



# **Surveillance Manual - Plant Pests and Diseases**

## **“Surveillance Techniques and Skills for Plant Health Technicians”**



By: Sumattie Gosine

A collaborative effort between the:  
Organization of the Eastern Caribbean States, and  
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### About the Author

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Ms. Sumattie Gosine has a MSc in Geoinformatics with Distinction and BSc in Agronomy (Upper Second Class Honours). She has been working in crop protection for over twenty years and was the core person in surveillance with the responsibility for coordinating surveillance activities on pest of quarantine importance, alien invasive species and pest of important crops in Trinidad and Tobago. She has over twelve years of experience in planning and executing surveys and drafting and producing public awareness material for invasive alien species. She has also assisted in planning emergency action plans for black Sigatoka disease, avian influenza, giant African snail, sweet potato weevil and citrus Haunglongbing disease.

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### Acknowledgement

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The author is grateful for the assistance of Mrs. Juliet Goldsmith, Plant Health Specialist, Caribbean Agricultural Health and Food Safety Agency and Mr. John Crowe, National Survey Supply Coordinator, USDA APHIS PPQ for assisting in training plant protection personnel in the OECS States and the wider Caribbean as well as their contributions in the preparation of this manual.

The material in this manual is an amalgamation of principles, facts, techniques, methodologies, etc. that can be found elsewhere. Every effort was taken to refer the user to the original material as reference of further reading, where applicable.

This manual is not intended for sale but to be used by governmental plant protection personnel/plant health technicians in the conduct of their duties to protect the OECS States and the wider Caribbean biodiversity from alien invasive species of plant quarantine importance.

**Preface by George Alcee, Programme Officer Agriculture, OECS Commission**

The Surveillance Manual – Plant Pests and Diseases “Surveillance Techniques and Skills for Plant Health Technicians” was developed because of the urgent need within the Caribbean plant health systems, for a nationally coordinated and targeted surveillance system that supports the early detection of new pests; reporting of pest free areas and areas of low pest prevalence; and enhances pest incursion responses.

The International Plant Protection Convention (IPPC) requires countries to report on the occurrence, outbreak, and spread of pests with the purpose of communicating immediate or potential danger. National Plant Protection Organizations (NPPOs) have the responsibility to collect pest information by surveillance and to verify pest records collected. The provision of reliable and prompt pest reports confirms the operation of effective surveillance and reporting systems within countries.

Currently, there is no formal comprehensive system of surveillance in operation in the Caribbean region. No single organization is dedicated to plant pest survey activities for the detection, delimitation or monitoring of established pests, or for the detection of new pests that may be introduced. Consequently there are major gaps in pest records and a sparsity of information on pest status. This limits the ability of the countries in the region to detect and respond to pest incursions as well as to fulfil their obligations to trading partners and international regulatory and standard setting bodies.

In light of improving the plant health systems in the region, the Organization of the Eastern Caribbean States (OECS) in collaboration with the Caribbean Plant Health Director’s (CPHD) Forum with funding from the European Union (EU) endeavoured to fill this important need within the Caribbean by producing this manual. The Surveillance Manual covers key areas such as: What is Surveillance, Need for Surveillance, Planning a Survey, Developing Survey Protocols, Developing Field Procedures, Developing Laboratory Procedures, and Preparing Public Awareness Information.

The Manual has a user friendly design and incorporates many pest and disease examples that significantly impact the region, up to the time of documentation.

This Surveillance Manual does not pretend to have an exhaustive list of all the surveillance activities, but represents a start towards educating the stakeholders in the region.

Finally we would like to acknowledge the United States Department of Agriculture Animal and Plant Health Inspections Services International Service (USDA APHIS) Office in Trinidad for the logistical support for the completion of this manual.

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## Chapter 1 Surveillance

### 1.1 Why do we need to do surveillance?

We need to do surveillance for many reasons; amongst these are the fulfilment of our international obligations as signatories to international treaties and conventions, namely:

- ◆ The International Plant Protection Convention (IPPC) and the Convention on Biological Diversity (CBD) require its signatories to develop surveillance capabilities to facilitate transparency in international trade and preserve their biodiversity.
- ◆ The CBD is a global, comprehensive agreement addressing all aspects of biological diversity: genetic resources, species, and ecosystems. Signatories are obligated to uphold Articles 7(c), 8(l), 8(h) and 8(k) of the CBD, with regards to surveillance activities. That is, signatories must:
  - ❖ identify/ monitor/ regulate or manage activities that are likely to have significant adverse impacts on the biological diversity;
  - ❖ prevent the introduction/ control or eradicate alien species which threaten ecosystems/ habitat/ species; and
  - ❖ develop or maintain necessary legislation and/or other regulatory provisions for the protection of threatened species and populations.
- ◆ The IPPC is an international treaty to secure action to:
  - ❖ prevent the spread and introduction of pests of plants and plant products, and
  - ❖ promote appropriate measures for their control whilst minimizing technical barriers to trade.

The IPPC is governed by the Commission on Phytosanitary Measures, which adopts the International Standards for Phytosanitary Measures (ISPMs). The IPPC is one of the three standard-setting bodies whose rules are accepted by the World Trade Organization's (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) (Article 3 and Annex A: 1(c)). The IPPC is recognized as the only international standard setting body for plant health.
- ◆ The World Trade Organization (WTO) operates a system of trade rules that provide the legal ground-rules for international commerce between nations at a global level. The WTO is the successor to the General Agreement on Tariffs and Trade (GATT) which had been in existence since 1947 as the organization overseeing the multilateral trading system.
- ◆ The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS), entered into force with the establishment of the WTO, concerns the application of food safety and animal and plant health regulations. Signatories responsibilities/obligations with regards to surveillance are outlined by IPPC Article I: 1 and 4; Article IV: 2(b), 2(e), 3(a) and 3(b); Article VII: 2(a), 2(g), 2(i), 2(j), 3, 4 and 6; Article VIII: 1(a), 1(b), and 1(c), and ISPMs No. 4, No. 6, No.8, No.10, No. 19 and No. 22.

## 1.2 What is surveillance?

The IPPC's ISPM No. 5 defines:

Surveillance as:

*An official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures.*

A survey as:

*An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area.*

Monitoring as:

*An official ongoing process to verify phytosanitary situations.*

A monitoring survey as:

*Ongoing survey to verify the characteristics of a pest population.*

### Box 1 Definition of surveillance

Pest surveillance

- ◆ involves
  - ❖ looking for and
  - ❖ recording the
    - presence
    - absence
    - distribution and
    - population levels of a pest;
- ◆ is conducted
  - ❖ over a period of time
  - ❖ in a scientific manner.

## 1.3 What is the purpose of surveillance?

The aim of surveillance is to supply current data on:

- ◆ pest presence and distribution, to fulfil the measures under the SPS Agreement, namely:
  - ❖ to prevent technical barriers to trade (Relevant IPPC Articles: IV 2(b), IV 2(e) and VII 2(j) and ISPMs: No. 6 and No. 8), and
  - ❖ to minimize interference with international trade in fulfilment of the:
    - transparency clause (Relevant IPPC Articles: VII 2(c), 2(i) and 2(j), VIII 1 and 1(a));
    - pest listing clause (Relevant IPPC Article VII 2 (i) and ISPM No. 19); and
      - early detection of pest
      - generate and maintain pest list;

- ◆ pest free areas (pest free places of production (PFPPs) and pest free production sites (PFPS)) and areas of low pest prevalence (Relevant IPPC Article II and ISPMs: No. 4, No. 8, No. 10 and No. 22) and reporting obligations.

IPPC's ISPM No. 5 defines:

A pest free area as:

*An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained.*

An area of low pest prevalence as

*An area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures.*

The main objectives of surveillance are to:

- ◆ conduct surveillance on:
  - ❖ pests of quarantine importance
  - ❖ alien invasive species
  - ❖ pests of economic and/or farming importance, and
  - ❖ natural enemies of pests
- ◆ develop and maintain a database and geographic information system which captures vegetation/ pest species surveyed and inspection details.
- ◆ provide information
  - ❖ on pest presence/absence (pest lists: national, regulated non-quarantine, quarantine)
  - ❖ for the conduct of pest risk analyses
  - ❖ to establish pest free areas
  - ❖ to facilitate transparency in international trade in fulfilment of obligations as a signatory to the WTO SPS Agreement.

The expected outputs of surveillance are to:

- ◆ develop a database on pest presence/absence (current pest lists: national, regulated non-quarantine, quarantine)
- ◆ illustrate the distribution of these pests presence using Geographic Information Systems
- ◆ illustrate and report on pest free areas
- ◆ provide pest incidence and distribution information to assist in determining management strategies for pests and
- ◆ promote transparency in trade thereby increased confidence with trading partners.

**Box 2 Benefits of surveillance**

1. Early detection of pest which allows for a rapid and effective response:
  - a. Eradication – the application of phytosanitary measures to eliminate a pest from an area.
  - b. Containment – the application of phytosanitary measures in and around an infested area to prevent spread of a pest.
  - c. Management or suppression - the application of phytosanitary measures in an infested area to reduce pest populations.
2. Expanding market access as markets requires evidence of:
  - a. Pest free area – illustrate the absence of pest.
  - b. Area of low pest of prevalence - An area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures.
3. Improved pest management as surveys allow one to detect population levels to determine when economic threshold is reached to warrant intervention.
4. Informed pest status -
  - a. Commodity pest list - A list of pests occurring in an area which may be associated with a specific commodity.
  - b. Host pest list - A list of pests that infest a plant species, globally or in an area.
  - c. National pest list – compilation of a list of pest occurring in a country.
  - d. Quarantine pest list - compilation of a list of pest not present in a country or may be present but under eradication or control.
  - e. Natural enemies presence/absence
5. Examine for quarantine breaches - high risk sites - where pest will spread upon breaching quarantine.

**1.4 What are the ISPM's that provide general guidelines/standards for surveillance?**

The WTO Member Countries must abide by the trading rules set by the WTO and the three recognised standard setting bodies: the Office International des Epizooties (OIE, which is the World Organisation for Animal Health), the Codex Alimentarius (which protect the health of consumers and ensure fair practices in the international food trade) and the IPPC (which regulates plant health in international trade). These standard setting bodies set standards/ guidelines to conduct trade safely with the least risk to Member Countries.

The Commission of Phytosanitary Measures of the IPPC has adopted a number of international standards/ guidelines that Member Countries must follow to trade in plant and plant products. The following ISPM's are those that must be adhered to with respect to surveillance and aspects of surveillance that impact on pest presence and distribution:

- ◆ ISPM No. 6 - Guidelines for Surveillance  
[http://www.acfs.go.th/sps/downloads/13717\\_ISPM\\_6\\_E.pdf](http://www.acfs.go.th/sps/downloads/13717_ISPM_6_E.pdf)
- ◆ ISPM No. 8 - Determination of Pest Status in an Area  
[http://www.acfs.go.th/sps/downloads/13730\\_ISPM\\_8\\_E.pdf](http://www.acfs.go.th/sps/downloads/13730_ISPM_8_E.pdf)

- ◆ ISPM 26 - establishment of pest free areas for fruit flies (tephritidae)  
[http://www.acfs.go.th/sps/downloads/133631\\_ISPM26\\_2006\\_E.pdf](http://www.acfs.go.th/sps/downloads/133631_ISPM26_2006_E.pdf)
- ◆ Fruit fly trapping Annex 1 to ISPM No. 26  
[http://faperta.ugm.ac.id/perlintan2005/puta\\_files/attach/ISPM%20DraftAnnex1FFtrapping.pdf](http://faperta.ugm.ac.id/perlintan2005/puta_files/attach/ISPM%20DraftAnnex1FFtrapping.pdf)
- ◆ Draft ISPM for member consultation: Establishment of areas of low pest prevalence for fruit flies (tephritidae)  
[https://www.aphis.usda.gov/import\\_export/plants/plant\\_exports/downloads/draft\\_ispm\\_ff\\_alpp.pdf](https://www.aphis.usda.gov/import_export/plants/plant_exports/downloads/draft_ispm_ff_alpp.pdf)

## 1.5 What are the types of surveillance activities?

ISPM's 5 and 6 defines various types of surveillance activities:

- ◆ General survey  
*is a process whereby information on particular pests which are of concern for an area is gathered from many sources, wherever it is available and provided for use by the NPPO.*  
For example, in compiling the national pest list the NPPO reviews literature from many sources: published literature (journals, books); unpublished literature (departmental records: annual reports, diagnostic reports); trade reports (interception records); etc.
- ◆ Specific surveys  
*are procedures by which NPPOs obtain information on pests of concern on specific sites in an area over a defined period of time.*  
For example, the conduct of a survey for a particular pest – giant African snail - in a specific area – high risk areas - over a specific time to determine pest presence/ prevalence. Specific surveys are conducted for regulated quarantine pests (that is, pests not known to be present in a country).
- ◆ Targeted or Detection survey  
*Survey conducted in an area to determine if pests are present.*  
For example, the conduct of a detection survey for a specific pest on a specific commodity grown in a particular area from which the commodity to be traded is grown. Targeted or detection surveys are used to monitor regulated quarantine pests at high risk sites to confirm that pest is still absent or if it has been recently introduced.
- ◆ Delimiting survey  
*Survey conducted to establish the boundaries of an area considered to be infested by or free from a pest.*  
For example, upon the detection of a pest, a survey is conducted around the index case to establish the extent of infestation. Delimiting surveys are used upon detection of a new introduction of a regulated quarantine pest or alien invasive species.
- ◆ Monitoring survey  
*Ongoing survey to verify the characteristics of a pest population.*

For example, upon the imposition of a control measure on a target pest a survey may be conducted to collect data on the pest's response. Monitoring surveys are used to determine the pest population characteristics upon the introduction of a biological control agent to reduce the pest population.

Other types of surveillance are:

◆ Active surveillance

*deliberate, coordinated effort by pest management personnel looking for the pest.*

For example the conduct of any of the surveys listed above.

◆ Passive surveillance

*activities where public, farmer, other industry personnel notify on pest presence; usually done through hotline, emails, farm visit records, interception reports, diagnostic reports.*

◆ Pathway surveillance

*surveys that target high risk sites to look for pests at specific intervals/time.*

## 1.6 What are the key terms used in surveillance?

Standardization of the terms used in reporting surveillance results allow for better communication between the plant regulatory personnel of trading partners. The vocabulary as defined by ISPM 5 used in surveillance is:

1. Endangered area - an area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss.
2. Ecosystem - a dynamic complex of plant, animal and micro-organism communities and their abiotic environment interacting as a functional unit.
3. Entry of a pest - movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled.
4. Establishment of a pest - the perpetuation, for the foreseeable future, of a pest within an area after entry.
5. Habitat - part of an ecosystem with conditions in which an organism naturally occurs or can establish.
6. Host range - species capable, under natural conditions, of sustaining a specific pest or other organism.
7. Incursion - An isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future.
8. Introduction (of a pest) - The entry of a pest resulting in its establishment.
9. Occurrence (of a pest) - The presence in an area of a pest officially recognized to be indigenous or introduced and not officially reported to have been eradicated.
10. Outbreak (of a pest) - A recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area.
11. Pathway - any means that allows the entry or spread of a pest.

12. Pest - any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products.
13. Pest introduction - the entry of a pest resulting in its establishment.
14. Pest record - A document providing information concerning the presence or absence of a specific pest at a particular location at a certain time, within an area (usually a country) under described circumstances.
15. Pest risk - the probability of introduction and spread of a pest and the magnitude of the associated potential economic consequences.
16. Pest status on an area - presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information.
17. Quarantine pest - a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.
18. Regulated area – An area into which, within which and/or from which plants, plant products and other regulated articles are subjected to phytosanitary regulations or procedures in order to prevent the introduction and/or spread of quarantine pests or to limit the economic impact of regulated non-quarantine pests.

## Chapter 2 Planning a survey

### 2.1 What are the requirements needed to plan a survey?

In order to conduct any type of survey, one must devise a survey plan or protocol. The survey protocol must be practical, scientifically and technically sound, feasible and cost effective. The survey protocol tells the surveyor how, where, what, how many, when and why. The survey protocol consists of:

- ◆ Field manual
- ◆ Laboratory manual
- ◆ Stakeholder awareness
- ◆ Data sheets

In Chapter 3 we will devise the survey protocol. This chapter describes what needs to be considered in devising the survey protocol.

#### Box 3 What the surveyor and diagnostician need to know?

How to:

conduct the survey; collect sample; package sample; transport/ship sample; process sample in laboratory

Where to:

look for sample; take or collect sample; send sample

What:

to look for in a sample; to do in collecting the sample; precautions to take so as not to be an agent of spread of pest and disease; questions to ask (data to collect); are the objectives of the survey

How many:

samples to collect; farms to sample; host to sample

When:

to conduct the survey

Why:

the survey is being conducted

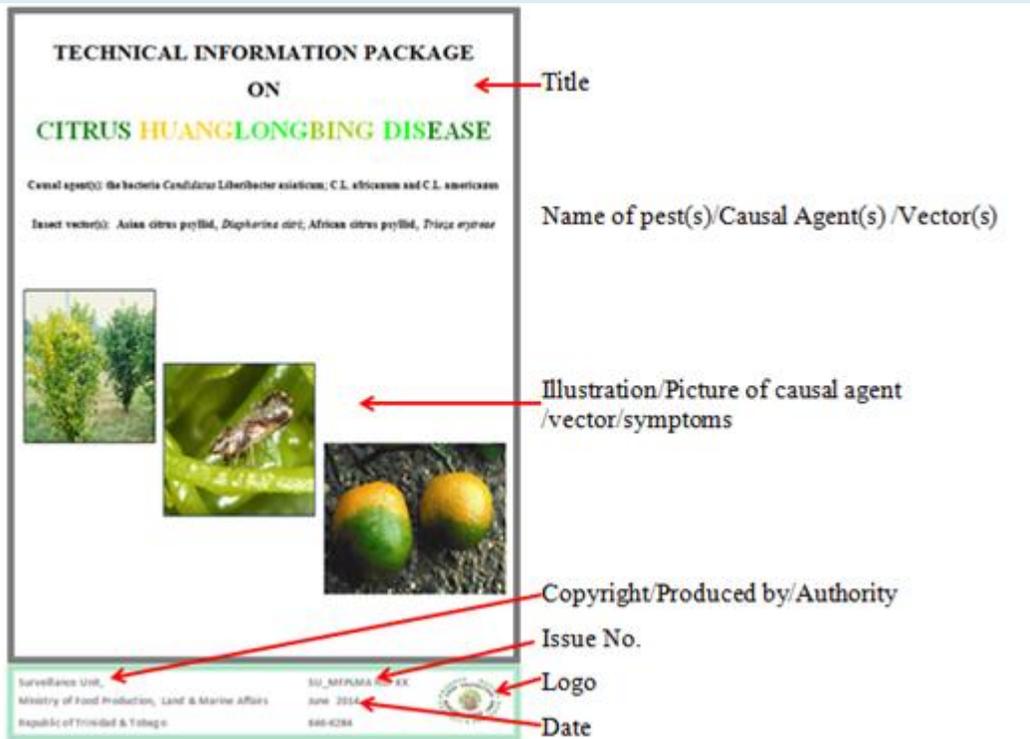
Basic information required for planning any type of survey is:

- a. Technical package on the pest (biology, life cycle, host, habitat, natural enemies, etc.)
- b. Host distribution (or list of farms or list of roads)
- c. Environmental data
- d. Population dynamics
- e. Map

a. Technical package

The technical package on the pest informs on the pest. It lays the foundation of sound science upon which the survey will be based. It provides justifications or rationale or reasoning for the particular actions in the protocol. It provides identifying details of the pest, its symptoms, means of diagnosing, and natural enemies. Box 4 list the contents a technical package should contain.

**Box 4 Technical Package: Cover and Table of Contents**



**Table of Contents**

- 1 Introduction
- 1.2 Summary of invasiveness (a brief description of why it is invasive - where it occurs, when it was first recorded as invasive, characteristics of its invasiveness e.g. ease of spread, establishment and impacts, impact on endemic or threatened native species / ecosystems)
- 1.3 Regulated pest/Alert list
- 2 Symptoms
- 2.1 Symptoms by affected plant part
- 2.2 Differentiation from other disease/nutrient deficiency symptoms
- 3 Host range (main, other, wild; aspects of host that predisposes them to attack; is host involved in international trade-thus likely to spread to other countries; pest stage associated with different plant parts)
- 3.1 Causal Agent
- 3.1 Vector
- 4 Spread (pathways of spread and rate – local/regional/international)
- 4.1 Transmission (biotic, abiotic, international, accidental)
- 4.2 Dispersal (natural, vector, international, accidental)

4.3	Affected plant parts
4.4	Affected plant stages
5	<u>Control</u>
5.1	Biological Control
5.2	Chemical Control
5.3	Cultural Control
6	<u>Impact</u>
6.1	Economic (quantitative details, frequency, severity)
6.2	Environmental (impact on species – competition, hybridization, parasitism, predation, etc.; habitat, ecosystem-processes: nutrient cycle, soil protection, waste decomposition, etc.; sites affected)
6.3	Human/Cultural (impact on human health/norms, livelihood, aesthetics, etc.)
7	<u>Phytosanitary risk</u> (risk of introduction to new areas through the various pathways of spread including accidental introduction or deliberate introductions associated with any intended uses; risk or likelihood of pest being a vector for or carrier of other diseases; listed as a quarantine pest elsewhere)
8	<u>Biology and ecology</u> (habitat; life cycle; environmental tolerances and adaptability – temperature, relative humidity, rainfall, soil type and conditions, water and water conditions, pH; reproduction, reproductive capacity/propagules; motility; pioneering; competitive ability; nutrition –range)
8.1	Causal Agent
8.2	Vector
9	<u>Names of disease</u>
10	<u>Taxonomy of causal organism</u>
10.1	Taxonomic position
10.2	Notes
11	<u>Morphology/Description</u>
11.1	Causal agent
11.2	Vector
12	<u>Similarities to other species</u>
13	<u>Detection and inspection methods</u>
14	<u>Diagnostic /Identification methods</u>
15	<u>Geographic distribution</u> (native or introduced/alien/exotic; deliberate or accidental introduction, invasive; localized/widespread; domesticated/bred in captivity; presence or absence; area of origin, date of introduction to other areas; pathway of introduction)
15.1	Causal Agent
15.3.1	Vector
15.3	Free areas
16	Current work
17	Legal (laws, regulations governing the pest)
18	<u>Bibliography</u>

#### **Box 5 Sources of literature to assist in developing technical packages**

- i. USDA\_APHIS New Pest Response Guidelines  
<http://www.aphis.usda.gov/wps/portal/aphis/resources/manualsandguidelines/>
- ii. Diagnostic Protocols, Handbooks and Contingency Plans from Plant Health Australia  
<http://www.planthealthaustralia.com.au/wp-content/uploads>
- iii. National Centre for Integrated Pest Management <http://www.icar.org.in/files/>

b. Host distribution / list of farms

The distribution of host plants will indicate the potential areas the pest may be found or can spread.

c. Environmental data

Environmental data indicate the potential areas that will favour the pest's establishment, thus where the pest can be found or spread.

d. Population dynamics

Some pests may be introduced through ethnic population or visitors. Identifying ethnic settlements can assist in identifying high risk areas of likely introduction of exotic pests.

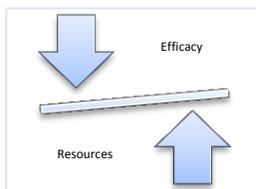
e. Map

Human and financial resources are usually the limiting factors that determine the intensity of sampling in the survey design. Geographic information systems allow us to combine the various factors to be considered in designing the survey methodology in a systematic manner to inform on the minimal areas that may be sampled to inform on pest presence and distribution based on sound science.

In its simplest form, a map of the country is used and each factor is overlaid, like a transparency, and the intersecting areas of pest-host-environment-human interaction illustrate the sites most suitable for the pest's introduction, establishment and spread.

## 2.2 How the factors affect the survey design?

When developing the survey design, it is critical to strike a balance with needed efficacy and available resources. Many factors will influence the survey design: ranging from the biology of the pest to available resources and political/administrative processes. This manual is to be used as a framework for the planning and executing of surveys. The goal of this section is to provide the information in determining the needed resources to accomplish the survey.



Factors that affect the survey design include those of the:

a. Pest-host-environment-human interaction that determine the sites (HIGH RISK AREAS) that a particular or target pest may be introduced, established, found or spread such as:

- ◆ Host – number of, distribution, commercial management practices affect survey design.
  - The number and distribution of host plants influence the survey sampling design as it informs on the sites to be sampled. It will assist in determining what will be the smallest unit of area to be sampled. For example, the smallest unit to be sampled vary with the pest, viz:
    - ❖ pest of food crops – farm;
    - ❖ pest of storage crop – warehouse, supermarket;
    - ❖ pest of ornamentals – malls, parks, etc.;

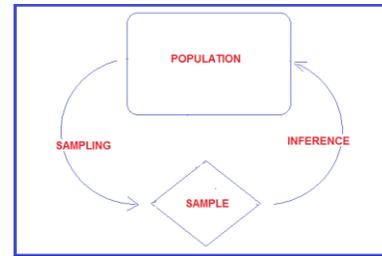
- ❖ pest of a backyard plant - a household plot along roadway.
  - ◆ Pest – ecology, habitat conditions, biology and life cycle, previously reported presence and distribution, transmission and spread, natural enemies and their distribution – influence the areas at risk. The mode of attack, time of attack, prevalence, and symptomology displayed influence the timeline for the survey.
  - ◆ Environment - climatic and physical suitability that will allow for the sustenance of the pest and completion of its life cycle.
  - ◆ Human – explores the likelihood that the pest can be dispersed by human through the commodity pathway (seed/planting material suppliers, nurseries, packing house, ethnic settlement or visits, marketers, workers/equipment operators/extension officers, waste disposal). This risk is usually summarised in the PHYTOSANITARY RISK - PATHWAY OF INTRODUCTION. The phytosanitary risk is usually rated ‘low’, ‘moderate’ or ‘high’ and is based on the probability of the pest’s entry, establishment, spread and consequences. The overall risk rating is also used to prioritise pest for surveillance, when resources are limited.
- b. Laboratory processing capacity - The size of the laboratory’s physical infrastructure determines the space available for processing samples for identification. For example if rearing is required, the area will determine the number of jars that can be accommodated, hence the number of samples that can be processed at a given time.
- c. Human resources available for conducting survey and processing samples in the laboratory. Usually there are more personnel available to conduct the survey, but the laboratory staff may be restrictive and therefor the survey duration will be restricted by the laboratory’s processing capacity.
- d. Timeline available for conducting survey which is determined by the pest life cycle; pest-host interaction: when pest most actively found or symptomology expressed; pest management programmes; and climatic conditions – some pests are best observed during a dry spell others require high moisture and humidity.
- e. Economics – of the survey is the economic/political/social impact of the pest versus the cost of surveillance.

## **2.3 Developing the sampling methodology**

### **2.3.1 What are key terms used and their meaning?**

Standardization of the language used in the conduct of surveillance will facilitate better understanding and thus trust amongst plant regulatory personnel of trading partners. The common terms used in describing the survey design and their meaning and relevance are listed below.

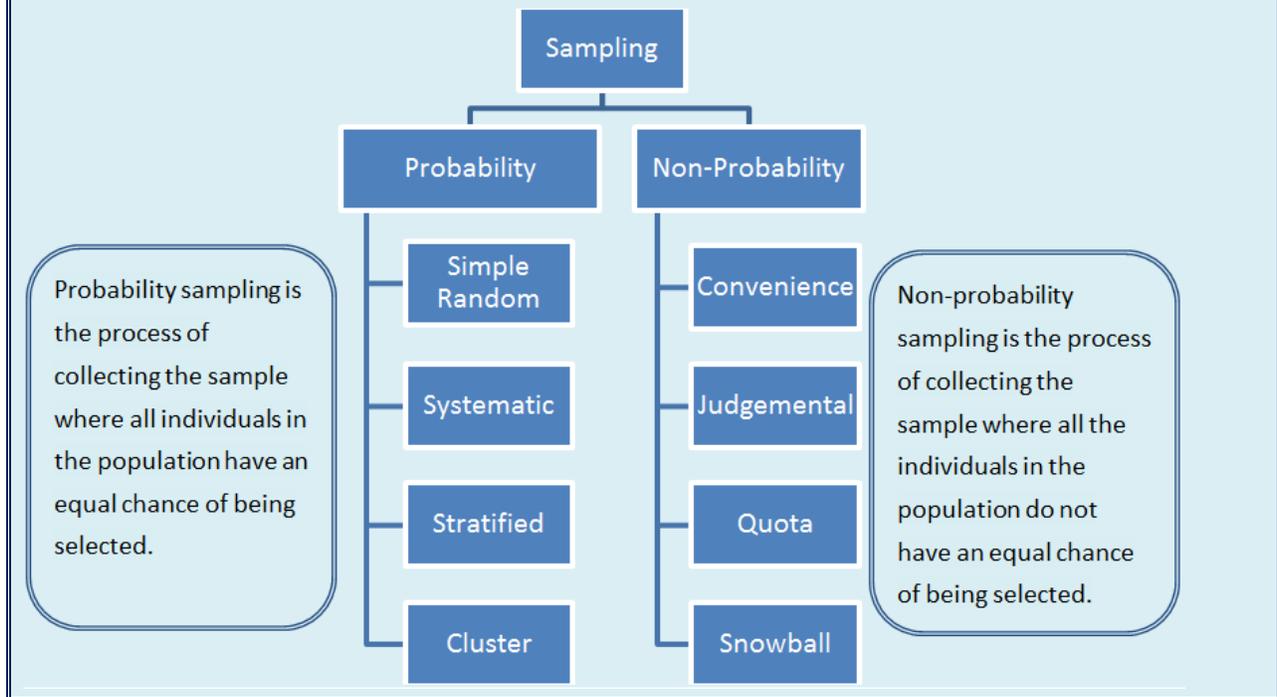
1. **Sampling** – is an information collection tool. It refers to the process of selecting a representative portion of the population. The observations obtained from studying the sample are then used to make inferences about the population. Sampling determines how credible and/or reliable the results are.
2. **Sample** – is a subset of the members from a larger population that is collected and analysed to make inferences. For example, in studying Black Sigatoka Disease on *Musa* – the sample is the infected *Musa* leaf.
3. **Sampling unit** – can be defined as the smallest unit, from which information is gathered. In the above example, is the smallest unit the *Musa* plant or the farm? Usually, the smallest unit is the farm as a number of leaves from different varieties can be selected from the farm. The data will be analysed on a per farm basis and not a per plant basis.
4. **Size of Sample** – is defined as what constitute a sample. What will be collected: a whole leaf, part of the leaf or five leaves to study from the farm? The size of the sample can be stated as: collect 3 pieces (6'' x 6'') of infected leaf from each *Musa* variety present on the farm.
5. **Sample size** – is defined as the number of members of the population being studied or the number  $n$  of sampling units that are selected from the population; for example 350 farms from the 1000 sweet potato farms registered will be examined for *Megastes*. Generally, the larger the sample size, the greater the likelihood the sample will be representative of the population.
6. **Sampling intensity** – is defined as the proportion/ percentage of the population being sampled.



### 2.3.2 Sampling techniques

The sampling methodology is a major part of the survey design. The sampling method or technique chosen is determined by the aim or purpose of the survey, factors affecting the survey design as stated in Section 2.2 and the resources available. Sampling techniques can be broadly classified into probability and non-probability sampling as described in Box 6. Each sampling technique is briefly described and an example of how it can be applied in plant protection is given.

**Box 6. Classification of sampling techniques**

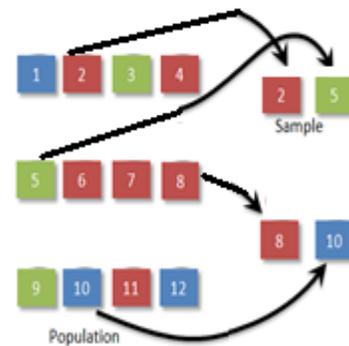


Random sampling - Each individual of the study population has an equal probability or chance of being selected.

Assign each individual a number:

- ❖ place in a hat, draw the required number of samples out; or
- ❖ select the required number of samples using a random number table; or
- ❖ select the individuals randomly generated by the computer.

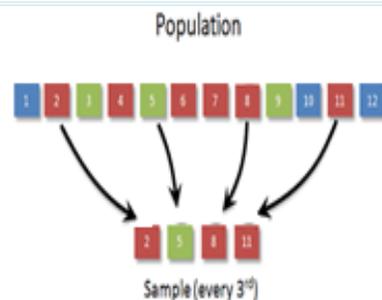
This is the most frequent method used in all surveys.



Systematic sampling - the  $N^{\text{th}}$  individual is sampled.

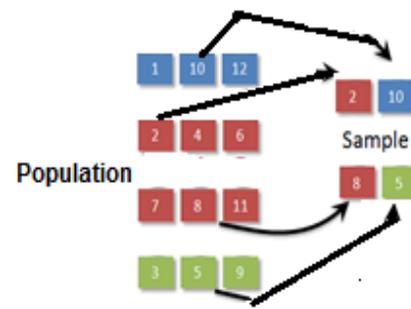
Assign each individual a number, randomly select the starting point then, every  $N^{\text{th}}$  occurrence, for example every 3<sup>rd</sup> individual. Care must be taken to ensure that the list does not contain any hidden order – age, sex, variety, etc.

This method is often used in surveys where the host (a backyard plant or ornamental) is distributed along the roadways. The survey design usually is a  $x$  km grid and in each grid the major or minor road is selected to be surveyed depending upon the distribution of the host. A random point along the road is chosen to begin the survey and thence every  $N^{\text{th}}$  km thereafter.



**Stratified sampling** - is achieved by separating the population into non-overlapping groups called strata and then obtaining a proportional simple random sample from each group. The individuals within each group should be similar in some way.

Assign each individual in a group based on a characteristic, for example: varieties, land owners and squatters; pesticide use and organic farming, then randomly select individuals from each stratum.



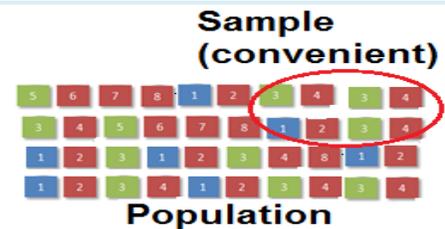
**Cluster sampling** - is achieved by selecting all individuals within a randomly selected collection or group of individuals.

This technique is used when the population is already broken up into clusters or groups and each cluster represents the population. The clusters are numbered and then clusters are randomly selected. Usually practiced in inspecting imports at the port – only a number of crates/boxes (clusters) are opened and inspected; not all.



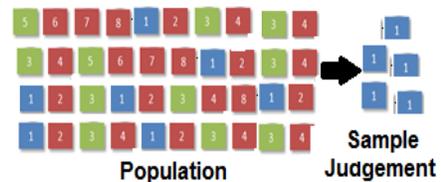
**Convenience sampling** is using the most convenient or readily available individuals to get a gross estimate of the results.

The individuals are chosen because they are present, for example, all solanaceous crops received in the laboratory for diagnosis will be used in the study.



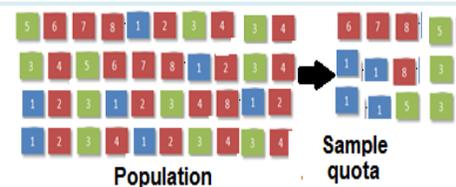
**Judgment sampling** selects the sample based on judgment.

The sample is chosen because it has the desired trait that is being studied. This is used in detection surveys. The sample is chosen because it exhibits the typical symptoms/damages expressed by the pest infestation.



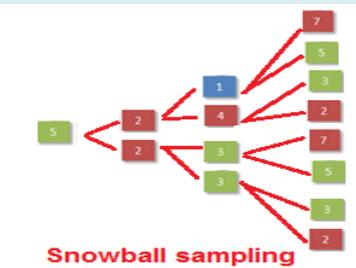
**Quota sampling** selects the first individual that meets the criteria until the desired number of samples is obtained.

The criteria are set by the surveyor as in judgement sampling. But here, only a specified number of samples or quota is desired. For example, 5 leaf samples displaying the desired symptoms from each variety of *Musa* present on the farm will be collected.



Snowball sampling relies on referrals from initial subjects to generate additional subjects. A small number of individuals with the desired trait is identified and used to find others who in turn identify others.

It is used in surveillance when performing trace backs and trace forwards to track the origin of the pest infestation and predict the further spread, respectively.



**Box 7. Easy Reading in sampling and sampling techniques**

- i. <http://www.statpac.com/surveys/index.htm#toc>
- ii. <http://stattrek.com/survey-research/sampling-methods.aspx?Tutorial=AP>
- iii. <http://www.dissertation-statistics.com/sample-size.html>
- iv. <https://faculty.elgin.edu/dkernler/statistics/ch01/1-4.html>
- v. <https://explorable.com/what-is-sampling?gid=1578>
- vi. <https://www.surveymonkey.com/mp/sample-size-calculator/>

**2.3.3 Detection tools**

The survey protocol must also consider the type of detection practices that are required with respect to the target pest. Detection practice can differ with the type of pest: pathogen, insects, weeds, mollusc, mites etc. In detection surveys, for most types of pest, visual inspections – where the surveyor is actively looking for the signs/symptoms/damages caused by the pest - are necessary, however for mobile pests – gastropods and insects - active and passive capture methods are used. Box 8 summarizes the detection practices for the various types of pests.

**Box 8. Detection practices commonly used for the various pest types**

PEST	DETECTION PRACTICE
Pathogens (fungi, bacteria, viruses, viroids, phytoplasmas, mycoplasmas)	Visual inspection looking for characteristic symptoms
Nematodes	Soil sampling
Weeds	Visual
Insects	Visual and/or active or passive capture
Gastropods (slugs and snails)	Visual and/or active or passive capture

For a listing of many USDA approved survey methods: <http://caps.ceris.purdue.edu/approved-methods>

Active capture is defined as a method which requires the surveyor to be involved in the collection process. Passive capture is defined as a method which doesn't require a collector. Each method has some equipment needs associated. Thus in planning the survey design, it is important to consider the collection

methods because it will impact on the diagnosis and resources required. Below is a small list of commonly used equipment.

a. Active capture equipment

1. ASPIRATOR – used to capture small and fast moving insects, which is easily collected by hand. It comprise of a vial, a tight fitting cork, rubber stopper, or other cap with two metal tubes running through it (Fig. 1). One tube has a rubber hose with a mouth piece on the outer end and a piece of fine mesh covering the end inside of the vial which prevents insects being sucked into ones mouth. The other tube is longer and flexible to collect the insect. To collect insects, one sucks air through the rubber hose and points the other tube at the insect - the insect is sucked into the vial

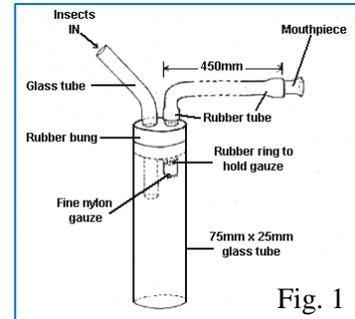


Fig. 1

2. NETS - There are three types of insect collecting nets: aerial, sweeping, and aquatic. A collecting net is composed of a net bag made of cloth or fine mesh that is attached to a wire hoop, affixed to a wooden or metal pole.

- i. Aerial nets are used for flying insects or those perched on something (bees, wasps, dragonflies or butterflies). The bag is made of a white or black tightly meshed material - soft nylon, organza, cotton or silk (Fig. 2). Larger hoops are used for large and fast moving insects; and smaller hoops are used for smaller insects. The handle is made of lightweight aluminium or wood and is extendable.



Fig. 2

- ii. Sweep nets are used to sweep through vegetation to collect insects not easily seen (Fig. 3). The bag is made of unbleached, heavy, sturdy, close-weave twill material - sailcloth, canvas, muslin - to drag through dense vegetation without being damaged. The handle needs to be more sturdy and heavy and usually made from wood.



Fig. 3

- iii. Aerial/ sweep nets are multipurpose general collecting nets that can be used as aerial nets or sweeping through dense vegetation (Fig. 4). The bags are made from a combination of heavier cloth with the apical third of the bag of fine mesh material.



Fig. 4

- iv. Aquatic nets are used to drag through the substrate of aquatic habitats, collect and deposit the material into a large white pan to sort through. The bags are made from a heavy duty material. The hoop is a D-shaped, square to triangular thick wire hoop. The handle is long and heavy.



Fig. 5

3. BEATING TRAY – is a simple white heavy duty cloth stretched over a circular hoop or over a frame of two crossed sticks (Fig. 6 and 7). It can be placed on the ground or held in place under branches, shrub etc. to catch the insects. The vegetation is “beaten” with a stick or shook to

displace the insects to fall onto the beating tray. The insects are then collected by hand. It is used to collect insects which are difficult to spot by casual observation. Beating sheets can vary in size, but a typical beating sheet would be about 3 feet square.



#### b. Passive capture equipment

Passive capture usually employs a trap with an attractant. The attractant manipulates the insect's sense of sight and /or smell. Some insects have an affinity for light or scent for finding food or mating. Traps can be an important tool in plant protection for pest detection or in its management. When traps are used as a tool to control pests, trap efficiency is related to the mobility of the pest, pest population density, attractiveness of the attractant and the trapping density. Trap design is influenced by the biology of the target pest. The basic types of traps used in general agriculture practices are listed below.

1. PITFALL traps - are tools for detecting walking and crawling soil and litter inhabiting arthropods. A pitfall trap is a container that is sunk into the ground so that its rim is flush with the soil surface. It is covered to keep rain out (Fig. 8). Insects and other arthropods are captured when they fall into the trap. Pitfall traps usually contain a killing / preserving agent such as anti-freeze, soapy water, or ethyl alcohol to prevent the pest from escaping or preying on each other.

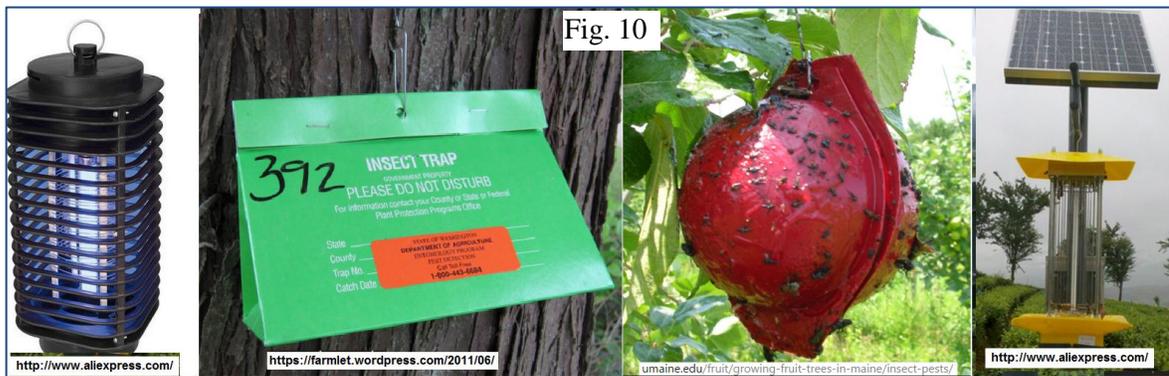


For more on pitfall traps: [http://pubs.ext.vt.edu/444/444-416/444-416\\_pdf.pdf](http://pubs.ext.vt.edu/444/444-416/444-416_pdf.pdf)

2. BAIT STATION OR ATTRACT AND KILL traps – uses a food source (proteins, sugars, fruit, piece of plant tissue, or yeast) as the attractant to lure the pest in. The success of the bait trap depends on the bait, the better the bait, the better the catch; poor sanitation will negate its effectiveness. Bait stations are often used in control activities (fruit flies) but for animals like snails and slugs they can be used in detection or delimiting surveys. Traps vary in shape, size, construction, and colour based on the behaviour or ecology of the target pest. It consists of a bait or lure, entry and some mechanism for holding the pest. The trap doesn't have to be expensive. It can be constructed of simple materials as shown in Fig. 9.



3. LIGHT traps – uses different wavelength of light as the attractant to lure the pest in. Traps vary in shape, size, construction, light source/colour based on the behaviour or ecology of the target insect and some mechanism to trap the pest – soapy water in a bulk container or glue on sticky traps (Fig. 10). For example, brightly coloured sticky traps can be: covered or enclosed structures; has soapy liquid or insecticide or an adhesive that causes the insect to stick on the panel. Traps can be suspended or placed on the ground. Traps with green light are used for *Eusepes postfasciatus*.



4. PHEROMONE traps – uses semiochemicals to attract the target insect. Pheromones are semiochemicals produced and released by the organism that enable it to communicate with other members of its own species to affect their behaviour; some examples are:

- ◆ Alarm pheromone – cause the insects to move toward the region of increasing concentration, their response changes to one of alarm, then they hasten their efforts to remedy the disturbance – examples seen in ants and honey bees.
- ◆ Trail pheromone – release to map /guide the others members of its own species especially in searching for food.
- ◆ Repellent pheromones – release to keep other insects away.

- ◆ Queen pheromone – release by queen bee to induce the workers to feed and groom her; inhibit the workers from building queen cells and rearing new queens; and inhibit ovary development in the workers.
- ◆ Aggregation pheromones – usually male-produced sex attractants which causes both sexes to arrive or aggregate to the site. Found in many family Coleoptera, Dictyoptera, Hemiptera, Homoptera and Orthoptera.
- ◆ Sex pheromones – released by the female to attract its mates.

The majority of lures used in agriculture are based on sex pheromones. It can be used to attract the males or caused confusion in the males, that is, "disrupt communication" thus preventing the sexes from meeting and mating. Sex pheromones can be used in surveys for early detection and in delimiting surveys. Sex pheromones are sex-specific and will not attract males and females; will not attract immature insects and are specific to a certain group of insects. Sanitation is key to its success.

Pheromone traps can be constructed as in the light trap but the pheromone is usually hung inside the trap to attract the insect inside. The pheromone is impregnated in a release device often a septa, capsule or string. Soapy water or insecticide can be used to kill the insect. Sticky glue surface can also be used to hold/trap the insect. Fig. 11 shows an assortment of traps using pheromones to attract the pest.



Traps may utilise any combination of techniques above. Box 9 gives a listing of references to assist in determining the pest capture method.

**Box 9. Sources of literature to assist in determining the most appropriate insect capture method/tools**

- i. Insect collection methods by Joe MacGown available at [http://mississippientomologicalmuseum.org.msstate.edu/collecting.preparation.methods/Collecting.methods.htm#.Vfy\\_St9Viko](http://mississippientomologicalmuseum.org.msstate.edu/collecting.preparation.methods/Collecting.methods.htm#.Vfy_St9Viko)
- ii. Insect trapping guide by A.J. Gilbert et al available at [https://www.cdfa.ca.gov/plant/pdep/Insect\\_Trapping\\_Guide/docs/Insect\\_Trapping\\_Guide\\_web.pdf](https://www.cdfa.ca.gov/plant/pdep/Insect_Trapping_Guide/docs/Insect_Trapping_Guide_web.pdf)
- iii. <http://www.pherobase.com/>

Box 10 is an example of how traps can be used to perform surveillance or monitor for fruit flies. Box 11 provides links to references that may be helpful in planning and executing surveys on fruit flies.

### Box 10. Fruit fly trapping

The IAEA's Trapping Guidelines for Area-Wide Fruit Fly Programmes states that Tephritid fruit flies cause devastating losses to fresh fruits and vegetables, thus having a great impact on international trade in agricultural produce. As major quarantine pests, control of fruit flies usually involve area-wide national or regional (trans-boundary) control programmes. The IAEA's trapping guidelines provides strategic guidance and direction on where and how to implement surveys in support of fruit fly control and quarantine activities. Some of these are briefly described below for fruit flies trapping in the OECS and the wider Caribbean.

#### ◆ Traps used in fruit flies trapping

1. Mc Phail - is a glass trap with a water reservoir (Fig. 12). Flies enter from below through the opening and drown in the solution. The trap is baited by filling the reservoir with water and Torula yeast and borax pellets. Water is added to the level just below the inside lip of the trap so that minor tilting of the trap will not cause spillage. Usually used against *Anastrepha spp.*, *Bactrocera spp.*, and *Ceratitis spp.*
2. Multi Lure - consists of an inverted funnel base and a transparent cover on top with a plastic dispenser holder for the pheromone (Fig. 13). A soap water solution is placed inside the inverted funnel. Insects are attracted to the pheromone and enter the trap through a hole in the inverted funnel base. Once inside the trap, insects fly around the cover, until exhausted and fall into the soap water solution contained in the inverted funnel base where they drown.
3. Jackson – consists of a body made of waxed cardboard material that is folded into a delta shaped object (Fig. 14). The lure is placed on a cotton roll wick, supported inside the trap by a wire wick holder. A sticky insert on the bottom captures flies. The wire hanger placed at the top of the trap body is used to suspend the trap.



#### ◆ Lures/attractants used in fruit flies trapping

Lures that can be used are food and pheromones. It can be used alone or in combination.

- a. Torula yeast - is a food attractant, high in protein. It is a tool widely used in detection and delimiting survey, for all fruit flies.
- b. 3-component lures are synthetic food lures that attract primarily female flies. They consist of

putrescine, ammonium acetate, and trimethylamine patches. They are used for Mediterranean fruit fly (Medfly) trapping.

- c. Tri Med Lure – is a synthetic female pheromone that disrupts communication such that the males cannot find the females to mate with. It is expensive and quite volatile. It is used in detection and delimiting survey for Medfly.
- d. Cue lure - is a synthetic kairomone, a very potent male insect attractant for *Bactrocera* fruit flies. It has not been isolated as a natural product. However, it is the most potent attractant for *B. cucurbitae* males and used worldwide in its detection.
- e. Methyl eugenol – is a kairomone used to attract many species of the Subfamily Dacinae. The chemical occurs naturally in more than 450 plant species. It is widely recognized as the most powerful male lure currently in use for detection, control, and eradication of any tephritid species. It is used to detect infestations of *Bactrocera dorsalis* or for male annihilation or killing of sexually immature males before they were able to mate with females.

The lure, trap and target pest association can be summarised as:

Lure	Trap	Target
Torula Yeast Pellets	McPhail trap Multi Lure trap	<i>Anastrepha</i> , <i>Bactrocera</i> , and <i>Ceratitis</i> spp.
3 Component	Multi Lure trap	<i>C. capitata</i> and <i>C. rosa</i>
Tri Med Lure	Jackson trap	<i>Ceratitis</i> spp.
Cue lure	Jackson trap	<i>Bactrocera</i> spp. ( <i>B. albistrigata</i> , <i>B. cucurbitae</i> , <i>B. tryoni</i> , <i>B. facialis</i> )
Methyl Eugenol	Jackson trap	<i>Bactrocera</i> spp. ( <i>B. dorsalis</i> , <i>B. zonata</i> , <i>B. correcta</i> ).

- ◆ Lure preparation is a critical step to the process and care must be taken to ensure both the lure's quality and that toxicants are added at appropriate levels. Liquid lures are sold in bulk or can be brought as impregnated plastic matrix plugs that are individually wrapped and ready to use. Liquid lures are cheaper but care (safety precautions) must be taken in handling them. General direction for its mixing are:
  - a. Torula yeast pellets - Mixed with 10% Propylene Glycol and water.
  - b. Cue lure - Liquid is mixed with 5% dibrome before adding to cotton wick.
  - c. Methyl Eugenol - Liquid is mixed with 1% dibrome before adding to cotton wick.
- ◆ Ordering traps and lure
 

There are two critical areas that need to be considered when ordering traps and lures - quality and quantity. Material and supplies when combined, likely make up less than 10% of the overall cost of the survey when travel cost, salaries and other expenses are added. In passive detection methods like trapping the success of survey hinges on the quality of the traps and lures. Quality is critical because issues like lure purity can have a large negative impact on efficacy of traps.

Quantity – in addition to calculating the number needed based on the survey design, a percentage loss must also be taken into consideration. Losses result from wind, people, and animals. Other factors to consider in calculating the quantity of lures and traps required are:

- ◆ duration of the survey
- ◆ number of sites
- ◆ number of traps at each site
- ◆ lures needed per trap
- ◆ lure longevity: storage life and length of effectiveness in field
- ◆ trap longevity or storage life.

It is impossible to cover calculation or cost of supplies for multiple countries in a single manual. It is up to the NPPO to consider as many factors as practical when considering purchasing traps and lures.

#### **Box 11. Reading on fruit flies trapping**

- i. Trapping guidelines for area-wide fruit fly programmes by International Atomic Energy Agency available at [http://www-pub.iaea.org/MTCD/publications/PDF/TG-FFP\\_web.pdf](http://www-pub.iaea.org/MTCD/publications/PDF/TG-FFP_web.pdf)
- ii. Development of Improved Attractants and Their Integration into Fruit Fly SIT Management Programmes by Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture [http://www-pub.iaea.org/MTCD/publications/PDF/te\\_1574\\_web.pdf](http://www-pub.iaea.org/MTCD/publications/PDF/te_1574_web.pdf)
- iii. [http://www-pub.iaea.org/MTCD/publications/PDF/TG-FFP\\_web.pdf](http://www-pub.iaea.org/MTCD/publications/PDF/TG-FFP_web.pdf)
- iv. California Department of Food and Agriculture Insect trapping guide [https://www.cdffa.ca.gov/plant/pdep/Insect\\_Trapping\\_Guide/docs/Insect\\_Trapping\\_Guide\\_web.pdf](https://www.cdffa.ca.gov/plant/pdep/Insect_Trapping_Guide/docs/Insect_Trapping_Guide_web.pdf)

## **2.4 What data needs to be collected?**

Determining what data are required aids in developing the Data Form. The Data Form consists of two components:

- ◆ Field, and
- ◆ Laboratory.

The Data Form should be kept simple and reduce the amount of writing to be done. If possible, a list of choices should be given to allow the surveyor to tick the most appropriate. Instructions should be simple, clear and easy to follow.

ISPM 6 recommends that the data form should contain the following information:

- ◆ scientific name of pest, family/order
- ◆ scientific name of host plant part affected
- ◆ means of collection (e.g. attractant trap, soil sample, sweep net)
- ◆ locality, e.g. location codes, addresses, coordinates

- ◆ date of collection and name of collector
- ◆ date of identification and name of identifier
- ◆ date of verification and name of verifier
- ◆ references, if any
- ◆ additional information, e.g. nature of host relationship, infestation status, growth stage of plant affected, or found only in greenhouses.

The layout must be logical, keeping like things together making it easy for the surveyor to gather and record the data required. Data Form can be sectionalised to such as:

- |   |  |
|---|--|
| <ol style="list-style-type: none"> <li>1. Field             <ul style="list-style-type: none"> <li>◆ Enumerator</li> <li>◆ Location</li> <li>◆ Cultivation</li> <li>◆ Symptoms</li> </ul> </li> <li>2. Entomology Laboratory             <ul style="list-style-type: none"> <li>◆ Laboratory Technician</li> <li>◆ Presence of insect observed</li> <li>◆ Counts</li> </ul> </li> </ol> | <ol style="list-style-type: none"> <li>◆ Diagnosis</li> <li>◆ Certifying Officer/Entomologist</li> <li>3. Plant Pathology Laboratory             <ul style="list-style-type: none"> <li>◆ Laboratory Technician</li> <li>◆ Symptoms observed</li> <li>◆ Test performed</li> <li>◆ Diagnosis</li> <li>◆ Certifying Officer/Plant Pathologist</li> </ul> </li> <li>4. Natural enemies</li> </ol> |
|---|--|

Box 12 gives an example of a data form for entomological use and plant pathological use.

**Box 12. Data Form – Detection survey protocol for sweet potato weevils/Black Sigatoka Disease**

**Detection survey protocol for weevils in sweet potato**

**16 Data Sheet**

**Field: (Fill in the Blanks)**

Officer:..... Date:..... Sample # C...../D...../F.....

**Location: (Fill in the Blanks or Tick where appropriate)**

Farmer/Owner:.....

Address:.....

.....

Easting:..... Northing:.....

District:..... Parish: .....

Plot size:.....acres

**Symptoms/pest observed (Tick as much as possible):**

1. Sweet potato weevils:  Cylas adults       Euscepes adults       larva
2. Damage seen:  shot holes leaf       shoot damage       puncture holes on stem  
 puncture holes tuber       tunnels tuber

3. Other insects:  Yes:       No      Names:.....  
 .....  
 .....

4. Comments.....  
 .....  
 .....  
 .....

**Detection survey protocol for weevils in sweet potato**

**Entomology Laboratory: (Fill in the Blanks)**

Date:.....      Receiving Officer:.....      Entomologist:.....

**Presence of insect observed (Fill in the Blanks or Tick as much as possible):**

1. Sweet potato weevils:  Cylas adults       Euscepes adults       larva
2. Damage seen:  shot holes leaf       shoot damage       puncture holes on stem  
 puncture holes tuber       tunnels tuber

3. Other insects:  Yes:       No  
 Names: .....  
 .....  
 .....

**Counts**

Date	No. Sweet potato weevils			Natural enemies (Name and No)		Comments
	Eggs	Nymphs	*Adult			

\*C= Cylas; E= Euscepes

Identification Confirmation:  Yes  No      Samples shipped to.....

Findings:.....

Entomologist signature: .....      Date: .....

**Data Form for Black Sigatoka Disease**

APPENDIX 1

**RECONNAISSANCE SURVEY FOR BLACK SIGATOKA DISEASE IN TRINIDAD  
FIELD DATA FORM**

Officer: ..... Date: d: ..... m: ..... Y: 04      <sup>1</sup>Sample Point#.....

Location: (Fill in the Blanks or Tick where appropriate).

Road: Random starting point from Boundary of County:  0km       0.5km       1km

Location of County Boundary: .....

House #.....      LP#.....      mm.....      Distance from Sample Pt 1 .....

Landmark .....

Road .....      Village .....

Town: .....      District .....      County .....

Market:  Wholesale     Retail       Plantain farm     Road:  Residence     Other

Musa farm:  Monoculture     Mixed      Size of Musa plot: ..... acres..... stools

Cultivation: (Tick where appropriate)

Sanitation:     none       pruning       removal of debris       pruning and removal

Cultural practice:  manure       fertiliser       nematicide       insecticide       fungicide

none

Plant/Disease: (Do not leave any blanks)

	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10
Varieties										
# of stools										
# of plants/stool										
# of leaves on plant										
# of infected leaves on plant										
Youngest leaf infected (YLI)										
Stage of lesion on YLI <sup>2</sup>										

Varieties: Plantain    Lacatan<sup>3</sup>    Silk    ~~Sucrier~~    Gros Michel    Moko    ~~Matabora~~    Other (state).

<sup>1</sup> Unique number for each sample point

<sup>2</sup> 2, 3, 4, or 5 as described in manual.

<sup>3</sup> Lacatan includes giant Cavendish, dwarf Cavendish, Robusta, cooking fig.

**RECONNAISSANCE SURVEY FOR BLACK SIGATOKA DISEASE IN TRINIDAD  
LABORATORY DATA FORM**

(Fill in the Blanks or Tick where appropriate).

Date received: d: ..... m: ..... yr: 04      Receiving officer: .....

Plant Pathologist: .....

Most advanced stage of lesion found on leaf # 3:     none    1    2    3    4    5

Spores found: (Tick where appropriate)

	Black Sigatoka	Yellow Sigatoka	<i>Cordana</i>	<i>Chloridium</i>	<i>Ramichloridium</i>	<i>Cladosporium</i>	<i>Zygothiala</i>
Plantain							
Lacatan							
Silk							
<del>Sucrer</del>							
<del>Croa</del>							
Michel							
Moko							
<del>Mataborap</del>							
Other (state)							

Comments:

.....

.....

.....

.....

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.....

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.....

.....

.....

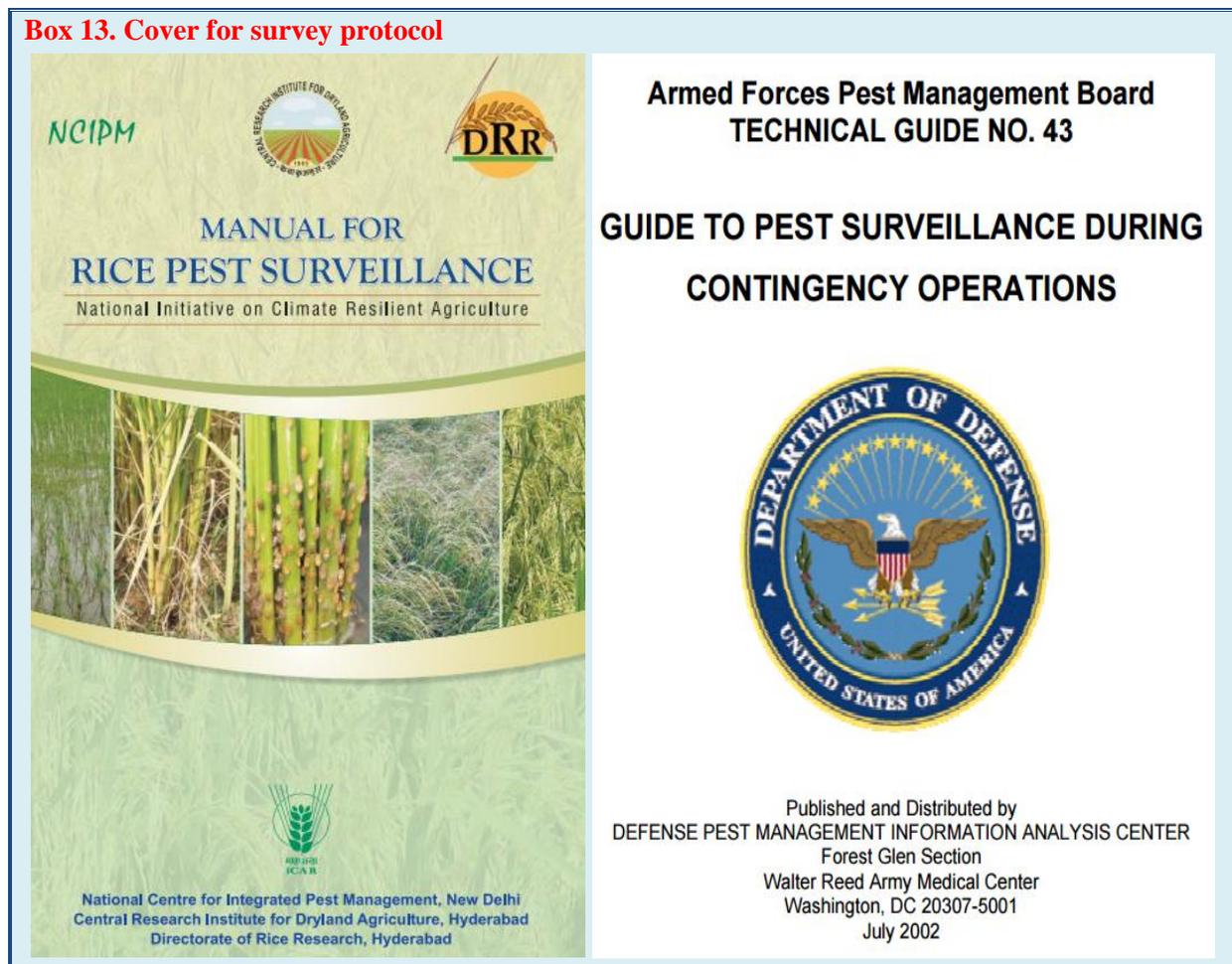
### Chapter 3 Developing the survey protocol

The survey protocol document consists of:

1. Title - which inform on type of survey to be done and the survey area, that is, identifying the scope (e.g. geographical area, production system, and season).
2. Author(s) – gives credibility to the survey design, procedures and pest identification.
3. Name of the target pest – both the scientific name and local name.

These are usually found on the cover of the document as show in Box 13.

#### Box 13. Cover for survey protocol



4. Justification for the survey – The justification describes the economics of the commodity (host), impact of the pest, purpose of the survey (e.g. early detection, assurances for pest free areas, information for a commodity pest list) and the specification of the phytosanitary requirements to be met.
5. Outcome of the survey – what is hoped to be achieved at the end of the survey.
6. Assumptions made in design the survey protocol, identification of timing (dates, frequency, duration indication of the statistical basis, (e.g. level of confidence, number of samples, selection

and number of sites, frequency of sampling, assumptions)). The statistical basis will differ from country to country, list of hosts and resources available.

An example of this is demonstrated in Box 14.

**Box 14. Justification, purpose and assumptions in the detection survey for weevils in sweet potato**

**INTRODUCTION**

Sweet potatoes are native to Central and South America and are one of the oldest vegetables cultivated as evidenced by sweet potato relics dating back 10,000 years that have been discovered in Peruvian caves. The sweet potato is cultivated in tropical and sub-tropical regions with abundant sunshine. It is a highly tolerant crop as it tolerates range of: temperatures, soil types, soil fertility, and water levels ranging from drought to floods. It is used for human food, animal feed and the production of alcohol and starch. The sweet potato contributes to food security as it is valued for its tubers as a source of starch and its leaves as a vegetable (like spinach) which is rich in proteins, vitamins and various minerals. FAO reported that 115 countries produced 106,569,572 tons of sweet potato in 2010; 82.3% of global production being in Asia, 14% in Africa and ironically a little more than 2% in its native Central and South America. Sweet potato weevils are serious pests that hinder sweet potato production.

***Cylas formicarius***

The sweet potato weevil, *Cylas formicarius* (Fabricius) is native to Indonesia and is the most important pest of sweet potato in the tropical and subtropical regions of Asia, the Pacific, the Caribbean (recently introduced into Venezuela and Guyana), the USA and several African countries. Infestation ranges from 20 to 50% and can even reach to 100% depending on the season and variety. Crop losses can range from 5 to 80%.

The adult weevil is 5-7 mm long, slender, smooth and hard-bodied ant-like insect with a distinct snout, metallic blue head, forewing and abdomen. The legs and thorax are reddish brown.

***Euscepes postfasciatus***

The West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) is native to the West Indies and has caused extensive damage to roots both in the field and in storage for as long as sweet potato has been grown. It is a major pest of sweet potato in the Pacific, Caribbean, and some countries of South America. It can cause 100% loss. Yasuda (1997b) determined the control threshold level of stems during the growing season to be 5%. The adult weevil is small - 5 mm long, has a prominent, downward-curving snout, is covered with short bristles, is reddish brown to near black.

Sweet potato weevils are nocturnal pests which are difficult to detect as they spend most of their lifecycle within the plant or underground. 80–90% of the weevil population is below the soil surface. Whilst sweet potato is the main economical host, *Cylas formicarius* has been recorded feeding on at least forty nine other members of the Convolvulaceae on seven genera in six tribes within the family. It has also been recorded as feeding on members of the Acanthaceae, Cruciferae, Euphorbiaceae and Umbelliferae suggesting that *Cylas* is a polyphagous pest. *Euscepes* has been recorded on fewer hosts. No sweet potato species are known to be resistant to infestation. Weevils cause damage in the field, in storage, and are of quarantine significance. Weevil's damage increases the longer the crop remains unharvested. In Hawaii, Sherman and Tamashiro (1954) showed that damage increased sharply between 24 and 30 weeks after planting.

*Cylas* is present in most Caribbean countries. Island X has been conducting surveys since 2010, and to date it has not been detected. *Euscepes* is native to the West Indies. Surveys conducted since 2010, did not reveal its presence. Even low-level infestations of these weevils reduce marketable yield because sweet potato roots produce furano-terpenoids and coumarins in response to weevil feeding that make even slightly damaged roots unpalatable and thus unfit for consumption. Island X is main exporter of sweet potato, averaging 70 thousand metric tonnes annually. The economic impact is considered to be High.

The establishment and spread potential of these weevils in the island are considered to be High due to the widespread availability of wild and ornamental hosts and the fact that this pest is native to the tropics. Most life stages (except adult) are flightless/legless thus stay within the plant/farm. Localised spread (between plants and within farms) can occur as a result of the flying adults which are known to fly as far as one mile. Longer distance spread can also readily occur from infested plants through human assisted movement – trade of the storage roots and planting material.

At the end of the survey, information will be available on:

1. presence/absence of both weevils in the island
2. if the pests are present:
  - a. districts which have infected fields
  - b. the pattern of spread (if one is present) can be displayed and
  - c. baseline information that will guide in formulating management strategies.

The assumptions made in this protocol are:

1. Null hypothesis: Both weevils are absent in the island. Alternative hypothesis: Both weevils are present.
2. The island is subdivided into agricultural districts and this is the unit being sampled.
3. Weevil found on a single sweet potato in a location means that the pest is present in that location.
4. Weevil found in a single location in a district means that the pest is present in that district.
5. 95% confidence of finding the pest in a given location (sample 10 plants/location; sample 10 farms/district).

7. Pest-host information which describes in details and illustrate with colour photographs the following:
  - a. symptoms of pest damage,
  - b. all stages of the pest life cycle
  - c. host affected
  - d. natural enemies
  - e. pest distribution
  - f. means of transmission and spread of pest
  - g. phytosanitary risk or summary of pathway of introduction

This is illustrated in Box 15.

#### **Box 15. Pest – host information in the detection survey for weevils in sweet potato**

##### **SYMPTOMS**

All stages of the weevils infest under-and-over-ground parts (buds, leaves, stems and storage roots). Whilst the adult feed on buds, leaves, stem and storage roots; the larva is the most destructive stage tunneling and feeding on mature stems and storage roots. The plant responds by producing furano-terpenoids and coumarins, which imparts a bitter taste and unpleasant odour that make even slightly damaged roots unpalatable. Symptoms include:

- ✓ small, shallow ovipositional punctures on stem/root surface
- ✓ *Cylas*: small, round, deeper feeding punctures on stem/root surface  
*Euscepes*: sunken feeding areas on stem/root surface
- ✓ *Cylas*: large, chewed, emergence holes on the roots/stems
- ✓ small scraped patches on stem
- ✓ tunneling of the stem
- ✓ tunneling of the storage roots

- ✓ enlarged, malformed, thickened, drying and cracked stems
- ✓ discoloured and wilted damaged vines
- ✓ pale green leaves
- ✓ growth and overall vigour of the plant adversely affected in first month after planting
- ✓ *Cylas*: occasional symptoms:
  - chewed away of portions of leaf lamina
  - small scraped patches of major veins and petioles
- ✓ unpleasant odour in damaged root
- ✓ bitter taste of affected roots
- ✓ storage root becomes:
  - dark in colour
  - light in weight
  - spongy in appearance
- ✓ rotted storage root (starting from the top)
- ✓ secondary infection by bacteria and fungi



Image: John Ruter, Univ Georgia, Bugwood.org; Agricultural Research Council of South Africa



Image: Institute of Plant Biotech for Developing Countries, Ghent University; Kohama, T. Okinawa Prefectural Gov't Fruitfly Control Project Office



Image: Extension Entomology, Department of Entomology, Texas A&M University



Image: Extension Entomology, Depart of Entomology, Texas A&M

**Cylas (spw)**



Image: Dick Nichols District Park, USA

- ✓ 5-7 mm long, slender, smooth and ant-like
- ✓ distinct snout
- ✓ metallic dark blue head, fore wing and abdomen
- ✓ thorax and legs bright red-orange-brown
- ✓ females differ from the males
  - ◆ antenna - terminal club ovoid in female, cylindrical in male
  - ◆ body size - female larger than male.
- ✓ adult emerges from the vine or storage root
- ✓ initially feed on above ground parts
- ✓ active in the field as soon as host plants are available
- ✓ as plant stem enlarges and becomes woody, females prepares to lay eggs
- ✓ enter the soil through cracks (cannot dig into soil)
  - punctures storage roots/base off stem to lay eggs
- ✓ female lays 100 -250 eggs
- ✓ eggs laid individually in holes in roots, and base of stems
- ✓ egg cavity is sealed with a protective, grey fecal plug
- ✓ eggs ovoid 0.65 mm long and 0.46 mm wide
- ✓ newly laid eggs are translucent white and soft with rugose surface



Image: James Costner, Univ Fds

**Euscepes (WI spw)**



Photo: G. McCormack, The Cook Islands Natural Heritage Trust, 2007

- ✓ 4mm, reddish brown to near blackish grey
- ✓ compact body covered with short bristles, arranged in parallel rows on the abdomen
- ✓ head is small and protrudes little from the thorax
- ✓ prominent, downward-curving snout
- ✓ adult emerge in the pupal chamber
- ✓ emerged adults are sexually immature
- ✓ adults stay within tissue > 10 days
- ✓ emerge from the host after sexual maturation by chewing exit holes
- ✓ pre-oviposition period 9-13 days at 25°C
- ✓ lay fertile eggs over 4-6 months
- ✓ 106 eggs per month
- ✓ live for up to 6 months
- ✓ eggs laid singly in shallow cavities in root/stem
- ✓ egg cavity is sealed with a protective, grey fecal plug
- ✓ ovoid, yellow to greyish yellow, 0.35mm in diameter
- ✓ 106 eggs per month
- ✓ hatch after 7-9 days, 24-27°C

- ✓ eggs about to hatch, are creamy with small brown irregular specks
- ✓ eggs hatch less than a week after being laid

***Cylas* (spw)**

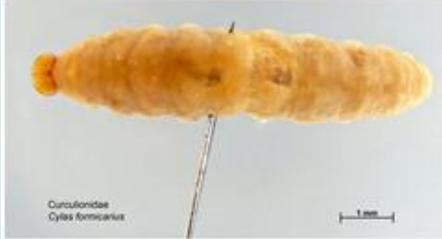


Image: Caroline Harding, MAF, Australia

- ✓ cream-coloured, legless, brown subglobular head and reddish brown gut
- ✓ abdomen is subconical for the first and second instars and subcylindrical from third to fifth instars
- ✓ newly hatched larva is slightly larger than egg; mature larvae 7-8 mm
- ✓ larva tunnels and feeds into stem or roots 2 to 3 weeks (1-3 instar)
- ✓ third instar larvae return to the plant surface nearest the soil line to pupate
- ✓ 5 instars

***Cylas* (spw)**



Image: Art Cushman, USDA Systematics Entomology Laboratory Bugwood.org

- ✓ creamy white, sub-ellipsoidal, 5-6 mm
- ✓ exarate type
- ✓ last abdominal segment has two outward and backward curved tubercles
- ✓ eyes, wing pads, and legs turn dark brown and the rest of the body pale yellow before emergence
- ✓ pupation takes place within the larval tunnels 1 week
- ✓ 4 days later weevil emerges from pupal sac.

***Euscepes* (WI spw)**



Image: Kohama, T. Okinawa Prefectural Government, Fruitfly Control Project Office

- ✓ white, legless
- ✓ newly hatched larva is slightly larger than egg; mature is less than 1 cm in length.
- ✓ grow within the host plants
- ✓ 5 instars
- ✓ develop into pupae after 18-30 days

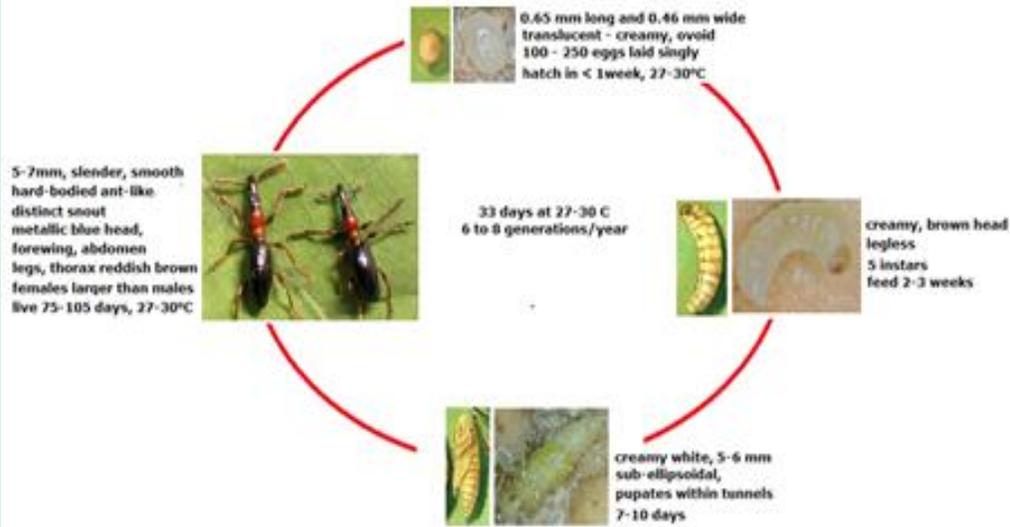
***Euscepes* (WI spw)**



Kohama, T. Okinawa Prefectural Government, Fruitfly Control Project Office

- ✓ creamy white
- ✓ Pupation occurs within a small chamber prepared by the larva
- ✓ pupal stage lasts 7-10 days

**Life Cycle of *Cylas formicarius***



**Life Cycle of *Euscepes postfasciatus***

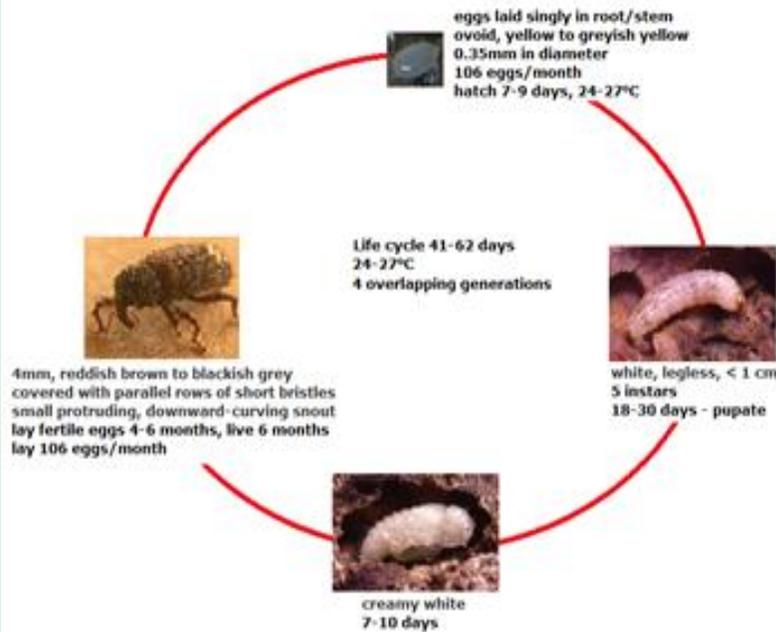


Image: Kohama, T. Okinawa Prefectural Government, Fruitfly Control Project Office; Art Cushman, USDA Systematics Entomology Laboratory, Bugwood.org

**DETECTION**

Detection of weevil infestation is difficult as adults are mostly active by night. Infestation can be detected early by checking the base of the plant for feeding punctures and exit holes. Plants with such damage should be dug up to check for damage to the storage roots. *C. formicarius* can be trapped by baiting with commercially available female sex pheromone to attract males.

The pheromone is so sensitive that their failure to catch weevils is a good indication that the pest is not present. There is no sex pheromone available to attract *Euscepes*, however recent work with green light-emitting-diode (LED) traps and pitfall traps have been used successfully to monitor this species.

The key features of the trap include:

1. attractant:
  - a. sex pheromone lure which is impregnated on a dispenser (e.g. rubber septa); a high dose is used to mass trap the weevils - *Cylas*
  - b. green LED light - *Euscepes*
2. a deep catchment container with a soap/insecticidal solution for catching and killing weevils that enter and to prevent the weevils from escaping
3. an adjustable frame to suspend the trap just above the canopy height as the crop grows
4. sufficient air vents to allow the diffusion of the scent/light to spread across the field.

The trap should be placed in the field as soon as planting is completed at a density of two traps /hectare of sweet potato. Traps must be placed just above the canopy; monitored, adjusted and moved around the field every week; and pheromone bait changed every 8 weeks.



**NATURAL ENEMIES**

Predators, parasitoids and biological agents have been found attacking sweet potato weevils.

Some of these are:

- A. Fungi: - attack and kill adult weevils
  1. *Beauveria bassiana* (80-90% mortality)
  2. *Metarrhizium anisopliae*
  3. *Isaria* sp (Java, Indonesia)
  4. *Fusarium*

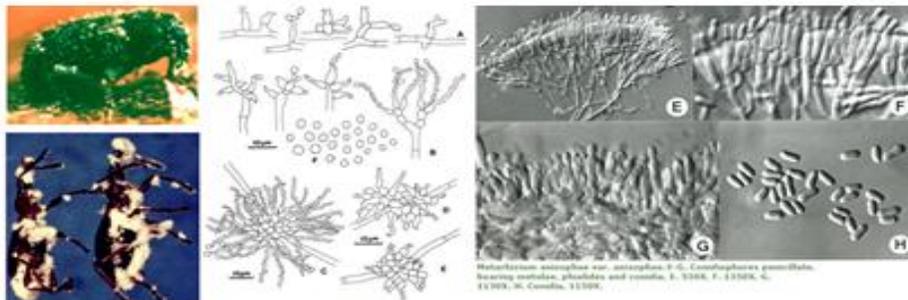


Image: S. S. Tzean and L. S. Hsieh 'Vuill. Bull. Soc. bot. Fr. 59

B. Bacterium:

5. *Bacillus thuringiensis*

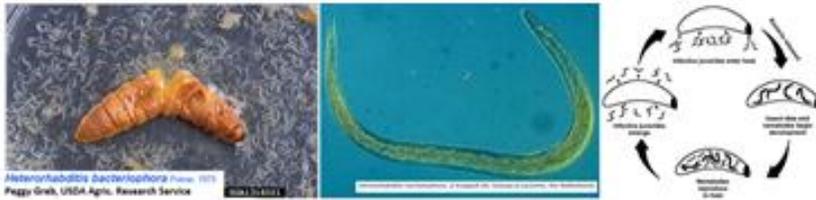


Larva infected with *Bacillus thuringiensis*  
Image: D. Amalin

C. Nematodes: kill the larvae, but don't persist in the soil

6. *Heterorhabditis* spp. *Heterorhabditis bacteriophora*, *Heterorhabditis heliothidis*

7. *Steinernema* spp. *Steinernema carpocapsae*, *Steinernema feltiae*



*Heterorhabditis bacteriophora* Pagan, 1971  
Peggy Greb, USDA Agric. Research Service

D. Predators:

8. Ants - very effective in Cuba. Both ants (a and b) cannot co-exist in same field.

a. Hymenoptera: Formicidae: *Pheidole megacephala* (big headed ant)

b. Hymenoptera: Formicidae: *Tetramorium guineense* [*Pheidole guineensis*]

c. Hymenoptera: Formicidae: *Iridomyrmex humilis* (Argentine ant)



*Pheidole megacephala*  
formicidbase2.blogspot.com

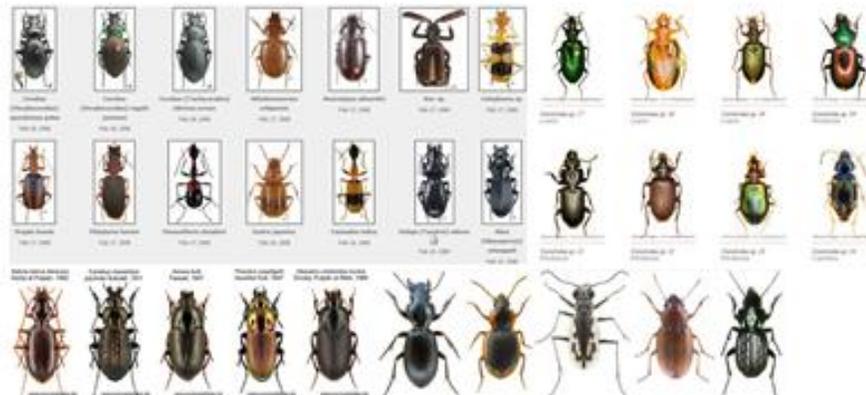
1mm

*Iridomyrmex humilis*.wiki.org

9. Diptera: Hybotidae: *Drapetis exilis*

10. spiders

11. Carabid beetles



12. earwigs



Earwigs. Image: E Vasquez

Wasps parasitoids (15 reported from India, Philippines and USA but not effective)

13. Hymenoptera: Braconidae: *Bracon yasudai* Maeto et Uesato sp (south-west islands of Japan)



Takumi Uesato *Entomological Science* **10**: 55-63.

14. Hymenoptera: Braconidae: *Bracon cylasovorus* (Microbracon cylasovorus)

15. Hymenoptera: Braconidae: *Bracon mellitor* Say



16. Hymenoptera: Braconidae: *Bracon punctatus* Muesebeck (Microbracon punctatus)

17. Hymenoptera: Braconidae: *Metapelma spectabile* Westwood



18. Hymenoptera: Braconidae: *Rhaconotus menippus* (China, India, Malaysia, South Africa, Thailand, Uganda)

19. Hymenoptera Braconidae: *Bassus cylasovorus* Rohwer (Phillipines)

20. Hymenoptera: Eulophidae: *Euderus purpureas* Yoshimoto

21. *Cerocephala* sp (Peru)



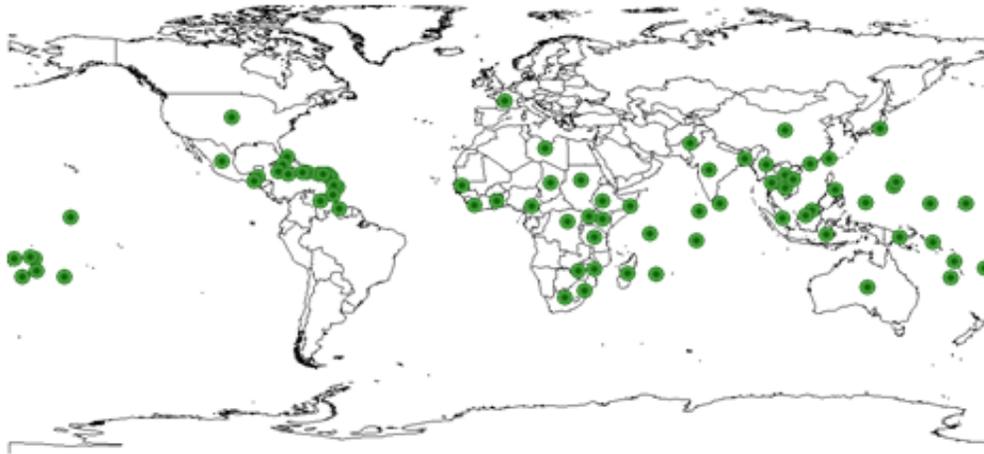
22. *Heteroschema* sp (Peru)

23. *Eupelmus cushmani* (Hawaii)



**DISTRIBUTION**

*Cylas* spw



Images: Cabi, EPPO

*Euscepes* Wlspw



The sweet potato weevil, *Cylas formicarius* is native to Indonesia and can be found in the tropical and subtropical regions of Asia, the Pacific, the Caribbean (recently introduced into Venezuela and Guyana), the USA and several African countries. The West Indian sweet potato weevil, *Euscepes postfasciatus* is native to the West Indies and can be found in the tropical and subtropical regions of the Pacific, Caribbean, and some countries of South America.

#### SPREAD

The entry potential of *Cylas* and *Euscepes* into the island is High as it is established in most Caribbean countries and could potentially enter via trade of sweet potatoes for consumption.

The establishment and spread potential of *Euscepes* and *Cylas* is considered to be High due to the wide host range and their widespread availability and suitable environment as these pests are native to tropical environments. Most life stages (except adult) are flightless/legless thus stay within the plant/farm. Localized spread (between plants and within farms) can occur as a result of the flying adults which are known to fly as far as one mile. Longer distance spread can also readily occur from infested plants through human assisted movement – trade of the storage roots and planting material.

#### HOST

Whilst sweet potato is the main economical host, *Cylas formicarius* has been recorded feeding on at least forty nine other members of the Convolvulaceae on seven genera in six tribes within the family. It has also been recorded as feeding on members of the Acanthaceae, Cruciferae, Euphorbiaceae and Umbelliferae suggesting that *Cylas* is a polyphagous pest. *Euscepes* feeds on fewer hosts.

»

<i>Cylas</i> spw		<i>Euscepes</i> Wlspw	
Scientific	common	Scientific	common
<i>Calystegia sepium</i>	great bindweed	<i>Ipomea alba</i>	
<i>Calystegia soldanella</i>		<i>Ipomea barlerioides</i>	
<i>Calystegia soldanella</i>	Sea bindweed	<i>Ipomea cordata-triloba</i>	
<i>Colocasia esculenta</i>	taro	<i>Ipomea hederacea</i>	
<i>Cuscuta</i>	dodder	<i>Ipomea hederifolia</i>	
<i>Daucus carota</i>	Carrot	<i>Ipomea horsfalliae</i>	
<i>Dichondra carolinensis</i>		<i>Ipomea imperati</i>	

<i>Cylas</i> spw		<i>Euscepes</i> Wlspw	
Scientific	common	Scientific	common
<i>Ipomea alba</i>		<i>Ipomea indica</i>	
<i>Ipomea barlerioides</i>		<i>Ipomea lacunosa</i>	
<i>Ipomea cordato-triloba</i>		<i>Ipomea macrorrhiza</i>	
<i>Ipomea hederacea</i>		<i>Ipomea obscura</i>	
<i>Ipomea hederifolia</i>		<i>Ipomea pandurata</i>	
<i>Ipomea horsfalliae</i>		<i>Ipomea sagittata</i>	
<i>Ipomea imperati</i>		<i>Ipomea separia</i>	
<i>Ipomea indica</i>		<i>Ipomea setosa</i>	
<i>Ipomea lacunosa</i>		<i>Ipomea sinensis</i>	
<i>Ipomea macrorrhiza</i>		<i>Ipomea triloba</i>	
<i>Ipomea obscura</i>		<i>Ipomea tubinata</i>	
<i>Ipomea pandurata</i>		<i>Ipomea wrightii</i>	
<i>Ipomea sagittata</i>		<i>Ipomoea</i>	morning glory
<i>Ipomea separia</i>		<i>Ipomoea acuminata</i>	
<i>Ipomea setosa</i>		<i>Ipomoea aquatica</i>	swamp morning-glory
<i>Ipomea sinensis</i>		<i>Ipomoea batatas</i>	sweet potato
<i>Ipomea triloba</i>		<i>Ipomoea cairica</i>	five-fingered morning glory
<i>Ipomea tubinata</i>		<i>Ipomoea pentaphylla</i>	
<i>Ipomea turbinata</i>		<i>Ipomoea pes-caprae</i>	beach morning glory
<i>Ipomea wrightii</i>		<i>Ipomoea purpurea</i>	Tall morning glory
<i>Ipomoea</i>	morning glory	<i>Ipomoea quamoclit</i>	Cupid's-flower
<i>Ipomoea aquatica</i>	swamp morning-glory	<i>Ipomoea reptans</i>	
<i>Ipomoea batatas</i>	sweet potato	<i>Pharbitis nil</i>	Japanese morning glory
<i>Ipomoea cairica</i>	five-fingered morning glory	<i>Quamoclit multifida</i>	
<i>Ipomoea pentaphylla</i>		<i>Quamoclit vulgaris</i>	
<i>Ipomoea pes-caprae</i>	beach morning glory	<i>Raphanus sativus</i>	Radish
<i>Ipomoea purpurea</i>	Tall morning glory		
<i>Ipomoea quamoclit</i>	Cupid's-flower		
<i>Ipomoea reptans</i>			
<i>Jacquemontia curtissii</i>			

<i>Jacquemontia tamnifolia</i>	Small flower morning glory			
<i>Manihot esculenta</i>	cassava			
<i>Merremia dissecta</i>				
<i>Pharbitis nil</i>	Japanese morning glory			
<i>Quamoclit multifida</i>				
<i>Quamoclit vulgaris</i>				
<i>Raphanus sativus</i>	Radish			
<i>Stictocardia tiliifolia</i>				

8. Survey methodology - Detection methodology in sampling procedures (e.g. attractant trapping, whole plant sampling, visual inspection, sample collection and laboratory analysis). An example of the detection survey methodology is given in Box 16.

**Box 16. Active detection survey methodology for sweet potato weevils in sweet potato**

**SURVEY PROTOCOL**

Island X is divided agriculturally into parishes. Each parish is divided into agricultural districts which contains many farming pockets. To investigate presence/absence of weevils in sweet potato two methods will be used simultaneously:

1. Trapping - traps will be placed on 10 farms at a density of 2 traps/hectare farmed in each farming pocket in the island; and
2. Bulk sampling - at harvest, damaged storage roots will be bulked and 20-25 lbs. brought to the Entomology Diagnostic Laboratory for investigation.

Once a sample/trap is determined positive for weevils, that district is deemed infested.

Field

A. Trapping

Traps will be constructed, given consideration to the key features of the trap listed in Section: Detection. Two traps per hectare farmed will be placed on 10 farms in each farming pocket. Traps will be serviced weekly and pheromone changed every 8 weeks /battery changed as needed, throughout the life of the crop/survey. Weekly service will include:

1. Collecting all insects trapped and submitting same to the Entomology Diagnostic Laboratory for identification – Strain soap/insecticidal solution from the catchment container into a bucket to collect insects on the strainer. Using an artist brush, place insects in a vial with alcohol and label vial.

2. Bulk sampling - at harvest, damaged storage roots will be bulked and 20-25 lbs. brought to the Entomology Diagnostic Laboratory for investigation.

Once a sample/trap is determined positive for weevils, that district is deemed infested.

Field

A. Trapping

Traps will be constructed, given consideration to the key features of the trap listed in Section: Detection. Two traps per hectare farmed will be placed on 10 farms in each farming pocket. Traps will be serviced weekly and pheromone changed every 8 weeks /battery changed as needed, throughout the life of the crop/survey. Weekly service will include:

1. Collecting all insects trapped and submitting same to the Entomology Diagnostic Laboratory for identification – Strain soap/insecticidal solution from the catchment container into a bucket to collect insects on the strainer. Using an artist brush, place insects in a vial with alcohol and label vial.
2. Refilling the catchment container with the strained soap/insecticidal solution, topping up if necessary.
3. Adjusting the frame to suspend the trap just above the canopy height as the crop grows.
4. Moving the trap(s) around the field.

B. Bulk sampling

At harvest, visibly infested storage roots will be bulked, 20-25 lbs. collected and submitted to the Entomology Diagnostic Laboratory for investigation.

Labeling samples:

1. Trapping

Label the vial with the date; unique sample number (Sample # P...../D...../F.....); farmer; address; and surveyor’s name.

2. Bulk sampling

Affix a 3x5 blue border adhesive label to the feed/garbage bag for each farm. Label with:

Date: .....	Sample # P...../D...../F.....
Name of farmer/owner/property: .....	
Address.....	
Eastings: .....	Northings: .....
District: .....	County: .....
Host: .....	Variety: .....
Name of surveyor.....	

Sample No is a Unique number where P= Parish Initials; D= First three letters in District; F=Farm  
1 to 10

Transporting samples:

Place vials with insects and/or feed bag with storage roots securely in a cooler for transporting.  
Submit to the Entomology Diagnostic Laboratory for investigation. All samples must be submitted on same day before 2:30 pm. The accompanying data sheet for each sample must be fully completed.

Laboratory:

The data sheet accompanying each sample should be signed and dated as having received the sample.

9. Good Sanitation practices during surveillance  
Farms with good biosecurity will limit visitors' admission. Visitors, workers, extension officers pose a risk of introducing pests into the farm. This risk increases with extension officers visiting multiple farms sequentially per day in the conduct of their duties. Officers must therefore take precautions so that they do not become an agent of pest spread. Box 17 provides an example of good sanitation practices in the conduct of surveys.

**Box 17. Sanitation**

**SANITATION**

When visiting farms to conduct surveys/collect samples, strict measures to minimise the risk of spread of plant pests and pathogens between crops/ properties must be adhered:

- Use a 5 percent solution of common household bleach:
  - disinfect pruning shears between cuts and after/before use on each property
  - spray boots after/before each property.
- Wash hands with soap and water. Change gloves after each property.

10. Field procedure - detail and stepwise description of survey methodology and quality management; the procedure would be determined by the biology of pest and/or purpose of survey – discussed in Chapter 4.
11. Laboratory procedure – good practices and diagnostic procedures - discussed in Chapter 5.

## Chapter 4 Developing the field procedure, list of material required and budget

The field procedure combines the survey protocol and information on the data form into detailed step-wise instructions to be followed to conduct the survey; gather data; and collect, package, label, transport and submit the sample to the laboratory. The procedure may be split into two components:

- ◆ Passive surveillance
- ◆ Active surveillance

### 4.1 Passive surveillance

Passive surveillance is setting up a system to receive reports from the wider stakeholders – the public. It can consist of a telephone hotline or email contact. The components in the procedure for passive surveillance are how and what to do and say in the following sections:

- ◆ Receiving calls/reports/pictures
  - ◆ Recording calls – data sheet
  - ◆ Contacting the caller
  - ◆ Visiting the caller
  - ◆ Sending samples and e-photo to plant pathology or entomology for diagnosing
  - ◆ Reporting
- } Investigations

Box 18 gives an example of a protocol for passive surveillance, including a telephone protocol for responding to callers making reports.

#### Box 18. Protocol for passive surveillance of the sweet potato weevils in sweet potato

##### PASSIVE SURVEILLANCE METHODOLOGY – HOTLINE REPORTS

###### A. Receiving calls

1. All calls must come through the hotline: XXX\_XXXX where a unique code will be assigned to each call.
2. All calls to other offices/numbers/emails must therefore be immediately redirected to:
  - hotline: XXX-XXX
  - faxed to XXX-XXXX
  - emailed to XXXXXXXX@gmail.com
3. On Mondays, the reports will be collated and sorted (by parish) from the previous week. List of sorted calls will be e-mailed/faxed to the respective parish offices by noon.
4. **ALL CALLS SHOULD BE INVESTIGATED OR AT LEAST A RETURN CALL MADE WITHIN 24 HOURS OF RECEIPT OF THESE LISTS.**

###### B. Contacting the caller

5. At the parish office, the officers handling the call must attempt to contact the caller on at least four consecutive days before concluding the caller cannot be located.

6. **DO NOT VISIT THE CALLER WITHOUT FIRST ARRANGING A VISIT.**
7. When contacting the caller, please state clearly:
  - Your name
  - Your post
  - Your institution – Ministry of XXXXXXXX, and office.
8. Ask the following questions:
9. May I speak with Mr./Ms. ....? (the caller whose name appears on the list).
10. Did you call the ministry to make a report of weevils in sweet potato?
  - If Yes, go to Item 12 below.
  - If No, go to item 11 below.
11. May I speak to the individual who saw the weevils in sweet potato?
  - If said individual is available or not, go to Item 12 below.
12. Can you please tell me what symptoms you are seeing that you are concerned about?  
Symptoms of weevils in sweet potato can be described as (see Appendix 2 – Symptoms of the sweet potato weevils and Appendix 3 – Images of sweet potato weevils):

- ✓ small, shallow ovipositional punctures on stem/root surface
- ✓ *Cylas*: small, round, deeper feeding punctures on stem/root surface  
*Euscepes*: sunken feeding areas on stem/root surface
- ✓ *Cylas*: large, chewed, emergence holes on the roots/stems
- ✓ small scraped patches on stem
- ✓ tunneling of the stem
- ✓ tunneling of the storage roots
- ✓ frass-filled tunnels
- ✓ enlarged, malformed, thickened, drying and cracked stems
- ✓ discoloured and wilted damaged vines
- ✓ pale green leaves
- ✓ growth and overall vigour of the plant adversely affected in first month after planting
- ✓ *Cylas*: occasional symptoms:
  - o chewed away of portions of leaf lamina
  - o small scraped patches of major veins and petioles
- ✓ unpleasant odour in damaged root
- ✓ bitter taste of affected roots
- ✓ storage root becomes:
  - o dark in colour
  - o light in weight
  - o spongy in appearance

✓ rotted storage root (starting from the top)

✓ secondary infection by bacteria and fungi

Symptoms not associated with weevils in sweet potato are outlined in Appendix 4 – Symptoms of other pests/diseases/disorders of sweet potato.

If the symptoms do not fall within those of weevils in sweet potato – the caller /farmer can be advised as to what pest/disease he/she may have (refer to: Appendix 4 – Symptoms of other pests/diseases/disorders of sweet potato) and advised to submit a sample to the Entomology Diagnostic Laboratory for confirmation and management advice.

If the symptoms described are similar to that of weevils in sweet potato **a visit to the caller is necessary**. Make an appointment to visit the caller within the week at the caller's earliest convenience.

#### C. Visiting the caller

13. At the site, greet the caller and present your Ministry's identification before entering the premises to look for symptoms of weevils in sweet potato.
14. Compare the symptoms to the photographs in (Appendix 2 – Symptoms of the sweet potato weevils and Appendix 3 – Images of sweet potato weevils) to assist in collection of suitable samples to submit for laboratory diagnosis.
  - ◆ Collect at least 5 suspect tubers and 2 infested stems per site – tag the area in the field from where samples were taken.
  - ◆ Place in a clear plastic bag, seal and label with: date, caller's name and unique code, variety of sweet potato, your name and contact.
  - ◆ Question the caller/farmer and completely fill the Entomology Diagnostic Form (attached). Clearly write the **unique code** in the top right hand corner of the form.
  - ◆ If weevils in sweet potato are suspected, **DO NOT INFORM** the caller. Positive identification must be confirmed from the Entomology Diagnostic Laboratory.
15. It is the Entomologist's responsibility to confirm the suspected samples are **infected with the weevil**. The Entomologist will inform the caller and implement the next step in the sweet potato weevil management protocol.

#### D. Sending samples and e-photo to the Entomology Diagnostic Laboratory

16. Take clear views of the suspect tubers/stems to email to XXXXXXXX@gmail.com
17. All photos emailed should be accompanied with the **UNIQUE CODE** and **CALL PARTICULARS**
18. Place sample in a cooler with ice for transporting to the Entomology Diagnostic Laboratory.
19. Double check that samples are labelled with the **UNIQUE CODE** and **CALL PARTICULARS**.
20. Ensure both samples and completely filled diagnostic form reach the laboratory within 24 hours.

21. The Entomologist will respond within 1-7 working days.

**E. Reporting**

22. The Parish Officer must report by Friday 10:00 a.m. on the status of all calls on the list by emailing XXXXXX@gmail.com or faxing XXX-XXXX.

23. The Entomologist is responsible for collating all calls and investigations to generate the weekly report on the status of weevils in sweet potato hotline calls programme and the presence and distribution of the weevils in sweet potato Island X.

**Telephone Protocol For Answering Pest Hotline Calls (XXX-XXXX)**

1. Good morning/afternoon. Identify yourself from Ministry of XXXXXX. How may I help you?
2. If the call is to report a pest, please proceed to 3, otherwise direct call as required.
3. May I please have your name, address and contact number so that an officer can arrange a visit at our mutual convenience
4. Please note the above in Sheet A and assign the next consecutive number to the call. THE COMPLETE NAME, ADDRESS (PO/HOUSE/LP/MM #; STREET/ROAD/TRACE; VILLAGE; TOWN) AND TELEPHONE CONTACT CONSTITUTES A CALL.
5. Sir/Madam (whichever is appropriate), can you please describe the symptoms you have observed that you are worried about? (Note these symptoms in the correct column in sheet A).
6. Could you please assist by tagging the trees/plants on which you observed the symptoms to make it easier to locate when the officer visit?
7. An officer will call you at (please repeat number/s for confirmation) within 2 working days to arrange a visit.
8. Thank you for assisting us in managing pest that may harm our environment.
9. **Recording calls from voice mail:**
  - i. Ensure all information that constitutes a call is there before assigning the next consecutive number to the call (Complete name, address -PO/House/LP/mm #; Street/Road/Trace; Village; Town- and telephone contact).
  - ii. If any information is lacking, please dial the number recorded –
    - a. Identify yourself from Ministry of XXXXXX is calling in response to your pest report.
    - b. Proceed from 3 to 7 above.
    - c. If no one answers the telephone:
      - please call back the number back at least twice per day for two days
      - if there is still no answer, do not assign a number but place number in Sheet B.

Please Note Significance of Colour Coding:

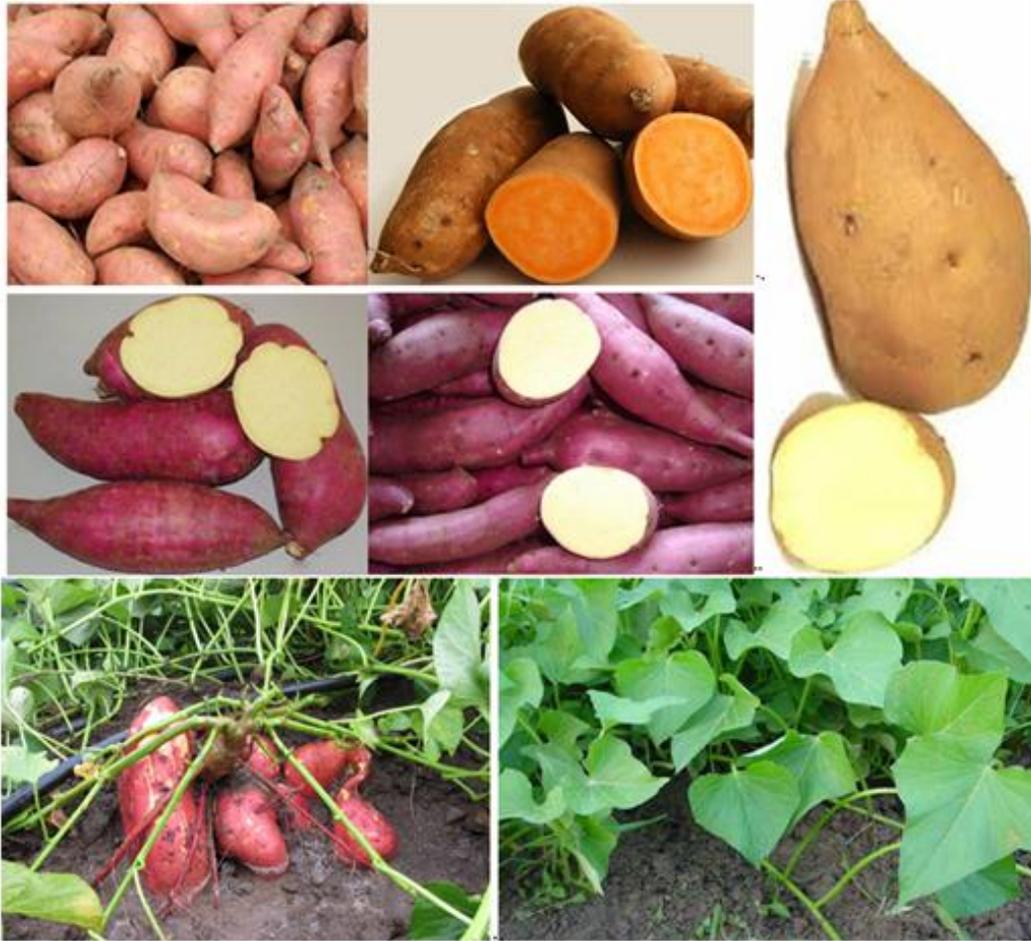
Red: Instructions

Blue: Actual Words

Green: Data to be recorded

**Appendix 1 - Healthy sweet potato tubers, vines, leaves:**

Images: microfarmgardens.com, galleryhip.com, microfarmgardens.com, galleryhip.com, www.pingminghealth.com, www.goodhousekeeping.com; urbanacresfarmstead.com, www.huffingtonpost.com



**Appendix 2 – Symptoms of the sweet potato weevils**



Image: John Ruter, Univ Georgia, Bugwood.org; Agricultural Research Council of South Africa



Image: Institute of Plant Biotech for developing Countries, Ghent University; Kohama, T. Okinawa Prefectural Govt Fruitfly Control Project Office



Image: Extension Entomology, Department of Entomology, Texas A&M University



Image: Extension Entomology, Department of Entomology, Texas A&M



Image: James Castner, Univ Fda

### Appendix 3 – Images of sweet potato weevils *Cylas* (spw)



Image: Dick Nichols District Park, USA

### *Euscepes* (WI spw)



Photo: G. McCormack, The Cook Islands Natural Heritage Trust, 2007



Image: Caroline Harding, MAF, Australia



Image: Kohama, T. Okinawa Prefectural Government, Fruitfly Control Project Office



Image: Art Cushman, USDA Systematics Entomology Laboratory Bugwood.org



Kohama, T. Okinawa Prefectural Government, Fruitfly Control Project Office

Appendix 4 – Symptoms of other pests/diseases/disorders of sweet potato

Images: keys.lucidcentral.org, www.cardi.org, http://cipotato.org/, http://biopbee.in/

Spodoptera



Megastotes



Leaf feeding insect eg beetles



Whiteflies, Bemisia



**4.2 Active surveillance – detection survey**

The field procedure of the survey methodology splits the methodology into its components and provides stepwise instructions - detail descriptions - of what needs to be done, how, where, and how often for each component. The survey protocol and information on the data form is combined into detailed step-wise instructions to be followed to conduct the survey; gather data; and collect, package, label, transport and submit the sample to the laboratory. It should tell the surveyor:

- ◆ how to select the smallest unit area to be sampled
- ◆ what pest capture method to use, how to set it up, how to maintain same
- ◆ how to/ where/what symptoms/pest to look for
- ◆ how to collect the pest/symptoms on infected/infested tissues
- ◆ how to preserve sample in field as it is collected
- ◆ how to package, label and transport the sample to the laboratory
- ◆ how often the survey needs to be done or visits need to be made
- ◆ what data needs to be collected

The survey methodology can be sectionalised in tabular format into:

- ◆ Survey unit/methodology
- ◆ Data collection
  - ❖ Location
  - ❖ Cultivation
  - ❖ Pest
- ◆ Sample collection
- ◆ Packaging sample
- ◆ Transporting sample

An example of the field procedure in a detection survey methodology is illustrated in Box 19.

**Box 19. Detection survey methodology for the sweet potato weevils in sweet potato**

DETECTION SURVEY METHODOLOGY	
Survey Components	Description of Actions
1. Survey unit and methodology: Sweet potato farms >3000 sq. ft. cultivated in sweet potato) in the farming pocket	<ul style="list-style-type: none"> <li>◆ Randomly select 10 farms in each farming pocket. Establish two traps/hectare farmed or 1 trap/farm (if less than 1 ha).</li> <li>Service traps weekly:                             <ol style="list-style-type: none"> <li>1. Collect all insects trapped and send same to the Entomology Diagnostic Laboratory for identification – Strain soap/insecticidal solution from the catchment container into a bucket to collect insects on the strainer. Using artist brush, collect insects and insert in a vial with alcohol, seal vial. Label vial. Submit to the Entomology Diagnostic Laboratory for identification.</li> <li>2. Refill the catchment container with the strained</li> </ol> </li> </ul>

	<p>soap/insecticidal solution, top up if necessary.</p> <p>3. Adjust the frame to suspend the trap just above the canopy height as the crop grows.</p> <p>4. Move the trap(s) around the field.</p> <p>♦ Examine hosts for symptoms of presence of weevils: shot holes on leaves; stem damage, puncture holes on stem. Record weekly.</p> <p>♦ At harvest: bulk infested storage roots, collect 20-25 lbs. per farm in a feed bag, and submit same to the Entomology Diagnostic Laboratory for investigation.</p>
2. Data collection	<p>♦ Sample # P..../D...../F..... (Unique number where P= Parish Initials; D= First three letters in District; F=Farm 1 to 10)</p> <p>♦ Address: Road, Village, Town, District, Parish; GPS reading in easting northing set at WGS 84</p>
a. Location	
b. Cultivation	<p>♦ Host, variety, plot size (area cultivated with sweet potato)</p> <p>♦ Cultural practices: use of Manure/ Fertiliser, Nematicide, Insecticide, Fungicide</p>
c. Pest	<p>♦ Presence of pest: larva, adult</p> <p>♦ Symptoms seen: shot holes on leaves, puncture holes on stem, stem damage</p>
3. Sample collection	<p>Trapping - weekly:</p> <p>♦ Wear gloves, strain soap/insecticidal solution from the</p>
♦ Insect trapped weekly	
♦ Bulk infested storage roots at harvest	<p>catchment container, use brush take up insects and place in labelled vial with alcohol.</p> <p>Bulk sampling – at harvest:</p> <p>♦ Bulk infested storage roots, collect 20-25 lbs. per farm in labelled feed bag.</p>
4. Packaging sample	<p>♦ Place samples in vial or bag</p> <p>♦ On each sample affix an adhesive label and label: date, sample #, farmer/owner, address, variety, surveyor's name.</p>
5. Transporting sample	<p>♦ Placed bagged and labelled samples in a cooler.</p> <p>♦ Submit to laboratory before 2:30 p.m. on same day</p>
6. Laboratory investigation Entomology	<p>♦ Examine sample within 24 hours for presence of and count all stages weevils, natural enemies</p> <p>♦ Rear all weevils stages for emergence of natural enemies</p>
7. Recording of information	♦ Record all information on Data Form, input in Access database
8. Analysis of Data	♦ Analyze data using
9. Presentation of Data	♦ Report with maps

### 4.3 Developing the budget

The survey methodology with the detailed descriptive instructions is used to generate a list of material that is required for each step in the methodology (Box 20). It also serves as a check list to ensure all materials required are packed before leaving the office/station to conduct the survey.

Prices for each item on the list are obtained to generate the budget required. The timeline indicates when the material is required and therefore how purchases can be staggered to ensure the material is available when needed.

#### Box 20. Developing the budget

14 DEVELOPING THE BUDGET						
Survey Components	Description of Actions	Materials required	Time Schedule			
1. Survey unit - Sweet potato farms (>3000 sq. ft. cultivated in sweet potato) in the farming pocket	◆ Sample 10 farms that were randomly selected per farming pocket	◆ Trained surveyor and labourer with a district extension officer ◆ For each surveyor a Survey manual, which contains: ✓ Introduction of pest ✓ Survey protocol ✓ Description of the symptoms ✓ Pictures illustrating symptoms ◆ Data Forms and Labels, pencils	Nov	Dec	Jan	
	◆ Place at least two traps/hectare farmed					
	◆ Collect insects trapped weekly					
	◆ At harvest, bulked infested storage roots, collect 20-15 lbs. per farm					
2. Data collection	◆ Sample # C..../D..../F.....	◆ Knowledge of the area ◆ Hand held GPS receiver, knowledge on how to use it				
	a. Location					
	◆ Name of Farmer ◆ Address: Road, Village, Town, District, Parish, GPS reading					
b. Cultivation	◆ Host, variety, plot size ◆ Cultural practices, use of Manure/ Fertiliser, Nematicide, Insecticide, Fungicide	◆ Answers provided by owner / manager / and observations				
c. Pest	Examine plants for presence of weevil: insect, symptoms of leaf, puncture holes on stem, stem damage. Record. ◆ Presence of pest: larva, adult ◆ Symptoms seen: shot holes on leaves, puncture holes on stem, damage on stem	◆ Knowledge in recognition of sweet potato weevils and symptoms				
3. Sample collection	Trapping: ◆ Strain soap/insecticidal solution from the catchment container. ◆ Using an artist brush, collect insects and insert in a vial with alcohol, seal vial. Label vial.	◆ Knowledge in recognition of sweet potato weevils and symptoms ◆ Latex gloves, strainer, bucket, artist brush, vials, alcohol, water, soap/insecticidal solution ◆ Hand sterilizing solution, paper towels ◆ Feed bags, clear vials				
	Bulk sampling ◆ Collect 20-25 lbs. infested storage roots per farm in a feed bag.					
4. Packaging sample	◆ On each sample affix an adhesive label and label: sample #, date, variety, surveyor's name, farmer, address.	◆ Adhesive labels 3x5 ◆ Pencils				

5. Transporting sample	<ul style="list-style-type: none"> <li>◆ Placed bagged and labelled samples in a cooler</li> <li>◆ Submit to laboratory on same day</li> </ul>	<ul style="list-style-type: none"> <li>◆ Styrotex Cooler</li> <li>◆ Transport</li> </ul>				
6. Laboratory investigation Entomology	<ul style="list-style-type: none"> <li>◆ Examine sample within 24 hours for presence of and count all stages weevil, natural enemies</li> <li>◆ Rear all weevil stages for emergence of natural enemies</li> </ul>	<ul style="list-style-type: none"> <li>◆ Entomologist, Laboratory assistants</li> <li>◆ Identification keys weevils, natural enemies</li> <li>◆ Rearing jars and material/microscope</li> </ul>				
7. Recording of information	<ul style="list-style-type: none"> <li>◆ Record all information on Data Form</li> <li>◆ Access database</li> </ul>	<ul style="list-style-type: none"> <li>◆ 8 ½ x 11 paper</li> <li>◆ Computer, UPS, Access database</li> </ul>				
8. Analysis of Data	<ul style="list-style-type: none"> <li>◆ Analyze data using</li> </ul>	<ul style="list-style-type: none"> <li>◆ Analysis software</li> </ul>				

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## Chapter 5    Developing the laboratory procedure

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General surveillance and specific survey activities must be supported by appropriated diagnostic services. It is at the diagnostic laboratory that the results of the survey is generated and without the ability to rapidly diagnose pests, surveillance activities to both quantify the magnitude of an incursion and determine and execute an appropriate response, would not be effective.

In addition to being critical to any eradication effort, diagnostic capacity also supports many of the management practices involved in the production and trade of plant products. Pest management programs, including the selection and application of registered pesticides, rely on the accurate identification of pests.

The IPPC recommends that each NPPO provides appropriate diagnostic services to support general surveillance and specific survey activities, or ensure access to such services. Characteristics of the diagnostic services should include:

- ◆ expertise in disciplines relevant to pest (and host) identification,
- ◆ adequate facilities and equipment - access to specialists for verification where necessary,
- ◆ facilities for record keeping,
- ◆ facilities for processing and storing of voucher specimens, and
- ◆ use of standard operating procedures, where appropriate and available.

An essential component of the survey protocol is therefore a laboratory protocol which should include the pest diagnostic protocol. This document should clearly outline the operating procedures to be followed when the survey samples reach the laboratory, through preparation and handling, to diagnostics and reporting. Each protocol will be unique to a particular survey as it will relate directly to the pest species of concern, the purpose of the survey and the expected results. **A protocol for handling a quarantine pest, for example, will include procedures to prevent material from leaving the laboratory**, a requirement which may not apply to handling a pest species that is widely distributed in the country.

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### 5.1    General (good) laboratory practices

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Good laboratory practices promote the production of quality data and ensure a sound approach to the management of laboratory studies; including conduct, reporting and archiving. These practices make sure that the data submitted are a true reflection of the results that are obtained during the survey.

They ensure that the data is traceable and promote international acceptance of tests and include:

- ◆ Standard Operating Procedures  
It is not reasonable to include all the technical details of the study in the survey protocol. The details of all routine procedures should be described in the Standard Operating Procedures (SOPs) which are part of the documentation system of the diagnostic laboratory. They define how to carry out protocol-specified activities and are most often written in a chronological

listing of action steps. SOPs contribute to reducing bias in studies by standardizing frequently performed techniques. Laboratories also need to standardize certain techniques to facilitate comparison of results between studies. It is important that SOPs be reviewed regularly and modified as necessary to reflect any advances in the science.

◆ **Sample tracking and data management**

It is very important that the connection between a set of diagnostic data and the samples from which they were obtained be maintained. Good laboratory practices ensure that a system of data entry and retrieval is in place to record and manage information relating to each sample received. The system should be sufficient to link samples received to particular field/collection/survey activities and to laboratory observations and diagnostic results.

Irrespective of whether the data are collected in notebooks or on data forms, if the numbers need to be analyzed statistically or a report has to be written, the information will need to be transferred into a computer program of some sort. A database program may be created for each survey, particularly if the survey is to be large and data entry will be repetitive. Each laboratory must decide what information is pertinent to the effective delivery of service for that institution and include such in the database design.

**TIP!** Possible headings for this data base are:

- ✓ Sample Number
- ✓ Host
- ✓ Location
- ✓ Collector
- ✓ Survey or routine submission
- ✓ Observations
- ✓ Diagnosis
- ✓ Diagnostician.

◆ **Laboratory equipment and facility maintenance**

For the proper conduct of laboratory studies, appropriate equipment must be available. All equipment should be suitable for its intended use, and it should be properly maintained to ensure reliable performance. This includes all equipment in the laboratory, whether it is used directly in diagnosis such as a microscope or used to maintain standard conditions such as a refrigerator. Records of repairs and routine maintenance and of any non-routine work should be retained. Routine maintenance should be documented in such a way that users of equipment can be assured that it is reliable and not outside its service interval. A label attached to the equipment or the provision of a clear service plan may ensure this. Particular care must be taken with equipment and facilities where quarantine or suspected quarantine plant pests are concerned. Clean-up and decontamination of spills, or after accidents must also be carried out to ensure no spread of

**TIP!** Clean microscopes, objective lens and light sources as necessary or as recommended by the manufacturer.

**TIP!** Fungal spores and small insects such as crawlers are easily spread outside the laboratory if not carefully handled.

infectious agents. There should be routine cleaning and decontamination of benches after use, and routine cleaning and decontamination at the end of each day.

- ◆ Scientific literature  
Maintain easy access to all relevant scientific literature. Use reference collection and consult with other taxonomists/scientists.
- ◆ Sample management  
In order to avoid possible contamination, confusion and misdiagnosis it is advisable to work on only one sample at a time. As much as is possible, clear the work area of all other material, especially specimens with which the study material can be confused. Do not mix taxa from different samples in the same Petri dish or on the same slide, except to make side-by-side comparison.

## 5.2 Preparing a laboratory protocol

Laboratory procedures to be followed in managing the samples received for diagnosis or other laboratory procedures can be outlined in the protocol as a series of chronological steps involving sample receipt, preparation and processing, diagnosis, storage and archiving.

### Step 1: Laboratory sample receipt

Samples collected during the survey and taken to the research institution for processing may include plant material (leaves, flowers, stems, fruits or roots), soil, live or dead insects. In surveys where traps are used, the samples may be presented in dry traps, on sticky cards or in wet traps. It is critical that the link be maintained between the samples collected and the final diagnostic. To ensure this a number of quality assurance procedures must be followed.

- ◆ Sample Registration  
At the diagnostic laboratory, each sample should be registered as being received, given a unique identification number and marked for submission to the relevant diagnostic laboratory. This can be done with the use of a simple registration form, designed for the purpose and representing the first point of entry of the sample into the laboratory system. The assigned identification number will follow the specimens throughout the handling and diagnostic process and into permanent storage.

The registration form may be generic document, prepared by the laboratory manager as a part of the SOP documentation. As such it may not be specific to a particular diagnostic laboratory or a specific survey. The laboratory protocol should however refer to the registration process and the use of the form as an initial reference point for each sample received.

Box 21 gives an example of a registration form. Each institution/researcher will design their own registration form to meet their particular laboratory standards. The reference number will also be unique to the institution.

<b>Box 21. Sample registration form: calendar year 2015</b>			
<b>Unique Identification No.</b>	<b>Sample Received</b>	<b>Laboratory of Dispatch</b>	<b>Signature: Diagnostician / Laboratory Technician</b>
SI-2015-09-23-01	live citrus psyllids (adults in jar)	Entomology, Molecular diagnostics	Dr. Lepi Doptera / Ms DN Andrews

In the example, the reference number (SI-2015-09-23-01) begins with a fictitious initial (SI) representing the institution, has the year (2015), the month (September) the date (23) and the number 01, indicating that this is the first sample received that day. The citrus psyllid samples received were sent to both the entomology and to the molecular biology laboratory for morphological identification molecular diagnostics. Sample registration forms can be kept in a binder and may be stored in a database.

◆ Specimen submission forms

For each specimen received at the diagnostic laboratory, certain auxiliary information must be known (Box 22). This information may be captured on a “specimen submission form”. This form could be the field data sheet used by the survey team collecting the samples or it may be designed to capture the information collected in the field in addition to that generated in the laboratory (Box 23). This specimen submission form is a critical document and must be appended to your laboratory protocol.

**Box 22. Auxiliary information to be collected for each sample received**

- ❖ Survey name
- ❖ Collector’s name
- ❖ Sampling site number or name
- ❖ Locations, such as by GPS readings (Eastings and Northings), of sampling sites
- ❖ Symptoms of the pest or host
- ❖ Pest life stage
- ❖ Treatments applied to site

**Box 23 Example of a generic sample submission form used in a diagnostic laboratory**

SPECIMEN SUBMISSION FORM			
Log Number:		Priority: <input type="checkbox"/> (1) Urgent <input type="checkbox"/> (2) Routine	
Date Received:	Receiving Officer		
Host Plant Scientific Name:			
Host Plant Common Name:			
Signs/Symptoms:			
Date Collected: March 19, 2012	Collector:		
Date Sent: March 19, 2012	Sender:		
Owner, Nursery, Grove or Apiary Yard Name:			
Address or Location:			
Country: Jamaica		E-mail Address	
Telephone Number		GPS Coordinates:	
Fax number			
Total Number of Plants Involved:		Total Acres Involved:	
Total Number of Plants Affected:		Total Acres Affected:	
Infection or Infestation Intensity: <input type="checkbox"/> (1) Slight <input type="checkbox"/> (2) moderate <input type="checkbox"/> (3) Severe	Part Involved: <input type="checkbox"/> (1) Bark <input type="checkbox"/> (2) Flower <input type="checkbox"/> (3) Fruit <input type="checkbox"/> (4) Leaves <input type="checkbox"/> (5) Roots <input type="checkbox"/> (6) Seed <input type="checkbox"/> (7) Stem <input type="checkbox"/> (8) Gall <input type="checkbox"/> (9) Mine <input type="checkbox"/> (10) Litter <input type="checkbox"/> (11) Soil <input type="checkbox"/> (12) Other	Stage or Organism: <input type="checkbox"/> (1) Egg <input type="checkbox"/> (2) Larva <input type="checkbox"/> (3) Pupa <input type="checkbox"/> (4) Adult <input type="checkbox"/> (5) Nymph <input type="checkbox"/> (6) All Stages <input type="checkbox"/> (7) Unknown	Collecting Technique: <input type="checkbox"/> (1) Beating <input type="checkbox"/> (2) Black Light <input type="checkbox"/> (3) Hand Catch <input type="checkbox"/> (4) Jackson <input type="checkbox"/> (6) Multi-Lure <input type="checkbox"/> (7) McPhail <input type="checkbox"/> (8) Reared <input type="checkbox"/> (9) Sticky Board <input type="checkbox"/> (10) Sweeping <input type="checkbox"/> (11) Other
Diagnosis:			
Determiner	Date Completed		Signature
Report Sent <input type="checkbox"/> Yes <input type="checkbox"/> No	Verbally <input type="checkbox"/>	E-mail <input type="checkbox"/>	Signature
	Telephone <input type="checkbox"/>		Date

◆ Sample integrity check

It is often useful to check the integrity of the samples received prior to storing. Depending on the material to be collected during the survey, the laboratory protocol should provide information on procedures for checking the integrity of the samples. The objective is to make sure that the material presented at the laboratory is what is expected based on the survey protocol and that anything that might compromise the integrity of the sample is excluded. For example, you may need to examine samples of plant material for rotting or spoilage. If specimens are in liquid preservatives, check that the fluid levels are sufficient to adequately preserve the material until

diagnosis. Integrity check may also need to be done on the documentation accompanying the samples (field data sheets, sample labels, etc.) to ensure that the required information is present.

### Step 2 Sample preparation and processing

The sequence of activities here will depend to a large extent on the pest(s) of concern, the type of material collected and the diagnostic method to be used. As such, it should be considered in conjunction with the diagnostic protocol discussed in Step 3 Diagnosis of a sample. The laboratory protocol should provide detailed information on how the samples are to be prepared for diagnosis and may include guidelines on:

- ◆ **Sorting and selection**

This activity determines how and what material is selected for further processing. Where insects or other arthropods are collected on plant material, instructions should be provided on best practices for recovering these organisms prior to storage. Mites, for example, may be recovered from plant material by washing with alcohol or water while small soft bodied insects such as mealybugs may require a soft brush or insect pin to remove them from plant material.

The survey may require the use of traps and the samples are delivered in some form of liquid bait or on sticky cards. The laboratory protocol should provide guidelines on how these insects should be removed, cleaned (if appropriate) and examined for morphological integrity before storage.

Sorting and selection also involves choosing the plant part or arthropod life stage(s) important for diagnosis. Where possible, it is advisable to collect a large number of specimens of all life stages. For example, with the adult stage, collect a number of specimens of varying size and colour depicting variation in the morphology of that species/biotype. There are some insect groups where a male is critical for species determination or where, in addition to having a male and female, a larval form is required. The diagnostic protocol (see Step 3) will include details on any such requirement and this should also be included in this section.

In case where only immature individuals are collected during the survey but adults are needed for reliable diagnosis, the laboratory protocol should include the relevant rearing methods.

If stems and/or leaves appear to be attacked by nematodes, these may have been collected along with soil samples and sent to the laboratory. The plant material should be removed from the bag and examined as soon as possible to avoid rotting of the tissue and stored separately from soil and/or root samples.

Finally, sorting and selection includes recording some observations. Plant material, insects and mites tend to change colour during storage, especially if stored in liquid preservatives such as alcohol. This is particular true of caterpillars and becomes critical in some groups such as tephritid fruit flies where coloured bands on the wings are important for identification. It is a

good idea to record the colours observed and even better if pictures can be taken. In the laboratory protocol provide guidance on the observations to be recorded and that will be useful during pest diagnosis.

◆ Temporary storage

After the samples have been received and sorted, time is often not immediately available to prepare the specimens for taxonomic identification and or permanent storage. The specimen must then be temporarily stored and proper storage is critical to maintaining the integrity of the specimen. There are several methods of storing specimens, depending on the type of sample material, the pests of concern and the length of time they will be temporarily stored.

❖ Refrigeration and freezing

Medium to large insect specimens may be left in tightly closed bottles for several days in a refrigerator and still remain in good condition for pinning as will smaller specimens if left overnight. Some moisture must be present in the containers so that the specimens do not become “freeze-dried”. Plant material can be placed in sample bags and also stored in the refrigerator for a short while. Fungal and bacterial samples should be stored in a refrigerator at 2–5°C. Note however, that some pathogens do not survive cold conditions. If this is true of the pest of concern, the protocol should identify the appropriate storage conditions. Soil samples can be stored in a refrigerator at 4–8°C for several days without severe deterioration or alteration in relative composition of the nematode population.

❖ Liquid preservative

Larvae and most soft-bodied adult insects and mites can be kept almost indefinitely in liquid preservatives such as alcohol, alcoholglycerin-acetic acid (AGA) solution (Thrips and most mites), kerosene-acetic acid-dioxane (KAAD) (larvae). Larvae of most insects should be boiled in water to "fix" their proteins and prevent them from turning black, then placed in alcohol.

❖ Storage for molecular diagnosis

Specimens for molecular work should be collected in 95% or absolute (100%) ethanol (ethyl alcohol). It is best if specimens are thoroughly dehydrated by changing the alcohol at least a couple of times before the specimens are stored for any length of time. It is also advisable to keep specimens cold (frozen if possible).

### Step 3 Diagnosis of sample

The main objective of taking survey samples to a diagnostic laboratory is for species identification. The method of diagnosis used is specific to the pest species of concern and must be detailed in the laboratory protocol. Each diagnostic protocol should contain the methods and guidance necessary for the pest(s) to be detected and positively identified by an expert (i.e. an entomologist, mycologist, virologist,

bacteriologist, nematologist, weed scientist, molecular biologist) or competent staff who is specifically trained.

The methods included in diagnostic protocols should be selected on the basis of their sensitivity, specificity and reproducibility. In addition, the availability of equipment, the expertise required for these methods and their practicability (for example ease of use, speed and cost) should be taken into account when selecting methods for inclusion in the diagnostic protocol.

There are a number of diagnostic protocols that are published as international, regional or national standards (Box 24) and these may be used and included in the laboratory protocol. Where no published diagnostic protocol is available, the diagnostician must prepare a protocol based on the scientific literature.

**Box 24. Links to some Regional and International Diagnostic Protocols**

<http://archives.eppo.int/EPPOStandards/diagnostics.htm>

<http://plantbiosecuritydiagnostics.net.au/resource-hub/priority-pest-diagnostic-resources/>

<https://www.ippc.int/en/core-activities/standards-setting/ispms/>

The following sections should be included in your diagnostic protocol:

- ◆ **Pest information** - The pest information section provides brief information on the pest, including, where appropriate, its life cycle, morphology and any variations (morphological and/or biological).
- ◆ **Taxonomic information** - This section provides information on name (current scientific name, author and year (for fungi, teleomorph name if known)), synonyms (including former names), accepted common names, anamorph name of fungi (including synonyms), acronym of viruses and viroids, taxonomic position (including information on subspecies classifications where relevant).
- ◆ **Detection methods** - This section of the diagnostic protocol should provide information and guidance on:
  - ❖ the host plant(s) and the part(s) of the plant on which the pest may be found
  - ❖ the signs and/or symptoms associated with the pest (characteristic features, differences or similarities with signs and/or symptoms from other causes), including illustrations, where appropriate
  - ❖ the developmental stages of the pest that may be detected, together with their likely abundance and distribution on the plant
  - ❖ methods for extracting, recovering and collecting the pest from the plants,
  - ❖ methods for indicating the presence of the pest in asymptomatic plant material or other materials (e.g. soil or water), such as ELISA tests or culturing on selective media.
- ◆ **Identification Methods** - This section should provide information and guidance on methods that either used alone or in combination lead to the identification of the pest.

The main types of methodologies used in diagnostic protocols include those based on morphological and morphometric characteristics, biological properties such as virulence or host range of a pest, and those based on biochemical and molecular properties. Morphological characteristics may be investigated directly or after culturing or isolation of the pest. Culturing and/or isolation may also be required for biochemical and/or molecular assays. Details should be provided when culturing or isolation procedures are necessary components of methods.

For morphological and morphometric identifications, details should be provided, as appropriate, on:

- ❖ methods to prepare, mount and examine the pest (such as for light microscopy, electron microscopy and measurement techniques)
  - ❖ identification keys (to family, genus, species)
  - ❖ descriptions of the morphology of the pest including illustrations of morphological diagnostic characteristics, and an indication of any difficulties in seeing particular structures
  - ❖ comparison with similar or related species
  - ❖ relevant reference specimens or cultures.
  - ❖ For biochemical or molecular identifications, each method (e.g. serological methods, electrophoresis, PCR, DNA barcoding, RFLP, DNA sequencing) should be described separately in sufficient detail (including equipment, reagents and consumables) to perform the test.
- ◆ **Records/Documentation** - As part of your quality assurance programme and in order to enable traceability of diagnosis results, laboratories should document all diagnoses conducted. The information which should be recorded as presented in Box 25.

**BOX 25. Information that should be recorded in a diagnostic report**

- ◆ scientific name of pest identified
- ◆ code or reference number of the sample (for traceability)
- ◆ nature of the infested material including scientific name of host where applicable
- ◆ origin (including the geographic location if known) of the infested material, and location of interception or detection
- ◆ description of signs or symptoms (including photographs where relevant), or their absence
- ◆ methods, including controls, used in the diagnosis and the results obtained with each method
- ◆ for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)

- ◆ for biochemical and molecular methods, documentation of test results such as photographs of diagnostic gels or ELISA printouts of results on which the diagnosis was based
- ◆ where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue) - the name of the laboratory and, where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- ◆ dates of collection of the sample, and of detection and identification of the pest where appropriate, state of the pest, alive or dead, or viability of its development stages.

In the report, the result of a diagnosis should be reported accurately, clearly, unambiguously and objectively. It should be capable of being verified later by a specialist expert. In case of a dispute on a diagnostic result, an accurate report is one of the key elements that will be considered. Box 26 gives an example of a simple diagnostic report.

**Box 26. Example of a simple diagnostic report**

**CROP AND PLANT PROTECTION UNIT: ENTOMOLOGY DIAGNOSTIC REPORT**

**DATE:** October 9, 2007

**SCIENTIFIC NAME:** *Thrips palmi*

**TAXONOMIC POSITION:** Thysanoptera: Terebrantia: Thripidae: Thripinae: Thripini

**NATURE OF INFESTED MATERIAL:** Cucurbitaceae: *Citrullus lanatus* Watermelon

**ORIGIN OF INFESTED MATERIAL:** St. Elizabeth, Jamaica

**OTHER LOCATION(S):** Old Harbour, St. Catherine

**STATE OF PESTS:** Live adults, nymphs and eggs

**DATE SAMPLES COLLECTED:** March 22, 2007

**DATE OF DIAGNOSIS:** March 26, 2007

**DIAGNOSTIC LABORATORY:** Bodles Research Station, Old Harbour, St Catherine

**DIAGNOSTICIAN:** Juliet Goldsmith

**REFERENCES:** Diagnosis determined with reference to diagnostic standard: *Thrips palmi*, Annex to International Standard for Phytosanitary Measures (ISPM) No. 27 (Diagnostic Protocols for Regulated Pests).

**SIGNS AND SYMPTOMS:**

- Silvery scars on leaf surface, Leaf bronzing, leaf chlorosis
- Stunted leaves and terminals.
- Deformed buds and fruits, fruits failed to develop.
- Small (yellow) fast moving insects seen with naked eye



Figure 1 *Thrips palmi* damage on cucurbit leaves

**MORPHOLOGICAL IDENTIFICATION**

• **Slide preparation**

Specimens were transferred from the leaf surface directly into a drop of Hoyers mounting medium on a glass slide. Wings and antennae were spread with a micropin and a cover slip added. Slides were placed on a hot plate (temperature just warm to the touch) overnight to facilitate clearing of the specimen and then examined using a compound microscope.

• **Characters of *Thrips palmi***



Figure 2 Antenna: seven segments; length of segment VI 42-48 micrometers

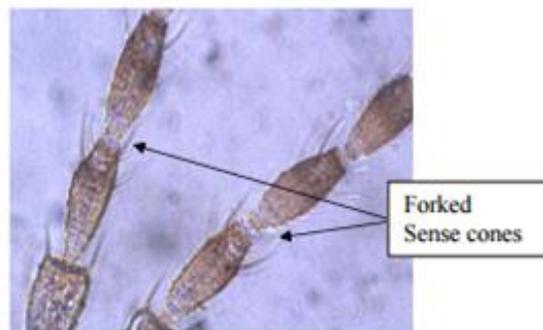


Figure 3. Antenna: segments III and IV with forked sense cones

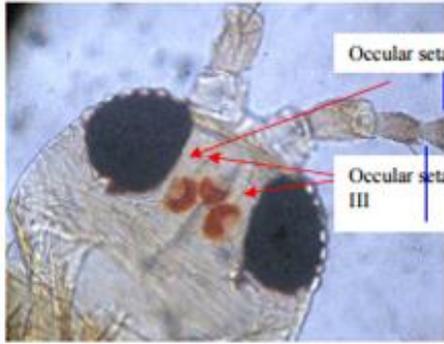


Figure 4 Head with two pairs of ocular setae

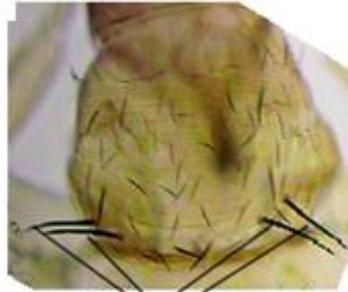


Figure 5 Pronotum: Two pairs of posteroangular setae

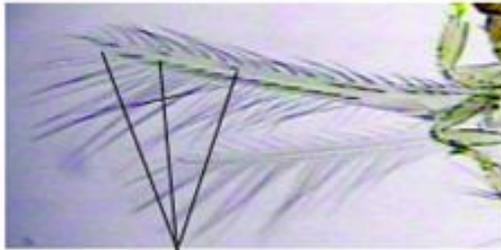


Figure 6 Forewing: first vein – row of setae with gaps in distal half

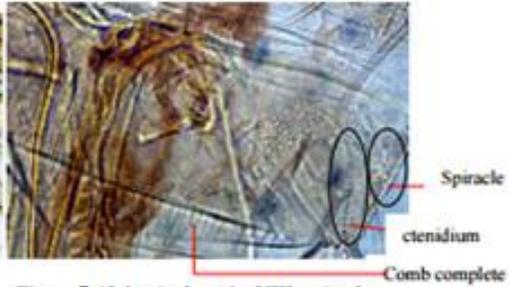


Figure 7 Abdominal tergite VIII: spiracle lying anterior and lateral to ctenidium

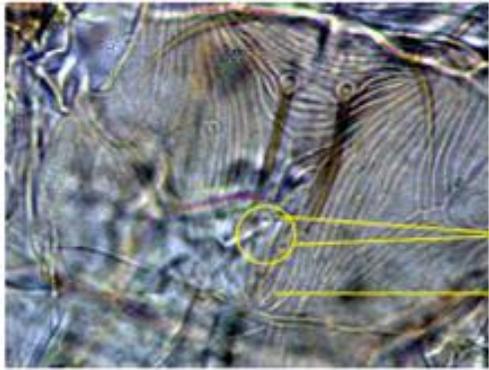


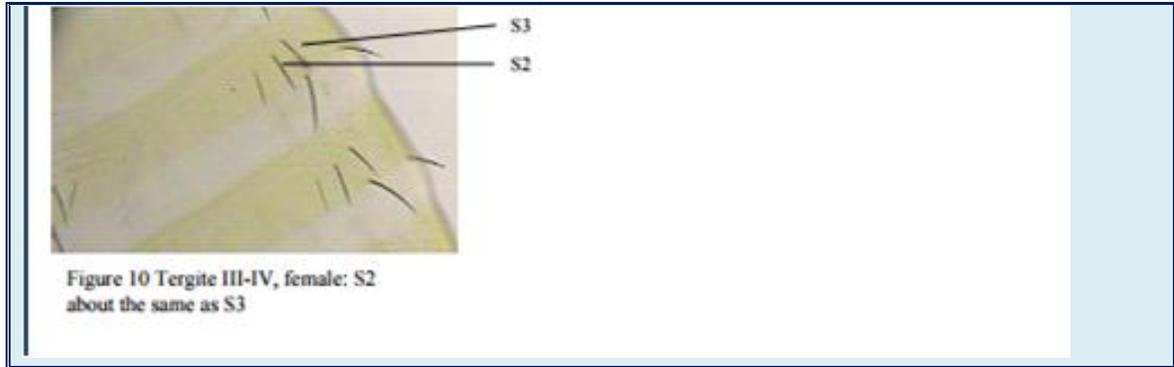
Figure 8 Metanotum

Metanotal pores  
Converging sculpture



Figure 9 Abdominal tergite II: four lateral setae

Four setae in a row



#### Step 4 Permanent Storage

Once taxonomic identification has been completed, it becomes essential that the specimen be properly stored in a permanent reference collection. The laboratory protocol should provide guidelines on the most appropriate preservation and storage methods for the pests concerned. These collections are very valuable as archives of biodiversity and as a scientific resource. They are the source of type and voucher specimens and are invaluable to taxonomic studies. There are many methods and materials used to permanently store specimens, depending on the type of organism:

- ◆ Dry collections - These mostly comprise pinned specimens that are usually stored in glass-topped drawers slotting into special wooden or steel cabinets. The drawers have tight-fitting lids to keep out museum pests, dust and moisture. The drawer bottoms are lined with a soft substrate into which the pins are inserted (Fig. 15).
- ◆ Wet collections - These are mostly collections of specimens stored in ethanol, or other preserving fluid, in glass vials with tight-fitting stoppers. Ideally, the vials should be of convenient containers for preserving specimens in liquid (Fig. 16).
- ◆ Slide mount collections - Slide mounts are stored in special commercially available slide boxes. These have grooves to hold the slides in position and apart from each other (Fig. 17). They are stored on shelves in an upright position, so that the slides inside lie horizontally, with the side containing the specimen facing upwards. Alternatively, cabinets made specifically for this purpose can be used.



Source: <http://sites.psu.edu/>

Collections should be stored in a safe place, adequately protected against fire and other hazards, and should be inspected on a regular basis for any sign of damage.

**Box 27. References on diagnostics and preserving samples**

- i. Collecting and preserving insects and mites by USDA ARS available at [http://www.ars.usda.gov/Main/site\\_main.htm?docid=10141&page=13](http://www.ars.usda.gov/Main/site_main.htm?docid=10141&page=13)
- ii. Basic requirements for quality management in plant pest diagnosis laboratories in EPPO Bulletin Volume 37, Issue 3, pages 580–588, December 2007
- iii. Documentation and reporting on a diagnosis in EPPO Bulletin Volume 36, Issue 3, pages 459–460, December 2006
- iv. Handbook: Good Laboratory Practices (GLP): Quality practices for regulated non-clinical research and development-2<sup>nd</sup> edition. 2009. WHO available at <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2338.2006.01042.x/epdf>
- v. ISPM 6 Guidelines for Surveillance available at , Rome, IPPC, FAO
- vi. ISPM 27, 2006 Diagnostic Protocols for Regulated Pests, Rome, IPPC, FAO
- vii. A listing of commonly used diagnostic tests in plant pathology has been shared by Sherrie Smith, the University of Arkansas and can be found at [http://wiki.bugwood.org/Virginia\\_Tech\\_Diagnostic\\_Manual](http://wiki.bugwood.org/Virginia_Tech_Diagnostic_Manual)
- viii. A basic key to identifying insects to the Order for any amateur can be found at <http://www.amentsoc.org/insects/what-bug-is-this/adult-key.html>

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## Chapter 6 Preparation of public awareness material

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Public awareness material doesn't have to be expensive and cut into your budget. Printed flyers can be produced in-house, photocopied and mass distributed, very cheaply, as inserts in your daily newspaper. Standardizing your printed material into pest fact sheets, pest alerts and pest advisories can, with time, sensitise your stakeholder to the purpose of your communication.

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### 6.1 Types of flyers

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Flyers provide meaningful information about a topic in a format that is extremely brief and easy to read. It can be used to provide an overview, recommendations and advice, and sometimes to convince the reader to change their behaviour. In surveillance flyers can be grouped into three types:

1. A **pest fact sheet** provides up-to-date, timely information on pest situations that are of plant quarantine/economic/agricultural importance. It delivers pest-related information (biology, ecology, distribution and control methods) to **facilitate detection, prevention and management**. It aims to promote good practices leading to reduced pest levels and pesticide use.
2. A **pest alert** serves as an early warning to citizens and travellers on plant pests not yet present in the country but which pose an imminent threat to the country's agriculture or the environment as they may be present in neighbouring countries or our trading partners'. It **facilitates awareness, early detection, prevention and thus early management** in the event of an introduction. It may also **advise on cautions/preventive/required measures that should be taken to deter the entry/spread** of potentially destructive plant pests.
3. A **pest advisory** aims to warn citizens on plant pests that are new to, or recurring pest with a seasonal explosion of population in your country that may pose a risk to the country's agriculture or the environment. It **advises and guides the response to this new plant pest or imminent threat and recommends appropriate actions**.

Box 28 gives two examples each of a pest factsheet, pest alert and pest advisory.

**Box 28. Examples of flyers****1. Pest factsheet**



Government of Trinidad and Tobago  
Ministry of Food Production

# FACTSHEET

## Red ring disease of coconuts

**What is it?**  
Red ring disease is the most important disease of coconuts. It is caused by a nematode, *Bursaphelenchus cocophilus* (Fig. 1). The nematode can be transmitted by the palm weevil, *Rhynchophorus palmarum* (Fig. 2).



Fig. 1. Red ring nematode (0.7-1.3 mm)  
Fig. 2. Male and female palm weevil (20 - 41mm)

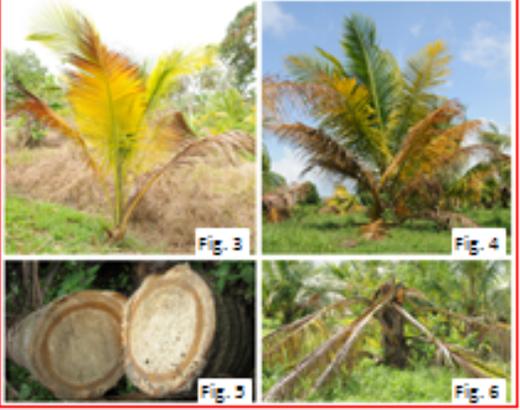


Fig. 3. Bronzing from the tips of the leaflets to the base and in the older to the younger leaves.  
Fig. 4. Petioles break at the base.  
Fig. 5. Brick-red to brownish-red ring 2.5 – 5 cm from the edge of the trunk.  
Fig. 6. Palm dies.

**Why should I care?**  
Red ring disease can be found on 17 palm species including oil palm, date palm, West Indian royal palm, gru-gru palm, Moriche palm, and cocurite palm. In Trinidad and Tobago coconut is the main palm affected. This nematode causes serious damage to coconuts, stunting and eventually killing the palm. Over 30% losses of young trees have been reported. Palms as young as 2.5 years can be infected.

**What do I look for?**

- Trees show a progressive yellowing and bronzing from the tips of the leaflets to the base and in the older to the younger leaves. (Fig. 3).
- The petioles break at the base and several yellowish or bronze leaves hang around the trunk (Fig. 4).
- Nuts fall prematurely.
- Inflorescences die.
- In 20 years or older palms, a little leaf symptom is seen. The palms produce progressively shorter leaves until only a leafless rachis is produced.

**What can I do?**

- Early detection, destruction and removal of infested palms are key to management.
- Aggressive phytosanitation is the best chance to halt the spread of red ring disease to nearby trees.
- Palms should be cut down.
- Then chopped into small pieces and sprayed with an insecticide (cabaryl) to control the larvae of the weevil.
- Trees should be removed and burned.

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Surveillance Unit,  
Ministry of Food Production  
Republic of Trinidad & Tobago

8U\_MFP No. 34  
August 2012  
848-8234, email: reportpest@gmail.com





Government of Trinidad and Tobago  
Ministry of Food Production

## FACTSHEET

### *Bursaphelenchus cocophilus*, the red ring nematode

#### What is it?

The red ring nematode, *Bursaphelenchus cocophilus* is very tiny, only 1mm long (Fig. 1). It causes red ring disease in palms. It is spread mainly through an insect vector. In Trinidad and Tobago this vector is the palm weevil, *Rhynchophorus palmarum* (Fig. 2).



Fig. 1

PHOTOS: PAREAN BOSENY

Fig. 1. Red ring nematode  
Fig. 2. Palm weevil



Fig. 2

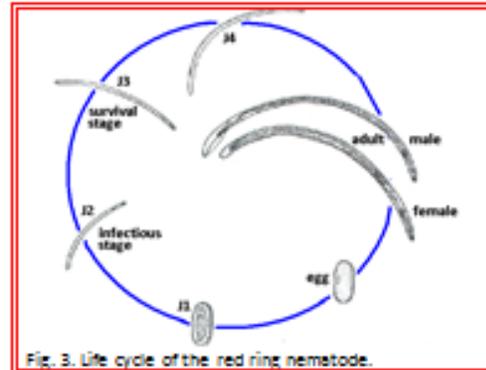


Fig. 3. Life cycle of the red ring nematode.

#### Why should I care?

Red ring disease can be found on 17 palm species including oil palm, date palm, West Indian royal palm, gru-gru palm, Moriche palm, and cucurite palm. In Trinidad and Tobago, the red ring nematode causes serious damage to coconuts, stunting and eventually killing the palm. Over 30% losses of young palms have been reported. Palms as young as 2.5 years can be infected.

#### How does it spread?

The red ring nematode has a plant parasitic life cycle. After mating, the female lays eggs in the plant issues which hatch into juveniles that undergo 4 molts before becoming adults. The first juvenile (J1) develops in the shell, the second juvenile (J2) is able to infect the vector. The third juvenile (J3) is carried by the vector to other healthy palms. It can survive in the insect without undergoing any changes. Upon infecting a new palm host J3 molts into the last juvenile (J4) and then into an adult (Fig. 3). The whole life cycle lasts 10 day.

The red ring nematode can spread to health palms in or on the bodies or faeces of the weevil. It may also be carried on seeds, seedlings, tools, vehicles, animals, or move by natural migration from infected to healthy roots. It can move 3.6 mm an hour in soil and almost 0.25 mm an hour in roots. Wood chippings, logs and the stump of cut infected palms also serve as infective material.

The red ring nematode does not survive in soil or on the body of its vector for more than 2-3 days and 10 days inside the body. It lives in rotten tissue of dead palms up to 90 days, 16 weeks in nut husks, 90 weeks in seedling tissue and a year in decaying roots. It prefers wet, poorly drained, swampy, or clay soils rather than sandy soil as it is susceptible to desiccation.

#### What can I do?

Treating infested palms with nematicide is difficult because the nematodes block the palm's waterways and reduce its water absorption. The most effective strategy is to destroy all infective material and control the vector. Infective palms should be cut down, chopped in small pieces and sprayed with an insecticide to control the weevil, and then burned to destroy both the weevil and the nematode.

Surveillance Unit,  
Ministry of Food Production  
Republic of Trinidad & Tobago

8U\_MFP No. 37  
8 September 2012  
848-8234, email:reportpest@gmail.com



## 2. Pest alert



Government of Trinidad and Tobago  
Ministry of Food Production, Land & Marine Affairs

## PEST ALERT

# Red Palm Weevil

## NOT PRESENT IN TRINIDAD AND TOBAGO

### What is it?

Red palm Weevil or Asian palm Weevil or Indian palm weevil, *Rhynchophorus ferrugineus* is the most dangerous and deadly pest of palms, including coconut, date, royal, and talipot (Fig. 1). It also infests agave and sugarcane.



Fig. 1

© Birgit Kremer, <http://www.iberia-natur.com>

### Where is it found?

Red Palm Weevil is native to Southeast Asia. Today it can be found in:

- **Asia:** Bangladesh, Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Pakistan, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, Vietnam
- **Africa:** Algeria, Canary Islands, Egypt, Libya, Madagascar, Malta, Morocco,
- **Middle East:** Bahrain, Georgia, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, United Arab Emirates
- **Europe:** Balearic Islands, Cyprus, France, Greece, Italy, Portugal, Sicily, Slovenia, Spain, Turkey
- **Oceania:** Australia, Papua New Guinea, Samoa, Solomon Islands
- **Americas:** Aruba, Curacao, USA.

### How is it spread?

Red palm weevil spread from one country to the next through the movement of infested palm material.

Within a country, the red palm weevil can easily spread as adult weevils are strong fliers and can fly up to 900m at a time and can travel up to 7 km in 3-5 days.

### Why should I care?

Red palm weevil damages include:

- tunnels on the trunk and base of the leaf
- oozing with a typical fermented odor from the tunnels
- drooping / yellowing of leaves
- trunk may break and the crown may topple (Fig.2)
- palm dies.

One weevil is enough to kill a tree as a female can lay 58-531 eggs.



Fig. 2

© <http://www.redweevil.com>

### What can I do?

- Avoidance is the best strategy.
- Do not bring any palm material from infested countries.

**HELP KEEP RED PALM WEEVIL  
OUT OF TRINIDAD AND TOBAGO**

Surveillance Unit,  
Ministry of Food Production, Land & Marine Affairs  
Republic of Trinidad & Tobago

SU\_MFPLMA No. 10  
December 2010  
646-6284





Government of Trinidad and Tobago  
Ministry of Food Production

## PEST ALERT

### Tomato leaf miner, *Tuta absoluta* Not present in Trinidad and Tobago

#### What is it?

The tomato leaf miner, *Tuta absoluta* is a tiny moth whose larva attacks solanaceous plants (like tomato, melongene, peppers, nightshade and jimson weed) and also beans. It is native of Central America and can also be found in South America, Europe and the Mediterranean.

#### What do I look for?

##### Damages:



Fig. 1-Gloplana; Fig. 2-Prof. Yamina Guenaou, University of Mostaganem, Algeria; Fig. 3 and 4- JM Cobos Suarez, Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid; and Fig. 5-Ghahram Farrokh, Iranian Research Institute of Plant Protection



The tomato leaf miner pierces into buds, shoots, stems, leaves, flowers and fruits. It feeds on the inner tissue, leaving the leaf transparent (Fig.1), as it feeds it deposits its excrements. Mines turn brown and become necrotic (Fig. 2). The inside of the stem appears hollow and brown (Fig. 3). Affected fruits show boreholes and mines under and around the sepal with heaps of dark, granular excrement on the surface (Fig. 4). Holes and mines serve as entry points for other organisms resulting in secondary rot (Fig. 5).

#### Why is it a problem?

It attacks the buds, stems, leaves, flowers and fruits. Affected fruits are unsuitable for sale. It can cause 100% loss. It is spread by infested fruits and packaging material. The population can grow very rapidly as it completes its life cycle in 24 days (having 12 generations per year) and each female can lay up to 260 eggs.

#### Insect:



JM Cobos Suarez, Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid



Eggs are creamy white to yellow and laid singly in the upper parts of the plant (Fig.6). The larva has four stages. The first stage is about 0.5 mm long and cream-coloured. It turns yellowish-green and has a black stripe at the back of their head (Fig.7). Full-grown larvae are about 7.5 mm and greenish pink. The pupa is light brown and in a cocoon (Fig.8). The moth is small 7mm, dark grey brown with dark spots on the wing, its antennae are long, and the legs and palps are ringed with black and brown (Fig.9).

#### What can I do?

- Report suspicious sightings to 646-6284
- email pictures to [reportpest@gmail.com](mailto:reportpest@gmail.com)

## 3. Pest advisory



Government of Trinidad and Tobago  
Ministry of Food Production

## PEST ADVISORY

### Bacterial fruit blotch, *Acidovorax avenae subsp. citrulli* A new pest in Trinidad

#### What is it?

Bacterial fruit blotch is a seed-borne bacterial disease that affects cucurbits (watermelon, cantaloupe, cucumber, and pumpkin) and some solanaceous plants (melongene and sweet peppers). It was discovered in Mayaro, Trinidad in 2012 on watermelons. It is known to occur in most watermelon producing countries.

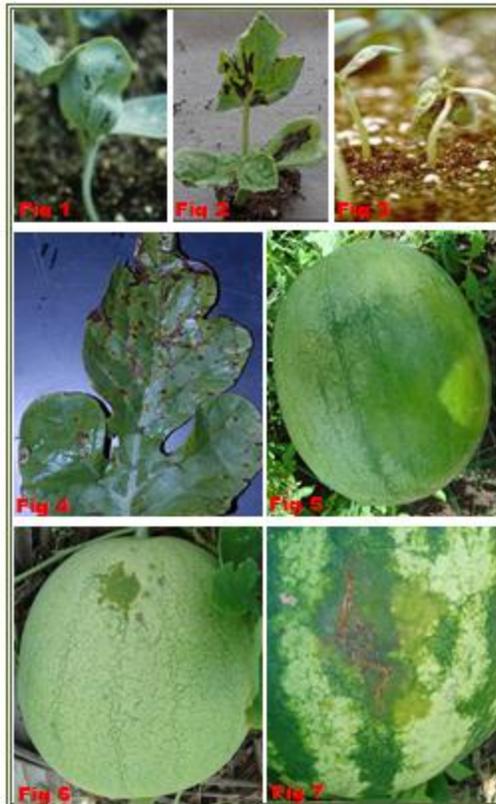
#### Why is it a problem?

Bacterial fruit blotch is devastating as losses reported are 80-100%. This loss is greater as symptoms may not be visible until two weeks prior to harvest and affected fruits are unmarketable. One infected plant is too much as the disease spreads rapidly!

#### What do I look for?

- Symptoms of bacterial fruit blotch can be seen on the seedling, mature leaves and fruit.
- On seedlings, water soaked areas on the underside of the leaf are the first sign (Fig. 1).
- Water-soaked lesions start as discrete spots, then coalesce, extend along the veins and may have a yellow halo (Fig. 2).
- In young seedlings, lesions can occur on the stem causing it to collapse and die (Fig. 3).
- In older leaves, lesions appear as dark angular spots (Fig. 4).
- The most characteristic symptom is dark, water-soaked lesions on the upper surface of the fruit (Fig. 5).
- Lesions begin as small, water-soaked spots with irregular margins that expand and coalesce to cover much of the fruit surface within 7 to 10 days (Fig.6).

- In advanced stages the rind ruptures and the fruit rots (Fig. 7).



Figures 1 and 3 taken from Univ of Florida; Figure 2 from Univ of Missouri and Figures 4-7 from Anthony St Hill, Ministry Food Production Trinidad.

**Report all sightings to 646-6284**  
**email us at [reportpest@gmail.com](mailto:reportpest@gmail.com)**

Surveillance Unit,  
Ministry of Food Production  
Republic of Trinidad & Tobago

SU\_MFP No. 38  
December 2012  
646-6284; [reportpest@gmail.com](mailto:reportpest@gmail.com)





Government of Trinidad and Tobago  
Ministry of Food Production

## PEST ADVISORY

### Bacterial leaf spot of lettuce A new pest in Trinidad

**What is it?**

Bacterial leaf spot is a new bacterial disease that affects lettuce. It was discovered in Trinidad in 2012. Bacterial leaf spot is devastating as losses reported are 80-100%.

**What do I look for?**

It appears as a water-soaked spot irregularly shaped, brown to black in appearance. Spots can coalesce to exhibit a blighted appearance.



Early symptoms: Numerous leaf spots on older leaves

Typical brown-black irregular spots – that begin to coalesce

Advance symptoms: Blighting of leaves

Photos: Anthony St Hill, Plant Pathologist, Ministry of Food Production

**Report all sightings to 646-6284  
email us at [reportpest@gmail.com](mailto:reportpest@gmail.com)**

Surveillance Unit,  
Ministry of Food Production  
Republic of Trinidad & Tobago

8U\_MFP No. 38  
January 2013  
646-6284; [reportpest@gmail.com](mailto:reportpest@gmail.com)



## 6.2 What are the basic guidelines in drafting flyers?

Flyers can be an effective tool to disseminate facts, send a message/illustrate your point, create awareness and solicit assistance. In drafting flyers, ask the questions:

- ◆ Who is the target audience?
- ◆ What will appeal most to this group?
- ◆ What reaction do you want?

Some general guidelines to follow in producing flyers are:

1. The flyer must be self-contained. It should obey the 6C's:
  - Complete - make sense on its own
  - Correct - defines your credibility
  - Clear
  - Concise
  - Conversational - easy to read
  - Constructive – stated in positive terms (avoid negative writing).
2. Target audience: determines the tone, style, pitch, angle and content of the message. It allows the assumption of the level of innate knowledge and what needs to be explained.
  - Know your audience – keep your language appropriate to the audience, write in layman's terms – avoid technical terms.
  - Give them all the tools to take action – who to contact, what to send, provide phone numbers/e-mail address.
  - Use an angle that would illicit the response desired - state facts, use 'shock' appeal.
3. Message/Content:
  - Keep it simple, clear and concise – easy to understand.
  - Must be relevant to the audience – tell them why they should care enough to take action.
  - Use pictures to effectively illustrate the message; keep words to a minimum - no one wants to read tons of information.
  - Write in present tense and active voice. Use short sentences.
  - Emphasize the importance in the first/lead paragraph.
  - Highlight the main message - what you want done.
  - Ensure it's factual, scientifically correct and current.
3. Layout (Box 29):
  - Must be attractive, eye catching, uncluttered - leave lots of white space. A coloured background can be distracting.
  - Make it readable - use at least 12-14 point font, non-cursive.
  - Must be logical, stated in a systematic and sequential order – easy to follow and read.
  - Organize the message in the 5 W's and H: What, Where, When, Why, Who and How.
4. Structure (Box 29): must contain
  - type of flyer
  - title,

- author/authority,
- referenced: number, year, copyright.

**Box 29. Structure and layout of a flyer**

**Country and Agency** (Government of Trinidad and Tobago, Ministry of Food Production)

**Type of flyer** (PEST ALERT)

**Title of flyer** (FROG SKIN VIRUS OF CASSAVA)

**Sub-Title of flyer** (NOT PRESENT IN TRINIDAD AND TOBAGO)

**What is it?**  
Frog skin virus of cassava is a virus-like disease that affects most varieties of cassava. It can be caused by a **phytoplasma** and a virus. It is found in Colombia, Peru, Costa Rica, Panama, Venezuela, and Brazil including the Brazilian Cassava Germplasm of EMBRAPA.

**What do I look for?**  
Some varieties may not show any symptoms above the ground. Some varieties exhibit leaf symptoms of mosaic, chlorosis, and curling which can easily be confused with damage from sucking insects, viral diseases, nutrient deficiency and herbicide (Fig 1).

**How is it spread?**  
Frog skin virus is spread by infected material, vegetative propagation and sucking insects.

**Why should I care?**  
Cassava yield loss of 90 - 100% is reported. Problem may not be visible as plants may appear healthy and vigorous. **Only at harvest little or no yield is obtained.**

**What can I do?**

- ✓ Avoidance is the best strategy. Check with Plant Quarantine Service before bringing any cassava planting material into Trinidad and Tobago.
- ✓ Do not bring any cassava material from infected countries.
- ✓ Select planting material by choosing those stems that produce healthy tubers.
- ✓ Contact your extension officer and check with Research Division of the Ministry of Food Production.
- ✓ Report suspicious symptoms to 646-6284 or email [reportpest@gmail.com](mailto:reportpest@gmail.com)

**Key Message** (Report all sightings to 646-6284 or email pictures to reportpest@gmail.com)

**Copyright, Author** (Surveillance Unit, Ministry of Food Production, Republic of Trinidad & Tobago)

**Issue No.** (56)

**Date** (May 2014)

**Logo, Identification**

**Box 30. Additional reading on effectively writing/drafting factsheets:**

- i. [http://www.programmed.eu/fileadmin/PROG\\_MED/Communication\\_projects/Communication\\_GUIDANCE/Factsheet\\_11.pdf](http://www.programmed.eu/fileadmin/PROG_MED/Communication_projects/Communication_GUIDANCE/Factsheet_11.pdf)
- ii. <http://www.onlinepclearning.com/brochure-make-a-brochure-or-factsheet-that-rocks-microsoft-word-and-powerpoint-2010/>

## Glossary

1. 3-component lures - synthetic food lures that attract primarily female flies.
2. Active capture - a method which requires the surveyor to be involved in the collection process
3. Active survey - deliberate, coordinated effort by pest management personnel looking for the pest.
4. Area of low pest prevalence - an area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures.
5. Aspirator – a tool used to capture small and fast moving insects, which is easily collected by hand.
6. Bait station or attract and kill traps – uses a food source (proteins, sugars, fruit, piece of plant tissue, or yeast) as the attractant to lure the pest in.
7. Beating tray – white heavy duty cloth stretched over a circular hoop or over a frame of two crossed sticks.
8. Cluster sampling - selecting all individuals within a randomly selected collection or group of individuals.
9. Collecting net - composed of a net bag made of cloth or fine mesh that is attached to a wire hoop, affixed to a wooden or metal pole.
10. Commodity pest list - a list of pests occurring in an area which is associated with a specific commodity.
11. Containment – the application of phytosanitary measures in and around an infested area to prevent spread of a pest.
12. Convenience sampling - the most convenient or readily available individuals to get a gross estimate of the results.
13. Cue lure - a synthetic kairomone, a very potent male insect attractant.
14. Delimiting survey - conducted to establish the boundaries of an area considered to be infested by or free from a pest.
15. Ecosystem - a dynamic complex of plant, animal and micro-organism communities and their abiotic environment interacting as a functional unit.
16. Endangered area - an area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss.
17. Entry of a pest - movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled.
18. Eradication – the application of phytosanitary measures to eliminate a pest from an area.
19. Establishment of a pest - the perpetuation, for the foreseeable future, of a pest within an area after entry.
20. General survey - a process whereby information on particular pests which are of concern for an area is gathered from many sources, wherever it is available and provided for use by the NPPO.
21. Habitat - part of an ecosystem with conditions in which an organism naturally occurs or can establish.
22. High risk areas – areas that the target pest may be introduced, established, found or spread.
23. Host pest list - a list of pests that infest/infect a plant species, globally or in an area.

24. Host range - species capable, under natural conditions, of sustaining a specific pest or other organism.
25. IncurSION - an isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future.
26. Introduction (of a pest) - the entry of a pest resulting in its establishment.
27. Jackson trap - waxed cardboard material that is folded into a delta shaped object.
28. Judgment sampling - selects the sample based on judgment.
29. Light traps – uses different wavelength of light as the attractant to lure the pest in.
30. Management or suppression - the application of phytosanitary measures in an infested area to reduce pest populations.
31. Mc Phail trap - a glass trap with a water reservoir.
32. Methyl eugenol - a kairomone used to attract many species of the Subfamily Dacinae.
33. Monitoring - official ongoing process to verify phytosanitary situations.
34. Monitoring survey - ongoing survey to verify the characteristics of a pest population.
35. Multi Lure trap - consists of an inverted funnel base and a transparent cover on top with a plastic dispenser holder for the pheromone.
36. National pest list – compilation of a list of pest occurring in a country.
37. Occurrence (of a pest) - the presence in an area of a pest officially recognized to be indigenous or introduced and not officially reported to have been eradicated.
38. Outbreak (of a pest) - a recently detected pest population, including an incurSION, or a sudden significant increase of an established pest population in an area.
39. Passive capture - a method which doesn't require a collector.
40. Passive survey - activities where public, farmer, other industry personnel notify on pest presence; usually done through hotline, emails, farm visit records, interception reports, diagnostic reports.
41. Pathway - any means that allows the entry or spread of a pest.
42. Pathway surveillance - surveys that target high risk sites to look for pests at specific intervals/time.
43. Pest - any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products.
44. Pest advisory - aims to warn citizens on plant pests that are new to, or recurring pest with a seasonal explosion of population in your country that may pose a risk to the country's agriculture or the environment.
45. Pest alert - serves as an early warning to citizens and travellers on plant pests not yet present in the country but which pose an imminent threat to the country's agriculture or the environment as the may be present in neighbouring countries or our trading partners'.
46. Pest fact sheet - provides up-to-date, timely information on pest situations that are of plant quarantine/economic/agricultural importance.
47. Pest free area - an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained.
48. Pest introduction - the entry of a pest resulting in its establishment.

49. Pest record - a document providing information concerning the presence or absence of a specific pest at a particular location at a certain time, within an area (usually a country) under described circumstances.
50. Pest risk - the probability of introduction and spread of a pest and the magnitude of the associated potential economic consequences.
51. Pest status on an area - presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information.
52. Pheromone traps – tools that use semiochemicals to attract the target insect.
53. Pitfall traps - are tools for detecting walking and crawling soil and litter inhabiting arthropods.
54. Quarantine pest - a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.
55. Quarantine pest list - compilation of a list of pest not present in a country or may be present but under eradication or control.
56. Quota sampling - selects the first individual that meets the criteria until the desired number of samples is obtained.
57. Random sampling - each individual of the study population has an equal probability or chance of being selected.
58. Regulated area – an area into which, within which and/or from which plants, plant products and other regulated articles are subjected to phytosanitary regulations or procedures in order to prevent the introduction and/or spread of quarantine pests or to limit the economic impact of regulated non-quarantine pests.
59. Sample - a subset of the members from a larger population that you collect and analyse to make inferences.
60. Sample size - the number of members of the population being studied or the number  $n$  of sampling units that are selected from the population.
61. Sampling - an information collection tool.
62. Sampling intensity - the proportion/ percentage of the population being sampled.
63. Sampling unit - the smallest unit, from which you are gathering the information.
64. Size of sample - what constitute a sample.
65. Snowball sampling - relies on referrals from initial subjects to generate additional subjects.
66. Specific surveys - procedures by which NPPOs obtain information on pests of concern on specific sites in an area over a defined period of time.
67. Stratified sampling - achieved by separating the population into non-overlapping groups called strata and then obtaining a proportional simple random sample from each group.
68. Surveillance - an official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures.
69. Survey - an official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area.
70. Systematic sampling - the  $N^{\text{th}}$  individual is sampled.
71. Targeted or Detection survey - conducted in an area to determine if pests are present.

72. Torula yeast - a food attractant, high in protein.
73. Tri Med Lure - a synthetic female pheromone that disrupts communication such that the males cannot find the females to mate with.