

1-2-3 *Of* **Fruit Fly Population Monitoring** (Agro-ecosystem Analysis)

Guidelines for IPM Farmers and Trainers



**Area-wide Integrated Pest Management of Fruit Flies
in South and SE Asia Project**
(<http://ipm.ait.asia>)

March 2011

1-2-3 of Fruit Fly Population Monitoring (Agroecosystem Analysis)

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Area-Wide Integrated Pest Management of Fruit flies in South and Southeast Asian Countries

Participating Agencies from Mekong River Basin Countries:



Project Partners:



Project Donor:



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1-2-3 OF FRUIT FLY POPULATION MONITORING

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About the project

“Area-wide Fruit Fly Integrated Pest Management in South and Southeast Asia” is a regional initiative coordinated by the Asian Institute of Technology with funding support from Taiwan’s ICDF (www.icdf.org.tw). The project has a focus on adaptation and adoption of fruit fly IPM practices among vegetable and fruit smallholder using Farmer’s Field School (FFS) in the Mekong river basin countries. While the Asian Institute of Technology (AIT; www.ait.asia); Bio-Control Research Laboratory (BCRL; <http://www.pcilindia.com/bcrl.html>), Bangalore, India, and the FAO Regional IPM Programme (<http://www.vegetableipmasia.org/>) are the collaborating project partners, the project is implemented by National IPM Programmes in Lao PDR, Cambodia, Vietnam; Department of Agriculture Extension (DoAE) Thailand, and Ministry of Agriculture and Irrigation (MAI) in Myanmar. The project is intended to test, promote and socialize among smallholder farmers a range of novel IPM options for fruit fly management within the context of ongoing IPM farmer training and action research programmes in the Mekong basin countries.

Contact

The Regional Office of the project is housed in its host institute’s Room # 207, AFE Bld. AIT, Bangkok, Thailand. Further information on project could be obtained from the following contact:

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Preface

Agro-ecosystem analysis (AESA) is a tool for making observations, assessing the various components of the crop agro- ecosystem and making informed decisions on crop management by IPM farmers. An Agro-ecosystem Analysis considers a number of biotic factors (insect-pests, natural enemies, neutrals) above and on the ground along with abiotic factors (temperature, humidity, soil and soil moisture condition). In Farmers Field Schools, farmers collect need-based information on weekly basis. The data gathered is then analysed and drawn pictorially to help the farmers visualize better, and later establish inter-relation and interdependence in relation to the overall crop health and development. This process is carried out as a group activity and the small groups' recommendations along with so called AESA drawing are later presented and critiqued during a plenary presentation and discussion session as part of the weekly farmer's field school curriculum. Since the data is taken from the same crop and field, the larger group arrives at a consensus on the management decision to employ after assessing all the components of the crop ecosystem. The process of monitoring fruit flies as part of this Agro-ecosystem Analysis is a new. Data to be gathered and methodologies were discussed at the AIT/FAO *Regional Training on IPM for Fruit Flies* held at the Southern Fruit Research Institute (SOFRI), Tien Giang, Vietnam from 07-14TH December 2010. This training was held under the auspices of the Asian Fruit Fly IPM Project (<http://ipm.ait.asia>). It is hoped that this addition to the already established and widely-used IPM monitoring tool *i.e.* AESA would help farmers and trainers to monitor the population dynamics of fruit flies and its natural enemies to continue to make better informed decisions on raising healthy and profitable fruit and vegetable crops.

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Background:

Agro-ecosystem Analysis (AESAs) is a robust tool commonly used by IPM farmers and trainers to monitor the population of pests, natural enemies, and weather and soil conditions along with other need-based details on crop ecosystems to make informed decisions on crop management.

Bactrocera dorsalis (Oriental Fruit Fly, OFF), *B. cucurbitae* (melon fly) and *B. correcta* (Guava Fruit Fly), are endemic pests of reproductive stages of a wide variety of fruits and vegetables and non-crop hosts in tropical and sub-tropical Asia. Due to the large number of hosts, wide dispersal and movements of fruit flies, individual field-based monitoring and consequently management cannot be employed. Therefore, IPM trainers and farmers need to adopt and adapt the AESA for FF monitoring (on an area-wide basis) to be able to accurately monitor fruit fly populations and plan and implement location and situation-specific “best-bet” management strategies.



What and how to monitor?

For all practical area-wide field implementation programmes, three (1-2-3) variables are suggested to be monitored in addition to the regular AESA observation:



1. Per cent Fruit Infestation:

- Adult female fruit flies have a needle-like ovipositor with which they puncture the skin of fruits to lay their eggs in the flesh. Flies usually prefer ripe fruit but often also lay their eggs in green or unripe fruit.



Fig.1 Fly ovipositing in ripe citrus
(Photo: Vijay Shanmugam)



Fig.2 Fly ovipositing in green chilli
(Photo: Vijay Shanmugam)

- The oviposition marks are at first difficult to detect but as the eggs hatch in 1-2days, each oviposition mark appears as a distinct spot with a brownish patch around it caused by microbial decay of the flesh within.



Fig.3 Fruit fly oviposition marks on Carambola
(Photo: Vijay Shanmugam)



Fig.4. Chili infested by fruit fly showing extensive larval feeding and microbial decay within (Photo: Vijay Shanmugam)

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Recording fruit fly oviposition marks on fruits/vegetables is a first step and a rapid way to assess the amount of fruit fly damage to a crop in an area. To make such a rapid assessment, do the following:

- Randomly pick from the tree a minimum of 100 ripe fruits for each crop. Where this is unavoidable, collect only freshly fallen fruits in addition to the fruit picked from the tree. Avoid old fallen fruits as these may be damaged by other insects in the ground.
- Do not mix different species of fruit/fruit vegetables together, but keep them separate
- Examine each fruit under good light and count how many show fruit fly oviposition marks as described above. The use of a hand lens will help detect fresh (less than 1 day old) oviposition punctures that are not visible to the naked eye.
- The percentage (%) fruit fly infestation for each fruit or fruit vegetable crop at the time of sampling is calculated as:

$$\frac{\text{Number of fruits showing fruit fly oviposition marks}}{\text{Total number of fruits collected and examined}} \times 100$$

(Note: For fruits that are harvested several times over the fruiting season, you should repeat the process at least three times during the season. For fruits that are harvested only once at the end of the season, the monitoring for percent infestation is done once just before the final harvest. In the case of fruit vegetables, the procedure should be done three times during the fruiting/harvesting season)



2. Number of larvae/adult emergence/parasitoids:

The eggs hatch in 1 -2 days time and the larvae burrow into the fruit flesh. There are 3 larval stages, of which the first stage or instar is about 1 mm long and very difficult to see with the naked eye. The 2nd instar larvae are slightly larger (2-3 mm) and more visible but the 3rd larvae are creamy yellow in colour, about 5 mm long and are easy to detect. The 3rd stage larvae also have the ability to “jump” and use this mechanism to exit the fruit to pupate.

Microbial decay of the flesh is associated with larval feeding and fruit fly infested fruit can be easily detected by noting this microbial decay.



Fig. 5 A cluster of fruit fly eggs in fruit surrounded by brownish microbial decay
(Photo: Vijay Shanmugam)



Fig.6 Creamy-yellow 3rd instar fruit fly larvae with extensive microbial decay of the fruit
(Photo: Vijay Shanmugam)



Fig. 7 Fruit fly infested guava
(Photo: Vijay Shanmugam)



Fig. 8 Melon fly infested bitter gourd
(Photo: Vijay Shanmugam)

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The 3rd stage larvae pupate for about 10 days in the soil and then emerge as adult flies. The eggs and larvae of fruit flies are also commonly parasitized by several species of Hymenopteran endoparasites belonging to the family Braconidae. The adult parasitoids lay their eggs in fruit fly eggs or larvae infesting the fruit and the parasitoid larvae then complete their life cycle and emerge from the fruit fly pupae as adult parasitoids. A single parasitoid emerges from each fruit fly egg or larva parasitized.

A second and more accurate way to determine the extent fruit fly damage as well as to measure the intensity of infestation is to count the number of larvae or adults/parasitoids that emerge from the infested fruits/fruit vegetables.

The protocol below describes how to do this.

Larval counts

- Randomly collect 100 ripe fruits or 50 fruits (in case of large fruits) from the field/orchard
- Record the weight of each fruit in grams
- Dissect the fruits and count the number of larvae to establish number of larvae/unit weight of fruit or fruit vegetable
- Any fruit, even with one fruit fly larva in it is to be considered infested
- The percentage (%) fruit fly infestation for each fruit or fruit vegetable crop at the time of sampling is calculated as:

$$\frac{\text{Number of fruits with fruit fly larvae}}{\text{Total number of fruits dissected}} \times 100$$

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Adult fly/parasitoid emergence

- This purpose of this exercise is to establish the number of adult fruit flies /unit weight of fruit
- Randomly collect 100 ripe fruits or 50 fruits (in case of large fruits) from the field/orchard and record the weight of each fruit in grams
- Prepare rearing containers from clear plastic containers to suit the size of the individual fruit. (i.e. each fruit should fit in lengthwise in bottom of the container with a little bit of space to spare). Remove the lid and place about 4 cm of insecticide-free sawdust (do not use sawdust from chemically treated or processed wood; use only sawdust from natural untreated wood) in each rearing container. Sieve raw sawdust to remove coarse matter and to obtain a fine even sized sawdust for your use.
- Place one fruit or vegetable in a container and use a fine mesh (about 1 mm mesh size) cloth (preferably rayon or similar synthetic material) and tightly cover each container with elastic bands so that nothing can escape or enter the container. Place the container in an airy room and protect from ants.
- *Drosophila* and other insects will be attracted to the fermenting fruit, so it is vital to ensure that the cloth mesh on each rearing containers is tightly secured around the edges to prevent any other organisms from entering the rearing containers, or adult fruit flies and parasitoids from escaping as well.



Fig. 9 Guava fruit on fine sawdust in individual rearing containers
(Photo: Vijay Shanmugam)



Fig.10 Rearing containers held on ant-protected shelves
(Photo: Vijay Shanmugam)

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- After 2-3 weeks, adult fruit flies and their parasitoids will emerge from infested fruit and can be seen moving around the inside of the clear plastic container. Feed the emerged flies a mixture of honey and water soaked onto cotton wool and placed on top of the cloth mesh of each rearing cage for 2-3 days to let their body harden and their colours develop fully to facilitate identification. Remove the water/honey after 2-3 days and leave for another 3-4 days after which flies and parasitoids will die and fall onto the sawdust surface. Remove the cloth mesh and count the number of dead adult flies and parasitoids. Carefully examine the bottom of the decayed fruits as pupae/fly will often be attached to these areas.



Fig.11 Fruit fly parasitoids
(Photo: Vijay Shanmugam)



Fig 12 *Fopius arisanus* – a common parasitoid of the eggs of fruit flies
(Photo: Vijay Shanmugam)

- Towards the end of study, the total number of all emerging adults should be summed up and expressed as 'number of adult emergences/unit weight of fruit' (for instance, 50 adult FF/500 grams of Sapota). The number of fruit flies per fruit is equal to adult flies plus parasitoids counted. One parasitoid represents one fruit fly that would have emerged if not parasitized.
- This process should be carried out for both IPM and non-IPM area. In case of fruits that are harvested many times, the process should be repeated at least three times (first fruiting, mid-season and end-season).

(Note: For fruits that are harvested several times over the fruiting season, you should repeat the process at least three times during the season. For fruits that are harvested only once at the end of the season, the monitoring for percent infestation is done once just before the final harvest. In the case of fruit vegetables, the procedure should be done three times during the fruiting/harvesting season)



3. Flies per Trap per Day (FTD)

A very useful and rapid method of monitoring the population of pest fruit flies in an area is to use male lures or attractants. These are chemicals that have a powerful attraction to some species of adult male fruit flies. Very widely used for *Bactrocera* species are the compounds Methyl Eugenol (ME) and Cue Lure (CUE). ME attracts both male Oriental Fruit Fly (*B. dorsalis*) and male Guava Fruit Fly (*B. correcta*), while the compound Cue Lure (CUE) attracts male Melon Fly (*B. cucurbitae*). ***Methyl Eugenol and Cue Lure do not attract the females.*** Since the ratio of male: female adult fruit flies on emergence from the pupae is always about 1:1 male lures in simple and cheap traps can be used to monitor fruit fly populations in an IPM program following the guidelines outlined below.

To do so:

- To make the trap, obtain a clear plastic mineral water bottle (1.5 liter size capacity) and make two windows on the side of the bottle so as to form a flap measuring 3 x 3 x 3 cm. Pull the flap outwards so that it forms an opening into the bottle with the flap serving as protection against rain entering the bottle (*see annex 2*);
- ME and CUE are provided separately in wooden blocks (by BCRL) that have been impregnated with the lure and contain 1 gram of either ME or CUE but do not contain any insecticide. Insecticide needs to be added to the blocks to kill male flies entering the traps
- Using disposable rubber gloves and forceps, remove a block from the foil packing and insert a wire through the hole provided. Add 5 drops of malathion insecticide to each block and let it absorb into the block. Use the pure undiluted insecticide, i.e. do not dilute with water. Do not worry about the strong smell of the malathion as adult fruit flies are not affected by this odour.

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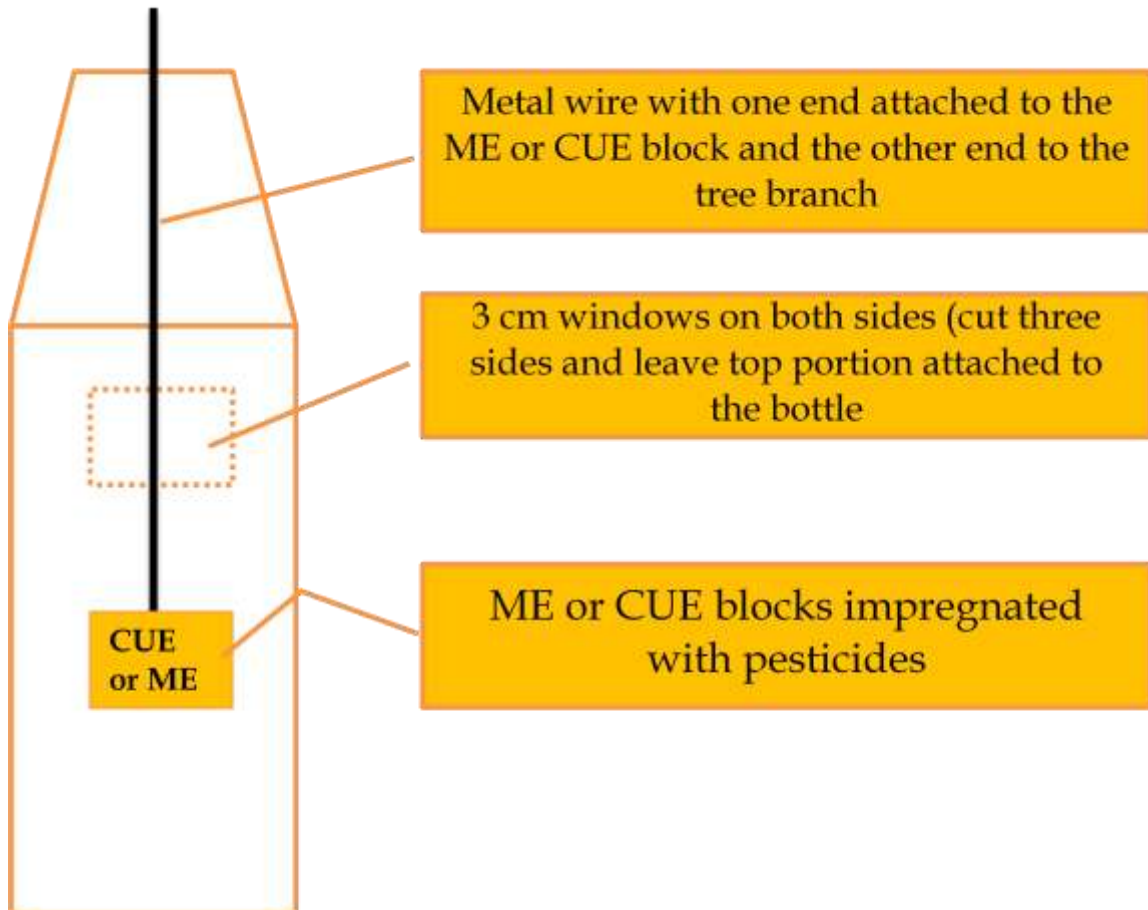
- Cross contamination between ME and CUE must be avoided, so always use separate gloves, forceps and equipment for each lure when handling these two chemicals. Do not mix them.
- Hang 1 ME or CUE block in each bottle trap. Number each trap with a marker pen. Hang the traps in the field at about eye level in the shade within a host tree at a density of approximately 1 trap per hectare. Thus if you have 6 hectares in your project site, you must use 6 traps.
- Set one trap/hectare (so **if the area-wide area is 6 hectares, use 6 trap**). Similarly, use another 6 traps in the non-IPM area;
- At weekly intervals, collect the adult catches, identify the species and count the numbers;
- After counting numbers, calculate the FTD and record in the data sheet; **Please remember to calculate the FTD for each species separately (see annex 3)**;
- Replace ME blocks in the traps monthly and the CUE every two months;
- Cross-contamination between ME and CUE is a common problem caused by handling. Avoid this by having different persons handling the ME and CUE traps.
- Collect the old blocks and bury them in the soil at a depth of more than 50cm
- If bottle of the traps are cloudy, mouldy or damaged, change it with a fresh one
- Wash your hands and all equipment with ethyl alcohol (about 50-70% strength) to remove traces of ME and CUE after handling these lure

Total number of flies trapped_(by species)

FTD = Flies/Trap/Day = _____

Number of trapping days x Number of traps

Annex 1: Simple design of a bottle trap



Annex 2: Hanging of bottle trap in a bitter gourd field



A bottle trap with BCRL Cue Lure block
(Photo: Vijay Shanmugam)

Annex 3: Suggested Data Sheet for recording FTD

Crop:

Village:

Date of start:

Species of Fruit fly: *B. dorsalis* / *B. correcta* / *B. cucurbitae* (encircle the species)

Location	Week 1 Date		Week 2 Date		Week 3 Date		Week 4 Date		Week 5 Date		Week 6 Date		Week 7 Date	
	IPM	Non IPM	IPM	Non IPM	IPM	Non IPM	IPM	Non IPM	IPM	Non IPM	IPM	Non IPM	IPM	Non IPM
Location 1 GPS coordinate														
Location 2 GPS coordinate														
Location 3 GPS coordinate														
Location 4 GPS coordinate														
Location 5 GPS coordinate														
Location 6 GPS coordinate														

Notes:

1. Please measure the GPS coordinate of the area-wide and also the location of the traps and enter the information in the data sheet
2. This data sheet is prepared assuming 6 hectare of area-wide IPM area and similar size of non-IPM area