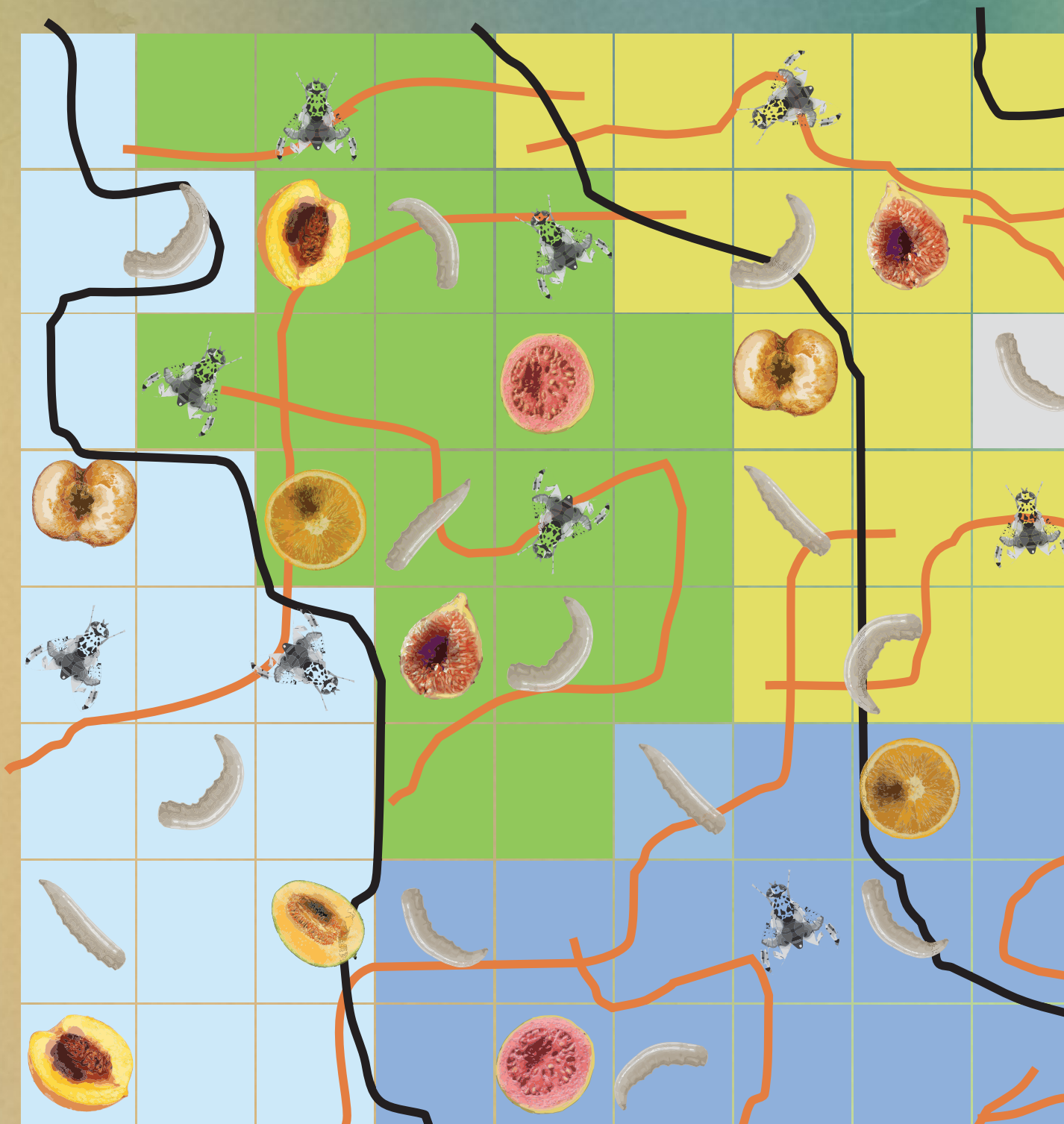
Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

# FRUIT SAMPLING GUIDELINES FOR AREA-WIDE FRUIT FLY PROGRAMMES



# Fruit Sampling Guidelines for Area-Wide Fruit Fly Programmes

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# Contents

# Foreword

Population survey is a basic component of any area-wide integrated pest management programme. Pest surveillance measures have to be practical, cost-effective and provide reliable information to action programme managers.

The most widely used surveillance measure for fruit fly pests is the trapping of adults by means of specific traps and attractants. Trapping provides useful information on the presence or absence of the pest, and on its relative spatial distribution and abundance. However, performance and thus effectiveness of trapping systems can be affected by extrinsic factors including changing environmental and ecological conditions. For example, trapping data might not reflect the real situation of a pest population in the field due to heavy rainfall for long periods of time, common in tropical and subtropical regions. This condition will reduce the movement of fruit fly adults, therefore, affecting the efficacy of the traps. A reduced availability of mature fruits in the field at the end of the fruiting season, will subsequently cause a substantially reduction of the pest population to a level where traps may not be able to catch adult flies. However, larvae could be more easily detected in the few mature fruits still available on the trees. In these and other situations, fruit sampling becomes as important a surveillance tool as trapping for population monitoring and detection. Fruit sampling also becomes an important pest detection tool in areas where sterile flies are being continuously released and where low density trapping is kept to avoid high sterile fly recapture rate and where traps are aimed basically at monitoring the released sterile flies.

When the aim of fruit sampling is to assess the presence or absence of a pest through a detection survey or to evaluate the impact of suppression and eradication actions through a monitoring survey, and given the large universe of fruit hosts normally available in the field, stratification of the fruit population becomes essential to increase the probability of detection.

The guideline follows the three categories of host status (Natural Host, Conditional Host and Non-Host) of fruit to fruit flies as per the International Standard of Phytosanitary Measures (ISPM) 37 “Determination of host status of fruit to fruit flies (Tephritidae).”

This guideline is aimed at facilitating the transfer of harmonized procedures to National Plant Protection Organizations and horticultural industry of FAO and IAEA Member States that want to apply fruit sampling procedures for fruit fly population survey in area-wide action programmes. The guideline will be useful also as a reference source to Appendix 2 “Guidelines for fruit sampling” of ISPM No. 26 “Establishment of pest free areas for fruit flies (Tephritidae).”

The officers responsible for this publication were W. R. Enkerlin and J. Reyes-Flores of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture.

# 1. Introduction

Fruit sampling in Area-Wide Fruit Fly Integrated Pest Management (AW-IPM) programmes, is applied to detect the presence or absence of the target fruit fly species in an area. It is also applied to monitor immature stages of the pest to determine those fruits that are hosts as well as fruit fly spatial and temporal distribution and abundance. This information is used by programme managers to plan and implement prevention, suppression or eradication activities. Fruit sampling can work as a stand-alone pest surveillance tool or it can be complementary to fruit fly trapping.

This guideline has been prepared to support decision making on the strategic planning and implementation of the fruit sampling as a component of a surveillance system in AW-IPM programmes. It is also aimed at serving programme managers as a guide to define the necessary resources that should be allocated to this important activity.

The guideline presents different fruit sampling scenarios commonly found, and it is also aimed at providing standardized operation procedures for fruit sampling in the field such as collecting suitable fruit hosts and for fruit processing in the laboratory including dissecting fruit hosts to find and extract larvae.

The guideline is based on a comprehensive review of fruit sampling manuals used in different fruit fly action programmes in Member Countries including Argentina, Brazil, Chile, Croatia, Dominican Republic, Guatemala, Israel, Mexico, Morocco, Panama, Peru, and Spain.

# 2. Fruit sampling in AW-IPM programmes

An effective fruit sampling plan can be design for an AW-IPM Programme if the following parameters are well known in advance: The pest control strategy, the size of the working area where the fruit sampling should be carried out and basic population ecology data such as preferred host, host phenology, and host distribution and abundance.

The different control strategies of an AW-IPM Programme are described in Appendix 1. It also presents the type of fruit sampling recommended for each specific control strategy. The specific phase of the strategy defines the intensity and frequency of the fruit sampling. Characterization of the area were fruit sampling will be conducted including size of the area is essential to quantify the needed resources to implement the activity.

## 2.1 Characterization of the fruit sampling working area

To implement an effective fruit sampling in AW-IPM Programmes it is essential to assess the size of the working area and to obtain the most precise agro-ecological information. This should include its geographical boundaries, either natural, as seashores, lakes, and mountain chains or artificial such as highways, roads, railroads, electric power lines, international borders, county delimitations, etc. Other important information for proper characterization of the area includes topography, human settlements (rural or urban areas), and species, density and spatial distribution of fruit fly hosts.

The programme should obtain the best available maps or imagery layers either printed or digitalized. Depending on the size of the working area maps can range from scale 1:5,000 to 1:100,000. Maps or imagery with topographic information, land-use and roads scale 1:50,000 are the most useful.

In addition to the use of maps, programme staff should explore in depth the working area to get full knowledge of it. It is imperative to travel throughout the working area by car, by walk, by boat (if needed) and by air to determine important landmarks that might not be included in the maps as dirt roads, paths, areas with no access by car, streams, mixed forests, agricultural and fruit commercial production areas, small villages and back yard orchards, fruit markets, areas without hosts and with potential wild hosts. The use of GPS is indispensable to record the geographical coordinates of these important landmarks.

It is recommended to divide the map into working areas using quadrants of 10 × 10 $km^2$ with geographical coordinates according to the Mercato cartography in order to organize the activities and prepare the sampling schedules for the fruit samplers. Fruit sampling routes are designed within the quadrants considering the available roads, urban and sub-urban areas, areas with or without fruit fly hosts, etc. These routes can be established either with or without trapping routes. The routes should allow a proper coverage of the whole working area.

The fruit sampling routes can be dynamic in space and time, not as the trapping routes which tend to be fixed to specific locations. With the information listed above, and with good knowledge of the presence, abundance, geographical distribution and fruiting phenology of the main hosts, a pre-established plan of fruit sampling routes can be drawn for each quadrant and for the entire sampling area.

## 2.2 Determination of the target fruit fly hosts

For determination of host status, this guideline follows the three categories of host status of fruit to fruit flies as per the International Standard of Phytosanitary Measures (ISPM) 37 “Determination of host status of fruit to fruit flies (Tephritidae).” https://www.ippc.int/static/media/files/publication/en/2016/05/ISPM_37_2016_En_HostStatus_2016-04-26.pdf.

***Host status (of fruit to a fruit fly).*** Classification of a plant species or cultivar as being a natural host, conditional host or non-host for a fruit fly species.

***Natural host (of fruit to a fruit fly)***. A plant species or cultivar that has been scientifically found to be infested by the target fruit fly species under natural conditions and able to sustain its development to viable adults.

***Conditional host (of fruit to a fruit fly).*** A plant species or cultivar that is not a natural host but has been scientifically demonstrated to be infested by the target fruit fly species and able to sustain its development to viable adults as concluded from the semi-natural field conditions set out in this standard

***Non-host (of fruit to a fruit fly).*** A plant species or cultivar that has not been found to be infested by the target fruit fly species or is not able to sustain its development to viable adults under natural conditions or under the semi-natural field conditions set out in this standard

To have the best knowledge of the hosts or potential hosts, preparation of fruit phenology charts as the one in **Table 1** is recommended. The table presents a list of fruits and their classification as fruit fly hosts as well as the fruit maturation periods. This table helps to know at a glance the availability of natural, conditional and non-hosts during the different seasons of the year and thus to assess the likely host sequence of the fruit fly pest. It also shows hosts with an erratic or out-of-season fructification. The fruit maturation periods are indicated and special attention is given to the early and final maturation periods of the fruits. Due to the low availability of fruits, fruit sampling is more effective during these periods since the probability of detection increases. This is one of the main fruit stratification measures used to reduce the fruit universe and increase the probability of larva detection.

It is also important to estimate the size of the area of backyard host fruits and commercial orchard area, categorizing and identifying the sites with presence of natural hosts, areas with absence of fruit hosts and sites with presence of natural hosts that are difficult to access, thus, that can serve as pest reservoirs. These areas should be clearly identified on a map using a GPS. This information is necessary to plan fruit sampling efforts and estimate more precisely the necessary resources to implement the activity.

**Table 1. Example list of Mediterranean fruit fly hosts found in a programme working area, with the host status and their ripening periods.**

| Fruit Fly Hosts | HS (1) | J | F | M | A | Ma | Jn | Jl | A | S | O | N | D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Figs | NH | | | | | | | | | | | | |
| Peaches | NH | | | | | | | | | | | | |
| Plums | NH | | | | | | | | | | | | |
| Pears | NH | | | | | | | | | | | | |
| Grapes | CH | | | | | | | | | | | | |
| Apples | NH | | | | | | | | | | | | |
| Apricots | NH | | | | | | | | | | | | |
| Rosa spp | CH | | | | | | | | | | | | |
| Wild Pomegranate | NH | | | | | | | | | | | | |
| Cherries | NH | | | | | | | | | | | | |
| Mandarins | NH | | | | | | | | | | | | |
| Oranges | NH | | | | | | | | | | | | |
| Grapefruit | NH | | | | | | | | | | | | |
| Loquat | NH | | | | | | | | | | | | |
| Papaya | Non-H | | | | | | | | | | | | |

(1) HS — Host Status, NH — Natural Host; CH — Conditional Host; Non-H — Non-host.

**Note:** Light colors in each fruit fly hosts maturation period represents the best times for the fruit sampling to increase probabilities of pest detection.

## 2.3 Fruit sampling organizational unit

A fruit sampling plan can be prepared using the information presented in **Sections 2.1** and **2.2**. However, to implement the plan an organizational structure has to be put in place.

Fruit sampling is an important part of the field activities of any AW-IPM programme. Therefore, it is placed within the organizational structure of the programme under the Coordination for Field Operations (see blue boxes in **Figure 1**).

The Fruit Sampling Section comprises two main activities: Fruit sampling in the field and fruit processing in the laboratory. The field activities are those executed to collect fruit samples from the working area. The laboratory activities comprise the reception, handling and dissection of all fruits from fruit samples and the identification of fruit fly larvae, pupae or emerged adults.

### *2.3.1 Fruit sampling in the field*

**Fruit sampler profile**

Staff dedicated to the activity of fruit sampling (regarded here as Fruit Samplers) should be well trained to collect good quality fruit samples. They can work individually or in brigades using a vehicle or other means (walk, horse, boat, etc.) depending on the accessibility of the working area.

Fruit Samplers should be able to drive vehicles (cars, pickups, motorcycles, boats, etc.), walk long distances and have basic technical knowledge of the fruit sampling activity. They should be well trained in the use of GPS, maps, fruit fly hosts recognition, fruit sampling procedures, and general knowledge

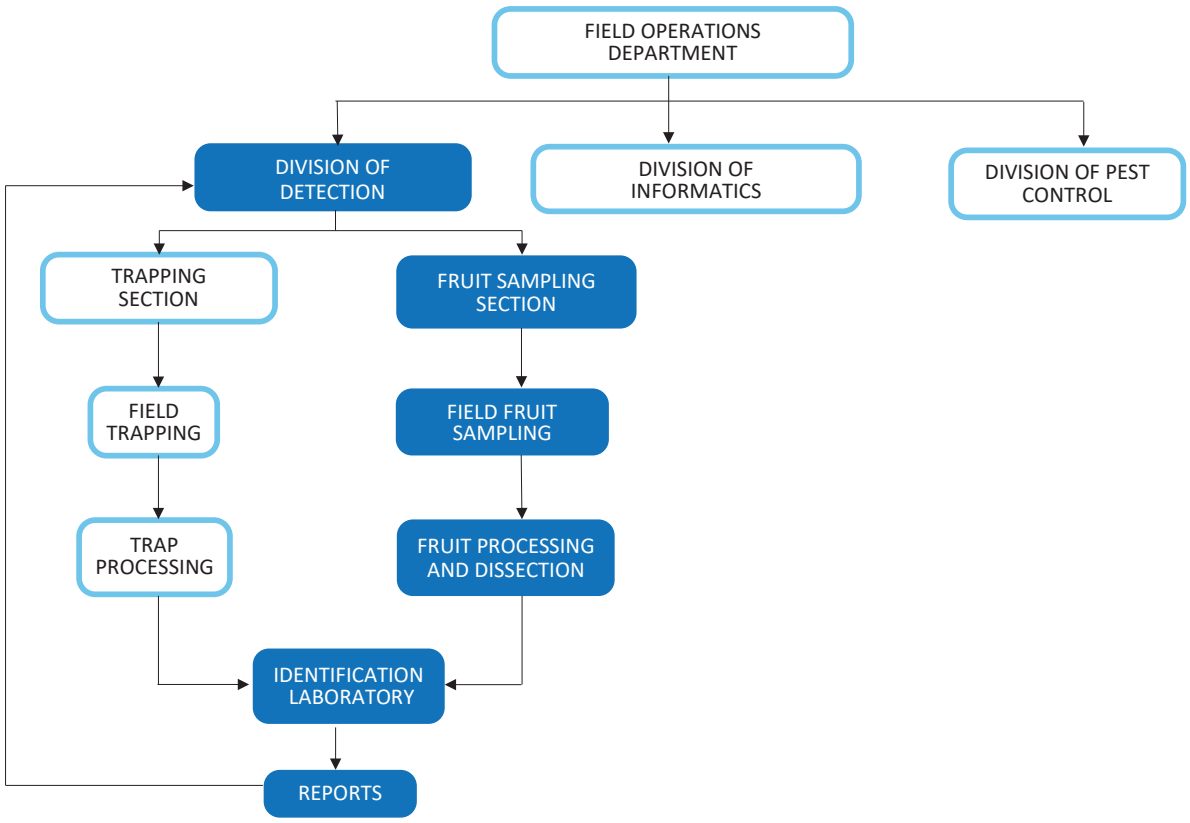


**Figure 1.** Organizational chart including the Fruit Sampling Section.
Source: Moscamed Programme DGSV-SARH Mexico, FAO (1983).

on detecting and identifying immature stages of target fruit flies. They should have a good relationship with people living in the working area and preferably they should be acquainted with the working area.

**Weekly activity**

Considering a working area with suitable characteristics for fruit sampling, i.e. abundant fruit hosts, easy access to host sites, etc., one Fruit Sampler in a vehicle can collect per day from 25 to 45 fruit samples weighing in average 1.5 kg, depending on the species of host fruit and availability of ripe fruits. The area covered during a working week can be from 20 to 50 $km^2$, visit from 50 to 100 sampling sites, and collect 125 to 225 fruit samples weighing in total from 187.5 to 337 kg.

When no vehicle is available or there is not access for vehicles to the sampling area, the Fruit Samplers can be hired in villages close to the sampling areas. They can easily carry out the fruit sampling by foot o by horse. In these cases, another sampler with vehicle can collect the fruit samples from the village in a predetermined specific day of the week.

### *2.3.2 Fruit processing in the laboratory*

The fruit processing laboratory is the place where the fruit samples coming from the field are handled and processed. The main goal of this laboratory is to detect inside the fruit and identify the target fruit fly pest. These can be done either at the larval, pupal or adult stages. Accurate identification of fruit flies is essential for appropriate and effective application of control measures. This laboratory and the trapping laboratory can be located in the same space.

The fruit samples should be handled and processed by trained staff. Well trained staff can handle 60 to 80 fruit samples per day or 300 to 400 per week.

Large scale AW-IPM programmes have a central laboratory with secondary laboratories strategically located within the working area.

### *2.3.3 Fruit sampling resources*

Estimates of the resources required for fruit sampling are presented as a guide in **Appendix 2.** A description of the implementation and evaluation of the fruit sampling activities is presented in **Appendix 3**.

# 3. Types of fruit sampling

A simple stratified random sampling is applied in area-wide fruit fly IPM programmes. The level of stratification of the sample unit (i.e. fruit host) will depend on the objective of sampling which can be for population monitoring, detection or verification of the impact of suppression and eradication procedures on fruit fly populations. Within this context, different fruit sampling types are used by programmes that apply the Sterile Insect Technique (SIT). In general terms, there are two distinctive types of fruit sampling: General Fruit Sampling and Targeted Fruit Sampling, with variations in its application based on programme objectives and the control strategy being used. The intensity of the sampling is influenced by specific agro-ecological and weather conditions, fruit fly hosts and available resources (**Appendix 4)**.

## 3.1 General Fruit Sampling

### *3.1.1 For population monitoring*

This sampling uses the lowest level of stratification and it is aimed at conducting a general survey to produce baseline information regarding fruit fly population ecology. The method consists on the collection of any soft skin fruit species likely to be infested by the target fruit fly throughout the entire working area and the whole year. The emphasis is on those fruits or vegetables that have been reported as fruit fly hosts in other areas or countries where the same or closely related fruit fly species occur and with similar prevailing environmental and agro-ecological conditions (**Appendix 4**).

The data gathered is analyzed and used to delimit the fruit hosts of the target pest based on susceptibility, establish their relative importance and assess spatial and temporal distribution of the pest. This type of fruit sampling is very useful to delineate general programme strategies, and for planning and implementation programmes activities.

There will be areas where only one species of fruit fly is present and other areas where two or more fruit fly species may occur. This general sampling will also help in providing information on the host status for each one of the occurring species as well as to assess those hosts that may be infested by more than one fruit fly species (ISPM 37).

The susceptibility of the hosts can be different according to the season, altitude and agro-ecological conditions of the area. Therefore, the General Fruit Sampling needs to be performed during the whole year and in the entire area so that the relationship between fruit fly species, type of host and characteristics of the area can be accurately determined.

General Fruit Sampling is also used to determine the geographical distribution of the pest, this is, the sites where the pest will most likely be found. In some fruit species and cultivars finding of larvae is more likely when the fruit is collected while still attached to the tree (coffee berries, citrus, apples, etc.) whereas, in other types of fruits, larvae are more easily found in fruits that have been collected from the ground (guava, mango, apricots, etc.). This is also affected by the population density, being more likely to detect larvae in the fruit attached to the tree in the case of low population densities and in fruit collected from the ground in highly infested fruit typical of high population densities. In addition, the general sampling also helps to determine host density (abundance), host spatial distribution (uniform or scattered), host sequence of the pest, host phenology (fruiting season) and in some instances the occurrence of fruit fly parasitoids.

All fruit samples collected in the field will be delivered to the fruit handling and dissection laboratory. In case of a larvae find in the fruit, larvae will be identified in this stage or reared to adult stage for identification. For some fruit flies of quarantine importance, there are reliable taxonomic keys for identification in third instar larvae that can be used so that the rearing process to adult is avoided and time saved.

In some instance, based on the records of negative detections or low pest occurrence by the general fruit sampling, the presence or absence of a pest can be assessed, and an area can be declared fruit fly free or area of low pest prevalence, according to ISPMs 26 and Annex 1 of ISPM 35, respectively.

### *3.1.2 For pest detection in free areas as part of a contingency response plan*

General Fruit Sampling is not applied in pest free areas. It is applied as part of the detection tools of a contingency response plan. It is enforced after an adult fly is captured in a trap. The main objective is to complement trapping in the process of delimiting a fly find and in assessing the magnitude of the infestation. Its application is discrete in time, being done only during the first estimated biological cycle of the pest which can range between 21 and 30 days depending on fruit fly species and temperature conditions. This sampling requires a low stratification level as all soft skin fruit susceptible to infestation available in the area of the detection are sampled. Nevertheless, an intensive and localized fruit sampling is required to increase the probability of detection (**Appendix 4**).

## 3.2 Targeted Fruit Sampling

Once the General Fruit Sampling has identified the relative relationship between the fruit fly species with its hosts, a list of natural hosts, conditional hosts and non-hosts is prepared and used as the basis for the Targeted Fruit Sampling. This type of fruit sampling can be applied for different aims.

### *3.2.1 Targeted Fruit Sampling as a monitoring tool*

This fruit sampling is applied as a complement of the trapping activities for pest monitoring. In some cases, sampling of hosts occurring in the working area is the only option to detect the target fruit fly when the trapping is less effective or cannot be applied due to the lack of specific attractants or traps.



**Figure 2.** Stratified fruit sampling collecting fruits from natural hosts and with infestation symptoms

This type of fruit sampling requires a higher level of stratification compared to the General Fruit Sampling. Stratification includes: Directing sampling efforts to the most susceptible natural hosts of the fruit fly species, to fruit species showing infestation symptoms, in areas with records of pest presence and during the time of the year when fruit host are susceptible to infestation (**Figure 2**).

Targeted Fruit Sampling can be as important as trapping in evaluating the level of efficacy of an area-wide fruit fly control programme. The assessment can be done by estimating the infestation of the pest in the area of interest before and after programme intervention. Comparison should be done using equal sampling schedules including same host species, sampling area and intensity of sampling (**Appendix 4**).

Records of negative detections or low pest occurrence by the Targeted Fruit Sampling, are used as part of the necessary technical information to declare a fruit fly free area or an area of low pest prevalence as well as to maintain its phytosanitary status.

### *3.2.2 Targeted Fruit Sampling as a detection tool*

**For pest detection in low pest prevalence areas used as buffer areas**

This type of fruit sampling is applied in buffer areas located between the infested areas and the pest free areas. In buffer areas the pest is present at very low density, therefore, it is regarded as a low pest prevalence area (Annex 1 ISPM 35). Since the population is present at low levels, in order to increase the probability of detection, fruit sampling also needs to be highly stratified. As for the targeted fruit sampling applied for monitoring purposes, stratification includes: Targeting sampling efforts to the most susceptible hosts of the fruit fly species, to host fruits showing infestation symptoms, in areas with records of pest presence following the population fluctuation of the pest and during the time of the year when fruit host are susceptible to infestation. However, stratification for this type of fruit sampling is even more rigorous, focusing only on the three most susceptible fruit hosts and collecting only fruits with clear infestation symptoms at specific times of the year (**Appendix 4**).

**For Pest detection in markets**

Movement of host material from infested areas to pest free areas can be done illegally for commercial purposes. Low volumes of fruit hosts with no or low commercial value that is highly appreciated by local people can also be mobilized from infested areas into small communities located within the pest free areas. The final destination of these fruits can be private houses, street shops or local markets. Over ripped fruit and fruit with larvae are discarded by the people selling or buying the fruit. The fruit is then rejected and disposed in trash cans in the houses and containers close to the market or in dumping sites. This is an opportunity to apply a targeted fruit sampling for detection purposes (**Appendix 4**).

Fruit Samplers can cover a good percentage of these local shops and markets in the working area, and coordinate with fruit sellers in order to obtain their support to collect the disposed fruits in special containers, from which fruit samples can be taken regularly. The programme can provide the containers to all shops and markets to increase the control of the disposed fruits, before they are taken to the dumping sites. Fruit Samplers are scheduled to visit regularly these selling points to collect samples for fruit fly detection purposes.

Direct purchase by the programme of this low value hosts that are highly appreciated by the local people is sometime recommended, either buying from the people that move the fruit from infested sometimes areas remote areas or from the fruit sellers in the street shop or local markets. This procedure should be based on a benefit-cost analysis.

In case of detection of fruit fly larvae in the fruits, immediate regulatory actions should be taken, although, the origin of the infested fruits is in most of the cases difficult to determine.

**For pest detection in fruit processing facilities**

Fresh fruit processing facilities offer an opportunity for pest detection in larval stage when the fruits are major hosts of the target fruit fly species. A typical example are facilities known as ‘coffee mills’ where

coffee berries are processed. Coffee berries are considered to be one of the preferred Mediterranean fruit fly hosts. Coffee plantations are extensive and during the harvest period massive amounts of berries are available, becoming impossible to conduct an effective sampling directly on the plantations. Under this condition, the concentration of coffee berries after harvest on the mills offer an opportunity for targeted fruit sampling for detection purposes.

Previously authorized by the coffee mill owner, programme personnel visits the coffee mills at the time of reception of the harvested coffee berries. Fruit Samplers collect small quantities of berries when they arrive at the reception site. To increase likelihood of detecting a larvae, stratification of the coffee berries include sampling only berries with infestation symptoms including those with dark red colour and a rugose skin. Sampled berries are placed in plastic bags labelled with the name of the sampler and specifications of the harvest site. Coffee samples are transported to the laboratory for fruit dissection and collection of larvae from the infested berries. In case of positive samples the coffee mill owner is notified and immediate pest control actions are enforced by the programme in conjunction with the coffee mill owner (**Appendix 4**).

**For pest detection in host fruit packing centres**

Fruit packing facilities from where fruit hosts are packed and transported into de low prevalence and free areas, need to be subjected to a targeted fruit sampling. In these facilities the low quality or damaged fruits are rejected and placed in containers for their disposal. This can be done from the field boxes, after the reception of the fruits, before the fruit enters the sorting process (washing tanks) and directly on the fruit sorting lines.

Upon authorization by the packing centre managers, programme personnel visit the centres during the harvest period of the target fruits. Stratification is done by conducting fruit sampling on the containers of rejected fruits and by selecting those with fruit fly infestation symptoms.

Fruit samples are collected in plastic bags and properly tagged with the sample information and transported to the fruit dissection laboratory. In case of a larvae find in the fruit of the fruit fly species of interest, and after notifying the packing centre manager, immediate pest control actions are enforced.

A unique opportunity for this type of fruit sampling is when the sampling is applied as a prerequisite to issue a phytosanitary certificate for fruit exports. Under the bilateral work plan agreed between the national plant protection authorities of the importing and exporting countries, fruit sampling protocols are established which include a statistical sampling procedure. This agreement can include the targeted fruit sampling from the pre-harvest period until the packing for exportation (**Appendix 4**).

The programme can offer support to the packing centres to conduct the activity of the phytosanitary inspectors for fruit certification purposes. This may include fruit sampling during the harvest periods in the orchards or at the packing centres upon reception of the fruits, on the fruit selection lines or from the fruit pallets before shipping. Fruit Samplers stratify the sample by collecting suspicious fruits in the amounts specified in the work plan.

Fruit samples are collected in plastic bags and properly tagged with the sample information and transported to the dissection and identification laboratory. In case of target fruit fly detection, the programme notifies the phytosanitary inspectors and the packing centre manager who immediately implements pest control actions.

# 4. Fruit sampling field procedures

## 4.1 Field procedures for the collection of fruit samples

### 4.1.1 Fruit Sampler

A fruit Sampler should carry out the fruit collection in the working area, following the specific criteria established by the programme and available in the fruit sampling procedures manual.

Each sampler should have detailed knowledge of the characteristics of the working area that has been assigned to him or her. The sampler should be familiar with fruit growers, owners of backyards, access roads, and land marks such as rivers, mountains, towns and villages. They must learn about the spatial distribution and abundance of specific host fruit (commercial and wild), fruit seasonal abundance, and maturation phenology. It is also of importance to be familiar with fruits considered to be the non-hosts (ISPM 37).

Based on the technical criteria established by the programme, the sampler together with his supervisor designs sampling routes taking into account access roads and other forms of access such as paths and rivers in order to properly cover the assigned working area. The fruit sampling routes are not fixed as in the case of the trapping routes. Sampling routes can be modified to cover sites where trapping is not carried out so that the working area where hosts are present is fully covered by either the trapping or the fruit sampling surveillance activity.

The fruit sampler should be trained to know with precision the target fruit fly hosts available in the assigned working area at a given time of the year. They must be familiar with the basic fruit fly infestation symptoms which can be different between fruit species and even cultivars as well as when fruits are collected from the ground or from the tree. Female oviposition stings surrounded by a small yellow-brown mark, rotten portions visible on the fruit skin, larvae exit holes, fruits with uneven colour and different maturation pattern compared with most of the fruits, are some of the fruit fly infestation symptoms that should be known.

Samplers should always have at hand the list of the most susceptible natural hosts which can be different depending on the season as well as the least susceptible natural hosts and conditional hosts. The programme must produce a list of hosts indicating its relative importance to the target fruit fly species as well as a field guide with images of the hosts and symptoms of infestation as a guideline for the Fruit Sampler.

The Fruit Samplers are also instructed to carry out their sampling routes, number of sites to visit and number of samples to be collected over a period of time (per day, week, month and year) on the assigned working area. They also must know the average number of fruit samples to be taken per site in a unit area which can be a square kilometre, a quadrant or a sector depending on the area unit being used.

Because fruit hosts are usually in private property, Fruit Samplers are constantly in contact with the public. Fruit Samplers should keep in mind that they are staff representing the programme so they must be polite at all times. It is recommended that samplers receive training on basic public relations skills (see **Section 4.2**).

### *4.1.2 Fruit sampling management units*

For planning of a fruit sampling schedule, the programme working area is divided into ‘management units’ (fruit sampling working area, quadrants, sectors and routs). The working area is presented in large-scale wall maps which are squared-off into a grid of units of the same size called ‘Quadrants’. Depending on the scale of the map the quadrants can range from 1 km² (1:10 000 scale; 1 cm = 100 m) to 100 km² (1:100 000 scale; 1 cm = 1000 m). The working area is also divided into sectors, which can be of different sizes (comprising one or several quadrants, or part of a quadrant), depending on political

or geographic delimitations and landmarks (roads, walking paths, creeks, rivers, swamps, etc.). These subdivisions are set for practical management uses and for the easy understanding by programme staff. **Figure 3** shows an example of a fruit sampling working area with six fruit sampling sectors (in different colors), daily sampling routes for the Fruit Samplers (main and secondary roads). One or two sectors are assigned to each Fruit Sampler, all sectors identified in the map should be covered and sampled in one week.

Size and delimitation of sectors are adapted to the programme strategic objectives and phase. Dimension and layout of sectors may also depend on the availability of personnel, vehicles and host seasonal abundance. All sectors should be covered every week, every two weeks or every month, depending also on the programme's strategic objectives and phase. For example, for planning the fruit sampling in an area of 120 km² (12 000 ha), the area is divided into 120 quadrants of 1 km² each and 6 sectors of 20 quadrants each. If the objective of the programme in this sampling working area is eradication, it is recommended that the area should be sampled once a week. If one Fruit Sampler can cover 2 sectors per week (40 km²), the programme needs 3 samplers.

## 4.1.3 Collection of fruit samples

The following is a standard procedure, adjustments can be made depending on the specific needs of the programme.

- The Fruit Samplers should receive the information from the supervisor quadrants, sectors and routes to cover and number of fruit samples to collect per day.

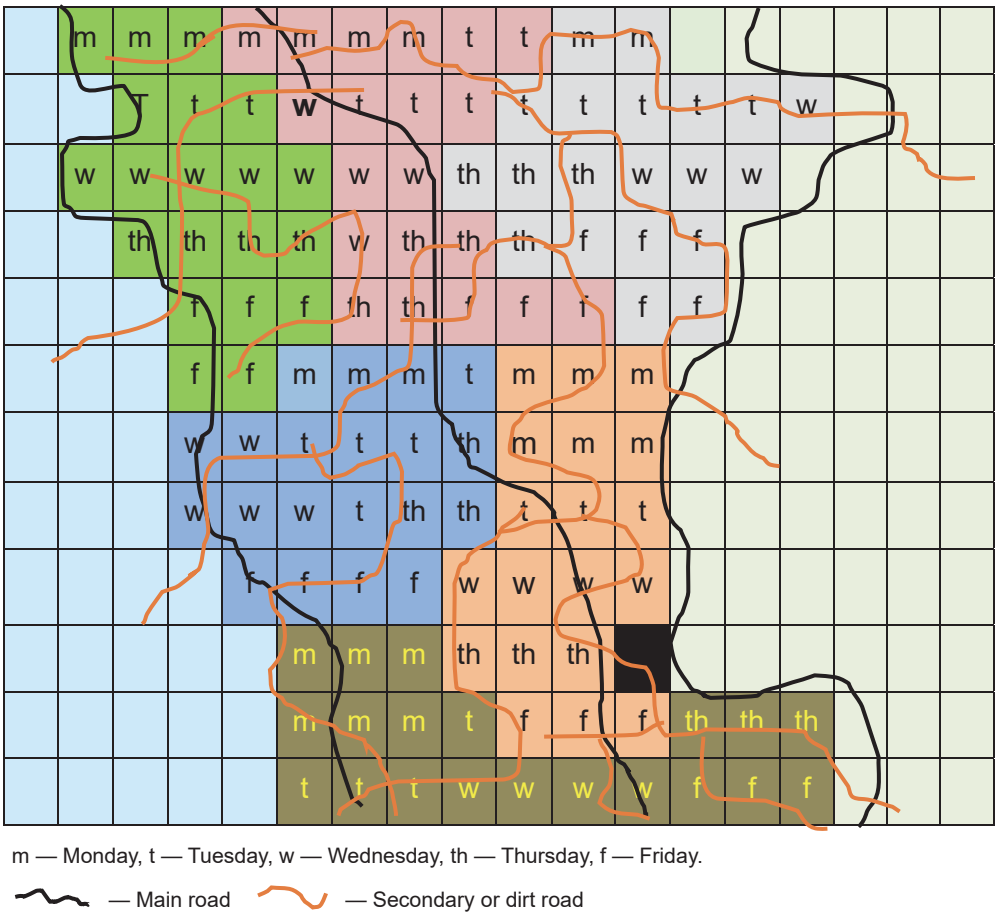


**Figure 3.** An hypothetical map showing a fruit sampling working area with corresponding quadrants, fruit sampling sectors, sampling routes and Fruit Sampler schedules. The map covers 120 km² (12,000 hectares). Quadrants are equivalent to 1 km² (100 hectares). Sectors are shown in distinctive colours.
Source: Moscamed Programme DGSV-SARH Mexico, FAO (1983).

- Fruit Samplers start the day with the inspection of the vehicle, fuel, materials and equipment and confirm the sampling area and priorities with his supervisor.
- To collect the fruit samples, the samplers use their experience, intuition and previous knowledge of the area to select the best sites, fruit species and trees.
- If the fruit host is inside of a private property the Fruit Sampler should ask for permission to the property owner. Once the permission is given, the sampler can enter the property, (orchard, home garden, backyard, etc.) to take the fruit samples.
- Fruit samples are collected in plastic or cloth bags (**Figure 4**) and kept in thermo boxes while transported to the laboratory. This will avoid overheating that would kill fruit fly eggs and larvae.
- The sampler should not mix different fruit species in the same bag. One fruit species per bag is recommended.
- The sample should be accurately labeled to avoid mistakes in locating an infested site if control measures have to be taken and to plot the site in a map. The label should include all relevant information as indicated in **Figure 5**. The label stays with the sample until final dissection of fruits.
- In some cases the Fruit Sampler is trained to dissect the fruit, therefore very ripe fruits or those that presents clear symptoms of fruit fly infestation can be dissected *in situ*. In these cases the Fruit Sampler can collect and keep the larvae in tagged vials noting the location of the sample and other key information. Larvae can be maintained in good conditions in vials with 50–70% alcohol. Live young larvae (1st or 2nd instar) can be kept in the fruits or transferred to less ripen fruits for few days until they develop to third instar. The larvae and fruit need to be kept in special sealed containers placed in thermo boxes and in cool conditions until they are delivered to the laboratory for extraction and identification.
- The size of a sample varies widely and depends mostly on the fruit availability and size of the fruit. It can range from 200 grams in the case of coffee berries, 750 g for medium size fruits such as guava or figs and up to 3 kg for larger fruits such as grapefruits (**Table 1**).
- Fruit Samplers deliver daily all fruit samples and collected larvae specimens to the laboratory for the immediate handling of the sample. The daily fruit sampling report adequately completed delivered to the supervisor. Fruit Samplers which do not return from the field to the laboratory the same day, should keep the fruit samples in the thermo boxes and in cool conditions to prevent overheating and death of the possible eggs or larvae present in the sampled fruits.

## 4.2. Public relations in the fruit sampling

- Good public relations is an important part of the programme but in the case of the fruit sampling it is critical. Public relations are more important in fruit sampling than in trapping because the sampler is taken fruits from a private property.


**Figure 4.** Fruit samples in properly labelled plastic bags

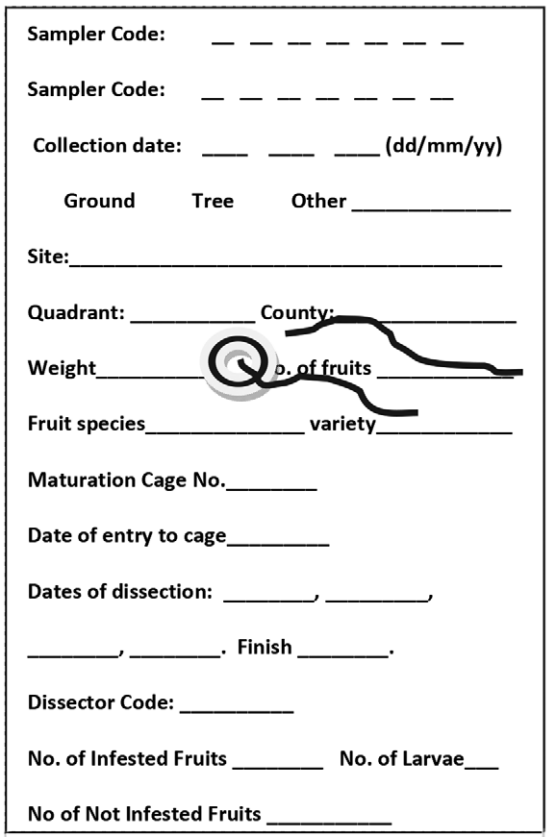


**Figure 5.** Fruit sample label.
Source: Moscamed Programme DGSV-SARH Mexico, FAO (1983).

**Table 1. Standard weights and number of fruits in fruit samples in AW-IPM Programmes**

| Fruit Fly Hosts | Average Weight Per Fruit Sample (Kg) | Average Number of Fruits Per Fruit Sample |
|---|---|---|
| Grapefruits and big mangoes | 2.5 – 3.5 | 6 – 8 |
| Oranges, normal mangoes, big mandarins | 2 – 3 | 10 – 15 |
| Peaches, guavas, figs, standard mandarins | 1.5 – 2.5 | 15 – 20 |
| Coffee berries, cherry fruits | 0.2 - 0.4 | 75 – 150 |

- The Fruit Sampler is constantly in direct contact with the public. Prolonged conversations should be avoided, instead, short cordial conversations concerning the work are desirable. Getting into arguments concerning the programme activities should be avoided.
- Fruit samplers should keep in mind that they are representing the programme, thus, good manners and courteous behaviour should be observed at all times. Personal appearance and actions should be appropriate so that Fruit Samplers make a good impression with the general public. Shirts and blouses that have illustrations on them should not be used. Official badges must be worn at chest level for easy identification.
- When entering a property for the first time, Fruit Samplers should always attempt to contact the owner or caretaker, explain the work briefly, and ask permission to take a fruit sample from the backyard or orchard. In the conversations with the public, fruit samples should be referred to as ‘samples for insect survey’. If no one is on the property, an official programme letter should be left kindly asking for collaboration and explaining the purpose and relevance of the action.

- Public information staff should always educate the general public on programme activities, either by organizing formal ‘talks’ to growers and residents of cities, towns and villages, or through using mass media including radio spots and local newspapers. Radio spots are required informing the public on the need to support the programme activities including routine trapping and fruit sampling in their properties.

# 5. Identification laboratory procedures

The laboratory activity begins with the reception of the fruit samples from the field delivered by the Fruit Samplers.

The laboratory staff should be trained in the handling of the fruit samples as well as in the identification of the immature stages and larvae of other insects occurring in decaying fruits. The laboratory should have a fruit fly taxonomist with experience in both immature and adult stages to provide the final diagnostic of the fruit fly specimens found in the fruit samples.

## 5.1 Laboratory infrastructure

The fruit sampling laboratory is comprised of:

**Fruit sampling reception room.** This room should be located close to the entrance of the laboratory to receive the fruit samples. The room should be isolated from the rest of the working areas to avoid Fruit Samplers entering into the laboratory.

In this room a laboratory table and adequate chairs should be available to the Fruit Samplers to place the fruit samples and to write and deliver the daily report. The laboratory supervisor or staff assigned by him should receive all samples and reports.

In the same reception room there should be another area equipped with a table, desk and balances for the laboratory staff to register samples, count fruits, weigh samples and classify them. Classification is made by fruit species using common names, sampled from trees or from the ground, samples ready to be dissected or unripe samples for placement in the fruit maturation room. The samples receive a consecutive number or code that is written on the sample label.

**Maturation room.** This is a room for fruit maturation and larvae development. The room should have enough space to set the fruit maturation cages in shelves (**Figure 6**). Here the samples with unripe fruits are placed in special cages to allow maturation of the fruits and development of larvae inside the fruits (**Figure 7**). First instar larvae or eggs in some fruits cannot be distinguished by the naked eye, being necessary to leave them in maturation cages to allow development for a number of days. The maturation room should maintain conditions for the optimum development of the insects to 3rd instar larvae to facilitate identification.

In some instances, in this same room there are conditions to rear the larvae to pupal stage, in order to obtain the adults for identification. This is particularly important when the identification of the fruit fly species of interest is difficult in the larval stage.

**Fruit dissection room.** A working space with laboratory tables and adequate tools for dissection and larvae extraction such as knifes and entomological tweezers. The samples can be from the fruit samples arriving the same day or from the maturation room. Mature fruits with visible symptoms of fruit fly infestations have priority for dissection.

**Identification room.** This room should be equipped with laboratory materials such as petri dishes, solvents such as acetone and microscopes. The identification of all larvae and in some instances adults from infested fruit takes place in this space. This room should maintain comfort temperature and humidity working conditions.

## 5.2 Procedures for handling of fruit samples

### *5.2.1 Reception of the fruit samples*

- Reception of fruit samples and vials from the field with fruit fly larvae.




**Figure 6.** Fruit holding and maturation room



**Figure 7.** Example of fruit and larvae maturation containers

- Revision of the information on the labels and that the daily report from the Fruit Samplers is adequately completed.
- Placement of the fruit sample inside the laboratory and opening of the bags with fruit for aeration.
- Registration of the data contained on the fruit sample labels in a specific data base, assigning each sample a unique consecutive number, which is written in the fruit sample label.
- Each individual fruit sample is weighed and the number of fruits counted. These data are written in the same label of the sample.
- Fruit samples are classified and separated in two groups. One is composed of the fruit samples for immediate dissection and the other is the fruit to be placed in the maturation room. The fruit samples or fruits within the fruit samples which are over ripe, starting to rot or have very clear symptoms of fruit fly infestation, are sent to the dissection room the same day. The fruit sample or fruits within a sample which are not fully ripe with no visible symptoms of fruit fly infestation, are sent to the maturation room.

- Labels with all necessary information should be kept together with the fruit sample throughout the process including those samples going straight for dissection and those going to the maturation cages.

### 5.2.2 Handling fruit samples in maturation containers

- All fruit samples or a portion of them are placed inside the maturation container in the room with controlled environmental conditions. Each maturation container has a unique consecutive number. This number is printed on the fruit sample label and registered in a logbook to avoid misplacing samples during handling.
- In tropical or sub-tropical areas with high humidity and temperature, it is recommended to immerse the fruit sample for 10 seconds in a solution of 4% Sodium Benzoate, to prevent fungus proliferation and reduce fruit decay.
- Every day the maturation containers are carefully inspected to look for larvae or pupae (**Figure 8**) at the bottom of the container. It is recommended not to use pupation media to allow for a rapid detection of the larvae or pupae. If larvae or pupae are present, these are collected and immediately sent for identification and a quick diagnostic. If the larvae is identify as the target fruit fly, the result should be reported immediately to the supervisor and emergency control actions should be implemented immediately.
- Fruit samples should remain in maturation containers for 5 to 15 days. This will be determined by the fruit species, degree of ripeness and environment conditions prevailing in the room. Once all fruits in the sample have reached an advanced maturation stage, final dissection is performed and the remaining larvae are collected and send for identification. After dissection fruit remains are incinerated or buried in deep holes to eliminate any immature stage that could have remained in the fruit. .
- During the fruit maturation process, fully ripened fruits in the container should be taken out for dissection even when some of the fruits in the sample are still unripe. Partial results are registered in the fruit sample label every time fruit is dissected.

Once all fruit from a sample have been dissected, the corresponding label should contain the complete information including: Sampling site, presence or absence of fruit fly larvae, number of fruit fly larvae, pupae collected, adults emerged, and other information according to Sections 4.1.3 and 5.2.1. The information on the labels should be recorded in a specific data base.

### 5.2.3 Dissection of fruit samples and collection of larvae

- Fruit samples are manually dissected by experienced technical staff.


**Figure 8.** Collecting larvae from the bottom of the container



**Figure 9.** Laboratory materials and equipment for fruit dissection



**Figure 10.** Fruit dissector inspecting a carambola (Averrhoa carambola) fruit sample

- Necessary materials and equipment should be available in the laboratory for fruit dissection, including: Tables, trays, knives, magnifiers, lamps with appropriate illumination to facilitate larvae detection inside the fruits (**Figure 9**).
- Fruit samples are dissected one at the time using gloves and knifes. Fruits should be fully dissected in slices of 1 to 2 cm width. The use of surgical gloves by the fruit dissector is recommended to protect the hand from the acidity of fruit juices and also to prevent possible knife cuts. Once the fruit has been completely sliced, the fruit dissector looks for any infestation symptom on the fruit pulp including changes in colour (often dark colour due to oxidation) and consistency of the pulp to find and collect the fruit fly larvae (**Figure 10**).
- The fruit dissector should carefully collect the larvae with entomological tweezers and place them alive in a Petri dish. The dish should be labeled indicating the consecutive number of the fruit sample available on the fruit sample label which indicates the origin of the fruit. The label on the dish should also indicate the date the larvae was collected. If larvae identification is difficult because of its small size, specimens should be placed back in the rearing room for further development or for pupation and adult emergence.
- If a larvae is found, the fruit dissector reports to the supervisor providing the complete information from the label. This is of particular importance in the case of a fruit fly free area being maintained.

The most common parameters used to report the level of an infestation are: The Larvae/kg of sampled fruits and the Percentage of fruits infested from total sampled fruits.

### 5.2.4 Rearing fruit fly larvae or pupae

If larvae identification is difficult or the larvae are already transformed into pupae, specimens should be placed back in the rearing room for further development into pupae and adult emergence. This may take from 12 to 20 days depending on the fruit fly species and the climatic conditions in the rearing room.

Fruit fly larvae or pupae for rearing are placed in plastic containers 13 cm high × 11 cm wide, with sand or vermiculite as pupation substrate and covered with fine cloth (**Figure 11**).

On a daily basis, the containers are carefully inspected to observe larvae or pupae development or adult emergence. If adults emerge they are collected and immediately sent for identification and a quick diagnostic. If the adults are positively identified as the target fruit fly, the result should be reported immediately to the supervisor and up the chain of command for implementation of emergency control actions in the field. The urgency of the actions is especially important when the aim of the programme is to protect the fruit fly free status of an area and a rapid response to a pest entry is crucial.

**Procedures for fruit fly larvae identification**

- The Petri dishes with live or dead larvae specimens collected directly from the fruit during dissection or from the maturation containers, should be orderly placed in the identification room. The Petri dishes containing larvae originated from fruit collected in priority areas such as pest free or low prevalence areas are given priority for immediate identification.
- A well trained and officially accredited fruit fly taxonomist with specific experience in identification of fruit fly immature stages should be responsible for the identification of the detected specimens.
- Live larvae are kept at 5 ºC during 30 minutes to immobilize them and facilitate identification. Larvae are placed in a Petri dish under a binocular microscope. Using an entomological tweezer or dissecting needles the morphological structures are exposed to facilitate identification.
- Dead larvae are kept in boiling water for 2 minutes and dipped in 50% alcohol solution for 15 minutes. After this procedure the larvae is transferred to a 70% alcohol solution. This will avoid shrinkage and discoloration of the dead larvae and will maintain in good conditions the morphological characters of the larvae. In this case for identification, the larvae are placed on a Petri dish with a black colour floor to create a contrast between the surface and the colour of the larvae facilitating larvae handling and identification. Black colored paraffin can be used for this purpose.



**Figure 11.** Plastic container with pupation media to allow adult emergence

- If a suspicious tephritidae larvae was not identified to species level, it should be send to a highly specialized tephritidae taxonomist and/or to a molecular analysis laboratory for identification through PCR procedures.
- Other non-tephritidae larvae commonly found in fruit samples belong to the families: Drosophilidae, Otitidae, Sarcophagidae, Muscidae, Nitidulidae and Staphilinidae.

# APPENDIX 1

# Fruit fly control strategies and fruit sampling

Fruit fly control strategies is defined in this context as a list of available options that the National Plant Protection Organizations (NPPOs) have at hand to control populations of fruit flies of economic and/or quarantine importance. Selection and implementation of such options will depend on the importance of the pest and objective of the programme. Control strategies include: Population monitoring, suppression, eradication, prevention, and containment of the target fruit fly species.

The fruit sampling is a basic activity in all fruit fly control strategies. It is recognized as a standalone tool for fruit fly detection, or as a complementary pest detection tool to the trapping activities. It is a valuable tool to evaluate AW-IPM Programmes including those that use the sterile insect technique.

## Fruit fly prevention in pest free areas

Prevention measures are applied in fruit fly free areas. It consists in the maintenance of a high-quality surveillance programme for early detection of non-native fruit fly species. The early detection of a non-native fruit fly is essential to immediately eradicate it and maintain the phytosanitary condition of fly free area. Trapping is permanently maintained in high, medium and low risk locations of the protected areas. This detection trapping is the main phytosanitary measure, but it can be complemented with a Targeted Fruit Sampling (see **Section 3.2.1**), which can be carried out seasonally or permanently at fruit production areas, export packing facilities and fruit markets within the protected area. The same type of fruit sampling can be used during time periods when trapping is not very reliable such as in extreme weather conditions, and fruit sampling can yield better results than trapping.

## Fruit fly eradication in pest free areas

An emergency action plan for eradication is applied around any non-native fruit fly detection site to immediately eliminate the possible establishment of the pest entry. In this situation, fruit sampling is applied for three main reasons: (1) To assess if the pest has infested fruits in and around the detection area (see **Section 3.1.2**), (2) To support the trapping activities to delimitate the area where eradication actions will be applied, and (3) To officially certificate the presence or absence of the pest during a period of time which is normally related to three biological cycles of the pest according to the national and international standards of phytosanitary measures. For verification of the phytosanitary status of an area, a Targeted Fruit Sampling for Monitoring is applied (see **Section 3.2.1**) aimed at sampling the most susceptible natural hosts present in the area subjected to eradication during the time period when the area is being verified as fly free.

## Fruit fly eradication in an infested area

Eradication of a fruit fly from an area is carried out in several phases, these are: Pre-eradication, eradication and post-eradication. After the pest has been eradicated, activities are aimed at preventing the re-infestation of the area. During the pre-eradication phase, fruit sampling activities are aimed at understanding the population biology and ecology including hosts and host sequence. For this objective a General Fruit Sampling is applied in order to generate the necessary baseline information (see **Section 3.1.1**). During the eradication phase, a Targeted Fruit Sampling is applied in areas under eradication as a fundamental basis to evaluate eradication actions (see **Section 3.2.1**) and in areas being used as buffer to protect pest free areas (see **Section 3.2.2.**).

## Fruit fly suppression

It is applied in fruit fly infested areas with important production of fruits and vegetables for commercial purposes and where fruit fly eradication is not feasible due to economic and/or agro-ecological factors. Interventions between the plant protection authorities in coordination with fruit growers in specific fruit production areas can lead to the establishment of low pest prevalence areas. A General Fruit Sampling is carried out to assess fruit infestation levels and to produce information about population trends which complements the information produced through trapping (see **Section 3.1.1**).

## Fruit fly containment barrier (buffer area)

It is a containment barrier (or buffer area) that is implemented to protect pest free areas from infested areas. Intensive suppression actions are conducted to keep populations at low prevalence levels reducing in this manner the population pressure towards the pest free area. A Targeted Fruit Sampling is carried out inside the containment barrier, focused on the most susceptible natural hosts during certain times of the year following host phenology (see **Section 3.2.2.**). The same fruit sampling can also be conducted in the pest free area contiguous to the barrier in a preventive manner. This can greatly improve early detection of a recent pest entries.

## Fruit fly monitoring

This survey is mostly applied to know the characteristics of a pest population which includes the abundance, spatial distribution and seasonal fluctuations and population ecology such as host sequence. A General Fruit Sampling is required in this case to complement the information on adult population obtained through trapping. This basic information is necessary to determine the best intervention strategy including the areas and timing of the control actions (see **Section 3.1.1**).

# APPENDIX 2

# Estimating resources for fruit sampling

The Programme should have a well-established procurement process for the acquisition of specific materials and equipment required for fruit sampling. Specific infrastructure may have to be built or lease and technicians hired. The costs for fruit sampling should include both, the collection of samples in the field and the laboratory activities.

Special attention must be given to the estimation of the personnel (salaries and benefits) and the vehicles (fuel and maintenance), which are the main costs incurred by this activity. These can add up to 80% of the total costs of the fruit sampling activity. The rest of the cost includes consumables, services and laboratory equipment.

As a basis to estimate personnel needs the first step is to prepare a list of hosts and its fruiting phenology as shown in Table1. The table provides information on the fruit fly hosts present in the working area, and their relative seasonal abundance which is part of the basic information required to prepare a fruit sampling schedule. This chart should be updated on a continuous basis to improve fruit sampling implementation and make the activity more cost-effective. Additional information such as the average production volumes per fruit species, the number of fruit trees in a specific area, the fruit varieties and its relative preference to the fruit fly species, is also used for planning and costing the fruit sampling activity.

The following is a simple example on how to identify and estimate fruit sampling resources.

## Basic information

### *Objective of the programme*

To establish a Mediterranean fruit fly free area through population eradication (eradication phase assumed).

### *Type of the fruit sampling*

Targeted Fruit Sampling (see **Section 3.2.1**).

### *Size of the working area*

There are 15 000 ha in total. With hosts, 8 000 ha of citrus commercial orchards, 4 000 ha of backyard host trees in urban and rural villages and 3 000 ha with no fruit fly hosts.

## Estimation of the Resources

### *Personnel*

One well trained fruit sampler is able to cover a total of 12 000 hectares. The area is divided into 120 quadrants of 100 hectares each (1 $km^2$ quadrants). The fruit sampler is able to cover all 120 quadrants in one month with an average 5 quadrants per day and with 35 fruit samples collected per day (7 samples per quadrant) or 840 per month in the total area. This means that one fruit sampler with a vehicle can survey the total working area of 12 000 ha once a month.

One well trained fruit fly taxonomist is needed. He or she can process 100 fruit samples per day, including handling of fruit samples, fruit dissection and larvae or adult identification.

### *Infrastructure*

One 4 × 5 $m^2$ room for laboratory activities with comfort working environment (air conditioning or heating).

### *Equipm ent and materials*

Field: One pick-up vehicle type or equivalent. Two telescopic fruit cutters, 5000 plastic recyclable bags of different sizes for the fruit samples, 4 thermo-boxes (50 × 40 × 40 $cm^3$) for fruit samples transportation, 1 GPS unit, labels for fruit samples data and other materials. Laboratory: 100 fruit maturation cages, 5 trays, 5 knives for fruit dissection, insect tweezers, lamps, cleaning materials, fruit conservatives (e.g. sodium benzoate), vials of various sizes, Petri dishes, stereo-microscope, desk, computer, fruit disposal containers and fruit handling tables.

### *Services*

Field: 150 liters of fuel per week and 3 maintenance services for the vehicle per year.

### *Laboratory*

Water, electricity, internet and telephone services.

### *Public information*

Spots in the local radio and TV stations and printing of pamphlets.

## Basic materials and equipment for fruit sampling

- Plastic bags of 3.5 and 10 kg capacity
- Extension poles fruit cutters
- Polyurethane Thermo Boxes (70 × 50 × 50 $cm^3$) to transport fruit samples
- Sticky Tags 3 × 2 $cm^2$
- Maps with sectors and quadrants of the working areas
- GPS handy equipment
- Marker, pens and pencils
- Notebook
- Reporting formats
- Handy magnifiers
- Formatted labels to identify fruit samples.

## Basic materials and equipment for the fruit sampling laboratory

- Maturation cages for fruit samples conservation
- Fruit dissection trays
- Knives and Entomological tweezers
- Sodium Benzoate
- Elastic cover cloths
- Vials 10 cc, 25 cc and 50 cc
- Tables for fruit dissection, shelves and desks
- Microscope and magnifying glasses
- Insect flasks
- Alcohol and cleaning materials
- Petri dishes
- Lamps

# APPENDIX 3

# Implementation of the fruit sampling

Once the Fruit Sampling Plan is technically and financially approved, the implementation of the activity is the next step. The designation of the fruit sampling field and laboratory personnel, the availability of the infrastructure and the equipment and materials as well as the public information campaign is considered to be already in place.

Newly hired technicians for fruit sampling activities (so called 'Fruit Samplers') should be trained for at least one month. Training includes theoretical as well as practices in situ covering basic lessons on fruit fly species, behaviour and life cycle, hosts preference and population ecology. It should also cover practical activities such as registration of field data and reporting. Fruit Samplers should also learn basic administrative procedures and working guidelines, including the proper use of equipment such as GPS, computers and vehicle and their preventive maintenance. Emphasis should be given to the technical importance of the fruit sampling as an integrated activity to the trapping and control activities. Trainees should receive enough written information such as: Technical documents and reports, procedures manuals and guidelines, regulations and report formats for their day to day use. Illustrated fruit sampling and fruit fly identification guides are important. A brief guideline indicating their working behavior when contacting the public in order to get permission to collect fruit samples from private property, and ways to create a good image of the programme.

Professionals with extensive experience in the field are the key to properly train Fruit Samplers on the know-how of the fruit sampling components. A good quality fruit sample becomes the main objective of the Fruit Samplers, especially considering the need to stratify as much as possible the immense universe of fruits available in the field, in order to increase the probability of pest detection.

The data produced by the various fruit sampling types, is recorded on specific electronic formats that feed a data-base. Data analysis contributes to technical and managerial decision making which improves programme management and pest control.

## Supervision

Constant supervision of the Fruit Samplers and laboratory staff is needed to assure reliable data. Poor supervision can be an important factor of programme failure or increased operational costs.

Supervisors should maintain up to date all records and reports with observations and recommendations made to the personnel. This will allow a close follow-up of staff performance and compliance with recommendations for improvement given by the supervisor.

### *Elements of supervision*

- Appropriate coverage of the sampling areas
- Selection of the proper fruit species and quantities to be sampled
- Selection of materials and equipment for fruit sampling
- Selection of the means of transport of fruit samples from the field to the laboratory to prevent mortality of immature stages within the fruits
- Data completion in the label of fruit samples
- Selection of the proper fruits to be sampled among those available on the ground or on the tree
- Use and maintenance of the vehicle assigned to fruit samplers
- Behavior of the Fruit Sampler when interacting with the public to collect fruit samples

- Accomplishment of the activity based on the schedule and times
- Accomplishment of the laboratory protocols for handling fruit samples
- Blind tests (or specimens seeding) as part of quality control procedures for fruit fly identification either as larvae or adult stages
- Correct capture of daily reports in the data-bases

## Periodic reports

The Fruit Sampler has to prepare and timely deliver a daily report. Daily field and laboratory reports should contain basic technical information and should be designed for easy completion. Be sure not to duplicate reporting formats.

### *Fruit sampling reports*

- Daily report of the individual Fruit Sampler. The report should contain the following information: (1) Name (initials) of the Fruit Sampler, (2) Number of fruit samples, (3) Samples from the ground or from the tree, (4) Quadrant and sector of origin of the fruit samples, (5) Site (or geographical coordinate) where the sample was taken, (6) Fruit common name and species, (7) Number of fruits per sample, (8) Date of collection, and (9) Sampler's signature (**Figure 3.1**) .
- Daily report of fruit sampling laboratory. The report should contain the following information: (1) Total number of fruit samples received per day, (2) Number of samples per fruit specie, (3) Samples collected from the ground or from the tree, (4) First and final fruit dissection results, (5) Number of samples in maturation cages, (6) Number of fruit fly larvae detected in samples, (7) Fruit common name and specie from where larvae was found, (8) Date of collection and identification of larvae, (9) Place of detection, (10) Coordinates and a map of location of the origin of the infested sample (**Figure 3.3**).
- Weekly and monthly fruit sampler and laboratory reports. The reports present a weekly or monthly summary of the fruit sampling parameters. The data are valuable for analysis and discussion by supervisors and managers of the weekly and monthly observed trends (**Figure 3.2** and **3.4**).

## Databases

A database specifically for the fruit sampling should be available in the programme. This is uploaded in the computers of the fruit sampling office for the daily capture of results, based on the daily reports from the Fruit Samplers and laboratory staff. With these information the database will be able to produce periodic reports with the technical and operative parameters (see following Section) which are needed to evaluate the fruit fly infestation or level of control as well as the operation of the fruit sampling activities. A trained computer technician should be responsible of the database management and its constant up-date and maintenance.

### *Basic information of the fruit sampling database*

- The consecutive numbers of the fruit samples
- The site, route, sector and quadrant of fruit sample collection
- The infested and non-infested fruit samples by sites, routes, sectors and quadrants
- The fruit species infestation index (per fruit species, per quadrant, per sector and total)
- The sampled and non-sampled quadrants per week
- The abundance and distribution of fruit species present per quadrant and total

## Technical parameters

The fruit sampling technical parameters provide infestation levels (technical indexes) in the working area or in any particular area or site. Infestation levels can be used to measure the degree of efficacy of the pest control applied in the working area. They are useful for routine analysis to support decisions to improve suppression or eradication actions. The common technical parameters are:

- Percentage infested kilograms of fruit (total, per sector and quadrant, per fruit species, per specific areas)
- Percentage of infested fruit samples (total, per area, sector and quadrant, per fruit species, per specific areas)
- Percentage of infested sites (per area, sector and quadrant)
- Percentage of infested fruits (total, per sector and quadrant, per fruit species, or per specific areas)
- Fruit fly larvae / kilogram of sampled fruits (total, per sector and quadrant, per fruit species, per specific areas)
- Fruit fly larvae per fruit and per sample (total per area, programme phase, per specific areas)

## Operative parameters

The fruit sampling operative parameters measure the management performance of the Fruit Samplers, laboratory staff, supervisors and managers. These operative parameters are periodically analysed by supervisors and programme managers to observe trends and conduct necessary adjustments to the activities. These are also used to support decision-making for planning and implementing fruit sampling activities. The most common operative parameters are:

- Kilograms of collected fruit per week or per any required period
- Number of collected fruit samples and number of sampled sites per period
- Kilograms of collected fruit from the ground and from the trees per period
- Kilograms, number of collected fruit, samples and sites per fruit species
- Kilograms of collected fruit placed in maturation cages and kilograms of dissected fruit
- Total target fruit fly larvae detected
- Total of larvae detected of other Tephritidae or other insect families
- Percentage of quadrants or sampling routes visited from the total area per period of time

## Use of Geographical Information System in fruit sampling

Fruit sampling is an activity aimed at timely monitoring and detecting a fruit fly pest in a specific location. High amount of base-line data has to be captured, processed, analyzed and mapped presented to programme managers and stored. The development and use of a specific Geographical Information System (GIS) for the programme should be done by integrating a GIS computer specialist with a fruit fly control specialist. This combination will produce the required information and reports for constructive feedback in support of decision making at the management level.

In the case of fruit sampling, the type of analysis conducted through the GIS helps to develop pest predictions in temporal-spatial variations. For example, the temporal-spatial variations of fruit fly populations using the technical index of larva per kilogram of sampled fruits. Thus, the final output of the fruit sampling-GIS is the availability of accurate and timely spatial and temporal information to achieve a cost-effective and precise control of the target fruit fly pest.

## Daily Fruit Sampling Report Form FS01

Name of the Programme:_____Country:_____ Year:_____ Page 1 of_____

Field Operation Center:_____Sub-Center:_____

Cuadrant:_____ Sector:_____ Closer Trapping Route:_____ Closer Trap (Number of trap) _____

Date:_____ Week: _____ **(This report is filled with the data of the Fruit Sampling labels)**

| No. | Fruit Sample Nº or Code | Fruit specie & variety | # fruits | Kg | From Tree/ Ground | Other source (1) | Trap Ref.(2) | AREA (3) | Place/Site/County |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | |
| 2 | | | | | | | | | |
| 3 | | | | | | | | | |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |
| 6 | | | | | | | | | |
| 7 | | | | | | | | | |
| 8 | | | | | | | | | |
| 9 | | | | | | | | | |
| 10 | | | | | | | | | |

Total number of fruit samples taken this day:_____ Total kilograms sampled this day: _____ Total sites sampled and visited this day:_____

Remarks:_____

(1) Other: Fruit sample taken from other source as a market place, fruit grow er, dumps, etc.

(2) Trap Reference: The number of the closer trap to the site the fruit sample w as taken

(3) AREA: C=Core; B = Buffer; I = Infested

Name/Sign:_____

**Figure 3.1.** Daily fruit sampling report (Form FS01).
Source: Moscamed Programme DGSV-SARH Mexico, FAO (1983).

## Weekly Fruit Sampling Report Form FS02

Name of the Programme:_____Country:_____ Year:_____

Field Operation Center:_____Sub-Center:_____

Cuadrant:_____ Sector:_____ Closer Trapping Route:_____

Week No. _____

**(This report is filled with the data of the Daily Fruit Sampling Reports)**

| Day | No. of Fruit Samples | No. FS from Tree | No. FS from Ground | Total Kg | Total Sites Visited | Fruit species (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Orange | Mandarin | Loquat | Fig | Peach | Sour Orange | Quince | Guava | Prickly Pear | Apricot | Plum | Pear | Apple | Other |
| M | | | | | | | | | | | | | | | | | | | |
| T | | | | | | | | | | | | | | | | | | | |
| W | | | | | | | | | | | | | | | | | | | |
| TH | | | | | | | | | | | | | | | | | | | |
| F | | | | | | | | | | | | | | | | | | | |
| S | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| Totals | | | | | | | | | | | | | | | | | | | |

Total number of fruit samples taken this week:_____ Total kilograms sampled this week: _____ Total sites sampled and visited this week_____

Remarks:_____

_____.

Name/Sign:_____

**Figure 3.2.** Weekly fruit sampling report (Form FS02).
Source: Moscamed Programme DGSV-SARH Mexico, FAO (1983).

## Daily Fruit Sampling Laboratory Report Form FS03

Name of the Programme:_____Country:_____ Year:_____ Page 1 of_____

Field Operation Center:_____Sub-Center:_____

Cuadrant:_____ Sector:_____ Closer Trapping Route:_____

Date:_____ Week: _____ **(This report is filled as a continuation of the Daily Fruit Sampling Reports)**

| No. | Fruit Sample Nº or Code | Fruit specie & variety | # fruits | Kg | Sample from Cage or Field | No. Fruits dissected | No. fruits infested w/ Cc. Larvae | Number of Larvae (1) | Larvae / Kilo | Area (C,B,I) (2) | No. Fruits to cages | Cage Number | Trap Ref.(2) | Other Larvae (3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | | |
| ETC | | | | | | | | | | | | | | |
| **Totals** | | | | | | | | | | | | | | |

Total No. of fruit samples processed this day:_____ Total kilograms processed this day: _____ Weekly larvae/Kilogram of fruit sampled this day:_____

Fruit Species infested: _____ ____% ; _____ ___%; _____ ____%; _____ ____% ; _____ ___% ; _____ ____ %

(Remarks:_____

_____.

(1) Larvae: Larvae of *Ceratitis capitata*

(2) Area (C = Core Area; B = Buffer Area; I Infested Area )

(3) Trap Reference: The number of the closer trap to the site the fruit sample w as taken

(4) Other Larvae: Detection of other fruit fly specie larvae than *Ceratitis capitata*

Name/Sign:_____

**Figure 3.3.** Daily fruit sampling laboratory report (FS03).
Source: Moscamed Programme DGSV-SARH Mexico, FAO (1983).

## Weekly Fruit Sampling Laboratory Report Form — FS04

Name of the Programme:_____Country:_____Year:_____Page_1_of____
Field Operation Center:_____; Sub-Center:_____Name of Supervisor:_____

Week No._____ From:____ to_____ of_____ 20___

| CONCEPT | CORE AREA | | | BUFFER AREA | | | INFESTED AREA | | | OTHER AREA |
|---|---|---|---|---|---|---|---|---|---|---|
| | from tree | from ground | other | from tree | from ground | other | from tree | from ground | other | |
| No. of samples taken | | | | | | | | | | |
| No. of Kilos sampled | | | | | | | | | | |
| No. fruit samples infested | | | | | | | | | | |
| % samples infested | | | | | | | | | | |
| Number of larvae | | | | | | | | | | |
| Hosts infested | | | | | | | | | | |

| Fruit Host | KG. | % | No. Fruit Samples | % | No. of Fruits | % | No. Fruits with Larvae | % Fruits w/ Larvae | NO. LARVAE | LARVAE / KG. |
|---|---|---|---|---|---|---|---|---|---|---|
| Orange | | | | | | | | | | |
| Mandarin | | | | | | | | | | |
| Pomelo | | | | | | | | | | |
| Loquat | | | | | | | | | | |
| Fig | | | | | | | | | | |
| Peach | | | | | | | | | | |
| Sour Orange | | | | | | | | | | |
| Quince | | | | | | | | | | |
| Prickly Pear | | | | | | | | | | |
| Apricot | | | | | | | | | | |
| Plum | | | | | | | | | | |
| Pear | | | | | | | | | | |
| Apples | | | | | | | | | | |
| Others | | | | | | | | | | |
| Totals | | | | | | | | | | |
| **Totals** | | | | | | | | | | |

Total No. of fruit samples processed this week:_____ Total kilograms processed this week: _____ Weekly larvae/Kilogram of fruit sampled:_____
No. of fruit samples infested / fruit species: _____
_____% ; _____ ____%; _____ _____%; _____ _____% ; _____ ____% ; _____ _____ %
(Remarks:_____ 38
_____

(1) Larvae: Larvae of *Ceratitis capitata*
(2) Trap Reference: The number of the closer trap to the site the fruit sample was taken
(3) Other Larvae: Detection of other fruit fly specie larvae than *Ceratitis capitata*

Name/Sign:_____



**Figure 3.4.** Weekly fruit sampling laboratory report (FS04).
Source: Moscamed Programme DGSV-SARH Mexico, FAO (1983).

For a detailed understanding of a GIS design and implementation please refer to the IAEA publication: "Designing and Implementing a Geographical Information System" A guide for Managers of Area-Wide Pest Management Programmes; Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. IAEA Vienna 2006. http://www-naweb.iaea.org/nafa/ipc/public/ipc-gismanual-web.pdf.

# APPENDIX 4

# Types of fruit sampling used by AW-IPM Programmes in relation to the programme objective and control strategy

| Programme Objective | Control Strategy | Type of Fruit Sampling | Intensity of Sampling |
|---|---|---|---|
| Infested area | Determination of fruit fly hosts. No Control. | General Fruit Sampling (simple low stratified random fruit sampling): Aimed at population monitoring. Collection of fruit samples year round. Any mature fruit with soft skin is candidate to be sampled. Focus fruit collection to fruits with infestation symptoms available on the tree or on the ground. | Low intensity fruit sampling. Four fruit samples from 4 sampling sites (16 samples) per km². Samples from 100 grs (small fruits such as coffee berries) to 3 kg (larger fruits such as grapefruits). Frequency: 14 to 28 days |
| Low prevalence area including buffer area | Fruit Fly Suppression Programme. | Targeted Fruit Sampling (simple stratified random fruit sampling). Aimed at evaluating control activities: Focused on areas with the most susceptible natural hosts including commercial fruit orchards and buffer areas if part of an eradication programme or containment barrier. Collection of fruit samples year round according to the fruiting phenology of primary hoswts. Focus fruit collection to fruits with infestation symptoms available on the tree or on the ground. | Medium intensity fruit sampling. Five to ten (0.2 to 3 kilos/sample) fruit samples from 4 sampling sites (20 to 40 samples) per $km^2$ per week in host areas including fruit orchards and in buffer areas.<br><br>During the ripening and harvest periods of the target commercial fruit, increase to 20 fruit samples per $km^2$ per week. |
| Low prevalence area under eradication | Fruit Fly Eradication Programme. | Targeted Fruit Sampling (simple highly stratified random fruit sampling). Aimed at pest detection complementing detection trapping. Collection of fruit samples year round. Focused on selected areas with the most susceptible natural hosts according to host fruiting phenology. | Same as above. |
| Pest free area (Contingency plan) | Emergency response to a pest entry or incursion | General Fruit Sampling (simple low stratified random fruit sampling). Aimed at characterizing a pest entry or incursion including delimitation of a fruit fly outbreak. | High intensity fruit sampling. Twenty to one hundred (2 to 10 kilos/ sample) fruit samples per km² per week, for every fruit fly detection or outbreak based on fruit availability and access to sampling areas. |
| Pest free area (Exclusion) | Areas subject to considerable risk of introduction of a quarantined fruit fly species | Targeted Fruit Sampling (simple highly stratified random fruit sampling). Aimed at early detection. Focus on sampling in packing and processing centres, markets and distribution centres according to a pathway risk assessment. | Low to medium intensity fruit sampling. Four to ten fruit samples per site per week. |

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# FRUIT SAMPLING GUIDELINES FOR AREA-WIDE FRUIT FLY PROGRAMMES

Population survey is a basic component of any area-wide integrated pest management programme. Pest surveillance measures have to be practical, cost-effective and provide reliable information to action programme managers. Fruit fly trapping provides useful information on the presence or absence of the pest, and on its relative spatial distribution and abundance. However, performance and thus effectiveness of trapping systems can be affected by extrinsic factors including changing environmental and ecological conditions. Under certain conditions, fruit sampling becomes a suitable tool for population sampling. For example, at the beginning or end of the fruiting season when fewer mature fruits are still available on the trees, larvae could be more easily detected. Fruit sampling also becomes an important pest detection tool in areas where sterile flies are being continuously released and where low-density trapping is kept to avoid high sterile fly recapture rate and where traps are aimed basically at monitoring the released sterile flies.

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