloprid (3, 4)	2.4 – 6.4 oz	12	0	6.4 oz		++
LORSBAN ADVANCED	Chlorpyrifos (1B)	2 - 12 pt	120	21-35	16 pt (2)	+	++
LORSBAN 4E	Chlorpyrifos (1B)	4 – 7 pt	120	21	15 pt (2)	+	++
MALATHION 57 EC	Malathion (1B)	2.4-7.2 pt	12	7	7.2 (1-3)		+
MICROMITE 80 WGS	Diflubenzuron(15)	6.25 oz	12	21	18.75	++	++
MOVENTO	Spirotetramat (23)	8 – 10 oz	24	1	20 oz	+	++
MUSTANG	Zeta-cypermethrin(3)	4.3 oz	12	1	17.2 oz		++
NEXTER 75 W	Pyridaben (21)	4.3-10.67 oz	12	7	21.34 oz (2)	++	+
PETROLEUM OIL	Petroleum Distillate	2 - 6 gal	4	0	-	+	+
PLATINUM***	Thiametoxam (4)	1.83 - 3.67 oz	12	0	3.67 oz		++
PORTAL/FUJIMITE	Fenpyroximate (21)	1-4 pt	12	14	8 pt (2)	++	+
PROVADO 1.6 F	Imidacloprid (4)	10 - 20 oz	12	0	40 oz		++
SEVIN 80 S	Carbaryl (1A)	3 ½ - 6 ¼ lb	12	5	25 lbs (8)	+	++
SPINTOR 2 SC	Spinosad (5)	4 - 10 oz	4	1	29 oz (2)		+
SUPRACIDE 2 E	Methidathion (1B)	1½ - 5 pt	72	14	20 pts (2)		+
VENDEX 50 WP	Fenbutatin-oxide(12B)	2 - 3 lb	48	7	6 lbs (2)	++	
VOLIAM Flexi	Thiam./Chlorantranilipole (4, 28)	4 – 7 oz	12	1	14 oz		++
VYDATE L	Oxamyl (1A)	2 - 4 pt	48	7	24 pts (6)	++	+
+ = provide some help, ++ = very effective							

Appendix 9.5. Texas Action Plan for Mexican Fruit Fly *Anastrepha ludens* (Loew)
July 10, 2013

I. ACTION STATEMENT

This action plan has been developed by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) and the Texas Department of Agriculture (TDA). This action plan is a guide to the major phases of an eradication program. Specific program actions may be modified based on the information available at the time of an infestation.

The Mexican fruit fly represents a major threat to Texas agriculture. It infests numerous species of plants and breeds and spreads rapidly. Because of this, a rapid response capacity is critical.

A chronology of action is detailed in the Appendix.

II. PEST PROFILE

Common Name: Mexican fruit fly
Scientific Name: Anastrepha ludens (Loew)

Order and Family: Diptera, Tephritidae

Description: The Mexican fruit fly is up to 0.4 inches long, slightly larger than a housefly. The adult has a pale orange-yellow body with two to three white bands on the thorax. The wings are clear with conspicuous yellow and brown banding. Around three times a day, the female uses her pointed, slender ovipositor to deposit clutches of 5-6 eggs beneath the skin of the host fruit. The larva is a legless maggot, creamy white in color, and may grow to a length of 0.4 inches within the host fruit.

History and Economic Importance: The Mexican fruit fly was first discovered in 1863. Initially found only in Central Mexico, the insect’s distribution and population levels have since expanded to reflect increased cultivation of fruit crops such as citrus, mango, guava, and avocado. Several crops such as apricot, avocado, grapefruit, nectarine, orange, and peach would be threatened by the introduction of this pest.

Distribution: The Mexican fruit fly is widespread from the southeast Texas border to the Yucatan Peninsula. It can be found sporadically as far south as Panama.

Life Cycle: A female deposits eggs in groups of 1 to 18 within a fruit, and may lay over 1,000 eggs in her lifetime. Larvae tunnel through the fruit, feeding on the pulp. They shed their skins twice, and emerge through exit holes in 11 to 30 days. Mature larvae drop from the fruit and burrow beneath the soil to pupate. In 12 to 100 days, adults emerge from the puparia. Newly emerged adults require from 6 to 34 days to mature before they begin laying eggs. Breeding is continuous, with 4 to 6 generations being produced annually.

Hosts and Damage: The Mexican fruit fly has been recorded infesting a number of commercial and dooryard fruit including oranges, grapefruit, peach, and guava. Fruit that have been tunneled through by feeding larvae subsequently are made susceptible to decay organisms and generally are unfit for human consumption.

III. ORGANIZATION, RESPONSIBILITIES, AND STAFFING

During outbreaks USDA and TDA will operate under Unified Command and each agency will designate an incident commander to be responsible for the overall project and administrative functions. USDA responsibilities include USDA administration, regulatory, sterile insect release, treatment and media. TDA may assist in trapping, treatment, larval survey, and establishment of quarantine areas.

If circumstances warrant, the Unified Command may request assistance of APHIS-PPQ's Incident Management Team. The project will use the Incident Command System in handling the project activities.

Technical Support Representatives

A. Scientific Panel: Consists of scientists recommended by TDA/USDA for their expertise on the pest. The panel advises the Unified Command on current research and technology as well as on the biological soundness of treatments and the detection program. The panel meets as needed to develop recommendations and submit them to the Commissioner.
B. Legal Counsel: State or federal attorneys who advise on the legal basis for enforcement decisions and the validity of claims, and who defend the program in court.
C. Medical Coordinator: A pesticide toxicologist who advises on public health implications of the treatment program.
D. Animal Health Coordinator: A veterinarian who advises on potential animal health risks of the treatment program and liaises with veterinary groups.
E. Industry Representatives: Technical representatives who advise on methods of treatment application.

IV. DELIMITATION PROCEDURES

A. Detection and Intensive Delimitation Trapping

PPQ and TDA maintain a cooperative trapping program for Mexican Fruit Fly to provide early detection of any infestation in Texas. The detection program uses either the McPhail trap, or the Multilure trap baited with Torula yeast/Borax, Nu-lure in water or 2 component (ammonium acetate plus putrescine). Traps are hung in host trees at specified densities. State and federal employees inspect these traps weekly throughout the year.

Intensive delimitation trapping, performed using the McPhail traps or Multilure traps as described above is triggered when a single feral fly is trapped. Following the confirmation of the specimen, trap density in the core square mile is increased within 24 hours. Trap densities in the remainder of the delimitation area are increased from the core outward within 72 hours of a find. Optimally, delimitation traps are placed over an 81-square-mile area in an 80-40-20-10-5 array.

Traps in the core mile are serviced daily for the first week. If no additional flies are found, the trap inspection frequency changes to weekly and intensive trapping continues long enough for 99.5% completion (mean degree-days for each stage + 3 standard deviations of the mean) of any immature stages (egg, larval and pupal) that might be present.

If a second fly is found during a trapping period, additional traps are deployed around the new fly find, trap servicing in the core area is increased to twice weekly, and increased emphasis is placed on servicing traps in the buffer areas in order to delimit the infestation more precisely. All traps in the buffer areas then are serviced weekly for three Mexican fruit fly life cycles beyond the date that last fly is detected. Traps may be relocated to available preferred host plants.

The existing trapping grid for Mexican fruit fly detection shall be modified in the delimitation phase of the program by extending the pattern of traps to approximate the optimal delimitation grid.

Following initiation of an eradication program, if no additional flies are trapped, intensive trapping ends after a temperature dependent developmental model indicates that the third complete life cycle following the last fly find has been completed.

B. Larval Survey

In order to survey for larvae on a property where an adult fly has been trapped, fruit will be inspected for possible small circular oviposition scars that occasionally are visible on infested fruit. In the absence of visible clues, 100 or more randomly selected fruit (if available) from preferred hosts in the immediate area may be cut open and examined for larvae. First and second instar larvae are tiny and may be feeding immediately under the surface of the skin; therefore, fruit cutting should be left to experienced personnel. Fruit on properties adjacent to a trap catch also may be inspected.

If two or more flies are trapped in proximity, fruit cutting may be extended to all properties in a 200-meter radius of the finds, concentrating on preferred hosts. Fruit must be inspected on the property.

V. ERADICATION ACTIVITIES

A. Triggers and General Approach

Criteria for Declaration of an Infestation:

1. Five flies within three miles of each other and within a time period equal to one life cycle of the fly: or
2. One mated female (known or suspected to have been mated to a wild male); or
3. Larvae or pupae.

The cooperative Texas Mexican Fruit Fly program begins an eradication project when the program determines on the basis of the preceding criteria that an infestation exists. The program may take up to 10 days after infestation criteria are met in order to further refine the extent and location of an infestation in order to better target eradication activities. Treatment will begin as soon as possible after an infestation is confirmed and will occur within 24 to 72 hours of notification to the property owners.

Insecticide/bait sprays are used to suppress fly populations until sterile flies can be reared in sufficient numbers to overwhelm the wild population. Consult current label(s) for conditions or restrictions to pesticide treatments. Treatments made using bait sprays generally will continue for at least two Mexican fruit fly life cycles after the last fly is detected. Treatments that include the use of sterile flies will continue for two life cycles past the last fly detection. A temperature dependent model of the fly’s life cycle is used to time the end of treatments.

The continued application of insecticide bait spray occurs only when the severity of an infestation warrants it. Continued and undiminished trapping of flies after one to two weeks of insecticide treatments by ground may trigger aerial applications of insecticide(s). Wide distribution of flies may also trigger aerial treatment.

B. Notification

The purpose of notification is to comply with state law and present accurate information in an understandable and non-threatening format to concerned groups. Local and state elected representatives of the residents in the treatment area will be notified and apprised of major developments before and during treatment. During ground treatment activities, any resident whose property will be treated with foliar sprays following the discovery of infested fruit on or near their property will be notified in writing prior to treatment. Treatment notices include the name of the pest to be eradicated, the material to be used, and a phone number to call in case of additional questions on project operations. Following treatment, a completion notice is left detailing any precautions the homeowner should take, including harvest intervals on treated fruit. Treatment without prior notification may be necessary on a small number of properties if active larvae are detected. However, reasonable efforts will be made to contact the homeowner.

During aerial treatment operations, notification will be made either by hand delivery or first class mail at least 72 hours before the first pesticide application begins, or in a declared emergency situation, at least 24 hours before treatment. The information contained in the notice will include that noted above plus the aerial treatment boundaries and the number of a toll-free hotline to answer health-related questions.

C. Treatment

1. Aerial Bait Spray

Before starting release of sterile Mexican fruit flies, at least one bait spray may be applied by air to the treatment area if conditions warrant. Insecticide/bait sprays will be applied at seven- to 14-day intervals, until sufficient numbers of sterile flies can be reared to overwhelm the wild population at the prescribed ratio. If the target ratio cannot be achieved, either because the distribution of flies is too wide or the numbers of flies are too great, aerial treatment may continue until two life cycles of the fly have passed with no new fly finds.

The bait spray will be applied in the core square mile (0.56 mile radius) surrounding the infestation. Consult current label(s) for conditions or restrictions to pesticide treatments.

2. Ground Bait Spray

If aerial treatment is not deemed necessary, the foliage of all host shrubs and trees within a 200-500 meter radius of each infested property will be treated with insecticide/bait sprays applied using hydraulic spray equipment. Residents and tenants on affected properties will be notified in writing at least 24 hours prior to treatment.

Completion notices are left following treatment detailing precautions to take and harvest intervals applicable to any fruit on the property. Treatments are repeated at 7- to 10-day intervals, unless significant rainfall justifies re-treatment. Consult current label(s) for conditions or restrictions to pesticide treatments.

3. Fruit Cutting

Fruit will be removed from all host trees on a known infested property and cut for examination. Fruit is placed in heavyweight plastic bags and removed to a landfill site to be buried under at least one foot of fill.

4. Sterile Insect Technique

This control method relies on flooding the area of an infestation of wild flies with sterile flies produced in rearing facilities. When the sterile flies mate with the fertile population, no offspring are produced. Gradually, the wild fly population decreases, while the sterile fly population is maintained through continued releases. When fertile female flies can find only sterile or no fertile male flies to mate with, the wild population becomes extinct. This technique is used only in combination with other control methods. In general, for the technique to succeed, a minimum ratio of 100:1 (sterile:wild) must be maintained. The sterile flies will be released within a 4.5 mile radius surrounding the detections sites that comprise the infested area and should focus on host areas. Scrub and brush land could be left out if large areas exist. If the infestation is within an existing Sterile Preventive Release program then the increased release area density with be out to a 1.5 mile radius area surrounding the infestation. The current minimum release density is considered to be 1000 bisexual sterile Mexican fruit flies per acre or 640,000 per square mile for eradication purposes in the core and 1st buffer square miles. This release density may be less for the other buffer areas within an 81 square mile quarantine zone.

D. Pesticide Monitoring

At the discretion of the program, a pesticide-monitoring program may be used to evaluate environmental impact.

Monitoring for detectable levels of pesticides in and around treatment areas may include sampling of air, foliage, food, crops, water, soil or other media. The evaluation must effectively address agency, cooperator, and public concerns.

E. Sterile Release Monitoring

In the event of a sterile release program, sterile:wild fly ratios are monitored in the treatment area using McPhail traps deployed at the rate of five evenly-spaced traps per square mile over the treatment area. McPhail traps are to be serviced in accordance with the National Exotic Fruit Fly Trapping Protocol.

F. Post-Treatment Monitoring

The success of the eradication program is monitored at intensive trapping levels. If pesticide sprays are used, intensive trapping levels are maintained during treatment. In a sterile insect technique (SIT) programs, intensive trapping protocol replaces the SIT monitoring system gradually over a three week period after the last release of sterile flies. Traps are serviced every week for one life cycle of the fly after the last treatment or after the institution of intensive trapping protocol. If no flies are caught during that time, trap densities return to pre-treatment detection levels.

G. Quality Control

Experienced personnel will monitor the quality of trapping by inspecting trap sites, trap placement, and servicing performed under the direction of the trapping supervisors or project leader. Personnel may accompany trappers, inspect trap lines or plant target insects. Reports of findings will be submitted to the project staff in charge of trapping.

VI. REGULATORY PROCEDURES

A. Hold Notices

After an infestation is known to exist, operations personnel will issue hold orders on all properties known to be infested with Mexican fruit fly.

B. Emergency Quarantine

An emergency quarantine shall be adopted if any of the infestation criteria listed in Section V. A. is fulfilled.

Quarantines

Mexican fruit fly quarantines shall be adopted by both the Texas Department of Agriculture (TDA) and the Animal and Plant Health Inspection Service (APHIS) as an emergency order if it has been determined that a Mexican fruit fly population has been detected. The TDA will adopt on an emergency basis an intrastate quarantine. Then APHIS will adopt a parallel interstate quarantine.

Quarantine Triggers

For the purposes of this Action Plan, the detection of a Mexican fruit fly population triggers the establishment of a quarantine. The following criteria are indications that a Mexican fruit fly population has been detected:

1) Five adult Mexfly within three miles (4.8 km) of each other and within a time period equal to one life cycle of the fly; or,

2) One mated female (known or suspected to have been mated to a wild male). A single mated female captured during and within an existing Mexfly sterile fruit fly release program area, in the absence of evidence to the contrary, is treated as if it has mated with a sterile male, and therefore is not an quarantine trigger in and of itself; or,

3) One or multiple eggs, larvae or pupae.

Area Under Quarantine

The area under quarantine will include that area which is within 4.5 miles (7.2 km) in all directions from each find site.

Length of Quarantine

The interior quarantine will be in effect until the declaration of eradication. This will consist of two life cycles of treatment and one life cycle of post-treatment monitoring past the date of the last fly find.

Public Outreach

Public outreach concerning the project will consist of press releases to the media to inform the general public, and direct targeted notification of regulated entities, e.g. growers, packing sheds, nurseries, wholesale distributors, retail outlets, homeowners, etc. either individually or through public meetings. Information discussed will include compliance agreement stipulations, regulatory treatments, safeguarding requirements and any other pertinent regulatory actions due to the quarantine.

Movement of host commodities outside of quarantine area

Regulated host commodities which have originated within the quarantine area may not move out of the quarantine area without having a limited permit or certificate issued in accordance with conditions outlined in the quarantine.

Post-harvest host commodity regulatory treatments

Treatment schedules for post-harvest treatments for host commodities are listed in the APHIS Treatment Manual found at:

http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf

Pre-harvest host commodity regulatory treatments

Procedures for the pre-harvest (Bait spray) premises treatment can be found in the treatment section of the Federal Domestic Fruit Fly Quarantine notice 7 CFR 301.32-10. Note: that this pre-harvest treatment is only applicable toward certification of host commodities found at least ½ mile from any Mexfly detection. This information can be found at:
http://ecfr.gpoaccess.gov

VII. PUBLIC INFORMATION

The quarantine is distributed to all state and local elected officials who represent the affected area; this includes mayors, state representatives, and state senators. It also is distributed to State and federal agencies that are concerned with eradication projects including, but not limited to, The Governor's Division of Emergency Management, Texas Department of State Health Services, Texas Parks and Wildlife Texas Commission on Environmental Quality, the United States Fish and Wildlife Service, Department of Agriculture, and Environmental Protection Agency. The quarantine notice may also be published in newspapers of general circulation that serve the affected area.

The purpose of a public relations effort is to inform the public of the need and plans for an eradication and quarantine program in order to secure their support or minimize opposition. Press releases are prepared by the Public Information Officer in close coordination with the Operations Chief. The Public Information Officer serves as the primary contact to the media. A telephone number for project information such as treatment schedules will be staffed by treatment personnel.

APPENDIX

CHRONOLGY OF ACTION

Once a fly has been detected, the chronology of action is as follows:

- 24 hours: Trap density increased to protocol levels within core area (Section III) around each fly find.
- 48 hours: First inspection of traps.
- 72 hours: Trap density increased to protocol levels in 81-square-mile area around each fly find.
- 1st week: Daily inspection of project traps in core area.
- 2nd week: Weekly inspection of project traps.
- Ground treatments conducted within a minimum of 200 to a maximum of 500 meters around the wild fly find begins as follows:
- 24-36 hours: Notification and larval survey begins
- 24-48 hours after notification: Pesticide treatment (bait spray) begins
- 1st week: completion of first pesticide treatment.

Any new treatment areas established due to additional fly finds will be handled within the same time frame as the first area.

TEXAS MEXICAN FRUIT FLY ACTION PLAN SUMMARY

Wild Mexican Fruit Fly detected:

A: 81 square mile delimiting survey initiated

B: Fruit cutting conducted within 200 meters of fly finds (larval survey) for 2 life cycles

C: Ground applied bait sprays within 200-500 meters of fly find conducted for 2 life cycles (optional)

D: Triggers not reached after three life cycles: no quarantine action or further survey/treatments

E: Triggers reached:

1) Initiate quarantine/regulatory action within 4.5 mile radius of fly finds
2) Conduct larval survey around fly finds for 2 life cycles
3) Conduct ground applied bait sprays within 200 to 500 meters of fly finds for 2 life cycles; or aerial applications of pesticides within a .56 mile radius of fly finds based on pest risk, terrain, topography, and available host for 2 life cycles, or sterile insect release within a 1.5 mile radius around fly finds for 2 life cycles. A combination of the three approved treatments may be used to increase program efficacy at the discretion of the SPHD/Incident Commander.
4) After three negative life cycles of trapping release area from quarantine and all corresponding regulations

Appendix 9.6. Quantifying Systems Approaches for Mitigating the Risk of Mexican Fruit Fly, *Anastrepha ludens* (Loew), in Citrus Fruit: Probabilistic Model Descriptions

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1. Introduction

The objective of this report is to explain the probabilistic models used to determine the likely effectiveness of different systems approaches for mitigating the risk establishment of Mexican fruit fly, *Anastrepha ludens* (Loew), in new locations via citrus fruit (grapefruit) from a domestic quarantine area. Here we describe the models in detail.

2. Standardized Methods

2.1. Model settings

The models were coded in spreadsheets and run using @Risk ver. 5.7 Professional (Palisade Corporation, 31 Decker Road, Newfield, NY 14867), a Microsoft Excel add-in. Unless otherwise specified below, simulation settings were as follows: number of iterations = 200,000; sampling type = Latin Hypercube; and random seed = 101.

2.2. Binomial processes

The binomial process is common to the models below. In this process, n independent, identical trials are run, each one with the same probability of success, p, producing some number of successes, s (Vose, 2000):

$$s = \text{RiskBinomial}(n,p) \qquad [1]$$

2.3. Probability of a mating pair

The probability of a mating pair (p_{mp}) being present depends on how many adult pests survive in the shipment. If zero or 1 adults survive, the probability is zero. Otherwise, the probability is calculated as follows (PERAL, 2005):

$$p_{\text{mp}} = 2^{\text{Asurv-2}} / 2^{\text{Asurv}} \qquad [2]$$

This formula simply reflects the idea that, given $A_{surv} > 1$, with random sorting of males and females and an equal gender likelihood (i.e., $p_{♀} = p_{♂} = 0.5$), only two possible combinations exist in which no mating pair forms: either all adults are males or all adults are females. Therefore, the probability of a mating pair forming is the number of possible combinations of males and females minus two, divided by the total number of combinations.

2.4. Mating pair formation

We modeled this as a binomial process (Eqn. 1), with $n = 1$ and the likelihood = p_{mp} (Eqn. 2). The function returns a 1 if successful (mating pair present) or a zero if not. The model tracks this process and the resulting mean (i.e., x successes divided by the no. of iterations) estimates the annual probability of a mating pair being present. We designate that mean value as p_{ann}.

2.5. Years to first mating pair

Once we have found the annual probability of getting a mating pair (2.4) we use the negative binomial function to estimate the number of years, Y, that will pass until the first mating pair arrives (Vose, 2000). The equation is as follows:

$$Y = 1 + \text{RiskNegbin}(1, p_{ann}) \qquad [3]$$

Note that the mean number of years until the first mating pair occurs is always equal to the reciprocal of p_{ann}.

3. Model specifications and results

3.1. Option 1: Quarantine area under routine SIT

3.1.1. Overview

In this option, the area quarantined was under routine sterile insect technology (SIT), in which 250-400 sterile male flies per acre ((PPQ Texas, pers. commun.; 160,000-256,000 flies per square mile) are dropped to interfere with females ability to mate (e.g., Stephenson and McClung, 1966; Thomas et al., 1999). The independent measures employed in this case (see main document) are area of low prevalence, regulatory trapping, a certified spray program, and limiting distribution of fruit to temperate states.

The model predicts the number of males and females in the entire quarantine area, the number in different portions of the area at three distances from the center (see below), the number surviving spray operations each week, the number of released sterile males and therefore the probability of a female encountering a wild male, the number of mated females and days they oviposited each week, the number of clutches laid and eggs laid, the number of viable larvae from hatched eggs, the probability of getting a mating pair in an infested fruit based on those numbers, the number of fruit with potential mating pairs, the likelihood of those fruit being misdirected to a citrus-producing state, and finally the number of years to the first misdirection of such fruit. We summarize below the parameter values and functions used, as appropriate (Table 1).

Table 1. Model nodes and parameters in Option 1, for fruit from quarantine areas under routine SIT

Node	Description	Function	Parameters	Source
1	Adult flies in field (no.)	Discrete	See Fig. 1	Separate simulation (see text)
2	Female adults in field	Binomial	*p*(being female) = 0.5	N/A
3	Male/female adults in different segments away from center (no. per area)	Binomial	*p*(dispersing to area) = Fig. 2	Hernández et al., 2007
4	Males/females surviving bait spraying (no. per area)	Binomial	*p*(surviving insecticide) = 0.01	Conway, Unpublished
5	Released sterile males (no. per area)	Uniform	[Min,Max] See Table 2	PPQ Texas, pers. commun.
6	Area adjustment to segments (proportion)	Arithmetic	0-100 m = 1; 150 m = 0.5; 200 m+ = 0.25	N/A
7	Proportion of wild males	Arithmetic	N/A	N/A
8	Mated females (no. per area)	Binomial	*p*(mating) = proportion of wild males	N/A
9	Mated females surviving/killed by bait spraying (no. per area)	Binomial	*p*(surviving insecticide) = 0.01	Conway, Unpublished
10	Days laying eggs (no.)	None or Discrete	Surviving females lay for 4 d per week, and killed females for some fraction of that time (uniform probability for 1 to 3d)	Berrigan et al., 1988
11	Clutches laid (no. per day)	Discrete	See Fig. 3	Berrigan et al., 1988
12	Total eggs laid (no.)	Discrete	See Fig. 4	Berrigan et al., 1988
13	Hatched eggs (no.)	Binomial	*p*(hatching) = 0.844	Vera et al., 2007 (*A. fraterculus*), corroborated for *A. ludens* by Celedonio-Hurtado et al., 1988
14	Viable larvae (no.)	Binomial	*p*(larval viability) = 0.666	Vera et al., 2007
15	One or more mating pair formed (N/A)	Binomial	*p* = Eqn. 2	PERAL, 2005
16	Years to first mating pair (no.)	Negative binomial (Eqn. 3)	*p* = annual likelihood of mating pair [mean of node #12]	Vose, 2000

Node	Description	Function	Parameters	Source
17	One or more boxes misdirected (N/A)	Binomial	*p*(being misdirected) = Triangle: Min = 0.0, Most likely = 0.0012, Max = 0.0061	PPQ; Mexican avocado shipping data
18	Years to first mating pair (no.)	Negative binomial (Eqn. 3)	*p* = annual likelihood of misdirecting a box with potential mating pair [mean of node #14]	Vose, 2000

<u>3.1.2. Description of nodes and parameters</u>

Adults in the quarantine area. Because detection trapping for Mexfly has been going on with no detections until those leading to quarantine, a breeding population of Mexfly is unlikely to be present. The adventive population is therefore highly likely to have gotten there via one of two ways: 1) a mated female flew across the border with Mexico, or 2) fruit infested with Mexflies were brought from Mexico and discarded, allowing adults to develop. Since the second possibility leads to the greater number of potential adults, that is the option we chose. It's also worth noting that despite the known limitations of catching Mexflies in detection traps, the historical numbers of flies caught during quarantines in the LRGV (not shown; PPQ data) do not seem to indicate that many hundreds of flies are present. So historical trapping data supports the proposed scenario and our analysis here.

Consequently, here we based the initial population size on one to six mangoes (uniform probability) being discarded. [Note that oranges were an option, but they ripen later in the year than mangoes and harbor fewer larvae.] We ran separate simulations (details available upon request) based on the number of adults likely to emerge from each fruit (Diaz-Fleischer and Aluja, 2003) and therefore the total number likely to emerge in the immediate area, and constructed a histogram for the number of flies in the core area. The resulting values (Fig. 1) ranged from 1 to 53, with a mean of 20.4, and 90 percent of the values sampled were between 5 and 38. We used that histogram in a discrete equation which returned one of the determined values at the assigned rate:

$$N_A = \text{RiskDiscrete}([n \text{ values}],[p \text{ values}]) \quad [4]$$

where N_A is the number of adults and the n and p values are two arrays (i.e., columns) of the values represented in Fig. 1.

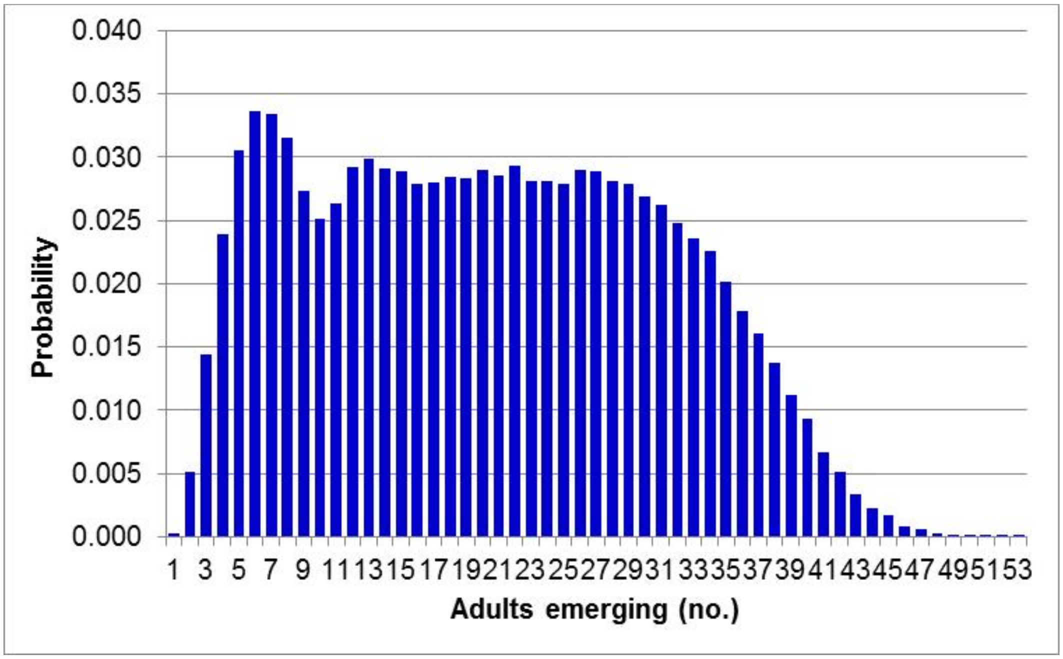


Fig. 1. Probability values for discrete values of the number of adult Mexflies in the field in Option 1.

Males and females in area. We predicted the number of males and females based on an equal sex ratio (i.e., p(being female) = 0.5). In single iterations sex ratios may not be equal, of course, but will be over many iterations. The number of females, $N_♀$, was a binomial (Eqn. 1), and the number of males, $N_♂$, was simply the difference between N_A (Eqn. 4) and $N_♀$.

Males and females in area segments. Mexflies tend to not disperse very far when suitable host fruit is available (Aluja et al., 2000; Bressan and da Costa Teles, 1991; Hernández et al., 2007; Plummer et al., 1941; Thomas and Loera-Gallardo, 1998). Dispersal within about 150m seems to be normal, with occasional movement beyond 200m (and further) (e.g., Baker and Chan, 1991; Baker et al., 1986; Flores et al., 2015; Hernández et al., 2007). We used image analysis (not shown) of the data in Hernández et al., 2007) to determine the probabilities of Mexfly moving outward in 50m increments (Fig. 2), or $p(\text{dispersal to segment})_i$, where i is 50m, 100m, etc. In Scenario 1, however, we did not need to evaluate each 50m-segment individually (computationally intensive) but instead evaluated only three: 1) inner 50m, 2) 50m-150m segment, and 3) a segment for 150m or more.

The calculations for male and female flies in each segment, $N_{♀,i}$ or $N_{♂,i}$, where i = distance from center, were binomials (Eqn. 1). However, we calculated them in a multinomial process so that correctly apportioned across each segment without exceeding the total number of flies. Hence, the number of male and female adults in the first segment (within 50m of center), was a binomial with $n = N_♀$ or $N_♂$, and $p(\text{dispersal to segment})_{50}$. In subsequent calculations, N_i was equal to the remaining number of flies and $p(\text{dispersal to segment})_i$ was equal to the value for that segment divided by the sum of all remaining p values (i.e., p_x / $(\Sigma(p_x, p_{x+50},\ldots, p_{200+}))$.

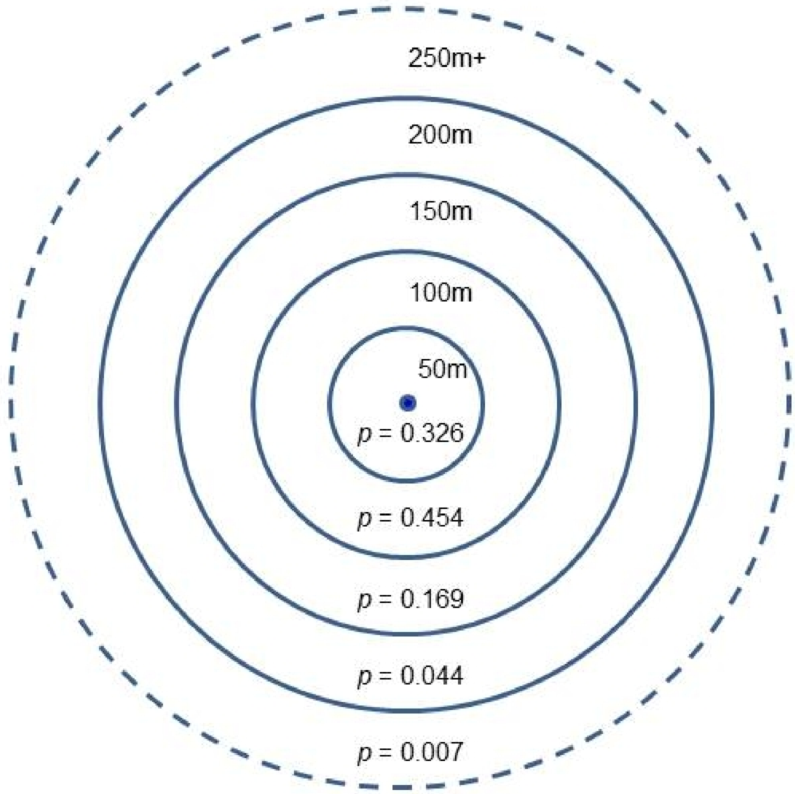


Fig. 2. Schematic showing the likelihood of Mexfly dispersing to different segments away from the apparent epicenter of the population (Hernández et al., 2007).

Adults surviving bait spraying. In bait spraying, flies (male and female) are attracted to an insecticide via bait, often protein and sugar, resulting in their death. We estimated efficacy, *p*(surviving bait spraying) to be 1 percent, based on results in Texas (Conway, Unpublished). In these systems approaches, treatments will be applied each week, with any surviving flies being susceptible to mortality at the next treatment. We calculated the number of surviving adults in each segment. The calculation for surviving adult flies ($N_{s♂}$ or $N_{s♀}$) was a binomial (Eqn. 1) with $n = N_{♂,i,j}$ or $N_{♀,i,j}$, where j indicates week (1-5), and *p*(surviving bait spraying).

The number of killed flies in each segment was the difference between the total in that segment and the number surviving.

Released sterile male flies. Under SIT, the program releases between 250 and 400 sterile male flies per acre (PPQ Texas, pers. commun.). That is the equivalent of 160,000-256,000 flies per square mile. For our simulation, however, we calculated the number of flies in each segment. This is important because mating occurs on scales much smaller than a square mile. In other words, a wild female in our situation is looking for a mate within a 50 or 100 m radius. Over larger areas—even the full outer segments—the potential number of sterile males overwhelms any likelihood of mating (of course, this is the management *intent* of SIT). Consequently, for the outer segments we restricted the estimate to half or a quarter of the area (see Table 2) to better represent the total number of males that a single wild female might encounter. The number of released males, $N_{R♂,i}$, at each distance i, was a uniform distribution (i.e., every value is equally likely) as follows:

$$N_{R♂,i} = \text{RiskUniform}(N_{Rmin,i}, N_{Rmax,i}) \quad [5]$$

where $N_{Rmin,I}$ is the minimum number at distance i and $N_{Rmax,i}$ is the same maximum value. The calculated parameter values for each segment are shown in Table 2.

Note that we did not simulate any impact of bait spraying on the sterile males. While that is highly likely to kill some sterile males, replenishment each week should keep populations relatively large and effective. Furthermore, we tested a version in which sterile males could be killed by the bait spray, and while the annual probabilities changed pretty dramatically (not shown), the overall conclusion of very low risk under the full systems approaches did not change.

Table 2. Minimum and maximum values of the number of sterile males in areas at varying distances from the center of the fruit fly infestation.

Distance from center	Default values		Adjusted values[a]	
	Minimum	**Maximum**	**Minimum**	**Maximum**
50m	485	776	485	776
100m	1,941	3,105	1,941	3,105
150m	4,367	6,987	2,184	3,494
200m	7,763	12,421	1,941	3,105
250m	12,130	19,408	3,033	4,852

[a] For outer segments the area considered was restricted by a factor of either 0.5 (150m), or 0.25 (200m+).

Proportion of wild males. The proportion of wild to total (wild plus sterile) males in the area is the probability that a female will mate with a wild male, *p*(finding wild male). Note that the value used here for number of wild males is the weekly total before accounting for deaths due to bait spraying. In other words, bait spraying only affects the number of wild males that will be available for mating *next* week. This is different than the effect of bait spraying on mated females (below), and was done so to make the weekly mating probability as great as possible.

Mated females. The number of mated female flies in each segment *i*, $N_{M♀,i}$, is a binomial (Eqn. 1) with $n = N_{s♀,i}$ and *p*(finding wild male), for each week (*j*, not shown).

Mated females surviving bait spraying. As above, the number of surviving mated female flies ($N_{sM♀}$) was a binomial (Eqn. 1) with $n = N_{M♀,i,j}$, where j indicates week (1-5), and *p*(surviving bait spraying).

Days laying eggs. Female Mexflies do not oviposit on every day of the week, even if mated (e.g., Berrigan et al., 1988). Although those authors described the frequency—"About 25% of the cohort laid eggs on 80-100% of the days following reproductive maturity, whereas over 30% of the cohort reproduced less than 40% of the days following the onset of reproduction"—this information was difficult to parameterize. Consequently, we created a small separate simulation model using those values (not shown; details available upon request), and determined that on average, mated Mexfly females would lay eggs on 4 days out of 7. Hence, any mated females not killed by bait spray in one week would lay eggs on 4 days, while killed mated females would lay eggs from 1 to 3 days, with a uniform probability for those options. We estimated that individually for each killed fly. Overall, then, the number of days laying eggs in each week, $N_{days,i}$, was the sum of days accrued by surviving and killed flies in that week (*j*).

Total clutches. Mated female Mexflies lay between 1 and 28 clutches per day (Berrigan et al., 1988). We digitally interpreted the displayed percentages in their Fig. 2 to create a probability distribution for the number of clutches laid each week, $N_{clutch,j}$ (Fig. 3). The mean of that distribution was 4.51 clutches per day and 90 percent of the values were between 1 and 9. The equation was a discrete distribution (i.e., only integer values selected) as follows:

$$N_{clutch,j,k} = \text{RiskDiscrete}([N_{clutch}\text{ values}],[p\text{ values}]) \qquad [6]$$

where *k* is the particular day on which eggs were laid.

Total eggs laid. Mated female Mexflies lay from 1 to 30 eggs per clutch (Berrigan et al., 1988). As above, we digitally interpreted Fig. 4 from that source to create a probability distribution for the number of eggs laid in each clutch, $N_{egg,l}$, where l indicates clutch number (Fig. 4). The mean of that distribution was 6.62 and 90 percent of the values were between 2 and 13. The equation was a discrete function (Eqn. 6) as above for clutches per day, but with values for N_{egg} (and associated *p* values) instead of N_{clutch}. The total number of eggs laid, $N_{egg,tot}$, was the sum over all clutches laid.

Hatched eggs and viable larvae. We estimated the proportion of eggs that hatch from data on *A. fraterculus* as 0.844 (Vera et al., 2007). Likewise, the proportion of viable larvae is 0.666 (Vera et al., 2007). Thus, we calculated the number of viable larvae, N_{larvae}, in each clutch as a binomial (Eqn. 1) with $n = N_{egg,l}$ and $p = 0.562$ (= 0.844 × 0.666).

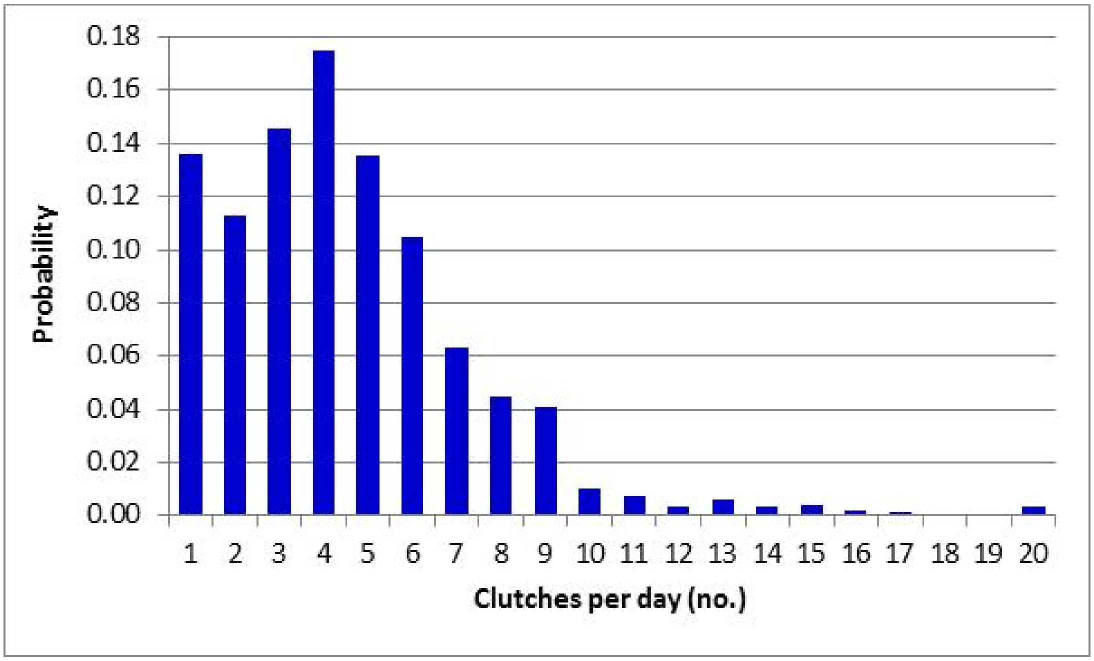


Fig. 3. Probability distribution for the number of clutches laid per day by mated female Mexflies, after Fig. 2 in Berrigan et al. (1988).

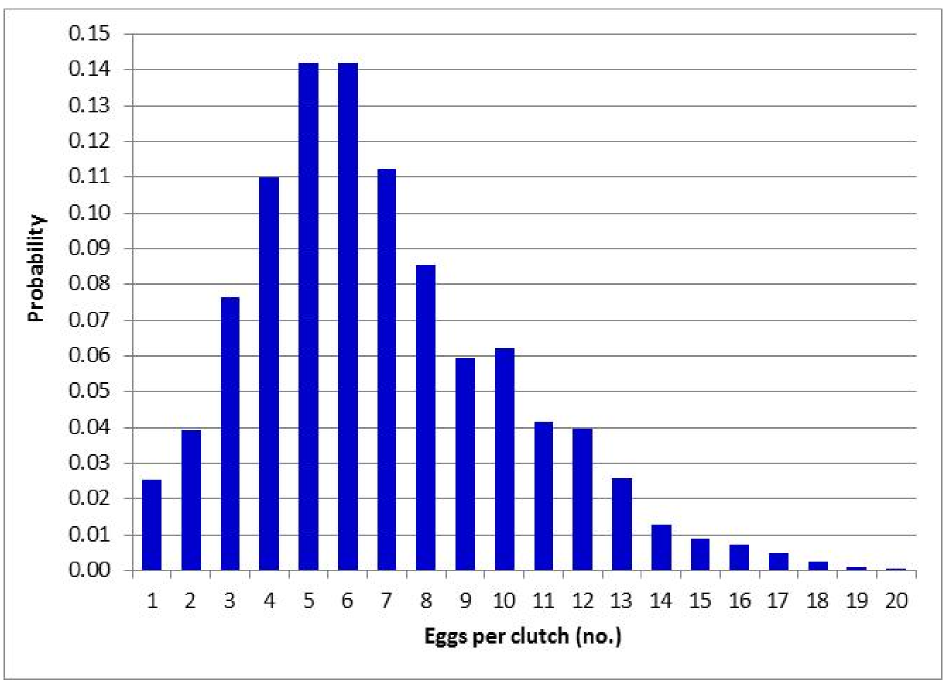


Fig. 4. Probability distribution for the number of eggs laid per clutch by mated female Mexflies, after Fig. 4 in Berrigan et al. (1988).

Mating pairs and years to first mating pair. We noted above that we assumed each clutch of eggs would be laid in a different fruit, in order to increase the number of infested fruit and therefore the likelihood of a misdirected infested fruit. Consequently, we calculated the probability of a mating pair (Eqn. 2) for each clutch (fruit) based on the number of viable larvae. Note that this assumes that each larva becomes a reproductive-capable adult. We then determined if a mating pair resulted in a binomial (Eqn. 1) based on that probability (§2.4), and summed the positives to find the total number of fruit with potential mating pairs.

Furthermore, once the values for p_{ann} have been calculated, we re-ran the simulation to find Y_{MP}, years to first mating pair (§2.5).

Boxes misdirected from distribution area. The above calculations estimate the risk of shipped fruit being infested with Mexflies, but if all those fruit stayed within the restricted shipping area, the risk of establishment would be negligible. Thus, we also estimated the number of those fruit that might be misdirected (e.g., smuggled, sent to a wrong destination) to somewhere more suitable for Mexfly establishment. Our estimate for this probability was based on data collected several years ago when avocados from Mexico had a similar restricted distribution (Table 3, Firko and Podleckis, 2001). The four points had no obvious trend, other than decreasing to small proportions in the final two years, so we simply calculated a mean and 99 percent confidence intervals, and used those values in a triangular distribution as follows:

$$p(\text{box misdirected}) = \text{RiskTriang}(p_{min},p_{ML},p_{max}) \quad [7]$$

where p_{min} is the minimum value, p_{ML} is the most likely value, and p_{max} is the maximum value. This distribution (Fig. 5) had a mean of 0.0024 and 90 percent of the values were between 0.00061 and 0.0049.

As above, in a binomial process with $n = 1$ and p(box misdirected) we determined if a misdirection occurred (Eqn. 1), and summed the positives to find the total number of boxes with potential mating pairs that were misdirected. We also calculated if any potentially infested boxes had been misdirected, which over all iterations estimates the annual probability of that happening. Using that value, we re-ran the simulation to find Y_{MD}, years to first misdirection of a box with a potential mating pair (§2.5).

For model results for Option 1, see section 5.2.1 in the main document above.

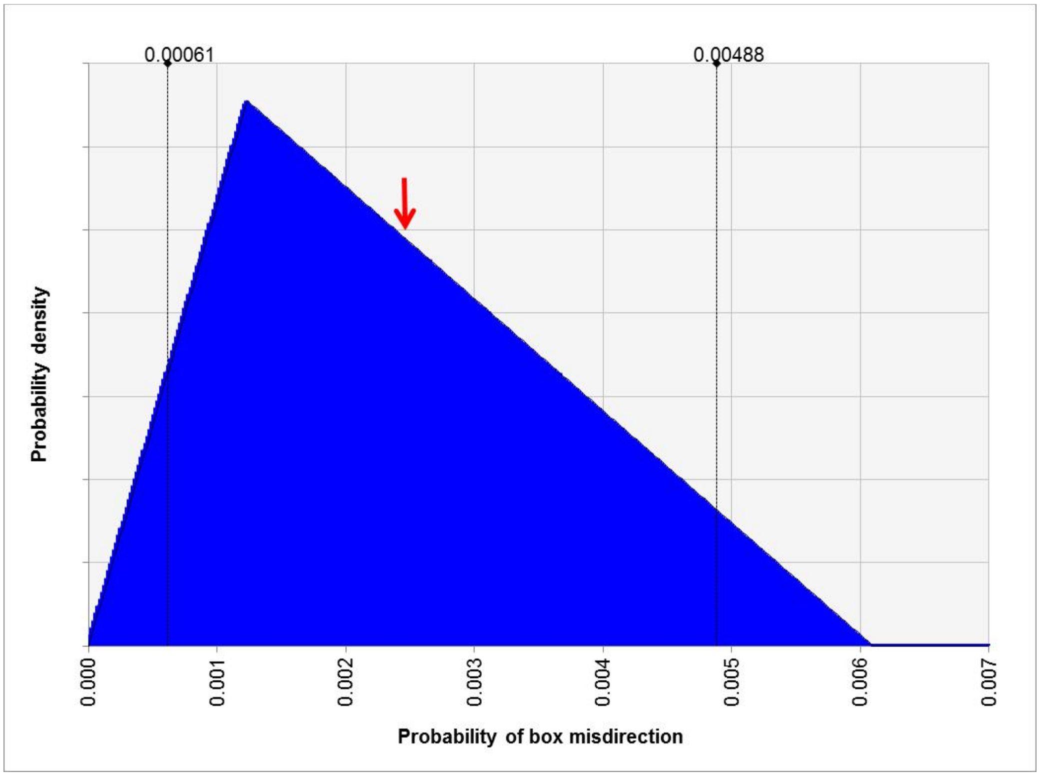


Fig. 4. Probability distribution for the probability of a box with a potential mating pair being misdirected, after Firko and Podleckis, 2001. The red arrow indicates the mean of the distribution and the vertical lines indicate the 5th and 95th percentiles.

Table 3. Data for misdirection of imported Mexican avocado fruit in the United States.

Years	**Boxes imported (no.)**	**Misdirected boxes (no.)**	**Proportion misdirected**
1997-98	537,850	668	0.00124
1998-99	868,000	3,114	0.00359
1999-00	1,036,950	45	0.000043
2000-01	895,900	54	0.000060
All (total)	*3,338,700*	*3,881*	*0.00116*

3.2. Option 2: Quarantine area not under routine SIT

3.2.1. Overview

In this option, the area quarantined was *not* under routine SIT. The independent measures employed in this case (see main document) are the same as in Option 1: area of low prevalence, regulatory trapping, a certified spray program, and limiting distribution of fruit to temperate states. In addition, we assumed that SIT would start after detection (PPQ, person. commun.). Because of the lack of SIT before detection, however, adventive Mexfly populations in these areas can develop nearly unhindered, which means fruit near the epicenter of the infestation are at greater risk.

The model is split into sections for predicting the number and impacts of flies before detection (i.e., before any mitigations start) and after detection. The first section covers 14 days before detection, and the second section covers the first week after detection. Survival beyond that point was very rare. We predict the number of males and females in the entire quarantine area, the number in different portions of the area at three distances from the center (0-100 m, 101-200 m, and beyond 200 m), the number surviving natural mortality (before and after detection) and spray operations (after detection) each week, the number of mated females and days they oviposited each week, the number of clutches laid and eggs laid, and the number of viable larvae from hatched eggs. After detection, we also predict the number of released sterile males and the number of mated females given the difference in the probability of finding a wild male. Finally, we predict the probability of getting a mating pair in an infested fruit (by week and overall), the number of fruit with potential mating pairs, the likelihood of those fruit being misdirected to a citrus-producing state, and finally the number of years to the first misdirection of such fruit. We summarize parameter values and functions used, as appropriate, below (Table 4).

Table 4. Model nodes and parameters in Option 2, for fruit from quarantine areas without routine SIT. Some items apply only for processes before or after detection, and some items apply to both.

Node	Description	Function	Parameters	Source
Weeks before detection				
1	Adult flies in field (no.)	Discrete	See Fig. 5	Separate simulation (See text)
2	Female adults in field	Binomial	*p*(being female) = 0.5	N/A
3	Male/female adults in different segments away from center (no. per area)	Binomial	*p*(dispersing to area) = Fig. 2 (for 0-100 m, 100-200 m, and 200 m+	Hernández et al., 2007
4	Males/females surviving natural mortality (no. per area)	Binomial	*p*(natural survival) = 0.712 (7 d), 0.808 (14 d)	Celedonio-Hurtado et al., 1988
5	Mated females (no. per area)	Binomial	*p*(mated) = [simulation results] see Table 5	after Novelo-Rincón et al., 2009
Week after detection				
6	Males/females surviving natural mortality (no. per area)	Binomial	*p*(natural survival) [21 d] = 0.619 (females), 0.75 (males)	Celedonio-Hurtado et al., 1988
7	Males/females surviving bait spraying (no. per area)	Binomial	*p*(surviving insecticide) = 0.01	Conway, Unpublished
8	Released sterile males (no. per area)	Uniform	[Min,Max] See Table 4	PPQ Texas

Node	Description	Function	Parameters	Source
9	Proportion of wild males	Arithmetic	N/A	N/A
10	Mated females (no. per area)	Binomial	*p*(mated) = [simulation results] × Proportion of wild males	
All weeks				
11	Days laying eggs (no.)	None or Discrete	Surviving females lay for 4 d per week, and killed females for some fraction of that time (uniform probability for 1 to 3 d)	Berrigan et al., 1988
12	Clutches laid (no.)	Discrete [Days ≤ 20], or Central Limit Theorem (Normal) [Days > 20]	Discrete: Fig. 3 Normal: Mean clutches/day = 4.5, standard deviation = 2.85	Berrigan et al., 1988 Berrigan et al., 1988; Vose, 2000
13	Eggs laid (no.)	Discrete	See Fig. 4	Berrigan et al., 1988
14	Hatched eggs (no.)	Binomial	*p*(hatching) = 0.844	Vera et al., 2007 (*A. fraterculus*), corroborated for *A. ludens* by Celedonio-Hurtado et al., 1988
15	Viable larvae (no.)	Binomial	*p*(larval viability) = 0.666	Vera et al., 2007
16	One or more mating pair formed (N/A)	Binomial	*p* = Eqn. 2	PERAL, 2005
17	Years to first mating pair (no.)	Negative binomial (Eqn. 3)	*p* = annual likelihood of mating pair [mean of node #16]	Vose, 2000
18	One or more boxes misdirected (N/A)	Binomial	*p*(being misdirected) = Triangle: Min = 0.0, Most likely = 0.0012, Max = 0.0061	PPQ; Mexican avocado shipping data
19	Years to first mating pair (no.)	Negative binomial (Eqn. 3)	*p* = annual likelihood of misdirecting a box with potential mating pair [mean of node #18]	Vose, 2000

<u>3.2.2. Description of nodes and parameters</u>

Adults in the quarantine area. The quarantine situation in Option 2 is the same as that in Option 1 (see above) except that SIT has not been ongoing in this area. Consequently, the adventive population is likely to have more time to develop before detection. Using the same scenario as above—one to six infested mangoes are discarded—we created a biological model (details available upon request) to predict population size of the first generation (rather than the adventive population size). That model predicted the numbers of males and females in Gen0 accounting for natural mortality, the number of mated females, days of egg laying, number of clutches laid, number of eggs laid, number of hatched eggs and viable larvae, and number of emerged adults (Gen 1), at which point the process repeated. The distribution of the number of emerged adults from Gen1 was our estimate of the adventive (and detected) population size for Option 2. That distribution ranged from 0 to 550 with a mean of 94.2, and 90 percent of the sampled values were between 38 and 194 (Fig. X). We used that data in a histogram function as follows:

$$N_A = \text{RiskHistogrm}(N_{Amin}, N_{Amax},[p \text{ values}]) \quad [8]$$

where N_{Amin} is the minimum number of adults, N_{Amax} is the maximum, and the p values are associated probabilities of each possible N_A value. The sampled real number value was rounded to the nearest integer.

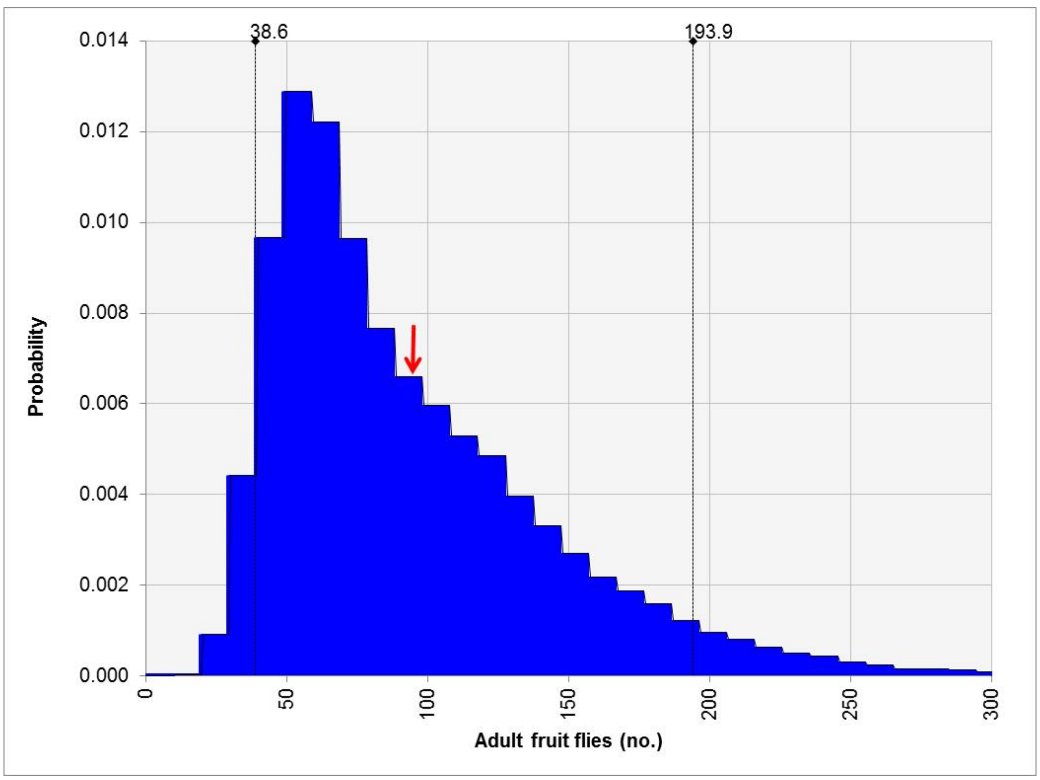


Fig. 5. Histogram for the number of adult Mexflies in the field in Option 2. The histogram extends to the value 550 in the model but was truncated here. The red arrow indicates the mean of the distribution and the vertical lines indicate the 5th and 95th percentiles.

Males and Females in each area and segment. We predicted these values just as in Option 1, except that the segments here were 0-100 m, 101-200 m, and 200+ m (technically only up to 250 m away from the epicenter in the model).

Released sterile male flies. This node only applies after detection. We estimated the number of sterile males released just as in Option 1, but the parameters were specific to the distances used here (Table 4).

Table 4. Minimum and maximum parameter values for the number of released sterile male Mexflies in Option 2.

Distance from epicenter	Released flies/area (no.)	
	Minimum	**Maximum**
0-100 m	2,426	3,881
101-200 m	4,124	6,599
0-200 m	6,550	10,480
200+ m	3,033	4,852

Mated females. Before detection, and the implementation of SIT, the likelihood of mating is unaffected by sterile males but depends on the natural ability to find mates. We predicted the likelihood of mating, *p*(natural mating), in that situation based on a separate simulation model created using data from Novelo-Rincón et al. (2009). Using rates for the likelihood of daily mating success by wild Mexfly males, and the number of females available (i.e., probability of finding a mate) that model predicted the number of females that mated over 21 d. The proportion mated was the estimate of *p*(natural mating). Because the male success rates were competitive, they may underestimate mating success, but that is balanced somewhat by not accounting for natural mortality over the 21 d, and by ignoring evidence that not every mature female will mate (Novelo-Rincón et al., 2009; but see Option 3). Closest to the epicenter, mating probability quickly rose over time to >0.95, while in the center segment (101-200 m) it rose more gradually (Table 5). Only in the outer segment (200+ m) did mating probability remain low over the whole period, reflecting the difficulty of finding the few mates that disperse that far.

Table 5. Mating probability over 21 d in the model for Option 2 at different distances from the epicenter.

Time period (d)	*p*(mated)			
	Distance from epicenter (m)			
	0-100	**101- 200**	**0-200**	**200+**
1	0.211	0.104	0.217	0.001
7	0.975	0.625	0.987	0.008
14	0.999	0.841	1.000	0.016
21	1.000	0.917	1.000	0.022

Consequently, the number of mated female flies in each segment *i*, $N_{M♀,i}$, is a binomial (Eqn. 1) with $n = N_{s♀,i}$ and *p*(overall mating), for each week (*j*, not shown). Before detection, *p*(overall mating) was equivalent to *p*(natural mating), while after detection and the initiation of SIT, *p*(overall mating) was the product of *p*(natural mating) and *p*(finding wild male) (see 3.1.2). Note that we calculated $N_{M♀,i}$ before predicting the effect of bait spraying.

Adults surviving bait spraying. In Option 2, bait spraying only occurs after the Mexfly population has been detected, so it doesn't impact populations before day 21. The calculation was done exactly as in Option 1, as a binomial with *p*(surviving bait spray) = 0.01, and applied in each segment to males, and separately to unmated and mated females.

Days laying eggs. The total number of days of egg laying by mated females was estimated exactly as in Option 1: unkilled females laid eggs on four days each week, and females killed that week laid on from 1 to 3 days. The number of days laying eggs in each week, $N_{days,i}$, was the sum of days accrued by surviving and killed flies in that week (*j*).

Total clutches laid/eggs laid/hatched eggs/viable larvae. We predicted these nodes exactly as in Option 1, above.

Mating pairs and years to first mating pair. We estimated these exactly as in Option 1, above. **Boxes misdirected from distribution area and years to first misdirected box with a mating pair**. Likewise, we estimated these as in Option 1, above.

3.2.3. Model results

In the main document, we summarized the results from the model for Option 2 over the entire time period (see section 5.2.2). Here, we provide some information about the risk before and after detection of the Mexfly population.

Beginning immediately in the simulation, two weeks before detection, the risks are very high in the two segments closest to the epicenter (Tables 6 and 7). Mean annual probabilities for a mating pair are equal to 1.0, and the mean probabilities for a misdirected box with a mating pair are greater than 0.3 for the innermost segment, and about 0.08 for the 101-200 m segment. Risks for each drop precipitously in week 0 when the mitigations (SIT and bait spraying [which only affects females]) begin. Hence, nearly all of the high risk associated with those areas results from Mexfly activities before detection.

Only in the outermost segment did pest risk remain low over the whole time period. Although mitigations are not in place, low dispersal rates lead to few individuals and therefore much less chance of mating than in the areas closer to the epicenter. The mitigations further reduce the risks when they begin, but fruit that was grown more than 200 m from the epicenter seems to pose little risk regardless of timing.

Table 6. Simulation results for Option 2 for the annual probability of shipped fruit containing a potential mating pair, by distance from the epicenter and weeks before detection of the Mexfly population.

Distance from epicenter (m)	**Week before detection (no.)**	***p*(mating pair)**	**Expected years to first mating pair (no.)**	
			Mean	**5th percentile**
0-100	2	1.0	1	1
	1	1.0	1	1
	0	0.0718	14	1
101-200	2	0.965	1	1
	1	0.956	1	1
	0	0.00315	318	17
200+	2	0.00307	326	17
	1	0.00209	480	25
	0	0.0	200,000	10,258

Table 7. Simulation results for Option 2 for the annual probability of shipped fruit containing a potential mating pair, by distance from the epicenter and weeks before detection of the Mexfly population.

Distance from epicenter (m)	**Week before detection (no.)**	***p*(misdirected box with mating pair)**	**Expected years to first misdirected box (no.)**	
			Mean	**5th percentile**
0-100	2	0.345	3	1
	1	0.299	3	1
	0	0.000915	1,093	57
101-200	2	0.0792	13	1
	1	0.0857	12	1
	0	0.000020	50,000	2,565
200+	2	0.000045	22,222	1,140
	1	0.000085	11,765	604
	0	0.0	200,000	10,259

3.3. Option 3: Quarantine area under routine SIT with 60-d delay

3.3.1. Overview

This option is similar to Option 2 but the focus is on finding mitigations that reduce the risk of the fruit in the inner segments (see above). Consequently, this systems approach adds a 60-d delay before harvest and shipping, to facilitate Mexfly larvae in cherries emerging from infested fruit before harvest. Fruit which had been infested could either not be harvested or be culled in the packinghouse, based on obvious damage.

Many of the modeling details are the same between Options 2 and 3, so here we discuss only on the new or changed features in the model for Option 3. We summarize relevant parameter values and functions used below (Table 8).

One important general change was that in Option 3 we did not need to estimate any Mexfly activity before detection. That's because, with the 60-d delay after detection in place, we can be confident that the greatest risk of larvae still being present in fruit at harvest is posed by those that developed from the eggs that were oviposited the latest. Eggs deposited two weeks before detection are likely to develop into larvae that emerge well before harvest. By contrast, eggs laid in the second week after detection are much more likely to develop into larvae that are still present in the fruit at harvest. Consequently, we started with the same potential Mexfly population size as in Option 2, but we only modeled the activity of that population after detection, over two weeks.

Another difference was that we did not simulate the effects of SIT, which we demonstrated in Option 2 did not sufficiently mitigate the risk for fruit in the innermost areas. Because Option 3 is more about developing systems approaches that can mitigate risk despite mated females having early access to fruit, here we only simulated the other standard parts of systems approaches. Consequently, our risk estimates here may somewhat overstate the true risk.

Finally, because of weekly bait spraying until harvest, the risk of successive generations—adult flies developing from pupated flies—within the quarantine timeline is negligible.

Table 8. Model nodes and parameters in Option 3, for fruit from quarantine areas without routine SIT and with a 60-d delay before harvest.

Description	**Function**	**Parameters**	**Source**
Days to pupate (no.)	Normal	See Table 9	NOAA, 2015; Thomas, 1997, 2012
Larvae in fruit at harvest?	Arithmetic	Threshold = 60 d	N/A
Mated females	Binomial	*p*(natural mating) = 0.85	Novelo-Rincón et al., 2009

<u>3.3.2. Description of nodes and parameters</u>

Days to pupation. The life cycle of a Mexfly takes 754 degree days to complete (Thomas, 2012), and about 267 for pupation in particular. Given that, we used weather data for 2008-14 to calculate how many days pupation would take given a particular starting date. We then found the mean days (and standard deviations) to pupation over all years of data (Table 9). We used those parameters in a normal function to predict the number of days pupation would take, N_P, in each week as follows:

$$N_{P,i} = \text{RiskNormal}(\mu_i, \sigma_i) \qquad [8]$$

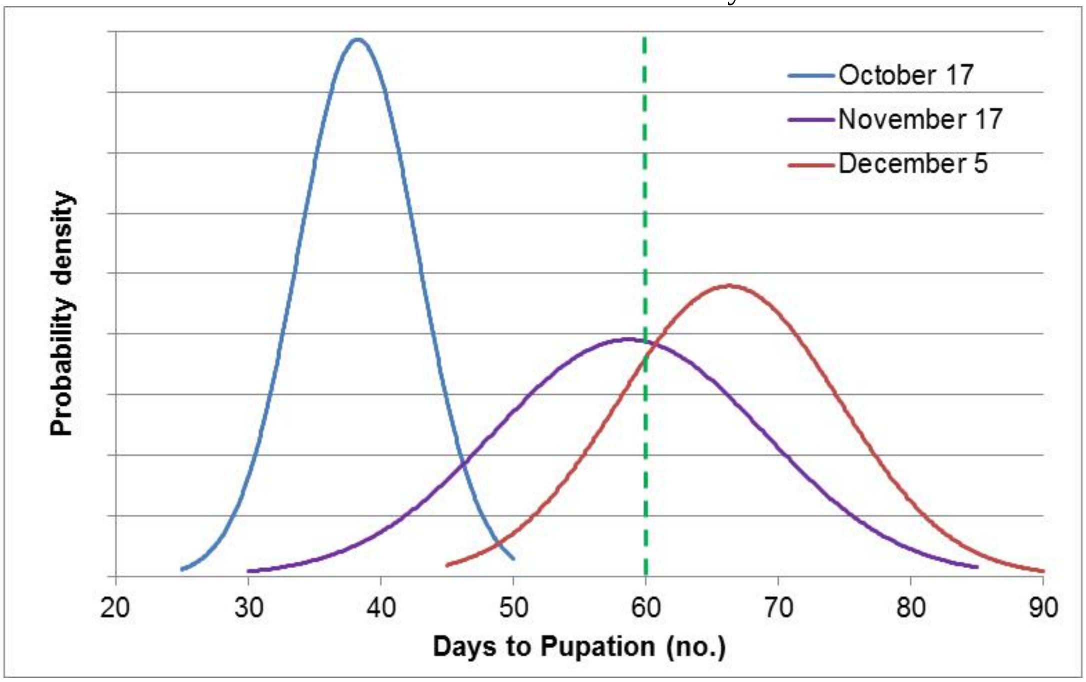
where μ_i is the mean N_P and σ_i is the standard deviation by week, i. Examples for earlier and later in the harvest season are shown below (Fig. 6ab).

Larvae in fruit at harvest? Based on the result for N_P we evaluated if the larvae would still be in the fruit at harvest, $N_P > 60$, or if the larvae would leave the fruit before harvest, $N_P < 60$. For the latter, no further calculations were needed, as the risk would be negligible. Consequently, we did not bother to simulate the risk for any weeks prior to October 17 (Table 9). If, however, larvae could be in fruit at harvest we calculated all nodes (mated females, days laying eggs, etc.) as in the previous options.

Table 9. Days to Mexfly pupation based on the start date of the quarantine, with standard deviations and probabilities of exceeding 60 d in a normal equation (see text). Data are based on weather data for 2008-14.

Quarantine start (week)	Harvest date	Mean days to pupation (no.)	Std. Dev.	Prob. >60
1-Aug	30-Sep	22.4	1.0	<0.001
8-Aug	7-Oct	22.7	1.4	<0.001
15-Aug	14-Oct	23.0	1.2	<0.001
22-Aug	21-Oct	23.9	1.3	<0.001
29-Aug	28-Oct	24.7	1.6	<0.001
5-Sep	4-Nov	26.0	1.9	<0.001
12-Sep	11-Nov	26.9	2.0	<0.001
19-Sep	18-Nov	27.6	1.9	<0.001
26-Sep	25-Nov	28.9	2.3	<0.001
3-Oct	2-Dec	30.6	1.6	<0.001
10-Oct	9-Dec	34.3	4.0	<0.001
17-Oct	16-Dec	38.3	4.5	<0.001
24-Oct	23-Dec	43.7	5.3	0.001
31-Oct	30-Dec	50.0	7.8	0.099
7-Nov	6-Jan	56.4	11.5	0.378
14-Nov	13-Jan	58.7	10.2	0.45
21-Nov	20-Jan	63.0	7.8	0.65
28-Nov	27-Jan	66.7	8.5	0.785
5-Dec	3-Feb	66.3	8.3	0.775
12-Dec	10-Feb	64.0	7.4	0.706
19-Dec	17-Feb	63.1	7.6	0.659
26-Dec	24-Feb	61.7	8.2	0.582
2-Jan	2-Mar	59.0	9.6	0.459

Fig. 9. Example normal distributions (truncated) for days to Mexfly pupation depending on the quarantine start date of (A) October 17, (B) November 17 or (C) December 5. The green dashed line shows the threshold for the 60-d delay.



Mated females. Our simulations above (§3.2.2) indicated that given about three weeks with no mitigation activities, the *p*(natural mating) for Mexflies is about 1.0. Rather than assuming every female would mate, however, we set *p*(natural mating) to 0.85, based on the fact that about 15 percent of mature females did not reproduce, regardless of mate availability, in the relevant study (Novelo-Rincón et al., 2009).

For model results for Option 3, see section 5.2.2 in the main document above.

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