TECHNICAL GUIDELINES FOR DETECTION SURVEY OF PLANT PESTS IN NEPAL

As endorsed by National Plant Protection Organization-Nepal on 2071/01/22 BS

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FOREWORD

Plant health, now-a-days, has become a major trade policy issue; knowledge of the health status of a country's agro-forest commodities has important applications. These include the development of robust quarantine policies and the management of endemic pests. Plant health problems affect society in many ways. Many areas of society may be affected by incursion of new pests, diseases and weeds into a community. The favorable health status of agro-forest industries provides a competitive advantage in accessing foreign markets.

Accession into WTO is taken as the era of export opportunities in Nepal. But the exports from Nepal have not expanded to the same extent as trade between the developed members. The developed countries have expanded exports by using the rules of the SPS Agreement. At the same time, the government of Nepal is experiencing pressure from her agro-forest producers and traders to use the SPS rules to exclude commodities that are posing threats to agro-forest industries within the country.

Nepal is still unable to provide an adequate description of the health (pest) status of her plant based industries, so we are in a disadvantage when negotiating access to foreign markets. Prospective importers of our agro-forest commodities will assess risk based on our knowledge of the pests' status within our country while we are seeking to export. Our trade partners interested to import our agro-forest commodities will assess the likelihood of introducing pests found in our country with the imported commodity. Prospective importers also assess our phytosanitary measures being practiced to reduce risk to an acceptable level. Extensive specimen-based records are the key for us to negotiate with developed countries on a fair trading system.

If we want to expand exports of agro-forest commodities under the rules of the WTO, we must have the wide collection of our pests in our reference laboratories like that of NARC, NAST and NHM. The development of specimen-based pest lists can be accelerated through structured surveillance programs, focusing on the pests that might be carried on the commodity to be exported. Pest collections are significant because they provide the most reliable evidence of the plant health status of a country. These records are the foundation for developing robust policies for domestic and international quarantine and for developing pest-management strategies at the field level. The collections have taken particular significance since the accession of Nepal into the World Trade Organization (WTO) in 2004. This

guideline has been prepared by the NPPO of Nepal with a view to helping plant-health professionals needing to undertake detection survey. This guideline is prepared for the scientists, working in the NARC or NAST; plant protection officers, working under the ministry of agriculture development; professors teaching in the universities or the foresters working under the ministry of forestry. This guideline will help them to devise survey program on pest detection and to transmit specimens to the laboratory for identification and preservation.

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ACRONYMS

ALPP: Area of low pest prevalence
CDO: Chief District Officer
DNA: Deoxyribonucleic Acid
DOA: Department of Agriculture
DPR: Department of Plant Resources
FAO: Food and Agriculture Organization
FNCCI: Federation of Nepalese Chamber of Commerce and Industry
AEC: Agriculture EnterpriseCentre
GIZ: German Technical Cooperation
GPS: Geographic Positioning System
IPPC: International Plant Protection Convention
ISPM: International Standard for Phytosanitary Measures
MOAD: Ministry of Agriculture Development
MOFSC: Ministry of Forests and Soil Conservation
NARC: Nepal Agriculture Research Council
NAST: Nepal Academy of Science and Technology
NEHHPA: Nepal Herbs and Herbal Products Association
NHM: Natural History Museum
NPPO: National Plant Protection Organization
NPQP: National Plant Quarantine Program
PFA: Pest Free Area
PPD: Plant Protection Directorate
PRA: Pest Risk Assessment
RPPO: Regional Plant Protection Organization
SPS: Sanitary and Phytosanitary Measures
VDC: Village Development Comittee
WTO: World Trade Organization

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BACKGROUND

Accession to WTO is taken as the era of export opportunities in Nepal. But the exports from Nepal have not increased to the same extent as trade between the developed members. The developed countries have escalated exports by using the rules of the SPS Agreement. At the same time, the Government of Nepal is obliged to use the SPS rules to exclude commodities that are posing threats to the related industries within the country. Nepal should provide an adequate description of the health status of plant based industries, while negotiating access to foreign markets. Prospective importers of Nepalese forest related commodities assess risk of introducing new pest based on the authentic pest information provided. Prospective importers also assess the phytosanitary measures being practiced in Nepal to reduce risk to an acceptable level. Extensive specimen-based records are the key for Nepal to negotiate with importing countries on a fair trading system. This document gives detailed guidelines for detection surveys of the plant pests in natural ecosystems, and is basis for specimen based records to be developed by the NPPO.

This document describes the components of survey for the purpose of pest detection in an area. The data generated from the survey is supplied for pest risk analysis. The data also supplies information to establish pest free area and to declare ALPP. To increase exports of forest related commodities under the rules of the WTO, there should be the wide collection of pests in the laboratories as of NARC, NAST and NHM. The development of specimen-based pest lists is made through well planned survey programs, focusing on the pests related to commodities in trade. Pest collections provide the most reliable evidence of the plant health status of the country. The collection and records of pests are the foundation for developing robust policies for domestic and international quarantine and for developing pest-management strategies at the field level. This guideline is prepared for the plant health scientists, plant protection officers, researchers and for the foresters working under different organizations. This document covers the procedures in detecting arthropod pests and plant pathogens in plantations, forests and natural ecosystems. This document have included and followed the ISPMs whenever possible. This document is not intended to guide the technicians to detect the pests in the consignments at the entry or exit points.

Under Plant Protection Act 2064, Clause 6 (2), survey and surveillance function and responsibility is designated to NPPO as per the sub clause (i). “To perform such other function as prescribed". Plant Protection Directorate of DOA is authorized as NPPO. This technical guideline to undertake survey program related to pest detection has been prepared by the NPPO of Nepal. This document also

guidesto transmit specimens to the laboratory for identification and preservation.The publication covers the planning of survey programs for building specimen-based lists of pest. The guidelinehelps design a survey program, emphasizing the need to carefully document the process. The guidelines also provide advice on how to approach the critical issues of designing a statistically valid program that will meet the most rigorous demands of trading partners and others who must have faith in the results. The glossary of ISPM terms that relate to surveillance is published in ISPM No. 5. The most relevant entries are reproduced in the terminologies of this guideline.

TERMINOLOGIES

Survey: Survey is an official procedure conducted over a defined period of time to determine the characteristics of pest population occur in an area. It includes regular systematic collection and identification of major pests, diseases and weeds as well as their natural enemies.

Surveillance: Surveillance is an official process which collects and records data on pest occurrence or absence by detailed study of the pests and beneficial insects. In other words pest surveillance monitors closely and regularly the development of plant pests, their natural enemies and other prevailing factors in an ecosystem.

Specific surveys: Procedures by which NPPO obtain information on pests of concern on specific sites in an area over a defined period of time. Pest list survey is one of the types of specific surveys within detection survey.

Detection survey: Survey conducted in an area to determine if pests are present [FAO, 1990; revised FAO, 1995] Note: The purpose of detection survey is to detect the presence or absence of the pest in a given area or production sites. These are more frequently carried out to determine pest status in an area and they follow a definite survey plan, which is approved by the Plant Protection Directorate/ (NPPO) or any other institute or organization authorized by PPD and are systematically organized. These surveys are carried out either seasonally or annually and/ or following the eradication measures applied to a pest in a given area or production sites. These surveys are organized following a definite survey methodologies based on statistical sampling, which are determined after taking into account the biology of the pest and employing appropriate detection techniques such as field diagnostic kits, traps etc. The results of survey are documented and communicated.

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 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.

National Plant Protection Organization (NPPO):

Official service established by a government to discharge the functions specified by the IPPC. The IPPC (1997), in relation to it's main purpose of "*securing common and effective action to prevent the spread and introduction of pests of plants and plant products*, (Article I.1) requires countries *to make provision, to the best of their ability, for an official national plant protection organization*," (Article IV.1) whose responsibilities include the following: *"…the surveillance of growing plants, including both areas under cultivation (*inter alia *fields, plantations, nurseries, gardens, greenhouses and laboratories) and wild flora, and of plants and plant products in storage or in transportation, particularly with the object of reporting the occurrence, outbreak and spread of pests, and of controlling those pests, including the reporting referred to under Article VIII paragraph 1(a)*…" (Article IV.2b).**ISPM 17.**

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 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.

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.

DESIGNING A DETECTION SURVEY

The collection and recording of pest information is fundamental to all WTO/SPS and trade related activities. NPPO should be in a position to validate declarations of the absence or limited distribution of pests. Particularly, NPPO should decide the objective and procedure of any specific survey to meet the country's requirement. It should guide the surveyors while collecting information about a pest in the field. It should provide the overall framework for planning any type of specific survey. NPPO should also develop specific protocols for survey of specific pest. Specific surveyis an official survey and so should follow a plan which is approved by the NPPO. Depending upon the bilateral trade requirements, both exporting and importing country's NPPO are in general, responsible for determining the process and the techniques of gathering the information on pest. When planning a new survey, the details of the design need to be carefully recorded and justified. Reasons and decisions need to be justified as the result of the survey requires approval from the NPPO. The survey plan should include:

- Properly defined purpose of survey (early detection, assurance for pest free areas, development of pest list, decision to apply phytosanitary measures, finding the extent of pest spread, biosecurity and incursion management, etc.)
- Identification of the target pests(their life cycles and identifiable characteristics, pest vector, host, etc.)
- Identification of scope (such as geographical area, production system, season)
- Identification of timing (dates, frequency, duration)
- In the case of commodity pest lists, the target commodity

A. Indication of the statistical basis (such as level of confidence, number of samples, selection and number of sites, frequency of sampling, assumptions). Description of survey methodology and quality management including an explanation of sampling procedures, sample collection and laboratory analysis, diagnostic procedures, and reporting procedures.

CHOOSING A TITLE

- The title should be simple, short and imparting the subject of survey.
- Name, address and the contact number of the person responsible for

producing the survey plan should be clearly written, so that the confusion happening in the field can be resolved by the survey team after contacting the planner.

- Name, designation and affiliated institution of survey team leader and composition of survey team
- Name and addresses of the institution and collaborating partner/s
- Defines period of time (days, weeks, months or years)
- Command potential survey areas or locations
- Estimated cost for survey

B. REASONING FOR SURVEY

There might be several reasons for pest survey. The survey plan should clearly explain the reasons for pest survey. The reason may include development of specimen based pest list and host list, to obtain data to support pest free areas(PFAs), pest free places of production (PFPP) or pest free production sites (PFPS), determination of pest status in an area, guidelines for pest eradication program, pest reporting, requirements for the establishment of areas of low pest prevalence, etc.

C. FIND THE INVESTORS FOR SURVEY

Survey needs adequate funding and therefore necessary to approach suitable investors/donors.. Preparation of the detail budget plan for survey is essential.

D. IDENTIFY THE TARGET HOST(S):

The importance of the host for nutritional or medicinal value and its significance to small communities, and its national or regional economic importance should justify the effort of the survey. The growth habits of host and any aspects of life cycle that are relevant to the diagnosis of the pests to be investigated should be recorded. Growing condition, growth habit, accessibilities to different parts, location characters, host's distribution pattern, and regional distribution, etc. should be clearly described before undertaking surveys. In applying for the PFA, the survey should provide information on the location and pattern of host plants distribution within the area of interest.

Host names:

List the common and scientific names of targeted host plants.

Value of host or commodity:

Describe the importance of the hosts; for example, their nutritional or medicinal value to local communities, and their national or regional economic importance.

Growth habits and life cycle of host plants:
Describe the growth habits of each host and any aspects of their life cycle that are relevant to the diagnosis of the pests to be investigated. List how and at what condition the host plants of interest are grown. Describehow tall and bushy does the vegetation grow,how much of the plant could be seen and accessed, ability to collect a specimen from the crown, the middle near the main stem, the tips of the growth, or at the base of the plant, etc.

Accessibility of the host plants:
While designing a survey, consider the vegetation and the areas in which the pestsare to be surveyed. Information about the accessibility of hosts is important for the future surveyor using the report as part of general surveillance; it should explain to the future surveyors why only certain places were surveyed. The plan should explain in detail on how the host plants are ordered?, and ability of surveyor to walk through the row or between the plants, could the surveyor see the entire plants in a row or in random?, where can the surveyor walk?, how much damage caused by walking through the crop would be accepted by the property managers?, how far the surveyor can expect that someone could see into the crop or forest?,what is the terrain like?,are there remote parts?, are there any dams, rivers or fences that may affect the accessibility of the site?, etc.

Regional distribution of the host:
A list of properties and sites with host plants should be developed from a number of sources, including industry and government records and personnel, local grower groups and cooperatives, packing operators and distributors, extension staff, researchers and property owners. Aerial photographs can be useful for identifying areas that are densely populated by hosts, such as production areas. The survey plan should consider all alternative hosts, as well as the susceptibility of endemic flora in forest, parklands, gardens and other areas close to the detection site.

Describe the distribution of the host in the country/region of interest. List all of the locations by name.Before going for the pest status survey, it is essential to have a report of host distribution survey performed earlier. If possible, consider the host distribution report of the departments under the Ministry of Forest.In applying for the PFA, the survey should provide information on the location and pattern of host plants distribution within the area of interest.

E. IDENTIFY THE PESTS OF THE TARGETED HOST

In case of pest-host specific survey, i.e. finding out the status of specific pest in specific host; or in case of known pests (more than one pests) are to be surveyed in a specific host, the following information on the specific pests of targeted host are required in the survey plan:

Find the sources of information:

Find the information on pests about their life cycles and identifiable characteristics by reference consultations or by browsing on the credible websites. The information sources may include: NPPOs, other national and local government agencies, research institutions, universities, scientific societies, producers, consultants, museums, the general public, scientific and trade journals, unpublished data and contemporary observations. In addition, the NPPO may obtain information from international sources such as, IUCN, Regional Plant Protection Organizations (RPPOs), etc. Other sources may include: existing PRA reports; either conducted by NPPO of Nepal or by agencies of other countries, reference collections of insects/ pests and plant pathogens of economic importance, and the pest interception databases from quarantine authorities. ISPM 8 has a basis for evaluating the reliability of a pest record that could equally be applied to assessing information sources to be used in developing the survey plan.

Record the Pest names:

Begin by creating a list of the scientific and common names of the pests that attack the host together with synonyms wherever applicable.

Record the Pest vectors:

Identify any vectors of the pests to be surveyed. If the pests have vectors, include them in the list of target organisms.

Record the possible Pest impacts:

Consider why these pests are chosen, are they regarded as major pests or pest threats? Do trade partners want more information on the status of specific pests in the targeted area? In general terms, describe how the pests would be likely to affect a host, production system or ecosystem, and the industry as a whole.

Pest characteristics:

The diagnostic characteristics of a pest, or symptoms of its presence, should be compiled. It is necessary to create a team of specialists and establish laboratories

that have experience with the pests and the diagnostic capacity to identify them, depending on pests intended for survey. Wherever host plants are involved, describe the parts of the plants most likely to be infested or infected, and parts of the plant that should be examined. Does the pest target a commodity, such as fruit or modified root? Is the pest associated with particular stages of a host plant's growth? Is the pest attracted by light or pheromones? Describe where the pest or the characteristic symptoms would be found on the host or commodity; for example, flying above a plant, bored into bark, the underside of leaves, at the base of plant, presence of curly leaves, etc. Are there any factors that affect symptom development, such as sub-species, growth stage, season, pesticide application and climatic conditions? Identify all the likely sites that the pest might have infested. Research the epidemiology of the pest, its means of survival, reproductive rate and life span. Include all available information about the pest's life cycle. In summary, prepare pest information sheet.Pest information sheets provide identifying details of pests that the survey team can refer to during the field survey.

When applying for PFPP and PFPS, the survey should provide the following essential information:

Natural spread habit: fast or slow, spreading long or over short distances;

Artificial spread habit: limited or high chance of the pest being spread artificially;
Host range: wide or limited;

Survival rate between seasons: high or low;

Rate of reproduction: slow or moderate or fast;

Detection: easily or hardly detectable;

Control measures: effective, practical and easily available or no measures.

Collecting reference specimens and images:

Having handout material that can be used in the field may be critically important, particularly if the pest has not been seen before by the surveillance team. Having a reference collection of pressed samples of plants or affected plants, or small specimen collections of invertebrate animals may also prove useful as long as they are not cumbersome and can be protected from damage. Electronic images or photographs of the concerned pests can be used as reference images. These can be used to create pest information sheets.

Alternative hosts:

Finding out the distribution of known alternate/collateral hosts of a particular pest in an area is called pest specific host distribution survey. It is done for delimiting the area of infestation by a particular pest. Listing of entire host range of a particular pest, including vectors, and surveying for the presence of hosts in an area is particularly important for declaringand managing PFA or PFPS or PFPP or for deciding any form of incursion management or for determining the buffer area where internal quarantine system would be implemented. Alternate/collateral host survey of specific pest is done with the objective to locate and destroy the hosts before declaring the PFA/PFPS or PFPP.

F. REVIEW THE EARLIER SURVEY PLANS

NPPO of Nepal and the concerned officialsin NPPO can have existing plans or can provide the contact with others who have designed survey plans. If the plan is connected with trade, the NPPO needs to involve as part of the process.

G. SELECT THE SURVEY SITE

There are usually six steps involved in site selection:

- Select the 'development region'.
- Select the 'district(s)' involved
- Select the 'VDCs' in the districts that could be surveyed.
- Select the 'field sites' within each place: forests, host communities, ports or markets.
- Select the 'sampling sites' within each field site. This could mean, individual plants, trees or produce, transects, or trees to which pheromone traps could be attached.
- Select the 'sampling point', which is relevant to choose specimens within a sampling site.

For undertaking detection survey for PFPP and PFPS, The following acceptable characteristics of the site need to be fulfilled:

- it should be a single property
- it should have clearly defined boundaries, including any buffer zones
- it should be sufficiently isolated from possible pest infestations
- there should be no other known hosts within the boundaries, including the buffer zone.

Methods of site selection:

There is no single best method for site selection. The main point is to transparently

document the choices and reasons for such choice.. The method can then be considered and discussed with other parties for agreement with the basis of the choice, given the circumstances.

Select the appropriate sites for survey:

Full sampling and blitz surveys are always recommended and universally accepted methods of providing the most detailed data for detection surveys in random population. If it is not possible to cover all sites at each level, itis necessary to select the site to cover. . To do this, use one or a combination of the following four tools:

1. Random sampling: This involves assigning all sites (of the uniform level), a number or symbol and then by using a random number generation method, the sites are selected and recorded.

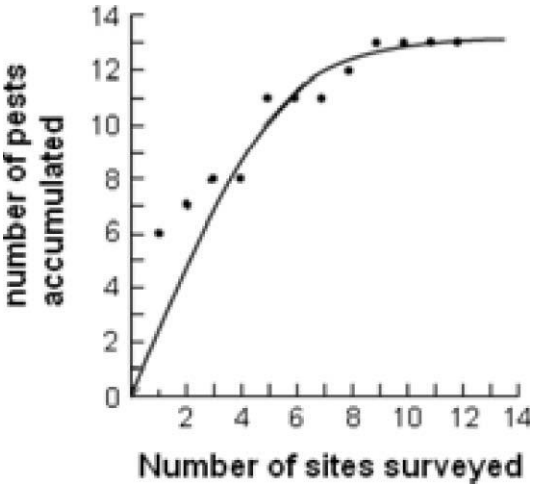
2. Systematic sampling: This involves selecting criteria to divide the sites into some form of regular intervals and then selection of sites accordingly. . For example, surveying every second site when listed by name in alphabetical order, setting up a grid of traps or parallel transects of a site.
3. Stratification sampling:This method 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. Example: 20 villages (level: place) are to be surveyed for diseases of *Sapindusmukorossi*(Ritha). Each village has 15 production sites (level: field sites), a total of 300 production sites. If 100 farms are to be surveyed, randomly choose the 100 from all 300. By chance, this may result in some villages having all their production sites 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.
4. Targeted site selection: The sites are chosen based on where the pest is most likely to be, thereby deliberately biasing the selection process in favor of finding the pest. Targeted sites can be in the field or forest where surveillance is focused on host plants or sites where the pest is most likely to be present. Field workers, property managers and others working at the places of interest may be able to provide local knowledge of where pests present may have been observed. With this method, it is possible to identify particular niches where the pests could be found.

Random number generation

Randomizing the order in which sites are visited, can be achieved by assigning each site a sequential number and carefully listing the sites and their numbers. Cards labeled with site numbers or names can be well shuffled and read off in the order they appear. These methods are useful unless the number of sites is more than a literal handful of cards. Using random number tables is the best of all methods of randomization. It is important to record the order of the sampling sites as species-accumulation curves need to be plotted to determine whether the survey is completed for detection surveys.

Species accumulation curves for detection surveys

After looking at a number of quadrates, the number of new species added to the list will be fewer and the increasingly smaller amounts of information gained need to be weighed against the effort. The sequential sites should be chosen by random selection so that they are unlikely to cluster together. Then the field sites should be stratified into sections.



A species accumulation curve is used to determine the number of sampling sites needed to survey. The process requires recording the number of new pests collected at each new site, then plotting the accumulated number of pest species—with sites across the X- axis and number of pests along the Y- axis. The number of new species will eventually decline with the increasing number of sites examined. A curve is then plotted to the data points. When the curve has flattened for example, five consecutive sampling sites, if no or few species are added with each additional site, the survey is complete.

This exercise should be repeated in different production areas or districts if there is reason, such as climatic variations, to suspect that the pest list may vary from site to site. Species accumulation curves can also be drawn for one location but over time. This means that surveyor would plot the number of new pests on the Y-axis against time intervals on the X- axis. Surveyor may wish to do this, if the pest distribution on a given host is seasonal.

Plan to install the static traps for insects for detection and monitoring

Insects can be caught by static traps that attract insects by light, color 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 sitting and density of traps is critical. Sitting 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.

Calculating sample size

Some main statistical parameters need to be understood by the planner.Parameterslike design prevalence or confidence etc. are expressed in percentages except for sample size which is in whole numbers.

Design prevalence

This is estimated prevalence (detection threshold) and usually based on a pre-survey estimate of the actual prevalence of the pest in the field, and used to determine the sample size.Clearly, for area freedom, the design prevalence and actual prevalence of a pest areexpected 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 the design prevalence greatly over-estimates the actual prevalence, the sample size calculated will be too small to detect the actual prevalence. If the design prevalence under-estimates the actual prevalence, then the sample size will be larger than needed to detect the actual presence, leading to over-sampling. If NPPO is unable to predict a meaningful prevalence, especially in case of detection survey to be done for the first time, it is necessary to choose a prevalence level that is acceptable to all parties. For detection survey, the design prevalence is usually taken as 1%.

Confidence

Statistical confidence is the probability that the actual prevalence will be within the range of the design prevalence.The relationship between confidence and sample size is simple;the more sites surveyed, the more certaintyof the accuracy of the estimated prevalence. As a general rule, a detection threshold of at least 95% confidence is considered acceptable. Confidence up to 99.9% can be necessary in some instances, for example in declaring PFA. Trading partners may require a particular level of confidence that the pest would be detected in a survey, independent of any logistical or financial constraints.

Sample size for detection surveys

Sample size is the number of sites that need to survey in order to detect a specified

proportion of pest infestation with a specific level of confidence, at the design prevalence. Formulas for calculating sample size are given below. These formulas are used when the survey is designed to detect a pest, and where the actual prevalence is likely to be rare.

A simple relationship exists between sample size, confidence level and detection threshold, where confidence is expressed as a percentage and detection threshold on a scale between 0 and 1.

Formula: Confidence level = $1 - (1 - \text{design prevalence})^{\text{sample size}}$

and therefore,

$$\textbf{Sample size} = \frac{\log(1 - \text{confidence level})}{\log(1 - \text{design prevalence})}$$

The following table presents the calculations performed on the basis of these formulas:

Confidence	1 – confidence	Design prevalence	1 – design prevalence	Sample size
0.95	0.05	0.01	0.99	298
0.95	0.05	0.02	0.98	148
0.99	0.01	0.01	0.99	458
0.99	0.01	0.02	0.98	228
0.95	0.05	0.001	0.999	2,994
0.95	0.05	0.002	0.998	1,496
0.99	0.01	0.001	0.999	4,603
0.99	0.01	0.002	0.998	2,300

Accuracy of methods

This deals with how well the survey will detect a pest when it is present.For example, if it is required to look for the pest on a row of trees but due to some reason the surveyorsplan to inspect only along a straight line then the method of inspection is not 100% accurate. It is expected that the observer cannot see all of the trees, if the foliage is dense or the symptoms or pests are not obvious. In such case, 80% can be specified as the accuracy of this method. Some methods can reasonably be expected to be 100% accurate. So if the surveyors are not going to follow the exact protocols of the observation, because of several reasons, then the sample size should be readjusted.

Adjusted sample size =(sample size above)/method accuracy size (generally 0.8).

H. IDENTIFY TIME FOR SURVEY

Pest survey:

The survey should be performed when the pest is most likely to be present and in an identifiable state. The timing of survey procedures is determined by: the life cycle of the pest, the phonology of the pest and its hosts, the timing of pest management programs, whether the pest is best detected on plants in active growth or in the harvested state (ISPM 6). If the best time to survey is not known for the pests, start by finding out its seasonal habits. At what stage of the host's life cycle do the pests prefer to infest it? How long will it persist? Does it die back; such as during the wet or dry season?, can it survive if the host is dead or dying? Does normal management of the forest control or eradicate the pest? How quickly does it reproduce and spread? How long does the pest survive under different conditions and at different stages of its life cycle? Are there weather conditions or weather events that may influence the pest's life cycle or survival? Other factors that may determine the timing of survey are:

- when the pest is most active
- accessibility and availability of vehicles
- time of local festivals or community events
- time of sowing, seedling emergence, flowering, fruit maturation and harvesting of hosts
- time of flowering for weeds
- time of obvious symptoms

Host survey:

The timing of a survey is particularly important when developing pest lists, as it is critical that host plants are examined throughout their life cycle since different pests prefer different stages of the host development. The minimum stages of development that should be surveyed are:

- seedling emergence
- vegetative flushing stage
- flowering stage
- fruiting stage
- post harvest stage
- Consider examining the host plants under different weather conditions.

Frequency of the survey

Some surveys need to be performed several times. For example, every 2 weeks when managing a pest in a plant species, or annually during harvest to support a pest-free-area status, or according to periods in the pest's life cycle. If trading partners are involved, the frequency of survey is based on mutual agreement. . Also, it is essential to revise the timing and frequency if they are dependent on weather conditions or events.The frequency of survey also depends on the trading partner's need, .The importing country may require that pest free area status be verified for 'one or more years' before the year in which export would commence, or simply from the year of export onwards. The frequency can be adjusted according to the perceived risk of the site.

I. PLAN FOR REQUIRED DATA

Tagging the site:

It is necessary to mark sampling sites in the field, even if, it is not intended to return to the same site. It is possible that a specimen or observation taken could be lost or destroyed.So with careful notebook entries and a marked site, the surveyor would be able to revisit the site, if needed. The quality of tags and inkshould be able to endure a variety of weather conditions.

Recording site details:

The location and unique identifying details of each site should be recorded in a notebook. Describing the sampling site would include information such as a GPS reading, a unique number, distances from visual cues, number or nearest number of plant in a row, or any distinguishing topographical features.

Field data to be recorded:

The most important is to record any information that could otherwise be forgotten, such as the dates of survey, the weather at the time, the site details, the names and contact details of the local informants involved, variations in the team member involved in survey, , and any other relevant details.While recording the data one should fill up the form provided in the Annex "1" that allows for recording all the information required for NPPO. For PFPP and PFPS detection survey 'may be required' of the harvestedcommodity at the production site. In this case, the additional information required depends on the demand of the importing country.

Units for data:

Data are normally reported in terms of a unit of measure, usually the number of pests per unit area. The number might be a direct count of the pests or could be a

scale of intensity of the pest that is recorded. For early detection or to support pest-free-area status, the pests will rarely be found. The pest count usually is zero, but it is still important to quantify the amount of effort expended for statistical purposes. For example, '600 trees were examined in each of 20 farms in an area, with no evidence of the pest'. In cases where the pest is numerous, or particularly for symptoms of plant pathogens, whole numbers of pests are not possible or useful. Instead, a scale of cover of the host or a standardized measure of the pest could be used.

Proving the negative data:

It is very important to record negative data, i.e. locations surveyed where the pest was not observed, so that there is a record of the effort expended to look for the pest. It is particularly important in surveys to support pest-free-area status. The validity of negative records depends on a number of factors:

- the pest is known to produce easily noticed signs or symptoms
- the host species is widely distributed and has high population levels
- the host is economically important and is likely to have been examined by plant protection specialists
- the pest is relatively easy to identify
- Environmental conditions are conducive to infection and pest development.

J. IDENTIFY THE COLLECTION PROCEDURE

Pest specimens should be collected and handled with the best possible care to preserve the diagnostic features for identification.If specimens are to be sent away for identification, consider collecting two or more specimens and preserve them adequately. One is to keep with surveyor and one is to send for identification. After identification, surveyor should provide the specimen to NPPO and that will be used for future reference. Specific protocols developed by NPPO for specific pest should be used for collection, handling and preservation.Insect and mite pests may be collected by netting, beating, aspirating and vacuuming, trapping, extracting, specialized collecting, etc. Similarly, there are several methods of getting disease specimen from different parts of the plant.

Basic specimen collection procedures:

- Sterilize any implements with 70% ethanol or 0.5% available chlorine solution before and after each sampling.
- If considered to be a root problem, include soil and crown (lower stem) tissues with root samples.

- It is essential that the time between sampling and dispatch of the sample for identification be kept to a minimum.
- If possible, sample from perceived area of minimal damage to perceived high damage within field and on individual plant

Insect samples (use specific protocols provided by NPPO):

- Where possible, collect a large number of specimens of all life stages.
- Collect specimens with appendages such as antennae, wings and legs.
- Use leak-proof alcohol and resistant plastic container with screw-top lid.
- If sending small and/or soft bodied insects (example: thrips, aphids, mites and larvae), place specimen in 65% ethyl alcohol and completely fill the container
- Do not remove mealy bugs or scale insects from the leaves or stems on which they are feeding as this will damage their mouth parts and make identification difficult. Instead, cut out leaf tissue around the insect and place this in alcohol.
- While sending hard-bodied insects (example: beetles, moths, grasshoppers and fruit flies), carefully fold specimen in tissue paper and place in crush-proof plastic tube or container with several holes in the lid for ventilation.
- Retain and store a spare sample in a secure, cool and dark location.
- If possible, store sample in freezer for 2 hours before dispatch to kill the insect.
- Clearly label all samples.
- Do not send live insects unless live sample is demanded by diagnostic laboratory.

Fungus and Bacteria (use specific protocols as provided by NPPO):

- Try to select the sample on the same day it is to be sent, to ensure freshness.
- For fungal and bacterial samples, store under 2–5°C until it is sent, but note thatsome pathogens do not survive in cold conditions.
- Select samples at the margin between the diseased portion of the plant and the healthy portion.
- Select a fresh, representative and generous sample covering the full range of symptoms.
- If considered to be a root problem include soil and crown (lower stem) tissues with root samples.
- Place samples in self-sealing ventilated plastic bags with some dry tissues or paper towel to absorb excess moisture.
- While submitting a succulent sample, wrap in dry tissues or paper towel and pack firmly in a crush-proof container.
- Do not send dead plant material.

Nematodes:

- Sampling of soils that are very wet or very dry should be avoided.
- The soil for the sample should be taken at least 5–10cm below the surface.
- Separate samples should be taken from the badly affected and normal areas
- Trees and vines should be sampled at the drip circle.
- Individual sample size should be about 250–300g.
- Roots should be either included in the sample or taken separately − about 25–100g, taken at random.
- Affected stems or leaf material should be kept separate from soil and/or root samples.
- Samples should be kept cool − do not leave in the sun or in a closed vehicle left in the sun.
- If immediate dispatch or processing is impossible then samples can be stored in a refrigerator at 4–8°C for several days.

Phytoplasmas and Viruses:

- Plant material that is suspected of being infected with a virus should be collected, cut them into pieces and place in a plastic container containing calcium chloride ($CaCl_2$) crystals or silica gel, but separated by cotton wool.
- Sterilize the scissors or safety blades in alcohol or a 10% sodium hypochlorite (NaOCl) solution between samples to prevent cross contamination.

Weeds:

- Select vigorous specimen, avoid insect or disease-damaged plants.
- Collect sufficient material to fill a herbarium sheet (ca. 450 × 300 mm) and still leave enough room for the label. Plants too large for a single sheet should be divided and pressed in a series of sheets.
- Where possible, include mature leaves, juvenile leaves, buds, flowers, fruits, and bark.
- Use specific protocols for collecting and pressing for trees, bulbs, rhizomes, cones, palms, vines, grasses, rosette plants etc.

Pressing and care of specimens:

Specimens should be pressed as quickly as possible after collection. If this is not possible, specimens can be stored in plastic bags, preferably wrapped in damp (but not wet) papers. Bags should not be packed tightly, and should be kept cool and moist. Make sure that each bag is correctly labeled. Place each specimen, with numbered tie-on tag attached, in a fold of several sheets of newspaper, and place in the press. While filling the press, try to keep it level to allow even distribution of

pressure. Close the press and exert pressure with the straps. The plants in the press should be dried fairly quickly, in a warm place. The specimens must not be left in damp papers otherwise they will go mouldy. It is therefore necessary to go through the press daily during the first few days and change the plants into dry newspapers. Then continue to inspect press daily and change newspapers as necessary until the plants are dry. Delicate plants and petals may be lost in changing and should be kept in tissue-paper.

K. LABEL THE SPECIMENS

Label the specimens in the field, at least in a temporary way until a full and appropriate label can be made. The following information should be included in the label:

- scientific name of host and plant parts affected;
- scientific name andlife stage or state of pest;
- family/order of the pest;
- locality, such as location codes, addresses, coordinates;
- date of collection and name of collector.

To submit specimens to a diagnostic laboratory or expert for identification, ask them about the required condition of specimen and format of details that must be submitted together. Annex 2 provides the general format to fill up before sending the specimen to diagnostic laboratory. Specimen details should be written withwater/alcohol proof inks. If the specimen is in a jar or container, the jar itself should be best labeled rather than the lid.Microscope slides should be labeled with small stickers on the upper side of the slide, away from the specimen itself.

L. TRANSPORT THE SPECIMENS

If the surveyor him/herself is transporting the specimens, it is necessary to ensure that they are being properly protected. If the specimens need to be sent by postal service, greater care should be taken in packaging them to cope with possible mishandling during transport. Take extra care when dealing with the following:

- **Live pests:** These require ventilation, so ensure that air can get in and the pest cannot get out. Keep plant specimens alive by wrapping in slightly damp paper and sealing in a plastic bag. Ensure that the specimens are protected from extremes of temperature during the journey.
- **Glass or breakable containers:** These should be packed carefully so that the glasses do not touch each other or hard surfaces and break. Such containers can be protected by packing them into a second container that is at least 2.5 cm larger on all sides, with packing material placed in the gap.
- **Multiple specimens:** If two or more specimens are to be packaged together,

make sure that each is well labeled.

- **Specimens preserved in alcohol:** The containers need to be leak-proof.
- **Timing:**Submit specimens as soon as possible after collection.
- **Postal or courier service requirements:** Check whether the postal or courier system has restrictions on sending particular volumes of alcohol, pests, container types or anything relevant to the specimen to be sent.

M. IDENTIFY THE HUMAN RESOURCE

Personnel involved in surveys should be adequately trained, and where appropriate audited, in sampling methods, preservation and transportation of samples for identification and record keeping associated with samples (ISPM 6).It is essential to think about the formation of the survey team involved in the field. Consider how experienced they are in recognizing the pest and if they need additional trainings. The team should be informed about the whole process, including the standard methods to be adopted to identify and record pests. Consistency in diagnostic skills of survey team members should be conformed before going into the field.

NPPO is responsible for all type of surveys, inspections and any other systems needed to verify pest status. The surveys should be carried out by NPPO personnel, or by experts authorized by the NPPO. For PFPS, PFPP and PFA, NPPO must certify the management, technical and operational skills of the producer to prevent the pest entering the place or site and their ability to manage the pest if it was detected on site. NPPO should provide the producer with trainings in pest-management systems when necessary. NPPO is also responsible for checking the regulations of the importing country and assisting the producer in establishing conditions that would lead to compliance with such regulations.

N. TAKE REQUIRED EQUIPMENTS TO THE FIELD

Below is the list of equipment to consider taking during field surveys .Depending on the objective and duration,the list of equipment can be reduced or increased.

Personal items:

- Light raincoat (in rainy seasons);
- Snake-proof boots, pants, and gloves;
- Drinking water and food; such as . glucose in the form of hard candies etc.;
- Mosquito repellent;
- Watch;
- First-aid kit: standard items plus bite cream, para-cetamol, anti-diarrheal medication, anti-allergy tablets, antiseptic (like Detol), rehydrating drink sachets (like Tang), chlorine water-purifying tablets (like Piyush); Mobile phone with SIM card;
- ID card.

Pest information sheets:

- Field data recording formats;
- Field guide.

Recording data:

- Water-proof/alcohol-proof pens;
- Field notebook;
- Water-proof paper may be needed to write on during rainy season.

Specimen-collecting equipment:

- Collectors tags;
- Plastic and paper bags;
- A magnifying glass/hand lens;
- Specimen tubes;
- Preserving alcohol (70–90% ethanol);
- Tissue paper;
- Parafilm;
- Tweezers/forceps/scalpel;
- Camera;
- Small pair of binoculars;
- Secateurs;
- Spade;
- Hand-held geographical positioning system (GPS) unit;
- Maps of the area;
- Compass;
- Diagnostic keys (identification, surveying, disease/pest rating scales);
- Permits for collection, survey andspecimen transport;
- Pen-knife;
- Gardening gloves;
- Random number table or pack of cards, dice etc.;
- Calculator, stapler, staples;
- Lighter/matchbox;
- Disinfectant wipes;
- Measuring tape;
- Spray paint;

- Brightly coloured ribbons/tape;
- Trowel or spade;
- Plastic zip-lock bags of various sizes;
- Axe;
- Portable icebox;
- Envelopes (Nepali paper- various size);
- Small buckets (to carry intact soil samples with plants);
- Survey bag-waterproof and non-leather;
- Hunting jacket and pant;
- A hammer and chisel;
- A pruning saw;
- Water spray(use where plant specimens are to be kept alive);
- Sturdy plant press, herbarium press and newspaper.

For entomologists:

- Sweep net;
- Pooter or aspirator;
- Lures or traps;
- Mounting boards and pins for insects;
- Cottonwool to place in tube with live insect (to prevent damage in field).

For plant pathologists:

- Spade and sieve for nematodes;
- Razor blades and scalpels (to section plant material);
- Culture plates;
- Specimen pots;
- Calcium chloride chips (to act as a desiccant);
- Ethanol;
- Ethanol flame lamp to sterilize scalpels, tweezers etc,.

Checking for consistency in diagnostic skills of surveillance team members

To assess if members in the team observe and record pests in uniform manner, begin by selecting five or more infested plants and number them. Each team member then assesses all the plants, recording details per plant on their own. Compare the results between team members , both per plant and as an average over the five (or more) plants. If there are differences in the records, inspect the plants together to develop a consensus on the results. Repeat the process with new plants or sites until consistent results are obtained within the group. If there is debate concerning the diagnostic characteristics, seek further information about their

appearance for the given conditions.

Obtain permits

Consider whether it permit is needed to visit villages, communities, or forests where it is intended to survey. Itis essential to inform and involve people, as appropriate, particularly those in charge of the area; for example CDO in the district. It is important to inform them on what dates the survey team would like to visit and give them a clear explanation of what the team will be doing and the expected results.. The timing may coincide with cultural or ritual events and so access may be denied. Itis wise to re-confirm permission before leaving. It may need to obtain release and transport permits from District Forest Office (DFO) and also quarantine permits for domestic and international transfer of specimens collected.

O. PERFORM THE SURVEY

The team should now be equipped with enough plans, information and tools to carry out the survey.

P. ANALYSE DATA

After survey, the surveyor should have a set of forms or data that are not processed or analyzed. The raw data can be used to:

- calculate basic statistics, such as the average and total numbers of pest;
- create a map of the pest distribution or spread;
- create pest records and the pest list of the surveyed host in the surveyed area. The standard format that can be used for pest record is given in the annex 3.

Q. REPORT THE RESULTS

If the survey is designed for trade-related and quarantine purposes, NPPO seeks for soft and hard copy of the report before acceptance.

Summary report

It is useful to have a simple summary to be provided as follow-up information for the people who were involved in the survey; from the team members to the local

property owners, rangers and community leaders. This acknowledges their assistance and show that their involvement are appreciated. Summary report should include the following sub-titles:

- the survey title and team members;
- the aim of the survey, including which pests, hosts and sites were targeted and why;
- what was found?
- what it means to the people who reads the report?

Final report to NPPO

It may be obligatory to submit the information according to the format and content of pest reports covered in NSPM 13 and 17 particularly,for situations involving trading partners. During the assessment and acceptance of the survey plan, the NPPO provides the format (if needed additionally) according to the objective of the survey. But in general condition, the report should provide at least the following information:

- The survey title and team members;
- The reason for surveying;
- Background information on the pest, host and sites surveyed, including discussion of any earlier, related surveys;
- The survey design methods in detail, including site selection, timing of the survey, the type of data and specimens collected;
- Process of data analysis and interpretation;
- Conclusions of survey findings and relation of finding with the objective. The report should provide the filled up information in the annexes 1, 2 and 3.
- Literature citation, if applicable, must be provided in the body text and at the "references" heading at the end of the report.
- The report should also have a brief abstract at the beginning and should include a glossary of terms and acknowledgments (such as from whom permission and funding were received).

ANNEXES

Annex- 1: Field datasheet

Field Datasheet											
1. Name of field/Site visited:											
2. Date/Time of visit:											
3. GPS reference point			Latitude:								
			Longitude:								
			Altitude:								
4. Locality:			Village & ward no.:								
			VDC:								
			District:								
5. Climate data of locality:			Average min. temp (in °C):								
			Average max. temp (in °C):								
			Rainfall (in mm)								
6. Survey/Field plot no.											
7. Host plant species inspected											
7.1 Description of habitat (such as . aspect, slope, vegetation type, soil type)											
7.2 Alternate host plant species/Variety inspected											
8. Phenologicalstage of the plant											
8.1 Main host											
8.2 Alternate host including vectors											
9. Sampling method											
10 Contact details of the local informant involved in the survey											
			11. Details of pest recorded								
SN	Scientific Name	Common Name	Category	Order	Family	Life Stages	Time	Plant parts affected	Symptom & Sign)	Behavioural notes	Intensity
10. Any additional information (including collection of specimens for investigation):											
11. Name/Signature of surveyor with date:											

Annex 2: Format for specimen forwardal?

Specimen forwarded for identification by Diagnostic/Referral Laboratory	
1. Collection number:	2. Date of Collection:
3. Submitting organization:	
4. Name/Address of the sender:	
5. Locality of collection (District/VDC/village):	
6. Reasons for identification:	
7. Name of the host plant species (Common/ Scientific)/variety and or/ commodity:	
8. Origin of host/commodity (where applicable):	
9. Plant parts affected:	*[ ] roots; [ ] stems; [ ] leaves; [ ] inflorescence; [ ] fruits; [ ] seeds/nuts [ ] others (_____)
10. Category of pest specimen/organism submitted:	*[ ] insects; [ ] mites; [ ] nematodes; [ ] fungi; [ ] bacteria; [ ] virus; [ ] others (_____)
11. Life stage of the pest (Applicable to insects):	*[ ] egg; [ ] larvae; [ ] pupae; [ ] adult;[ ] nymphs; [ ] juveniles; [ ] anamorphic [ ]; cysts; [ ]others (_____)
12. Type of pest specimen/organism submitted:	*[ ] preserved specimen; [ ] pinned/card board mounted specimen; [ ] dry specimen with host; [ ] culture; [ ] disease specimen (fresh); [ ] disease specimen (partially dry); [ ] slide mount; [ ] others (_____)
14. Number of specimens submitted per each collection:	
15. Signature/stamp/office seal of the sender with date:	
For identifier use	
16. Name &address of Diagnostic/Referral Laboratory:	
17. Remarks of identifier (condition of receipt of specimens):	
18. Pest identification (Common/Scientific name/Taxon):	
19. Description notes, if any:	
Place: _____	
Note: This form should be prepared in duplicate by the sender and forwarded to the identifier/referral laboratory along with each collection of specimen. The identifier should return the original copy after entering the particulars of the pest identified along with description notes and remarks if the identifier will retain any to the sender of the specimen and duplicate copy.	

Annex: 3: Pest record datasheet

Pest Record	
Reference number	Name of laboratory, address, catalogue reference number of the specimen in the pest library etc.
Scientific name of pest: Common name: Growth habit: Habitat characters: Species name: Family: Order:	
Life stage of pest:	[ ] Egg; [ ] Larvae (caterpillar/grub/maggot); [ ] Pupae; [ ] Nymph; [ ] Adult , anamorph, teleomorph, juvenile, dormant spore, etc (dependent on pest)
Scientific name of host: Variety: Common name: Species name: Family:	
Plant parts affected:	[ ] Leaves; [ ] Stem; [ ] Roots; [ ] Buds/Flowers; [ ] Fruits; [ ] Seed ; and [ ] Whole plant
Stage of plant:	[ ] Seedling stage; [ ] Vegetative Growth stage; [ ] Flowering stage; and [ ] Fruiting stage
Locality: Village: VDC: District: Province/State:	
Date,time and GPS location of pest collection	
Method of collection:	
Name of the collector:	
Species accumulation curve:	
Method of identification:	
Name of the identifier:	
Date of verification:	
Method of verification:	
Name of the verifier:	
Method of pest preservation (detail):	
Suitable for:	Taxonomic analysis or DNA analysis
Life stage of pest affecting alternate host:	[ ] Egg; [ ] Larvae (caterpillar/grub/maggot); [ ] Pupae; [ ] Nymph; [ ] Adult , anamorph, teleomorph, juvenile, dormant spore, etc (dependent on pest)
Scientific name of alt. host Common name: Species name: Family:	
Alt. host. plant parts affected:	[ ] Leaves; [ ] Stem; [ ] Roots; [ ] Buds/Flowers; [ ] Fruits; [ ] Seed ; and [ ] Whole plant
Stage of alt. host plant:	[ ] Seedling stage; [ ] Vegetative growth stage; [ ] Flowering stage; and [ ] Fruiting stage
Locality, Village, VDC, District, Province/State:	

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