NATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

NSPM:

Standard Technical Protocols for Collection and Handling of Disease Samples

2014

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1. INTRODUCTION

1.1 Scope

This standard describes the technical protocols for the collection and handling the disease samples to be used by the quarantine staff or field staff.It focuses on collection, preservation, mounting, labeling packaging and forwarding the specimen to diagnostic laboratory for identification.

NSPM preparation is based on guidelines and recommendations developed within the framework of the IPPC. This standard also adopted the principles, recommendations and format of ISPM to achieve international harmonization of phytosanitary measures with the aim to facilitate trade.

1.2 References

ISPM 5**.**Glossary of phytosanitary terms.Rome, IPPC, *FAO*

ISPM 20. 2004. *Guidelines for a phytosanitary import regulatory system*. Rome, IPPC, FAO.

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Lester W. Burgess et.al. 2008. Diagnostic manual for plant diseases in Vietnam. Australian Centre for International Agricultural Research

Natalie P. Goldberg and Jason M. French .2013 ??. How to Collect and Send Plant Specimens for Disease Diagnosis Guide Booklet H-158 . NM State University.

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S.L. Joshi, B.B. Karmacharya and B.R. Khadge. 1991. Training Manual on Plant Protection No. 14. Manpower Dev. Agri. Project (GTZ), Kathmandu, Nepal

TimothyS.Schubert,LisaL.Breman and Sarah E.Walker. 1999. Basic Concepts of Plant Disease and How to Collect a Sample for Disease Diagnosis Plant Pathology Circular No. 307 Fla. Dept. Agric. & Consumer Services

WTO. 1994. *Agreement on the Application of Sanitary and Phytosanitary Measures*. Geneva, World Trade Organization.

1.3 Definitions

Definitions of phytosanitary terms used in this standard can be found in ISPM 5 (*Glossaryof phytosanitary terms)* and Plant Protection Act,2006 and Regulation, 2009.

1.4 Outline of Requirements

The plant quarantine officers/plant health inspectors has the responsibility of inspecting consignments of plants & plant products moving in international traffic and, where appropriate, the inspection of other regulated articles particularly with the object of preventing the introduction and or spread of pests. Those provisions have been clearly specified in the Article IV. 2c of the IPPC 1997. The inspection determines the options to be used for the pest detected during consignment sampling. In such cases, if pestshave been detected by signsmay require specialized diagnosis in a laboratory or by a specialist before a decision or determination can be made on the phytosanitary risk of the pest.

A precaution also has to be taken for handling the quarantine pests and new organisms detected for sending the materials for diagnosis purpose in the referral laboratories. The accurate diagnosis depends upon the acquisition of appropriate sampling, packaging and timely delivery of specimen materials. The specimens are carefully handled so as to retain, as much as possible, their key diagnostic features. For these, the authorized officers or inspectors should have technical qualifications & competencies, especially in pest detection; collection& handling as different pests may require different sampling and handling techniques. Proper handling of disease sample is critical and need to take special precaution so that the quarantine pests are not escaped in the transit between sampling area containment area foe the inspection

Furthermore, a system for properly documenting and maintaining samples and/or specimens should be in place to ensure trace-back to the relevant consignment and to facilitate later review of the results if necessary.Being a signatory member of WTO, IPPC & APPPC Nepal has to follow all these guidelines enshrined under international documents in international trade. For this, the general guidelines for collecting and handling the insect specimens are provided in this standard to be followed by the NPPO/NPQP staff.

Sampling protocols are intended to be used by quarantine staff and staff performing field survey as part of phytosanitary measures. They are subject to review and amendment to take into account new developments in sampling technique. The standard also provides guidance on how these protocols will be initiated, developed, reviewed and published.

2. BACKGROUND

Plant disease is combined manifestation of pathogen, host and environment in a given time frame. Plant disease diagnosis is a technical job that requires highly technical skill.Proper diseasediagnosis is crucial for the appropriate application of phytosanitary measures. The correct diagnosis also is required to determine if pests should be regulated and the level of phytosanitary measures to be taken against them. New organisms detectedduring consignment sampling needs identification in a laboratory to justify the phytosanitary action.

The collection and proper handling of disease samples up to the diagnostic laboratory is very essential for proper diagnosis. Special care also needs to be taken to prevent the potential risk of escape of exotic pathogens to the environment.

The process of sample collection for disease diagnosis is of pivotal importance. Unless done correctly, the entire diagnostic process may be difficult, and recommendations for phytosanitory measure may be of limited use. Sample collection can be a difficult, time-consuming process, and there is no guarantee that the diagnosisproblem resolved with a single sampling. With all the variables involved in plant production, every plant problem demands different sampling techniques.

3. GENERAL REQUIREMENT

The following general provisions apply to the protocol related tosampling and handling:

- Laboratory tests may involve the use of chemicals or equipment which may present a certain hazard. In all cases, safety procedures should be strictly followed.
- Use of names of chemicals or equipment in this protocols implies no approval of them to the exclusion of others that may also be suitable.
- The procedures presented in the protocols may be adjusted to the need of individual case of collection and handling, provided that they are adequately validated.

3.1 Training of staff

The staff responsible for survey work and collecting and handling disease sample should have knowledge and skill of plant disease diagnosis. The staff should be trained for the skill and knowledge. NPQP should organize training program for the newly recruited staff before giving task of sample collection and handling. Refresher training should be organized to update new development in the subject regularly.

4. SPECIFIC REQUIREMENTS

4.1. Procedure for Sample Collection, Packagingand Submission

A suggested list of supplies to include in a field kit used for collecting diseased plant samples is given in Appendix 2. After deciding what to include in the sample, the following procedures for obtaining, packaging, and submitting the sample need to be followed:

4.1.1 Collecting plant samples

It is important to collect the best plant samples possible and to record all pertinent information for the diagnostic purpose. Following are general guidelines for collecting disease samples.

4.1.2 Examine the entire plant for symptoms

One or more pathogenic microorganisms may infect diseased plants, although they may also have an abiotic disease that does not involve a plant pathogen. Diseased plants often display a range of symptoms. Often, not all symptoms of a particular disease will appear on any one plant within a diseased crop, and more than one plant organ may be affected by a given disease.

All of the main plant parts/organs should be for disease symptoms: roots, stems, leaves, and blossoms. Samples from various plant parts/organs should be collected as needed. Plants may suffer from more than one disease simultaneously. Different types of plant parts with different symptoms should be segregated into different samples.

4.1.3 Multiple disease specimens

A single sample may not be enough to allow a correct diagnosis of the disease problem; several plant samples showing the range of symptoms or not having symptom may be needed. Samples with various stages of disease development should be selected (early and late stages). Samples should be as typical or representative of the overall disease problem as possible. The best plant tissues for diagnosis are the ones showing the symptoms in various stages of disease development, and adequate amounts of them are important, but submitting excessive amounts of leaves or soil should be avoided. Suitable plant material for varietal and or species identification should be included, since occasionally field identifications of the host variety or species may not be known.

4.1.4 Avoid dead plants or plant parts/organs

Dead plants or plant parts/organs may not be useful for diagnosis. Often their tissues have been invaded by saprophites and the original pathogens are no longer detectable. Always select plant samples from living tissues and focus your attention on plants or plantparts/organs that are in early stages of disease or are in the process of dying, and not already dead.

4.1.5 Collect the entire plant whenever possible

For the correct diagnosis with confidence correct plant parts should be submit; for example, some leaf symptoms of disease (wilting, for example) are the result of damaged or diseased roots that have rotted and are no longer functioning to support the plant; in such cases, a correct diagnosis often depends on having a sample of the roots. Plants should be carefully dug from the ground (not pulled out) so the root systems remain relatively intact. When the entire plant cannot be sampled, shipped, or submitted, collect the largest plant sample possible, or portions of each major plant organ (roots, stems, leaves, flowers). Branch specimens should include the diseased area and part of the healthy area and may be at least 8–12 inches long. Root samples should be taken from the affected plants. Roots of theadjacent weeds should be avoided. If entire plants cannot be sampled, photographs of affected plants where possible should be submitted.

For soil and root sampling, roots should be carefully lifted so as not to leave feeder roots or rotted roots behind. About a kilogram of soil fromrhizoshere should be taken for pH, soluble salts, and possibly a nematode identification.

Samples should be placed in appropriately sized plastic bags, including a paper towel (better sterilized) or a blotter if sample is very wet. Do not use only plastic bag for wet sample. Duplicate samples are recommended if the sample is succulent or fragile.

When whole plant sample is taken, wrap a wire twist-tie around stem at ground line to keep soil off of above-ground plant parts.

Samples should be accurately labeled and should be place in a paper bag or an unsealed plastic bag.

Samples should be kept cool and should be protect from crushing (Use icebox with sufficient ice to cool the sample in the transit). Sample should not be frizzed. Sample should be delivered promptly to the laboratory.

4.1.6 Provide backgroundand related information to the maximum extent possible

Good information contributes to a better understanding of the problem. A complete description of the problem and the crop’s history should accompany the sample. The name or number of the plant

sampled should be given. When the problem appeared and when the sample was taken should be indicated. The crop growing site/area should be examined carefully and the prevailing conditions should be noted. The conditions for the site such as elevation, flooding, previous crop history, etc. should be noted. Any observable pattern of disease occurrence should be noted (for example, in random patches, or uniformly throughout the crop). The worksheet information should be reviewedas required. This information is helpful in making a distinction between damage caused by different types of pathogen and damage caused by other factors.(Appendix 1.)

In short, the collector should make the following observation critically:

- Spatial and chronological pattern of disease in crop, on individual plants.
- Symptoms- different or consistent, uniform or scattered?
- Spreading disease- across crop or progressing on individual plants
- Frequency and intensity of the disease problem
- Signs of pathogen/causal agent- (fruiting structures, chemical residues, etc.).
- Vector (insects and others) and its different stages
- Any evidence of host recovery
- Symptoms of nearby plants (same or different)
- Internal symptoms/signs of disease (after cut-open)

4.1.7. Preserving plant samples

- After collecting the samples, do not expose the samples to direct sunlight.
- Keep them cool and do not allow them to dry out.
- Place samples in plastic bags in the shade or in a cooler until they are ready for delivery to the laboratory. Leaves may be pressed between newspapers in plant-press or between the pages of a book or magazine or wrapped in tissue.

4.1.8. Isolation of Pathogen in the field

Some fungal pathogens are too slow growing and get easily overtaken by saprophites in the sample. They need to be isolated in the field and kept viable in the transit. In the field portable isolation chamber (Fig.1.) can be used to reduce contamination by other organism. Sterilized petri plated with 15% water agar is prepared and sealed with parafilm and used to isolate the fungal or bacterial pathogens. The disease sample with the suspected pathogen is surface sterilized with 0.1 % sodium hypochroride, rinsed with sterilized distilled water three times and transferred to the petriplates with agar and resealed with parafilm tape. In the laboratory, the sample is used to isolate the pathogen in suitable selective or non-selective growth medium. The sample can also be used for other diagnostic purposes like DNA-analysis.

4.1.9. Root/Soil/plant sample for nematode

Field Crops

Nematodes do not necessarily occur uniformly throughout a field, therefore several sub-samples must be taken from across the field, and then combined. Twenty to thirty random sub-samples should be collected from each block of 1-2 hectares. Samples should be taken directly from the root zone. Sub-samples should be mixed thoroughly and place 500 g of soil, with roots, in a plastic bag, for laboratory analysis. Because nematode damage within a crop can be patchy, samples from healthy

plants, as well as from plants showing symptoms of decline should be collected. Samples should be kept separately and label them as.

Trees and Shrubs

Soil from around the rhizosphere containing feeder rootlets or intact root systems should be collected and place them in a plastic bag. Samples should be collected from the upper 20-30 cm depth around drip rings. Several sub-samples should be taken from each tree or shrub. A minimum of 500 g of soil, and 10 g or a handful of feeder roots, per sample is recommended. Thick woody roots are of little use for nematode analysis. Take Samplesshould be taken separately from healthy trees and from those showing symptoms of disease.

Nurseries and Greenhouses

About 500 g of soil including feeder roots should be. Leaf, stem, seed or other aerial plant material should be collected where symptoms are noticed and nematodes suspected.

4.1.10 Avoid cross contamination:

There is always chance of cross contamination from one sample to other. The chance of cross contamination should be minimized at the process of handling sample. The equipment like spatula, forceps, pruning knives, etc. used for collecting and handling sample should be disinfected before using to the new place or area. The collector should use disposable hand gloves and clean hand and equipment each time they star handling the sample. It will be safe to keep samples collected from one lot or area in one container like plastic bag or ice- box.

4.1.11 Labeling Samples

Labeling should be done properly so that identity of the sample does not get lost on the way. NPQP should develop coding system for the labeling so that uniformity is there and identification of the sample become easier (ex. d/h/t----). Labeling should be done right after collection. Labels should be there both inside and outer surface of the container. The label should be strong enough to withstand the rough handling. Permanent marker is not good for labeling soil and moist sample. Pencil written label on strong paper should be used to label samples. The label should be small and readable. The detail of the label should be noted on the note book or form designed. Blank labels with adhesive are available in the market. Such label should be tested before using for the sample.

4.1.12 Transportation of disease samples to laboratory

The sample should reach laboratory in minimum time possible. The sample should be transported in cool conditioned van because sample get contaminated or destroyed at high temperature. If cool van is not available, sample should be transported in icebox containing enough dry ice so that fresh samples reach to the laboratory in time. The sample should be categorized in fragile group and should not be staggered one above the other heavily.

4.1.13 Handling quarantine samples

The quarantine samples should be handled carefully so that pathogens do not escape in the transit to containment area. The sample should have warding sign in the sample that the sample may contain quarantine pest, while forwarding to the laboratory for diagnosis. In the laboratory the quarantine samples should be stored or preserved separately. The sample should be properly disposed after the lab work so that it does not escape the laboratory.

Appendix -1 : Worksheet for gathering information necessary for diagnosis of plant disease while collecting disease sample

Sample to be sent to:

❑Plant Pathology Laboratory

❑Soil Laboratory

Name and address .....

Date of sampling: .....

Area where sample was collected (e.g., District, Farm) .....

Elevation .....

Collector of sample .....

Host common name .....

Host scientific name .....

Type of grower, growing situation (check all that apply)

❑Commercial/Agriculture Field ❑Greenhouse ❑Potted ❑House plant

❑Home garden/landscape ❑Grounds (commercial) ❑Lawn/turf

❑Stored products ❑Other

Pesticide history

List pesticides used (with application date).....

Rates of application (concentration) .....

Frequency or number of applications .....

Compare with recommended rate: ❑High ❑Low ❑Normal

Fertilizer history

List fertilizers used .....

Amount of fertilizers used .....

Frequency and time of fertilizer applications .....

Compare with recommended rate ❑High ❑Low ❑Normal

Plants or area affected

Area (ha) ..... No. plants ..... % of plants …….

Spatial pattern of diseases plants (check one) ❑Uniform❑ widespread Random distribution ❑Clustered

Rooting environment

Plant parts affected (check all that apply)

❑Buds ❑Seeds ❑Stem ❑Trunk ❑Bark

❑Tubers❑Blossoms ❑Petioles❑Rootlets

❑Large rots ❑Growing tips ❑Fruits or nuts ❑Bulbs or corms

❑Branches, large ❑Branches, terminal ❑Leaves, upper surface

❑Leaves, lower surface

Plant symptoms (check all that apply)

❑Galls ❑Wilting ❑Stunting ❑Gumming ❑Yellowing ❑Root rot ❑Dieback ❑Shot hole ❑Leaf spot or blossom blight ❑Leaf fall or blossom drop ❑Fruit spot ❑Fruit rot ❑Mildew

History of disease problem (check one): ❑First time ❑Recurrent

Symptom appearance (check one) ❑Rapid ❑Gradual

❑Malformation ❑Tip burn ❑Discoloration ❑Sudden collapse

❑Slow decline

❑Leaf scorch❑Blight ❑Canker ❑Mosaic

❑Ringspot ❑Rust ❑Soft rot ❑Sooty mold

Environment information:

Temperature -------- Humidity---------- Ventilation----------wind ----------

lightning-exposure --------- light intensity ----------Air quality ----------

Method of heating and cooling----------Soil moisture ---------- etc.

Any addition information or comments that will be helpful for diagnosis.....

Appendix- 2 : Contents of a fieldkit for plant disease sample collection

Plastic and paper bags

Paper towels

Trowel, shovel, soil probe

Forms,

Pencil

Padded collection boxes

Maps

GPS equipments

Weather meter (Optional)

Pruning shears,

pruning saw, Knife, hatchet, , Rubber bands,

Labels

Camera (with magnify- lenses, macro lens and flash equipment)

Field manuals,

Reference material (compendia) ,

Wire twist ties,

Plastic/rubber gloves

Hand lens 10X and 20X

Vials/jars with lids

Large ice box/chest/cooler

Sodium hypochloride

Alcohol

Cotton

Sprit -lamp

Needle

Forceps

Appendix 3 : Sample collection and forwarding form

Sample collection and Forwarding Form								
Sample No	Date of collection	Name of Farm/ farmer	Locality	Identity (Name and designation). of collector	Type of Sample	Qty./No of units	Mode of packing	Remarks
Name & Signature of Forwarder: _____ Date					Forwarded to: ---------- Name of diagnostic Laboratory and address			

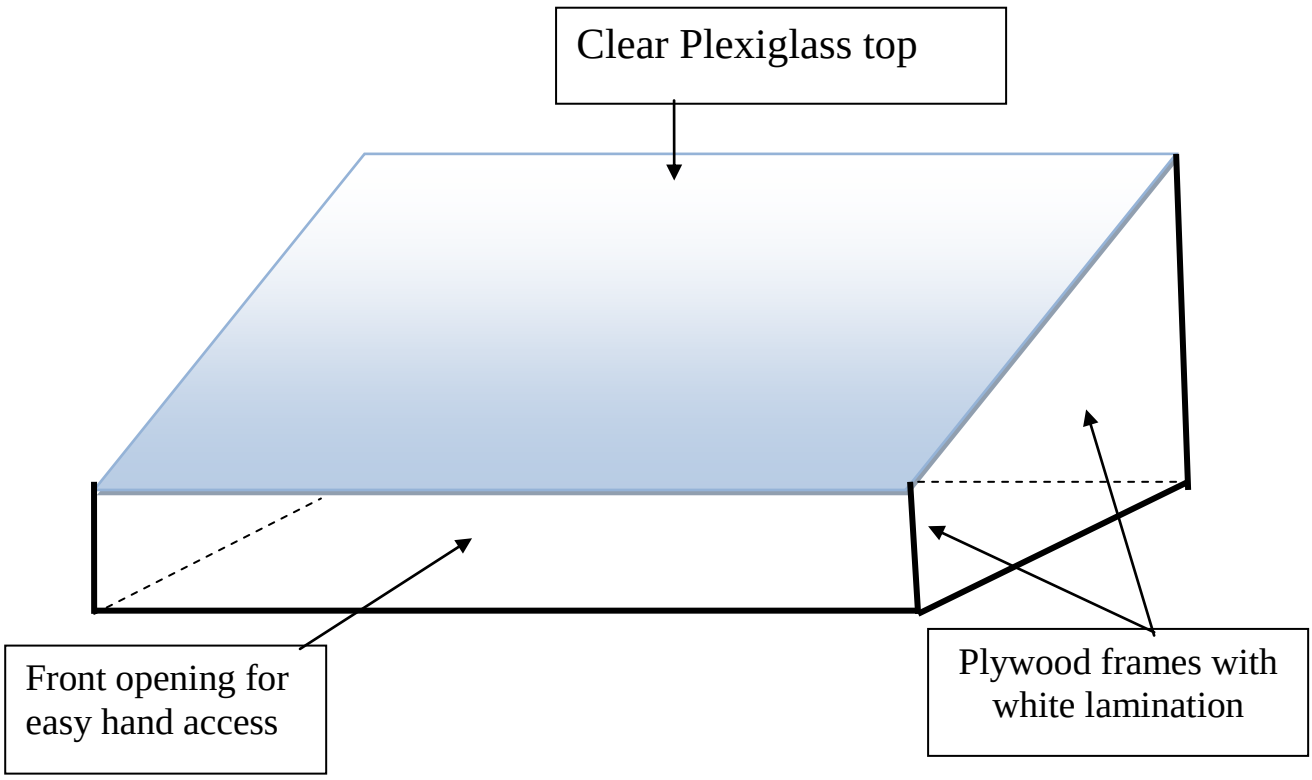

Fig.1. Portable isolation chamber