Agriculture Pest Surveillance Manual

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Foreword

Plant pest surveillance record is essential for the development of robust quarantine policies, management of endemic pests and for negotiating international trade for agricultural commodities.

Crop health problems that affect its yield and quality adversely harm not only the famers but also the society and consumers at large. Outbreak of Giant African Land Snails in the east; wide-spread of Armyworm; Corn blight in Maize; Rice Blast in Rice; Chili Blight in Chili; Potato Blights in Potato; Citrus Greening and Citrus Fruit fly in Citrus; and weeds like Congress grass and Shochum are some of the important examples of adverse socio-economic and environmental impacts.

In addition, the current system of agriculture development is mostly based on inclusion of exotic germplasm in hope of better quality and production. Regulation of quality and safety of agricultural products including foods were least concerned until the year 2000 when the Bhutan Agriculture and Food Regulatory Authority (BAFRA) was formally established. However, not much of rigorous quarantine actions can be initiated owing to the absence of robust surveillance system in the country. This is going to affect in availing competitive advantage in accessing foreign markets as well in progressing with Integrated Pest Management research and developmental impacts.

Therefore, the National Plant Protection Centre being mandated to carry out the pest surveillance activities in the country has initiated the development of National Pest Surveillance System which consists of national plant pest database and the database management tools.

In this surveillance manual, most of the principles and methods are adapted to Bhutan's need from various standards and guidelines such as "Guidelines for Surveillance for Plant Pests in Asia and the Pacific (Teresa McCaughey 2005), maintaining the prescribed standards of International Standard for Phytosanitary Measures (ISPM). Therefore, information generated henceforth will also qualify in meeting the global agriculture trading standards in addition to real-time information required for other applications, particularly for R&D. This surveillance manual is broadly divided into two parts:

1. General Surveillance, and
2. Specific surveys

The information generated through the general surveillance in most of the cases shall be the basis for conducting specific surveys by the researchers.

This manual shall be guiding the individual in conducting surveillance activities but need not necessarily replace entirely the pest specific surveillance formats. It is advisable that researchers and extensionists conduct the plant pest surveillance activities in line with the standard methods of pest surveillance described in this manual.

The manual shall be updated as and when necessary.

Thank you,

Yeshey Dema
Program Director

Glossary

Area: An officially defined country, part of a country or all or parts of several countries

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

Delimiting survey: Survey conducted to establish the boundaries of an area considered to be infested by or free from a pest

Detection survey: Survey conducted in an area to determine if pests are present

General *surveillance*: 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

International Plant Protection Convention (IPPC): An international convention deposited with FAO in Rome in 1951 and as subsequently amended

International Standard for Phytosanitary Measures (ISPM): An international standard adopted by the Conference of FAO, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the IPPC

International standards: International standards established in accordance with Article X paragraph 1 and 2 of the IPPC

Monitoring survey: Ongoing survey to verify the characteristics of a pest population

*National Plant Protection Organization (NPPO)***:** Official service established by a government to discharge the functions specified by the IPPC

Non-quarantine pest: Pest that is not a quarantine pest for an area

Pest: Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products

Pest free area (PFA): 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

Pest free place of production (PFPP): Place of production in which a specific pest does not occur, as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period

Pest free production site (PFPS): A defined portion of a place of production in which a specific pest does not occur, as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period and that is managed as a separate unit in the same way as a pest free place of production

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

Pest risk analysis (PRA): The process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it

Pest status (in 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 judgment on the basis of current and historical pest records and other information

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

Regional Plant Protection Organization (RPPO): An intergovernmental organization with the functions laid down by Article IX of the IPPC

Regulated pest: A quarantine pest or a regulated non-quarantine pest

Regulated non-quarantine pest (RNQP): A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party

Specific surveys: Procedures by which NPPOs obtain information on pests of concern on specific sites in an area over a defined period of time

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

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

Abbreviations

AEO	Agriculture Extension Officer
APPPC	Asia Pacific Plant Protection Commission
ARDC	Agriculture Research and Development Centre
ASEAN	Association of Southeast Asian Nations
ASEANET	South East Asian LOOP of the BioNET INTERNATIONAL
BAFRA	Bhutan Agriculture and Food Regulatory Authority
DAO	District Agriculture Officer
DoA	Department of Agriculture
EIL	Economic Injury Level
ET	Economic threshold
FAO	Food and Agriculture Organization of the United Nations
GPS	Geographical Positioning System
ICPM	Interim Commission on Phytosanitary Measures
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
MoAF	Ministry of Agriculture and Forests
NPPC	National Plant Protection Center
NPPO	National Plant Protection Organization
PFAs	Pest Free Areas
PFPP	Pest Free Place of Production
PFPS	Pest Free Production Site
PPO	Plant Protection Officer
PRA	Pest Risk Assessment
RA	Research Assistant
RNR EC	Renewable Natural Resources Extension Centre
RO	Research Officer
RSPM	Regional Standard for Phytosanitary Measures
SPS	Sanitary and Phytosanitary Measures

Table of Content

Foreword ..... ***i***
Glossary ..... ***ii***
Abbreviations ..... ***iv***
Chapter 1: Introduction: Concepts, Principle and Practices ***1***
1.1 Introduction 1
1.3 Objectives of Pest Surveillance 1
1.5 Types of Pest Surveillance 1
1.5.1 General Surveillance *1*
1.5.2 Specific Surveys *2*
1.5.2.1 Kinds of Surveys 2
Chapter 2: Conducting General Surveillance studies ***5***
2.1 Introduction 5
2.2 ePest Surveillance System 5
2.3 Other electronic tools/ system 5
Chapter 3: Specific Survey ***6***
3.1 Introduction 6
3.2 Survey sampling methods 6
3.2.1 Stratified random sampling *6*
3.2.2 Random sampling surveys *6*
3.2.3 Systematic sampling surveys *7*
3.2.4 Targeted site surveillance *7*
3.2.5 Blitz surveys *7*
3.2.6 Full sampling *8*
3.2.7 Farmers' observations *8*
3.2.8 Insect-trapping surveys *8*
3.2.9 Purposive and haphazard Sampling *8*
3.3 Designing specific surveys and Planning Process/ steps 9
Step 1: Choosing a Title *10*
Step 2: Reasons for surveying *10*
Step 3: Identify target pests *10*
Step 4: Identify target host(s) *10*
Step 5: Alternative hosts *11*
Step 6: Review of earlier survey plans *11*
Step 7: Identifying the survey area/regions *11*
Step 8: Identifying the survey Dzongkhag within the region *12*
Step 9: Identifying the possible survey places, field sites and sampling sites *12*
Step 10: Methods for choosing sites *12*
Step 11: Calculating sample size *13*
Step 12: Timing of the survey *16*
Step 13: Planning collection of data in the field *16*
Step 14: Methods of collecting pest specimens *16*
Step 15: Electronic data storage *20*
Step 16: People involved / farmers *20*
Step 17: Obtain permission to visit sites *20*
Step 18: Pilot survey/ validation survey *20*
Step 19: Perform survey (collect raw data and samples) *20*
Step 20: Analyze data *20*
Step 21: Report the results *21*
Chapter 4: General sampling guidance for weeds ***22***

4.1 Introduction 22
4.2 Sampling area 22
4.3 Sampling method 22
4.4 Time of survey 22
4.5 Sampling Pattern 22
4.6 Weed flora 23
4.7 Example of detection and delimiting survey in weed 23
Chapter 5: General sampling guidance for insects and diseases 25
5.1 Introduction 25
5.2 Sample size 25
5.3 Sample Unit 25
5.4 Sampling pattern 25
5.5 Sampling methods and devices 26
5.6 Methods for sampling diseases 27
5.7 Other methods 27
5.8 Insect's stage to be sampled 27
5.9 Number of sampling unit 27
5.10 Time to sample 28
5.11 Pattern of sampling 28
5.12 Other considerations 28
Chapter 6: Some sample survey guidelines for specific pests 29
6.1 General survey guidelines for citrus fruit fly 29
6.1.1 General Background 29
6.1.2 Fruit Fly Surveillance Methodology 29
6.1.2.1 Fruit Fly Lures 29
6.1.2.2 Traps 29
6.1.2.3 Materials Required for Survey 31
6.2 Survey Strategies and Guidelines for Detection Survey for Citrus HLB 32
6.2.1 Overview 32
6.2.2 Requirement 32
6.2.3 Survey procedures 32
6.2.4 Identification Guide 32
6.2.5 Survey Guidelines 33
6.2.6 Collection of samples for laboratory test 34
6.3 Potato Tuber Moth (PTM) monitoring with Pheromone Traps 34
6.3.1 Introduction 34
6.3.2 Survey Methodology 34
6.3.2.1 Requirement for Survey 34
6.3.2.2 Traps Set-ups 35
6.3.2.3 Counting the Moths 35
6.3.2.4 How to Interpret Tuber moth Trap Catch Numbers 35
6.3.2.5 Recognition features for tuber moth 35
Annexure 1: Pest Surveillance Reporting format 2016 37
Annexure 2: Some of the internet resources for pest information and specific methodology 38
References 39

Chapter 1: Introduction: *Concepts, Principle and Practices*

1.1 Introduction

Pest surveillance is an official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures. Through pest surveillance, up-to-date data on the pests' diversity, their distribution and damage to crops are maintained to provide advisories to the growers, for contingency planning and research purpose. It is therefore an essential pre-requisite of any IPM program to play a significant role in the ability to see the possibility for harm to the crops. A little extra time and resources spent on surveillance at the beginning will save a lot of time and money later on should a pest break out. Pest surveillance comprises three basic components:

- Determination of the level of incidence of pest species,
- Determination of what loss the incidence will cause, and
- Determination of economic benefits the control will provide.

It is very important to get a taxonomic support while conducting any surveillance studies or surveys without which a misidentification or incorrectly screening of target pest is high. A correct identification is also essential for various agencies/ individuals like Bhutan Agriculture & Food Regulatory Authority (BAFRA) for quarantine regulation, for breeders to explore resistance varieties etc…

1.3 Objectives of Pest Surveillance

The objectives of pest surveillance are; to

- monitor the presence of existing and new pest species (early detection)
- assess pest population and damage at different growth stage of crop
- study the influence of weather parameters on pest
- study changing pest status (minor to major and vice versa)
- assess natural enemies and their influence on pests
- effect of new cropping pattern and varieties on pest
- effect of pest management practices

1.5 Types of Pest Surveillance

According to ISPM 6, there are two major types of surveillance system practiced i.e. general surveillance and specific surveys.

In Bhutanese context, the general surveillance shall be carried out mostly by the extensionists or designated research officers/ assistants and also to some extent by regulators like BAFRA field inspectors. The specific surveys are highly technical and shall be carried out by the researchers mainly National Plant Protection Center (NPPC).

1.5.1 General Surveillance

The term "general surveillance" consists of two categories of activities; first collecting information about a pest and second to develop clear communication

between NPPC and other people/ agencies like BAFRA who have information about pests.

The main source of information shall be the general surveillance studies conducted by the extensionists or regulators using either the electronic platform (ePest Surveillance System) or the off-line surveillance system established & managed by NPPC. However, other relevant sources such as national and local government agencies, research institutions, universities, general public, journals, unpublished data and contemporary observations, including international resources etc. shall also be used to support in designing specific surveys. The general surveillance study shall:

- support NPPC and/ or BAFRA in declaration of pest freedom;
- aid early detection of new pests;
- report to other organization such as Agriculture Research & Development Centers (ARDCs), Department of Agriculture (DoA) and other international/ national organizations;
- Compile of host and commodity pest list and distribution records.
- Or can be used as one part of designing specific surveys.

1.5.2 Specific Surveys

It is the procedures to obtain information on pests of concern on specific sites in an area over a defined period of time. This survey can be of detection, delimiting or monitoring, which is officially conducted and therefore should follow a plan which is approved by the NPPC or in accordance with international standards set in.

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

- ✓ The overarching reasons why we survey for plant pests are for pest management, bio-security, trade and quarantine purposes.
- ✓ No control measures should be undertaken unless the presence of the pest is established.
- ✓ Avoid blind control schedule of pest to prevent unnecessary destruction of beneficial organism, resurgence of minor pests, development of resistance, and undesirable contamination of environment.
- ✓ *No control measures should be undertaken unless it is understood that the pest is in sufficient numbers to cause economic loss*.
- ✓ Use economic injury level (EIL) and the economic threshold (ET) as a tool to make decision in IPM.

1.5.2.1 Kinds of Surveys

The surveys are broadly categorized in to **qualitative surveys-** identification of different species and aims at pest detection e.g. for a newly introduced pest; and **quantitative surveys-** defining numerically the abundance of

an insect population in time and space. It is useful for enumeration of pest and to predict future population trends and to assess damage potentials.

The specific kinds of surveys; detection, delimiting, or/ and monitoring surveys are detailed below:

1.5.2.1.1 Detection Surveys

Conduct a detection survey in an area to determine if pests are present" (ISPM 5) in order to:

1. *Develop pest and host lists*- of pest and host (including alternative hosts)
2. *Establish pest free areas (PFAs), pest free places of production (PFPPs) and pest free production sites (PFPS).* If PFA/ PFPP/ PFPS status cannot be established for an entire area, it may be possible to identify the pest free status of smaller units, that is, Places and Sites within an Area.
3. *Early detection of established or native organisms becoming pests*
4. *Examine for quarantine breaches*- targeting at high-risk areas/ site where a pest has breached quarantine.

1.5.2.1.2 Monitoring Surveys

Undertake a monitoring survey in an area where a pest is known to be present to:

1. *Assist with pest management*- for application of management measures only when the pest number increases to EIL or when weather conditions pre-dispose crops to infections, etc…
2. *To establish and maintain an Area of Low Pest Prevalence (ALPP) status.* ALPP is an area, whether all of a country, part of a country, or all or parts of several districts, as identified by the competent authorities, in which a specific pest occurs at low (accepted) levels and which is subject to effective surveillance, control or eradication measures. However it is not PFAs.

1.5.2.1.3 Delimiting Surveys

Conduct a delimiting survey to establish/ determine the boundaries of an area considered to be infested by a pest rather than to define a PFA.

This will help in knowing the extent and distribution of a pest incursion, whether the pest is eradicable/ manageable and in evaluating the spread of a pest in light of quarantine purposes.

1.5.2.1.4 Other Important Type of Surveys

Roving and fixed plot surveys are other types of survey but they aren't discussed in this manual.

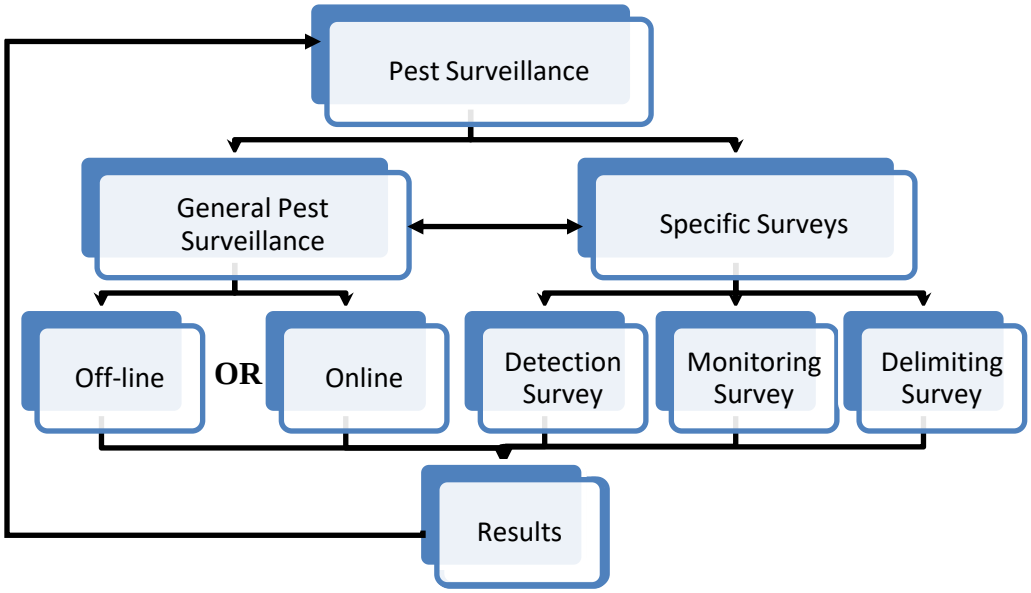

Figure 1: Pest Surveillance System-*a process flow*

Chapter 2: Conducting General Surveillance studies

2.1 Introduction

The conduct of general surveillance in Bhutan shall be as per the existing system and methodology of surveillance data collection i.e. either or both (when necessary) of the off-line or online system which are in line with the principles and practices of general surveillance.

The existing online system includes the ePest Surveillance System and other pest-specific systems such as SAARC Rust surveillance system. In addition to the general surveillance information, the ePest Surveillance System have the capacity to provide certain specific survey related information as well.

A separate offline surveillance reporting format is also being used which shall be subject to update from time to time (Annexure 1).

The preliminary information obtained through the general surveillance systems must be used (but not always) in conducting the specific surveys as per the protocols described in the Chapter 3 of this manual.

2.2 ePest Surveillance System

ePest Surveillance System was jointly designed and developed by Infronics Systems Limited, Hyderabad, India and NGN Technology (the then Peljorkhag Pvt. Ltd.), Thimphu, Bhutan, based on supports/ inputs provided by the Food and Agricultural Organization (FAO); United Nations and Ministry of Agriculture, Govt. of India. The system consists of two applications; the web application and an android application. The administrator manages and processes the information received from the field for consumptions.

The ToR developed for different stakeholders must be followed to implement the system. For guidance, the designated users must refer the manuals available in the website of the ePest Surveillance System (http://epest.moaf.gov.bt/)

2.3 Other electronic tools/ system

Other electronic tools/ system meant for pest surveillance are also being used which serves the purpose of general surveillance activities in the country.

Example: SAARC Rust Surveillance Toolbox

Chapter 3: Specific Survey

3.1 Introduction

Specific survey is to obtain information on pests of concern on specific sites in an *area* over a defined period of time for detection of new pest(s), delimiting or monitoring pest free areas or for low pest area.

The planning decisions (such as how to decide where to look, how many places to look in and what sort of data to collect including important considerations to make) to be made before going to field to look for pests are described in this chapter.

A survey plan needs to be robust, feasible both physically & financially and the results should represent the actual pest status of your area.

The steps chosen for designing survey must transparent and manageable. It is advisable that you get as much information as possible from other sources before conducting your survey (Annexure 2).

3.2 Survey sampling methods

Among many sampling methods for selecting survey sites or host plants, the most relevant for our situations is stratified random sampling. Some additional sampling methods are also discussed in brief in this manual. Further reading in external sources is suggested if in-depth knowledge including their advantages & disadvantages is required.

3.2.1 Stratified random sampling

This is one of the most appropriate sampling methods given the agriculture production system in our country with different agro-ecological zones and different cropping pattern. This can be used in combination with random sampling. This involves dividing the sites into logical categories and then systematically or randomly choosing sites from within the categories.

- Divide the host plant or sites into groups (strata) of sites/ hosts depending upon the shared attributes/ characteristics such as climate/ family/ etc…
- Apply random sampling within the groups to randomly select the sites/ host in each groups (Refer the Random Sampling Survey for more details).
- Decide the number of samples as per the calculated number of samples for the survey.

Example: 20 villages (level: place) are to be surveyed say for cardamom diseases. Each village has 15 farms (level: field sites), a total of 300 farms. If 100 farms are to be surveyed, we could randomly choose the 100 from all 300. By chance, this may result in some villages having all their farms surveyed and others having none. If it is important that all villages be surveyed, the selection of the 100 sites can be stratified by village such that, for example, five farms per village are chosen randomly.

3.2.2 Random sampling surveys

Usually, all sites and host plants cannot be examined and so a subset number of sites or host plants need to be chosen for surveillance/ survey. To avoid

selection biases, all hosts and sites need to be equally likely to be surveyed. In random sampling survey, the sites and plants are chosen by an impartial method that reduces the influence of human biases in the site selections. These impartial methods—methods to introduce randomness into a survey plan can be done in various ways but in this manual a simple way to select the random samples and decide the number of samples using MS Excel is detailed below:

Using Microsoft Excel to generate randomness in the samples or sites:

Open the MS Excel sheet\list the samples or sites to be randomly selected in the first cell (of the first column)\ select adjacent cell (of the 2nd column) to the corresponding first data: sample or site\ type "=ran" in the cell\ select and double-click the "RAN" function from dropdown list\ press enter\ copy the above cell along with resulting data in it\ paste in all the remaining cell below that corresponds to the list of samples or sites\ select that (2nd column) whole column and sort (A-Z or ascending order)\ select "Expand the selection"\ press "sort"\ take any samples or sites from any order or anywhere as per the number calculated for the survey. All the samples or sites are randomized.

3.2.3 Systematic sampling surveys

Systematic surveys involve mapping out a site and surveying at regular intervals of distance, area or host plant. For example, examining the plants of every tenth row; every third farm; every eighth square meter; setting insect lures in a grid pattern; two apples from every tree; or performing parallel sweeps of a site. Systematic sampling can also be viewed as having a random element if the intervals of the sampling are independent of the expected pest distribution. For example, regularly spaced sites should not coincide consistently with the presence or absence of the pest.

3.2.4 Targeted site surveillance

The sites are chosen based on where the pest is most likely to be, thereby deliberately biasing the selection process in favour of finding. However, targeting particular sites is designed to maximize the chance of finding the pest. Surveillance for early detection of exotic pests usually involves targeting sites that are the first point where exotic pests could arrive or infest. Goods and people that may carry pests enter a country by crossing borders or arriving at sea- or airports. In Bhutan, the places closer to border towns having to do mostly with import of new crop cultivars has higher chances to spots new pests.

Depending on the possible routes of arrival, these sites are targeted for surveillance. The intensity of survey sites is highest around the first points of entry and then is reduced in frequency with distance.

Targeting can also be done in the field where surveillance is focused on host plants or sites where the pest is most likely to be present (and thereby deliberately introducing bias).

3.2.5 Blitz surveys

The purpose of blitz surveys is to detect all pests present, even those in low numbers, and to identify less visible symptoms and newly emerging pests. These

surveys involve the intensive inspection of all plants in a given field site or at a set time, generating pest lists for a host or range of hosts. The survey may be restricted to a list of pests that have particular relevance or risk. This survey will be conducted only under the R&D mandates especially for outreach programs or pilot sites.

3.2.6 Full sampling

Full sampling involves examining all the sites at a particular level. This could be full sampling of all places right through to surveying all sampling sites at a field site. This term overlaps with blitz surveys which entail full sampling at the field site level. This sampling will be carried out if specifically instructed particularly for newly introduced varieties in an area or place.

3.2.7 Farmers' observations

Farmers/ crop managers should report to a central person (say an extension official) about the pests that they have seen during their work. The workers must recall *where*, *when* and *what* they observed. Alternatively, framers must show surveyors where they have observed pests or diseased plants and the information may save a great deal of surveying for early detection of pests.

3.2.8 Insect-trapping surveys

Insects can be caught by static traps that attract insects by light, colour or pheromones. The insects are then removed from the trap and identified. These traps are useful primarily for identifying whether or not a pest is present in the area.

The *siting* and *density* of traps is critical. Siting and density are determined by the trap type and the manufacturer's instructions, and applied according to the survey setting.

Traps are often used to estimate the prevalence of pests in the area. In some cases, the number of insects trapped is directly proportional to the true pest prevalence (e.g. 1 trapped fly could reflect 100 flies in the area).

3.2.9 Purposive and haphazard Sampling

Purposive sampling involves choosing places, field sites, sampling sites or even sample points purposefully when the surveyor (s) knows in details about the site/ sample.

In haphazard sampling surveyors collect 'random' specimens by mentally selecting sites sporadically. There is, nevertheless, a tendency for people to distribute sites uniformly, or choose sites based on an idea of a 'randomized' pattern. For example, people generally would not consider choosing clustered sites within a large area, and yet such a configuration can result if the sites are chosen randomly.

3.3 Designing specific surveys and Planning Process/ steps

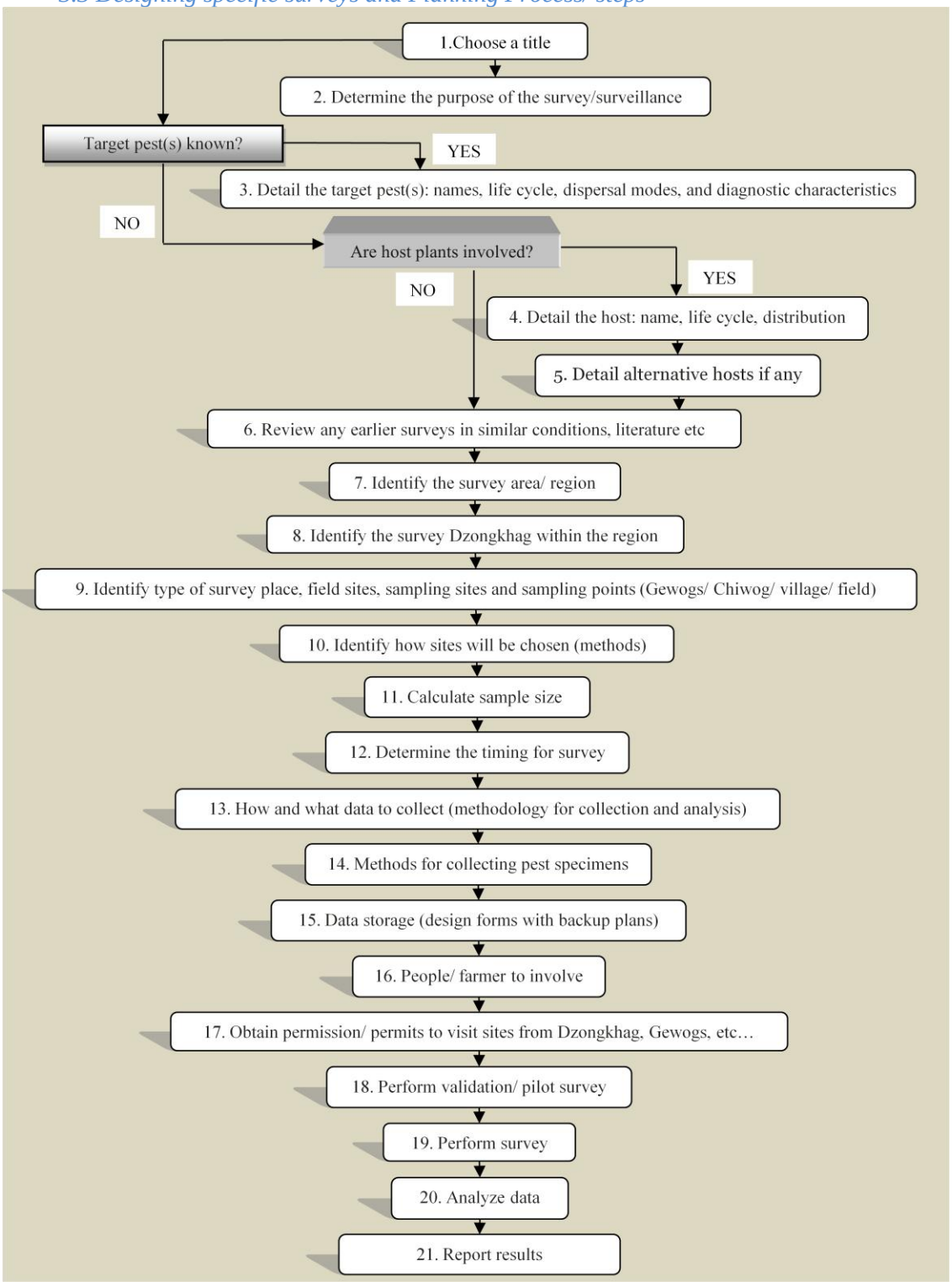

Figure 2 Process flows for conducting a specific survey

Step 1: Choosing a Title

- Choose a simple title for your survey
- Record the title of the survey and assign the author (s)
- Develop a proper survey plan: pests and host to be involved, schedule and coverage
- You may wish to revise it as you go along. It's the first step for proper planning process.

Step 2: Reasons for surveying

- Record the purpose/ rationale of your survey
- Some of the possible reasons may be:
    - to develop a list of pests or hosts present in an area
    - to demonstrate a pest-free area or places of low pest prevalence for trade purposes
    - to develop a baseline list of pests before ongoing monitoring for changes in pest status for pest management and control
    - for early detection of exotic pests
    - for early detection of established organisms becoming pests
    - to delimit the full extent of a pest following an incursion
    - to monitor progress in a pest eradication campaign etc…

Step 3: Identify target pests

(If the targeted pests are not yet known-for example, you intend to survey for new weeds—skip to Step 4: Host.)

If you know the pests you intend to survey, find the following information on targeted pests from sources such as pest database, leaflets, reports, text books, specimen collected, photographs, PRA studies etc. from NPPC, RDCs, Departments, research institutions, universities, museums, the general public, scientific and trade journals, unpublished data and contemporary observations, international sources such as FAO, IPPC etc…

- Record the scientific/ local/ common names of the pest.
- Record the importance/ impact of the pest.
- Record vectors if any
- Record the diagnostic characteristics of the pest, including the life cycle.
- Create any pest information sheets you will use in the field (scientific/common/local names, symptoms, mode of action, morphology, habitats, pictures/ image).

For the exotic pest likely to enter into the area, comprehensive search and finding for their identifiable characteristics is very important prior to field visit.

Step 4: Identify target host(s)

(If the targeted host is not known-for example, in the surveying of weeds or pheromone trapping of insects-skip to Step 6: Review similar literatures.)

- Record the scientific/ common/ local names of the host plants.
- Record the right cultivar and crop/ host information

- Record the value/ importance of the host/ commodity plants.
- Record the growth habits of the host plants (tall, bushy etc).
- Record the distribution of the host plants your area or region.

The list of internet sources may be referred for the pest/ host information as in Annexure 1

Step 5: Alternative hosts

- Record alternate hosts if any by talking with locals, referring publications, databases and internet resources as it is important both for early detection of exotic pests as well as delimiting the extent of a pest incursion.

Step 6: Review of earlier survey plans

- Collect and refer any accessible similar survey or surveillance plans/ reports to help your survey

Step 7: Identifying the survey area/regions

- Provide brief details on the climate, topography and geographic coordinates of the area where you would look for pests (Country, Dzongkhag, Gewog, Chiwog, Village).

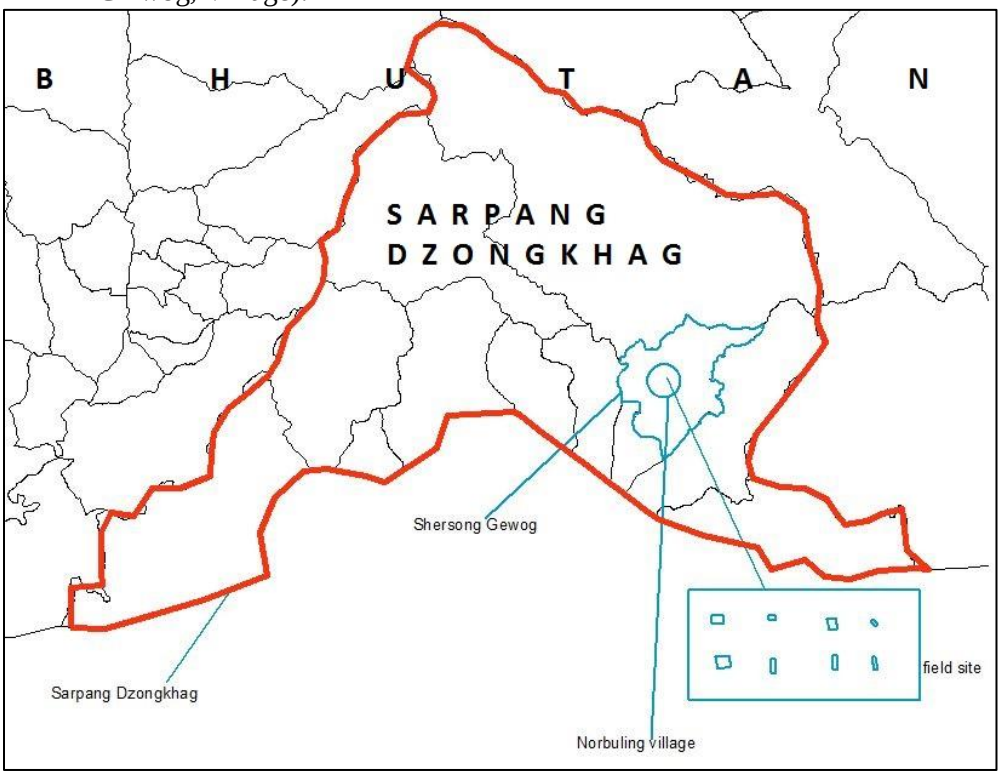

Figure 3: Diagrammatic map illustrating with example the concepts of area, district and field site

Step 8: Identifying the survey Dzongkhag within the region

- Identify the Dzongkhag to be covered by the survey that fall under different climatic zones and crop production pattern.
- Record the general information of the Dzongkhag

Step 9: Identifying the possible survey places, field sites and sampling sites

- Select the *gewog* (s) in the Dzongkhags that could be surveyed
- Select *chiwog* depending on the Agro-ecological zone if necessary
- Select the 'field sites 'within each *gewogs*/ *chewog* such as field, plantation, or orchards.
- Select the 'sampling sites' within each field site/ orchards such as trees, individual trees/ plant/ etc
- Select the 'sampling point', if required for choosing specimen sites within a sampling site. For example, select 20 plants and you will observe every 4th plant that will narrow down to 5 sample/plants.
- Based on the convenience, follow one of the sampling patterns as in Figure 4 (below)

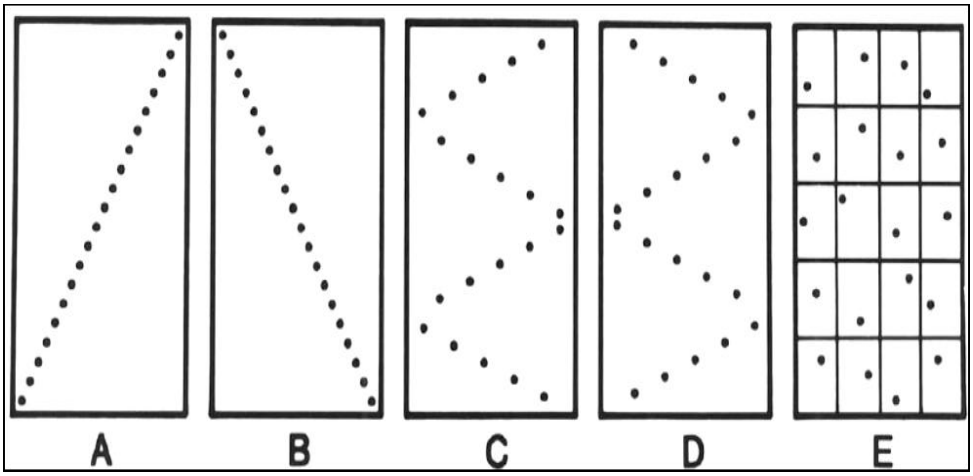

Figure 4 Sampling designs. Point represents sample site. A=right diagonal; B=left diagonal; C=right W; D=left W; E=stratified random. Source: Lin et al (1979)

Step 10: Methods for choosing sites

There is no single best method for site selection and it may not be possible to use the 'best' method, due to logistical or financial constraints. Therefore, transparently document your choices and reasons for the choices made.

- Apply the principle of selecting sites described in Chapter 3 (*Section 3.2: Survey sampling methods*)
- Record methods for choosing places, fields and sampling sites to survey.
- Tabulate all possible number of places, field sites and sampling sites being considered, providing these with individual identifiers.

Step 11: Calculating sample size

Sample size calculation depends on the purpose of the survey. The two approaches considered here are for *detection surveys and monitoring surveys*.

To calculate the sample size for the above surveys there are important parameters to understand first:

1. Design prevalence

- Unlike actual prevalence (true proportion of infested units in a population by one or more pests), the design prevalence is usually based on a pre-survey estimate of the likely actual prevalence of the pest in the field, and used to determine the sample size.
- It is expressed in percentage.
- Design prevalence and actual prevalence for an area free of a pest are expected to be near-zero.
- For surveys that monitor a pest that is known to be present, the design prevalence can range from near-zero to 100%.
- If you are unable to predict a meaningful prevalence, you need to choose a prevalence level that is acceptable to all parties.

The design prevalence estimation differs with survey types as detailed below:

Estimating design prevalence for Detection Survey

- This situation is when anticipated design prevalence is near-zero
- estimate pest prevalence start date when the presence of pest is very low in the survey area (could be from the date of last entry or eradication or the date when the quarantine measures were put in place)
- Estimate with evidences, the rate at which the small population of pest would multiply and spread over time in the area (as percentage of hosts (sites) affected).
- And predict the prevalence at the time of intended survey

Estimating design prevalence for Monitoring Survey

- When the predicted prevalence is thought to be greater than near-zero
- When we know that the pest is present at the intended survey area, we need to understand its available data and life cycles and other conditions like weather
- Based on these information, you can extrapolate (the observed rate of infestation using reports, journals, observations or trials), or compare pests with similar population dynamics to arrive at the design prevalence.
- Complex computer modeling can also be used

2. Confidence

- Statistical confidence is the probability that the actual prevalence will be within range of the design prevalence. The relationship between confidence and sample size is simple—the more sites you survey, the more certain you can be about the accuracy of the estimated prevalence.
- Confidence level is expressed in percentage.

- As a general rule, a confidence level of at least 95% is considered acceptable. However, in some cases, up to 99.9% may be necessary depending on the type of surveys, requirements from the trading partners, financial and logistical constraints in place.

3. Accuracy of methods (sensitivity)

- This deals with how well the survey will detect a pest when it is present.
- The accepted accuracy of methods to be used may be ranging from 80-100 %.
- Method accuracy has a direct effect upon the ability to detect the presence of a pest and must be considered when estimating sample size.

4. Sample size

- Sample size is the number of sites that you need to survey in order to detect a specified proportion of pest infestation with a specific level of confidence, at the designed prevalence.
- Sample size is expressed in whole numbers.

The calculation of sample size has been considered for a situation needed to calculate the proportion of sampling sites or sampling units infested with pests; for example, either the pest is present on a fruit or tree or is absent.

Formulas to calculate sample size for detection surveys

- Use when survey is designed to detect pest
- Where the actual prevalence likely to be rare
- Confidence is expressed as percentage
- Detection threshold (design prevalence) is taken on a scale between 0 and 1.
- Use the formula for Confidence level as:

$$\boldsymbol{Confidence\ level = 1 - (1 - design\ prevalence)^{Sample\ size}}$$

Therefore,

$$\boldsymbol{Sample\ size = \frac{\log(1 - confidence\ level)}{\log(1 - desgn\ prevalence)}}$$

Considering the method accuracy, the adjusted sample size is calculated using the following formula:

$$\textbf{Adjusted sample size} = \frac{\textbf{Sample size}}{\textbf{\% method accuracy}}$$

Table 1 can be used as reference in calculating the sample size for detection survey.

Situation	Confidence level (CL) (%)	1-confidence level	Design prevalence (DP) (%)	1-design prevalence	Sample size	Adjusted sample size (method accuracy < 95%; say 90%)
CL=95%; DP=1%	0.95	0.05	0.01	0.99	298	331
CL=95%; DP=2%	0.95	0.05	0.02	0.98	148	165
CL=99%; DP=1%	0.99	0.01	0.01	0.99	458	509
CL=99%; DP=2%	0.99	0.01	0.02	0.98	228	253
CL=95%; DP=0.1%	0.95	0.05	0.001	0.999	2994	3327
CL=95%; DP=0.2%	0.95	0.05	0.002	0.998	1496	1663
CL=99%; DP=0.1%	0.99	0.01	0.001	0.999	4603	5114
CL=99%; DP=0.2%	0.99	0.01	0.002	0.998	2300	2556

Table 1: Calculating sample size for detection survey

Formulas to calculate sample size for monitoring surveys

- In statistics, the known standard Z-scores is 1.96 for 95% confidence level; 2.58 for 99% confidence level; 1.65 for 90% confidence level and so on…
- Calculate sample size for monitoring survey with some exemplary scenarios as below:
- *Estimate the proportion of tree in an orchard infested by a pest:*
    - Where confidence level is 95% and Z-score for this is 1.96
    - Expected prevalence is >2%
    - Confidence interval width 5% (*but always expressed as decimal between 0 & 1*)
    - Design prevalence of 20% (*but always expressed as decimal between 0 & 1*)

$$Sample\ size = \left[\frac{Z}{confidence\ interval\ width}\right]^2 X\ Design\ prevalance\ X\ [1 - Design\ prevalence]$$

$$Sample\ size = \left[\frac{1.96}{0.05}\right]^2 X\ 0.2\ X\ [1 - 0.2]\ = 246$$

- For in-depth information on confidence interval/ width and other statistical methodology (ies), get help from statisticians/ experts.

Formulas to calculate sample size for delimiting surveys

- Unlike the detection and monitoring surveys, the delimiting survey don't need a statistical survey sample numbers but only need to choose a sampling structure, such as a grid of traps, that is statistically sound.
- Follow the trace back and trace forward methodology as also described in the general protocol/ guidelines for conducting weed detection survey

(example: *Potamogeton distinctus* in Bumthang, Bhutan) in Chapter 4 of this manual.

Step 12: Timing of the survey

- *When to survey*
  - When the pest is most likely to be present and in identifiable state.
  - In weed, usually early during the crop growth stage when weed are small and easy to count is preferred
  - Crop (host) and disease/ insect growth stages, phenology and life cycle are very critical while sampling. So, timing of sampling must coincide with the time of peak numbers of stage.
- *Frequency of the survey*
  - Decide the frequency of survey depending on the survey types and fund availability for the survey.
  - Decide according to the phenology and life cycle of crop/host and disease/ insect.
  - Some need every 2 weeks, some need annually
  - Sometime it also depends on the requirement by the trading partners involved.

Step 13: Planning collection of data in the field

- Identify the sampling sites and tag properly on the ground with weather-proof materials
- Decide on the methodology of data analysis
- Design form keeping in mind the data analysis methodology chosen
- Agree the survey planning among the team member (s) and team leader
- Include the followings among others (as per the need) while designing the form: pest and host details; data of field/ site such as location, lat/long, altitude, hints for exact location of the sample site or trees within the field (such as 20 m from the road/ first row/ first plant/ tree etc…), dates, weather, time, farmer/ local head's names etc…
- The data unit is usually in scale rating (Zero=0; 1-25%=1 (low); 26-50%=2 (moderate); >50%=3 (high); no. of pest per unit area; it could be direct count of pest or the scale of pest intensity; per tree; per crop; per kg; per quadrat; cover class (% cover); per sweep of net; per trap etc…
- Record any negative data (that is the area surveyed but not recorded any pest)
- Record all those information not captured by the designed form in separate note

Step 14: Methods of collecting pest specimens

- Collect specimens and handle with care as per the purpose of collection: example: for identification/ preference collection or herbarium
- Refer useful/relevant references for method of collecting plant pests
- Label clearly on the specimens to identify it later without any mistake
- Refer generic specimen collection protocols described below if you don't have any specific ones (Box 1, 2 & 3)

Generic specimen collection protocols (Adapted: Plant Health Australia, 2005)

General advice

- *Sterilize any implements with a sterilant (eg. 70% v/v ethanol or any other as appropriate) before and after each sampling.*
- *For root problem, include soil and crown (lower stem) tissues with root samples.*
- *Time between sampling and dispatch of the sample for identification must be to the minimum.*
- *For suspected exotic plant pest, do not move from site to site when sampling to decrease the potential spread of the pest.*
- *Where possible, sample from perceived area of minimal damage and perceived high damage within field and on individual plant.*

Insect specimens

- *Refer insect-specific protocols where available.*
- *Always collect duplicate specimens of all possible life stages such as adult and immature for better diagnosis and in good conditions i.e. complete with appendages, wing, antennae, legs, mouths etc…*
- *Use leak-proof alcohol resistant container such as glass bottle with air- and liquid-tight stopper, or plastic container with screw-top lid.*
- *For small/ soft bodied insects such as thrips, aphids, mites and larvae place them in 65% ethyl alcohol (methylated spirits can be used)–35% water and completely fill the container.*
- *Tape the lid securely to avoid accidental spillage.*
- *For insects such as mealy bugs or scale insects, cut out infested leaf tissue/ stems and place them in alcohol container to avoid the damage of mouth parts for better identification.*
- *For hard-bodied insects such as beetles, moths, grasshoppers and fruit flies, fold specimen in tissue paper and place in crush-proof plastic tube or container with several holes in the lid for ventilation.*
- *Store sample in freezer for 2 hours before dispatch to kill the insect.*
- *Clearly label all samples.*
- *Do not send live insects.*
- *Retain and store a duplicate sample in a secure, cool and dark location.*
- *Live materials may be sent in exceptional case for diagnostic purpose but under special arrangements.*

Pathogen specimens

- *Refer pathogen-specific protocols where available.*
- *Sample the specimens the same day it is to be sent to ensure freshness.*
- *Ensure the duplicate specimen as reference material.*
- *For fungal and bacterial samples, store sample in a refrigerator at 2–5°C until it is sent but some pathogens do not survive cold conditions.*
- *For suspected exotic pest store under appropriate conditions.*
- *Sample specimens from area with fresh, representative and full range of symptoms between the diseased portion of the plant and the healthy portion.*
- *For root problem include soil and crown (lower stem) tissues with root samples.*
- *Place samples in self-sealing plastic bags with some dry tissues or paper towel to absorb excess moisture.*
- *For fruit or vegetable sample, wrap in dry tissues or paper towel and pack firmly in a crush-proof container.*
- *Retain and store duplicate sample using the same methods described above.*
- *Do not send dead plant material.*
- *Do not add extra moisture or don't pack a sample that is wet.*
- *Do not allow sample material to dry out.*

Box 1: Generic specimen collection protocol: Insect and Pathogen

Nematodes specimens (Adapted: CABI Bioscience training manual: Ritchie, B.J., ed. 2003)

- *Avoid sampling of very wet or very dry samples.*
- *For soil, take at least 5–10cm below the surface as the nematodes congregate in the root zone.*
- *Crops with patches of poor growth, sample from the badly affected and normal areas to have better comparison.*
- *For tree crops such as citrus, sample the soil from drip circle area (area on ground where water from outermost leaves would drip) as surface roots are most abundant there.*
- *Individual sample size should be about 250–300g.*
- *Bulk and thoroughly mix the samples and take duplicate sub-samples of the same weight for analysis and for references.*
- *Roots may be either included in the sample or taken separately (if separated; 25g (for vegetables or citrus) to 100g (for large roots like banana) is sufficient).*
- *For suspected nematodes infestation, live material be removed and placed in polythene bags without soil samples but should be examined soon to avoid any rotting of tissues.*
- *The polythene bags with samples be properly labelled.*
- *Keep the samples in cool place and never in sun.*
- *Dispatch the samples as soon as possible.*
- *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 if not possible to dispatch immediately.*

Viruses specimens (Anon. 2005. Management of plant pathogen collections. Canberra, Australia, Department of Agriculture, Fisheries and Forestry)

- *Suspected virus infected plant material be collected and temporarily preserved using desiccators at temperatures of 0 to 4°C/ at ambient temperatures.*
- *Swab leaves with water or alcohol to clean off any dust or sooty mould or scale insects.*
- *Cut the leaves from near-center of lamina into 3 to 5mm squares with sterilized scissors or safety scalpel blade.*
- *Place 5 to 10 squares in a plastic container filled up to a third with calcium chloride (CaCl2) crystals/ silica gel but separated by cotton wool.*

Phytoplasmas (Anon. 2005. Management of plant pathogen collections. Canberra, Australia, Department of Agriculture, Fisheries and Forestry)

- *Phytoplasmas are obligate parasites and can't live freely in the environment, so not been successfully grown in culture.*
- *Identify phytoplasmas through resultant symptoms, host range, vector specificity, appearance under electron microscope of ultra-thin sections of diseased tissue.*
- *Prepare the specimens for DNA tests in the way it is prepared for viral specimens.*
- *Get experts advice for specimen collection and handlings.*

Box 2: Generic specimen collection protocol: Nematodes, viruses & phytoplasmas

Weeds specimens (Adapted: Australian National Herbarium)

- *Collect vigorous/ healthy plant which is representative of the population.*
- *All plant parts including leaves, flower, fruits, roots, bulbs, and other underground parts be carefully collected for better identification.*
- *Collect different types of foliage, flowers and fruits from the same plant.*
- *Collect material to fill a standard sized herbarium keeping space for labelling.*
- *For plants too large for a single sheet may be divided and pressed as a series of sheets.*
- *For woody plants bark and wood samples be collected as well.*
- *For bulky plants or parts, halve it or sliced before pressing.*
- *For bushy twigs, prune to make a flatter specimen*
- *For spiny plants, place the plant under a board and stand on the board before pressing in order to prevent the spines tearing the paper.*
- *Kill succulent plants and bulbs before pressing by soaking in methylated spirits for 15–20 minutes to avoid from sprouting.*
- *For water plants, float them in a dish of water, lift them out on a sheet of stiff white paper, dry excess water, press the plant with a piece of waxed paper over the top to prevent from adhering to the drying paper.*
- *For tall rosette plants and grasses, press complete by bending them once or more into the shape of a 'V', 'N' or 'M'.*
- *For dioecious plants should be represented by both sexes.*
- *Press the specimen as quickly as possible after collection.*
- *When impossible to press specimens immediately, it may be stored in cool, moist and loosely packed plastic bags, preferably wrapped in damp (but not wet) papers.*
- *Label the plastic bags properly.*
- *In warm places, specimens must not be left in damp papers or they will go mouldy.*
- *Change the newspapers when necessary until the plant are dried.*

Special considerations when collecting a new exotic pest

As some new exotic pests pose a great threat to industry or natural environments, extreme care must be taken when a pest is first sighted or suspected to be present. If the pest has windborne spores or is a winged insect, it may be best not to disturb it as it may spread further. If a specimen needs to be collected, a generic protocol below may be followed but additional hygiene and containment steps should be taken:

- *Leave vehicles outside the infested area.*
- *Sterilize all collecting equipment before and after collecting at each site.*
- *Start surveying from parts least likely to be infested to those most likely.*
- *Ensure all specimens collected are well-secured and contained.*
- *Do not throw away specimens suspected of cross-contamination with exotic pests.*
- *Label these specimens clearly so they can be destroyed appropriately.*
- *Disinfect vehicles as appropriate, if been in the infested area as the pest could have adhered to the vehicle (such as seeds, pathogens in soil or fungal spores) within the infested area to reduce the likelihood of transporting the pest.*
- *Consider using disposable clothing/ suites such as overalls, boot covers, gloves etc… and sterilize/ autoclave the clothes in sealed bag after the site visit.*
- *Use a fresh set of clothes at each survey site where the exotic pest has been found.*
- *The gloves, boots soles and hands can also be sprayed with methylated spirits.*
- *Pack securely and label the specimens properly (including the names of sender/ receiver, contact numbers and addresses with clear marking as urgent/ exotic plant pest, keep cool, etc…) before sending for identification.*
- *Do not send live insects unless specifically required for identification (such as fruit fly larvae in fruit)*
- *Notify the laboratory (NPPC) in advance about the suspected exotic plant pest for collection and identification of the specimen.*

Box 3: Generic specimen collection protocol: Weeds & exotic pets

Step 15: Electronic data storage

- Design a spreadsheet or database structure to electronically store data from the survey and if possible before going to field.
- The survey can be done either in paper forms or in the portable electronic devices such as laptop or tabs as per the convenience.
- The paper forms can be manually entered into the electronic database system/ spreadsheets once back from the field & save the data securely with robust backup plans.

Step 16: People involved / farmers

- Record the members of survey team and other people involved in design, data analysis, pest identification etc.
- Organize adequate information and training for the team and for farmers as well.
- Record other participants including farmers.

Step 17: Obtain permission to visit sites

- Obtain visa/ travel documents/ approval, permits and permissions needed during the conduct of survey from the relevant office/ personnel/ communities such as Dzongkhag, Gewog Administrations, etc…
- Communicate and arrange the survey visits in advance with the collaborator at fields to avoid any miscommunication.

Step 18: Pilot survey/ validation survey

- Have a look at the site.
- Meet and inform all the people involved.
- Practice (pilot) surveying and collecting specimens.
- Problems encountered/ experienced/ feedback gained during pilot survey be used to improve the quality of real survey.
- A pilot study can include a structured component; for example, what the expected prevalence of the pest would be.
- Experiments on team members' ability to detect pests could be performed in this stage as well.

Step 19: Perform survey (collect raw data and samples)

- Perform survey and collect data in the field.

Step 20: Analyze data

- Once raw data is collected, use data analysis tools that were selected during the stage of planning data collection (Step 13).
- Calculate basic statistics, such as the average and total numbers of pest.
- Estimate the confidence of the data collected.
- Create a map of the pest distribution.
- Examine changes in pest locations and densities if monitored over time.
- Store, tabulate and analyze the survey data.

- Draw species accumulation curve (Y-no. of species accumulated over time; X- no of sites surveyed); epidemiological curve (disease progression over time); or disease-gradient/ dispersal curve.
- Find prevalence of disease (total no. of diseased plants in a particular time in a farm/ orchard), etc…

Step 21: Report the results

- Report the results in standard format of summary, press releases, newsletter articles, basic report and formal reports, etc…

Chapter 4: General sampling guidance for weeds

4.1 Introduction

In order to get a reasonable idea of the status of a weed population in a field at a given point of time, obtain the most representative sample possible, taking into account time and manpower constraints, economic considerations, and other factors.

4.2 Sampling area

- Weed samples can be counted from any sampling area convenient for the particular vegetation being investigated
- Sample area:
    - A small quadrat (of $1m^2$ or less /per sample) is adequate for weeds of small and high weed populations (densely populated weeds)
    - A large quadrate (of $5m^2$ or less /per sample) is appropriate if the species is large or sparsely populated.

4.3 Sampling method

a. Weed counts (density)

- This method is accurate, allows direct comparison of different areas and different species, and is an absolute measure of the abundance of a plant but laborious and time-consuming, particularly if large numbers of plots are involved.
- Record the weed density, and weeds in accordance with the importance
- Low-density weed population needs a larger sampling area as compared to those with high-density weed population.
- Count the weeds by species and class them as broad leaved, grasses or sedges etc…

b. Visual estimation-visual estimates of the number of individual weeds in a stand can provide useful data. The bias can be reduced by sampling plots randomly, by plot number, with no regard to treatments imposed.

4.4 Time of survey

Weed counts are usually done *early during crop growth*, when weed competition is greatest and weeds are small and easy to count. A count of individuals is feasible only when plants are spaced far enough apart and have a discrete growth habit.

For creeping perennials, counts should be made of individual stems or shoots rather than of individual plants.

4.5 Sampling Pattern

Follow any of the following sampling patterns as per the convenience and shape of the field.

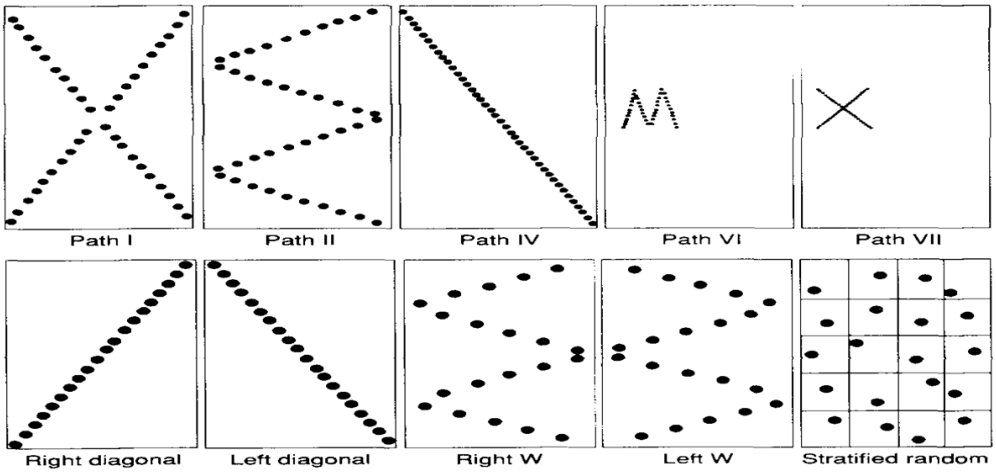

Figure 5: Sampling pattern; Source: Lin et al (1979)

4.6 Weed flora

Regardless of the method of weed assessment used, every effort should be made to identify all the weeds present in all plots and to list them in order of importance into the system. Pre-survey is required to list the important weeds species targeted for survey in a particular field.

4.7 Example of detection and delimiting survey in weed

Step 1: Choosing a title

Potamogeton distinctus A. Bennet (Shochum) in Bumthang: A surveillance study 2016

Step 2: Reason/ purpose of survey

To detect and delimit the spread of Potamogeton (Schochum) in Bumthang paddy fields and Provide a preliminary quarantine measures/ advices to farmers in the affected area

Step 3: Identify the target pest

Potamogeton distinctus A. Bennet (*Shochum*)

Step 4: Identify the host

Paddy field

Step 5: Alternate host

Marshy areas/ water bodies

Step 6: Review of earlier survey plans

Review of other reports (NPPC extension materials and reports)

Step 7, 8 & 9: Identify survey area/ region/ district/ fields

Paddy fields in Choekor gewog, Bumthang Dzongkhag

Step 10: Methods of choosing sites

The preliminary information on suspected fields in the gewog was obtained from the district officials and farmers. Then the sampling sites were purposively/ randomly selected in to 4 sites dividing the whole stretch of paddy areas into north, central and south.

Step 11: Calculating sample size

4 suspected sites were scouted using trace back and trace forward (scouting till the edges of the field from the point of detection until no Shochum was detected) with in the area consisting of multiple adjacent fields. 3 out of 4 sites turn positive to the presence of weed. Jalikhar field was divided into 3 different surveillance plots. 2 samples each was drawn from the plot of 10 terraces.

Step 12: Timing of the survey

Time was when the Potamogeton was in the field and when the turions are matured enough to see when dug (late summer); September, 2016

Step 13: Planning data to collecting in the field

The standard format/ form and protocols were developed before field visit.

Step 14: Method of collecting pest specimens

The Shochum were sampled with in delimited fields by throwing quadrate of 0.25x0.25 in two places randomly. The whole of the shochum plant including rhizome (turions) within the quadrate were careful dug washed and packed in plastic bags with proper labeling.

Step 15: Electronic data storage

The biomass weight of the samples both before drying and after drying were recorded in excel sheet; The interview questionnaire were also recorded in epidata

Step 16: People involved/ farmers

Pest surveillance and Weed & Vertebrate divisions of NPPC; Dzongkhag Agriculture Sector, and paddy growers of the Choekor gewog were involved.

Step 17, 18 & 19: completed

Step 20: Analyze data

Data analyzed using MS Excel and ArcGIS-based cartography

Step 21: Report of the Result

The back to office report submitted to NPPC with recommendations. Additionally, an article published in an annual magazine of Ministry of Agriculture & Forests, "*Sanam Drupdrey*"

Chapter 5: General sampling guidance for insects and diseases

5.1 Introduction

Specific method designed for particular pests and diseases are covered in detail in this chapter. The methods described here could be used for capturing the prevalence of pests and diseases observed in the field or elsewhere of new cases encountered.

5.2 Sample size

A sample should provide an acceptable estimate of population density or disease intensity. Obviously the larger the sample the more reliable is the estimate. However, if the resources are limited, a compromise must be reached between sample size and reliability. The relationship between precision (expressed in terms of standard error), number of samples and cost is shown in Figure 6.

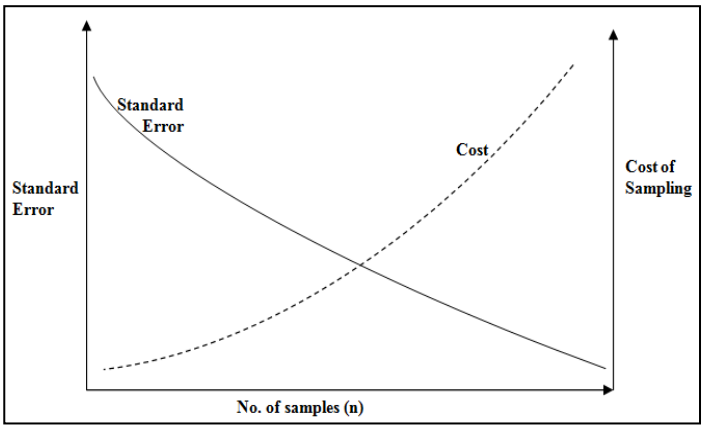

Figure 6: Relationship between no. of samples taken, standard error, and cost of sampling; Source: Karandinos, 1976

5.3 Sample Unit

Sample unit is the smallest element of habitat from which measurements are taken (Southwood, 1978). Numbers of spores per lesion, number of lesions per leaf, number of infected leaves per plant, number of insects per plant or leaves etc… are used for severity ratings.

5.4 Sampling pattern

Sample *size* is more important than sampling pattern when disease distribution is random. Sampling *pattern* is more important when disease distribution is aggregated. Therefore, depending upon pest dispersion in the crop and the surrounding habitat, an appropriate sampling patterns needs to be selected (Figure 7).

If the purpose of sampling is to monitor disease progression and to estimate insect population densities, weekly intervals usually are satisfactory (Gaunt 1985, Shepard et a1 1988). Estimates of insect density also may vary with time of day. For instance, fewer green leafhopper (GLH) *Nephotettix spp*. were estimated by sweep net sampling during 1400-1430 h than during 0730-0800 h or 1800-1830 h (Estano and Shepard 1988). It is likely that estimates of population densities of other rice arthropods also differ with the time of day samples are taken.

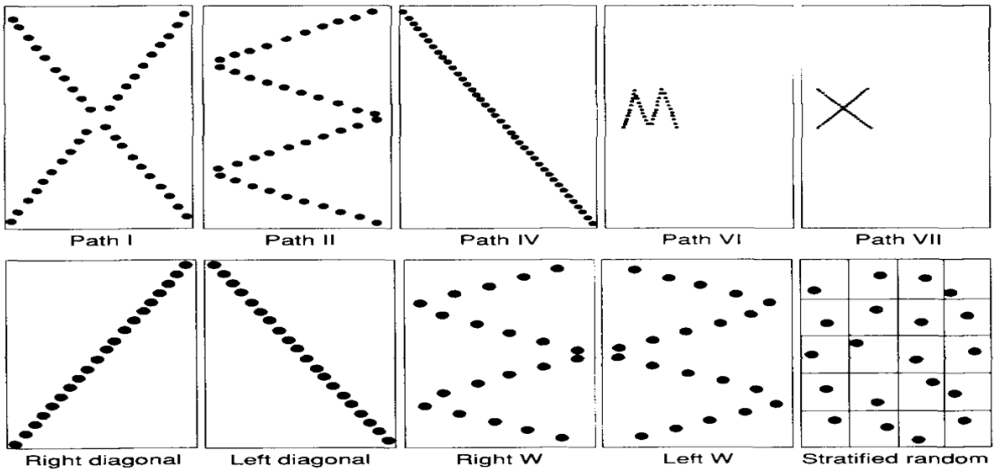

Figure 7: Sampling patterns; Source: Lin et al (1979)

For detection survey and pest prevalence, target sampling for specific pest at their peak time of occurrence for specific crops. Given the time, manpower and cost factors, it is advisable to conduct survey at least 2-3 times during each crop cycle.

5.5 Sampling methods and devices

Field sampling methods can be divided into three major types: absolute, relative, and population indices (Beardsley et a1 1979, Ruesink 1980).

Absolute methods

Absolute methods estimate density per unit area in a habitat. Presumably, all or virtually all the target species in the sample unit are collected. These methods are used in research to calibrate relative methods; they are too costly and time- consuming to be used in IPM.

For insects, absolute samples may be taken to:

- Determine the density of insect pests and their natural enemies, for in-depth studies of population dynamics.
- Establish economic thresholds.
- Estimate effectiveness of control measures.
- Evaluate resistance varieties.
- Calibrate relative techniques.

For diseases, basic studies are important in determining the relationship between a pathogen or disease, its host plant, the environment and human interference. These studies include:

- Quantification of disease epidemics in time and space.
- Crop or yield loss assessment.
- Evaluation of plant resistance to diseases.
- Evaluation of efficacy of control measures like fungicides, biological control agents, etc.
- Development and verification of predictive models and decision aids.

Relative Methods

Relative methods give density estimates based on sampling a unit of habitat, with only a proportion of individuals collected. Because these methods are selective and require less time and effort, they are the choice for IPM.

Population Indices

These are damaged plants or plant parts (e.g. Whiteheads, dead hearts, percent foliage area covered by a disease, etc.). For insects, it is normally not wise to recommend treatment when a certain level of damage is reached unless live insects are present. For diseases, population indices are widely used because it is more practical to assess disease incidence than presence of the causal agent.

With the limitation in the system, indices as such could be counted as pest quantity based on experience or search for the pest to get correct quantity.

5.6 Methods for sampling diseases

Two important parameters for assessing disease intensity in the field are prevalence/ incidence and severity. Incidence is the proportion of plants or plant parts infected with a disease. Severity is the amount or proportion of tissue damaged by the disease. Most methods for sampling diseases are based on severity, especially when information on quantitative relationship between the degree of infection and amount of yield loss is needed.

Ratings are based on visual differences between proportions of infected and healthy plants or plant parts. Before a sampling method is adopted, its accuracy (efficiency) and reliability (repeatability or precision) must be determined.

5.7 Other methods

Other methods include spore trapping or lesion counts and, in the case of insect-borne diseases, insect trapping and field sampling of the vector.

5.8 Insect's stage to be sampled

Mostly sample the insect's stage which is causing damage with long developmental time. However, a stage prior to the damaging stage may be selected so that early predictions can be made. For example, sampling the non-feeding adult moths can help predict the subsequent damaging armyworm larval population.

5.9 Number of sampling unit

The number of sampling units depends on the degree of precision needed and the cost. When greater precision is desired the number of sampling units may be increased.

Morris (1995) laid the following criteria for sample unit and sample size:

- The size of sample be such that all units have an equal chance of selection
- The sampling unit must be easily delineated in the field
- The sampling unit should be of such size as to provide a reasonable balance between the variable and cost.

5.10 Time to sample

Sampling time depends on pest as well as on crop phenology. It is most important for the sampling to coincide with the time of peak numbers of stage. Such timing may be predicted using degree day or other *phenological traits models* or by *trial sampling.*

For trial sampling, the samples are taken regularly (once or twice per week) until a peak is obtained from the cumulative data until the surveyor gain enough experiences and confidence.

Time of day at which samples are taken is also important e.g. flight activity of some insects may be at dusk or dawn, so samples should be taken during that activity period. Further, diurnal rhythms of insects may cause them to move from one part of habitat to another which should be considered while taking sample.

5.11 Pattern of sampling

Depending on the uniformity of the habitat and population dispersion, the sampling pattern may vary. Sampling units should be spread out over an area from where samples are to be taken at random. The edge of a growing area (first 5 meters) are usually avoided in making estimates unless species distribution demands so, for example in the case of *army worms, grasshoppers, stalk hopers, initial aphid incidence, etc*, samples may also be required to be taken from border areas. Refer Figure 7 for more details.

5.12 Other considerations

Sometimes, it may be necessary to examine weeds and other alternative hosts of the pests which in other cases, the pest may overwinter in the surrounding areas.

Chapter 6: Some sample survey guidelines for specific pests

6.1 General survey guidelines for citrus fruit fly

The general guidelines provided in this chapter can be used but it is advised to look for better and more recent methodology/ protocols/ technology in conducting the same.

6.1.1 General Background

A few species of fruit flies like *Bactrocera dorsalis*, *B. tau*, *B. curcubitae*, *B. zonata*, *B. tuberculata* and *B. minax* have already been recorded in Bhutan. No fruit fly species falling under the genus *Ceratitis*, *Anastrepha*, *Rhagoletis* have been recorded until now, but vigilance should be maintained as these pests qualify to be classified as important quarantine pests for Bhutan.

Figure 8: Citrus fruit fly (Adult)

Among the species, only *Bactrocera minax* in citrus is currently of economic importance in Bhutan. This does not mean that the other species of fruit fly are not important. *B. dorsalis*, *B. curcubitae*, *B.tau* and *B. zonata* are of economic importance in some countries and have the potential of inflicting economic losses in Bhutan too, particularly in the warmer regions of the country.

6.1.2 Fruit Fly Surveillance Methodology

For the surveillance of fruit fly, lures and traps are used for survey.

6.1.2.1 Fruit Fly Lures

Para-pheromones

In general mostly para-pheromone are used which only attract male fruit flies. The two most commonly used para-pheromones are methyl-eugenol (ME) and cuelure (CUE) which captures a large number of *Bactrocera* species. The lures emit certain chemical odours which the males take it as a potential female mate and would climb inside the traps and are captured.

Food Based Lures

Lures for capturing females are based on food (liquid protein baits protein) or host odours like fruit juice. These food based baits however are not as effective as the para-pheromone bait. Food based lures capture a wide range of fruit fly species. Protein baits (TY, HP) or Synthetic food lures (AA) are available.

6.1.2.2 Traps

Different traps are used to trap fruit flies. These traps are used along with the lure which attracts the flies to the trap and as a result the flies are trapped and killed after coming in contact with some insecticide. Traps used for fruit flies depend on the target species and the nature of the attractant. The most commonly used traps and lures are:

McPhail Trap: The conventional McPhail trap is a transparent glass or plastic pear shape invaginated container. This trap uses liquid food bait, based on hydrolyzed protein (Nu-lure, Staley's, Miller, etc.) or Torula yeast/borax tablets.

Steiner Trap: The Steiner trap is a horizontal, clear cylinder with a large opening at each end. This trap uses the male specific para-pheromone lures TML, ME and CUE. A wire hanger, placed on top of the trap body, is used to hang the trap from the tree branches. The lure is added by suspending, from the centre of the trap, a cotton wick soaked in 2-3 ml of a mixture of a chemical lure and an insecticide, usually malathion or dichlorvos. If the insecticide is not mixed with the lure, it is added on a strip of filter paper and placed in the trap.

Lynfield Traps: The Lynfield traps are usually used for trapping fruit flies in low rainfall areas. It is a non-sticky disposable pot type trap for trapping adult male flies. The trap consists of a modified clear 1 litre plastic container with a 100 mm base, a 90 mm diameter top and is 115 mm deep. It has a screw top lid, which may be white or yellow. There are four entry holes of 25 mm in diameter evenly spaced 15 mm below the lip of the trap. Two, three or four dental cotton wicks containing liquid lure are held together with a wire clip and hung from a wire loop under the lid of the trap. The hook holding the wick is formed by a wire inserted through the center of the lid, which extends about 25 cm above it so that it can be attached to the branch of a tree allowing the trap to hang freely.

Jackson Trap: Jackson traps, which are triangular traps, made up of thick paperboard with a wick impregnated with lure attached by a clip at the center with a complete opening on both sides and a sticky paper plate is inserted at the bottom of the trap. The most common lures used with the JT are: trimedlure (TML), methyl eugenol (ME) and cuelure (CUE). The lure is added by suspending, from the center of the trap, a cotton wick soaked in 2 to 3 ml of a mixture of the parapheromone and an insecticide, usually malathion, naled or dichlorvos (DDVP), when the trap is used with ME or CUE but without insecticide when the trap is used with TML.

6.1.2.2.1 Trap Placement

In order to place traps in important and strategic place for gathering precise data on fruit fly presence, abundance or its population dynamics, it is important to have a list of the primary, secondary, and occasional fruit fly hosts, their phenology, distribution, and abundance. One of the most important factors of trap placement is selecting a proper trap site. If possible, pheromone traps should be placed in mating areas. *Fruit flies normally mate in the crown of a fruit host tree or close to the host trees selecting semi shaded spots and usually on the upwind side of the crown*. Other suitable trap sites are resting and feeding areas in trees that provide shelter and protect flies from strong winds and predators. Protein traps should be placed close to fruit host trees, in a shady area. Traps should be placed 2-4 meters from the ground (this will also depend on the height of the host tree). It should be placed approximately half the distance from the trunk to the outer edge of the foliage. Traps should not be exposed to direct sunlight, strong winds or dust. It is of vital importance to have the trap entrance clear from twigs and leaves to allow proper air

flow and an easy access for the fruit flies. If no fruiting trees are available in the vicinity of grid, the traps should be relocated in adjoining fruit bearing tree or in its absence placed in trees with suitable foliage (i.e. broad leaved trees).

6.1.2.2.2 Trap Density

Trap density (number of traps per unit area) is critical for fruit fly surveys and it should be adjusted based on target fruit fly species, trap efficiency, and biotic and abiotic factors. Trap density also depends on the risk associated with potential points of entry. For surveillance higher densities are required in commercial production sites and lower densities at points of entry. Usually higher densities are set up in areas of high risks areas like national or international seaports/airports, train stations, tourist places, market places of metropolitan/municipal areas. Medium risk zones include growing orchards/production sites/processing places located in rural areas located in proposed areas; and low risk zones include sparsely vegetated or non-fruit fly host areas.

6.1.2.2.3 Trap Servicing

The frequency of trap servicing (maintaining and refreshing the traps) during the period of trapping will depend on the longevity of baits (attractant persistency); retention system; rate of catch & season of fly activity. Make sure that the lures and liquid baits does not come in contact with other areas or is spilled on the surface as this will reduce the chances of the flies being captured by the traps.

6.1.2.2.4 Trap Inspection (checking the traps for fruit flies)

The frequency of inspection during the period of trapping should depend on the level of fly activity and response periods required at different times of the year and the relative number of target and non-target fruit flies expected to be caught in a trap.

6.1.2.2.5 Record Keeping

- Fruit flies that could not be identified should be sent to NPPC for correct identification and record following the generic specimen collection protocols described in the Chapter 3 of this manual.
- Record all trapping data

6.1.2.3 Materials Required for Survey

- Traps and lures
- Pen and note book/ electronic forms/ apps
- List of properties of orchards and location site to be surveyed
- Pest information sheet
- Sample collection kit (if required to be sent NPPC)
- Metal or Plastic box
- Two or three) glass or plastic containers (screw capped) for storing the wicks soaked with lure and insecticide (Separate containers for different lure and insecticide)

- Two (or three) pairs of long-nosed tweezers (for changing each type of lure)
- One or two pairs of plastic gloves
- Two (or three) small camel-haired paint brushes (for handling specimens)
- Two (or three) medium stiff artist brush (for removing flies and cleaning each type of lure trap)
- One glass jar with screw capped lid for storing insecticide
- Screw-capped plastic vials with labels (for storing the dead fruit fly specimens)
- Field lenses (pocket type, 10 or 20 X)
- Flagging tape
- Plastic disposable bags
- One box containing soft tissue
- One screw capped bottle containing 80% alcohol
- Marking Pen
- Spare traps

6.2 Survey Strategies and Guidelines for Detection Survey for Citrus HLB

6.2.1 Overview

Carry out this type of survey to determine whether HLB is present or absent in a particular area or if the pysllid vector (s) are known to occur in the region/ Dzongkhag/Geog.

6.2.2 Requirement

Pest information Sheet: Surveyor must have information on the symptoms of the disease and should prepare a survey plan along with comprehensive pest information sheet.

Pre-survey: Pre-survey is required if the surveyor is not sure about the disease. Collect samples from sampling site and send to NPPC for diagnosis. Sample collection tools such as: plastic bags, marker pen, pencil, sample box, information recording sheet or field notebook, staplers, etc., are required.

6.2.3 Survey procedures

- Learn the symptoms of the disease prior to field visit
- Identify the place of survey and the sampling sites
- Follow the procedures for detection survey

6.2.4 Identification Guide

Symptoms

HLB symptoms are not specific and thus not a diagnostic tool of the disease. Many other factors including Phytophthora rots, trunk borer, citrus tristeza virus (CTV), greasy spots, phytoplasmas, nutrient or mineral deficiencies induce similar symptoms. Moreover, HLB can be difficult to detect because symptoms are not visible during the early stage.

Foliar Symptoms Associated with HLB include the following:

Figure 9: Yellowing of shoots; Source: Dr. Thinlay, NPPC

Yellow Shoot(s)

The yellow shoot may be present only in one section or a branch of a tree while the remaining portions appear healthy and bear good fruits. If the tree has been infected for a long time then the tree may show several yellow shoots. Infected portions of trees show sparse, small and upright foliage. These shoots may also bear leaves with zinc deficiencies (interveinal chlorosis: veins are green).

Figure 10: Yellowing/ mottled leaves; Source: Dr. Thinlay, NPPC

Yellowing of Leaves

Leaves may appear yellow or blotchy mottled. The mottling induced by HLB crosses the veins whereas mottling caused by nutrient deficiency is seen between the veins.

Fruit Symptoms

Fruits on infected shoot(s) or tree appear as small, lopsided with no proper colouration. *Normal fruit colour begins from the styler end, whereas on infected fruits colouration begins from the peduncular end/ Inverse colouration*. Infected fruits usually have aborted seeds and fruits taste bitter.

Figure 11: Inverse colouration; Source: Dr. Thinlay, NPPC

Other Symptoms: Infected plant or plant parts gradually decline due to heavy defoliation and therefore, twig or branch dieback is other symptom that is associated with HLB. Young plants infected early may die. Plants may become unproductive within few years of infection.

Figure 12: Aborted seeds; Source: Dr. Thinlay, NPPC

6.2.5 Survey Guidelines

Time of Survey

If surveying during flush periods, pay attention to older leaves as young leaves may not show symptoms yet. Avoid surveying during the main flush periods as leaf symptoms are not clear during these periods. Usually field symptoms are more apparent during August- November but may depend on local environment. Surveys may be conducted two times in a year (April-May & August- November).

Regardless of the presence of the psyllid vector(s), observe every row in the orchard. Observe the shoots for yellowing and look for yellowing or blotchy mottled leaves. Observe mature leaves. New flushes will not show the mottling

If there are fruits in the orchards, observe the fruits for its shape and coloration. Cut open these fruits to look for seed abortion. Record all observation in the add comments field. Sample the trees if symptoms are found for diagnostic test in the laboratory. Mark the tree (s) from where samples are collected with sample identification number (assign a number for the sample and mark the tree with the same number). Determine the geographical coordinates with the help of GPS. Observe and record other infestations such as trunk borer and Phytophthora rots.

6.2.6 Collection of samples for laboratory test

- Collect around 3-4 samples per tree (from the yellow branch) with 10-12 normal sized leaves per sample. Sample more leaves if leaves are small (example: 16 leaves)
- Leaves should not be too wet (leaves collected after rainfall must be dried with tissue paper or air dried).
- Put each sample in a plastic bag.
- Label properly (pencil) each sample with the following information: Dzongkhag; Geog & village; Farmer's name; Date of collection; Orchard age; GPS coordinates; Altitude; insect/mechanical damage/other diseases; vectors; general orchard management.
- Place the label inside the plastic bag (with the sample) and seal (double fold the open side and staple).
- Use cool storage e.g., cool box or other locally available materials like banana haulm & leaves for storage and transport. Do not freeze the samples.
- Submit samples to NPPC as soon as possible

It is important to confirm the disease prevalence in the area by getting the sample tested at NPPC lab. Currently centre is equipped with Real-time PCR and analysis protocols are developed and practiced.

6.3 Potato Tuber Moth (PTM) monitoring with Pheromone Traps

6.3.1 Introduction

Potato tuber moth or tuberworm or tobacco *Phthorimaea operculella* Zeller is primarily a pest of later summer and early fall. Its populations grow best in hot weather. In general, tuber moth trapping is most important in July or August through harvest.

Figure 13: Pheromone trap

6.3.2 Survey Methodology

6.3.2.1 Requirement for Survey

Delta-style corrugated plastic traps that should last 2-3 full seasons in the field, enough sticky liners and

pheromone capsules for the season, and a trap stand made of PVC conduit (Figure 10). The magnifying glass is essential to correctly identify tuber moths.

6.3.2.2 Traps Set-ups

Pheromone traps should be set up as shown in Figure 13. A plier or a strong finger and thumb will be needed to fit the trap hanger wire through the hole drilled in the PVC elbow. It is important to keep the traps low to the ground as shown because tuberworm move about very close to the ground. Pheromone capsules should be placed in the middle of the sticky liner. Use a pencil or other tool to move the lures so that you avoid spreading pheromone on the outside of the trap. We recommend at least one trap per field of one acre.

Placing of Traps

Traps should be placed on field margins, out of the way of machinery and away from dusty roads. Also be sure that irrigation water cannot get inside the traps - water fouls the glue on the sticky liner. We have no research to suggest a certain compass direction to be best, although many feel that the upwind side of a field would be best. Avoid setting up pheromone traps near bee hives or crops that are visited by large numbers of bees. Bees will be caught in the traps, fouling the traps and making them ineffective for catching PTM.

Checking the Traps

Traps should be checked every few days during the 8-10 weeks leading up to harvest. When moths are freshly caught, as will be the case with frequent checks, it is relatively easy to glance in each trap to check for new moths. Trap liners should be changed once a week or sooner if they become covered in insects and dirt. Pheromone lures should be changed once per month.

6.3.2.3 Counting the Moths

It is reported that PTM pheromone catches almost only PTM. However, there pose a challenge for farmers or researchers in identifying PTM amongst more than a dozen of its species that can be found in PTM pheromone traps. Fortunately, only 3 species are most common and they are quite distinctive (Figure 14 & 15).

6.3.2.4 How to Interpret Tuber moth Trap Catch Numbers

Traps are very effective at detecting moths in the vicinity, and for assessing the relative pressure from field to field. Clearly, more moths in or near your field means a greater risk to the crop harvested. Bear in mind that the tubers are infested near harvest time, and number of moths in the area is most critical just prior to field destruction until the harvest. It may be tempting to rely on the regional trapping network for your PTM information though the moth can have large populations on a very local scale as well.

6.3.2.5 Recognition features for tuber moth

Important recognition features for PTM (Figure 14 & 15):

Size: Moths are about 0.25” long.

Colour: The predominant colour is light brown, have grey and black scales on the wings, but most scales are brown. There are two large dark spots on each front wing. Most specimens have a third smaller dark spot on each front wing behind the large spots. The antennae are always light brown. Most of the non-PTM in traps has black or grey antennae. The thorax has three longitudinal lines (Figure 15) and shape as in the Figure 14.

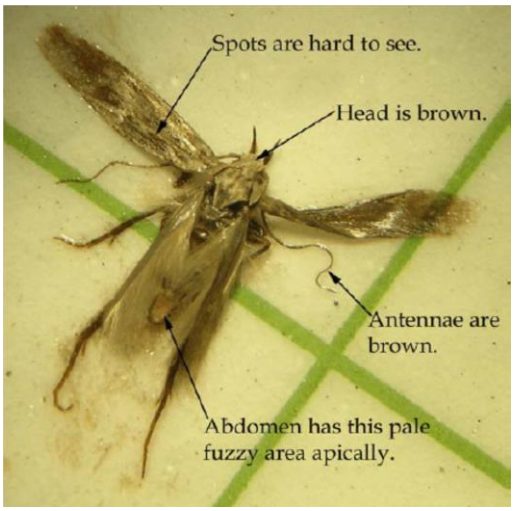

Figure 14: Tuber moth caught in glue with wings spread. Note that some features are hard to see in this specimen, while the unusual structure on the abdomen is visible.

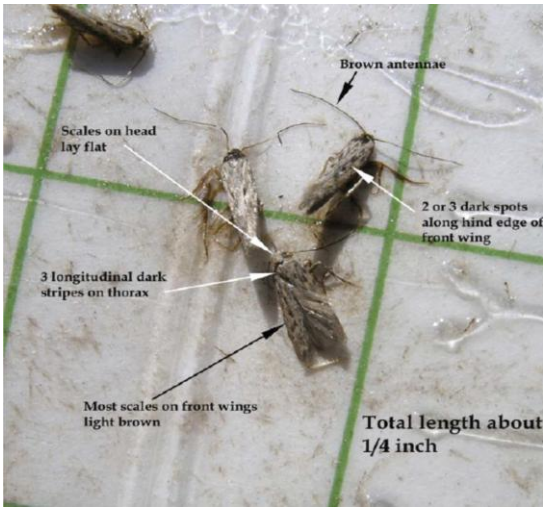

Figure 15: Tuber moths on a sticky trap, with important recognition features highlighted.

As noted above, there are dozens of species that might be found in PTM traps, but there are three that are most common (Figure 15).

Annexure 1: Pest Surveillance Reporting format 2016

Pest Surveillance Reporting Format 2016

~National Plant Protection Center (NPPC)

Dzongkhag Name : .....

Gewog Name : .....

Village Name : .....

Farmer Name : .....

Longitude/ longitude : .....

Altitude : .....

Name of reporter : .....

- *Contact No* : .....
- *Email ID* : .....

#	Crop Name	Crop Stage	Problem: Insect/ disease/ weeds/ abiotic/biotic stress	Affected area (acre)	Incidence of the problem (*no. of insects per m²; diseased plants per m²; no. of weeds per m²; or no. of any other pest per m² or per plant*)	When was it first noticed this year (dd/mm/yy)	Which year it was first observed (yy)
1							
2							
3							
4							
5							
6							

General Comments about the problem (if any):

.....

.....

.....

.....

Submit the report directly to: *pestinformation@gmail.com*

Or fax to NPPC at 351656 with attention to Pest Surveillance Division

Annexure 2: Some of the internet resources for pest information and specific methodology

- CAB International (CABI)/ CABI Crop Protection Compendium
  www.cabi.org
- Diagnostic Protocols (DIAGPRO)
  www.csl.gov.uk/science/organ/ph/diagpro
- European and Mediterranean Plant Protection Organization (EPPO)
  www.eppo.org
- Global Invasive Species Programme (GISP)
  www.gisp.org
- International Plant Protection Convention (IPPC)
  www.ippc.int/IPP/En/default.htm
- Invasive Species Specialist Group (ISSG)
  www.issg.org
- PestNet
  www.pestnet.org
- CAPS-Approved methods
  http://pest.ceris.purdue.edu/services/napisquery
- American Phytopathological Society (APS)
  www.apsnet.org
- National Plant Protection Centre
  www.nppc.gov.bt

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