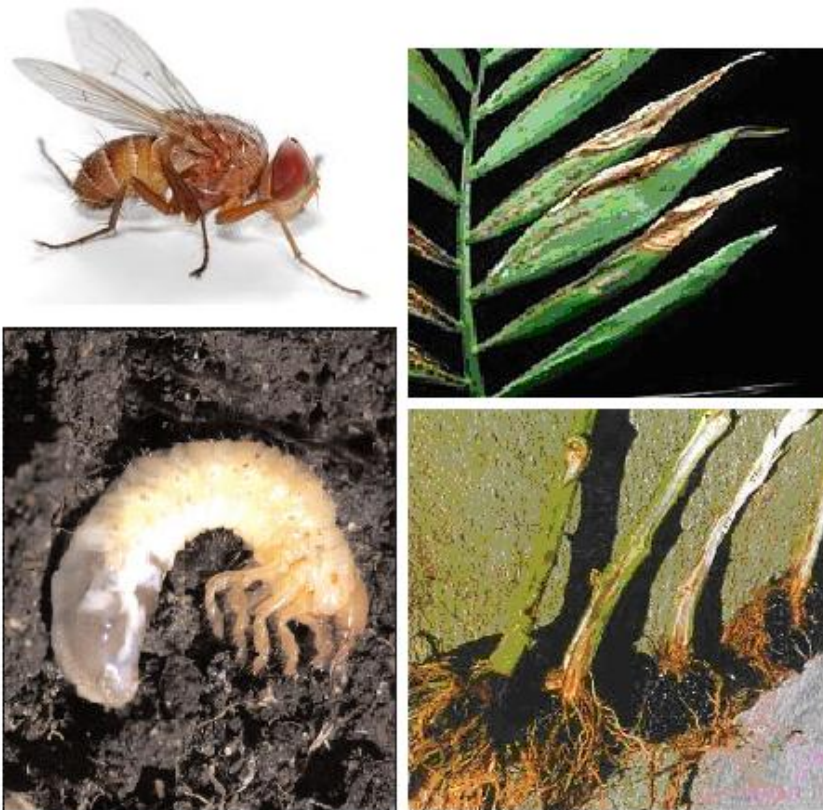


Ministry of Agriculture Development
Department of Agriculture
PLANT PROTECTION DIRECTORATE



SURVEY PROTOCOL FOR PESTS IN GINGER AND LENTIL

Submitted by:
Integrated Environment Awareness and
Community Development Centre
Lazimpat-2, Kathmandu
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FOREWORDS

A survey protocol is a comprehensive set of instructions which facilitates the examiner to determine the characteristics of a pest population or to determine its presence or absence of species in an area by monitoring or other officially agreed procedures. This includes instructions, considerations and costs for data collection, data management, analysis, and reporting of results. It can also be called as analogous to a study plan for conducting inventory and monitoring (I &M) tasks. Survey protocols, further facilitates the interested parties to know what, why, where, by whom, when, and how a survey is conducted.

The protocols describe procedures and methods for monitoring the pests that are relevant for international trade. They provide at least the minimum requirements for reliable and diagnostic protocols of pests. Detailed information and guidance for the detection of pests is provided on, for example, signs and/or symptoms associated with the pest, illustrations (where appropriate), developmental stages of the pest, and methods for detecting the pest. Diagnostic protocols are intended to be used by laboratories performing pest diagnosis as part of phytosanitary measure. This survey protocol for pests in commercial ginger and lentil production also provide detailed descriptions of methods for data collection, handling, and post-collection processing including pre-survey logistic checklists, descriptions for sampling unit/s, and methods for field and laboratory data collection, as well as processing and disposition of samples, documentation of reports, for future reference and disseminated to interested parties. Regular and timely dissemination of survey result is essential for making informed management decisions.

In this endeavor, technical and financial support provided by Plant Protection Directorate (PPD) to prepare this document on Survey Protocol for Pests in Commercial Ginger and Lentil Production is highly appreciated and E-ARCC would like to express sincere gratitude to PPD. **Dr Dilli Ram Sharma, Program Director of PPD** for his valuable technical support and encouragement is duly acknowledged. The technical support and contributions made by PPD's officials (Mr Dinesh Babu Tiwari, Mr Ram Krishna Subedi and Rajiv Das Rajbhandari) are also highly appreciated. The E-ARCC also acknowledges the contributions made by all Team Members: Mr BR Palikhe, Team Leader, and Mr Ganesh K. KC, Member, Dr BR Khadge, Member and Ms Nabin CTD Shrestha, Member to the preparation of this Survey Protocol. Finally, we would also like to extend our sincere thanks to all who contributed during the various consultation meetings held for relevant information collection.

We solicit from each of you corrections and of course references to update those errors contained in this manuscript. It is our hope that you will find this information useful in your work.

**Integrated Environment Awareness and
Community Development Centre (E-ARCC)**

Lazimpat-2, Kathmandu

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1 Survey Protocol for Rhizome Fly (*Mimegralla coerruleifrons*) in Ginger

1. Introduction

1.1 Background

The overall objective of this study is to detect pests while their populations are still below economic injury level and watch whether their natural controls—such as adverse weather, other insects, and diseases—will keep the population low enough to prevent damage. Specifically it is also;

- To predict and evaluate potential pest problems and non-target effects and provide info for designing appropriate control actions
- To evaluate effectiveness of management practice and establish a pest history for the specified period and commodity.

1.2 Target commodity detail

Nepal occupies third position in the world in terms of ginger production after India and China respectively. (B.K. Poudyal, 2011). Demand of ginger is increasing every year in the world market due to its diverse products and use (FAO, 2009). Ginger rhizomes (*Zingiber officinale* Rosc) are commonly used as vegetables or spices depending on the age of rhizomes. The young rhizomes are usually used as vegetables, while the old or dry rhizomes use as spices or medicinal plants. Nowadays, ginger rhizomes are used in various kinds of industry such as medicines, ready-made foods and cosmetics, therefore the requirements of ginger rhizomes in world markets are increasing year by year. Ginger, a medicinal and spices crop belonging to family Zingiberaceae. Ginger requires tropical and sub-tropical climate and prefers light shade for good growth. The production of ginger in Nepal is about 2, 35,033 MT (MOAD, 2013).

Ginger is one of the principle spice crop commercially grown in the mid hills of the Nepal and established as a cash/commercial crop. It is valued for its spicy properties. Ilam, Salyan, Palpa, Nawalparasi, and Doti are the major ginger growing districts of the country. The total ginger cropped area in Nepal is 19,376 ha and the production is 235033mt and the productivity is 12.13 Mt/ha (statistical information on Nepalese Agriculture 2012/2013 (2069/070)). Ginger rhizomes are popular as a spice and an herbal dietary supplement. The anti-inflammatory and anti-nausea qualities of ginger have applications in the pharmaceutical industry.

Scientific name and taxonomic information:

Taxonomically, it fall into two group viz; *Z. officinale* cv. group *Officinale*, which is cultivated throughout the tropics, and *Z. officinale* cv. group *Rubrum* ('haliya padi'), grown on a small scale in South-East Asia for medicinal use and as a spice. The latter differs from the former by having smaller, red rhizomes with a stronger and more pungent odour, the red coloring of the basal parts of leafy stems and petioles, larger leaves and the presence of a larger, scarlet-red mottled labellum.

Preferred scientific name

Zingiber officinale Roscoe

Taxonomic position

Domain: Eukaryota

Kingdom: Viridiplantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Monocotyledonae

Order: Zingiberales

Family: Zingiberaceae

Other scientific names

Amomum zingiber L. (1753)

Common names

Ginger

Common ginger

1.3 Survey area detail

Fill in the location (latitude and longitude) and elevation details with other information (See Table No.: 4). First identify the districts growing the ginger commercially and are having the fly problem.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1. Pest name including scientific name of pest and family/order

Scientific name: *Mimegralla coerruleifrons* Macquat

Family: -Mecropezidae

Order: - Diptera

1.4.2 Life cycle of the pest

Fliers are noticed in the fields in the months of August and September. Female fliers lay eggs singly or in clusters of 6-10 near the base of the plants under small lumps of soil, in cracks and

on the surface or soil. The incubation period lasts for 2-5 days. The larval period lasts for 13-18 days. The full-grown maggots pupate into rotten rhizomes. The pupal period lasts for 110-15 days. The pupal period lasts for 10-15 days. The total period of life cycle is about 4 weeks. The flies are fairly large with slender body and long legs. The body is black in color and wings are transparent with ashy spots. The wing expansion of flies varies from 13 to 15mm

Images of Rhizome fly



1.4.3 Damage symptoms

- The maggots feed on the rhizome as a result of which yellowing of plants and rotting of rhizomes takes place.
- Dead hearts due to primary injury, wilting and drying of aerial plants, can be seen.
- Rotting of rhizomes due to invasion of fungus, *Pythium* sp. through feeding injuries occurs.

1.4.4 Favorable conditions

- Warm and humid weather

1.4.5 Spread of a pest

Any means that allows the entry or spread of a pest (FAO 2010). The probability of spread considers the factors relevant to the movement of the pest, after establishment on a host plant or plants, to other susceptible host plants. Geographically this fly is distributed in the Asian countries-China, India, Indonesia, Malaysia, Philippines and Vietnam (CPC, 2007).

1.4.6 Target host/alternate host

- Ginger and
- Turmeric

1.4.7 Pathways for introduction (possible pathways of spread)

Seed rhizomes for planting are carrier of rhizome fly pupae. Thus the commodity pathway of this insect is seed rhizomes. Maggots harbor into and feed on rhizomes. Cut-open rhizome shows maggots feeding inside.

2 Purpose of survey

The purpose of survey is to monitor rhizome flies population development in fields to determine if and when control measures are warranted. Detection methods are the first tools to be used and inspection techniques in order to find incursions of pests in the field. This is often done visually in the first instance, with support from a laboratory for confirmatory testing and subsequent monitoring. Laboratory testing causes significant delays and action is only taken after getting the laboratory results. Thus, there is a real need for rapid, simple and robust detection methods that can be deployed by NPPO in the field with inspection services to enable early detection of pests.

3 Survey Methodology

A survey method should provide detailed descriptions methods for rhizome data collection, handling and post collection processing. This includes pre-survey checklists, descriptions for sample units/size in the sample frame, methods for field and laboratory data collection as well as processing and disposition of samples. The observations of traps from the selected units constitute the sample of the protocol.

3.1 Sampling unit

Area within the sampling universe from which measurements are taken, eg. Light Traps

3.2 Sampling Techniques

- Various sampling techniques can be used to quantify pest populations in the field: eg Trapping
- Trapping is one of the most important sampling techniques used for monitoring insect abundance and behavior

3.3 Pest Monitoring with Light Traps

Generally, monitoring includes similar elements and adds repeated measurements to detect changes in rhizome flies population over time. Monitoring is important to identify pests and to keep track of changes in population levels of fruit fly pests in order to indicate when or whether to implement controls. However, rhizome infestation is the best indicator for presence of pests or evaluation of pest controls. Among the various methods and devices used in pest monitoring, the most popular and widely used are light traps for flying species that are attracted to light (Appendix 1). While adult males are mostly caught in sex pheromone traps, adults of both sexes are trapped in light traps. Light trap for managing insects can be used for monitoring or mass trapping of harmful insect pests in the crops. Insect traps are useful tools for monitoring insect populations to determine the need for control or the timing of control practices (Appendix 2). Light traps are more efficient when placed soon after the adult moths start to emerge but before they start laying eggs

3.4 Sampling design

Proper sampling design supports accurate and efficient assessments of rhizome flies populations. It creates scientific credibility in surveys, makes management decisions defensible, and can save time and money.

3.4.1 Sample design of the study

When compared with other sampling methods, light-trap sampling is found to be more efficient for lepidopteran and dipterian population dynamics. However, many factors affect catches of insects in light traps. Trap design, the light source and its energy, and the attraction efficiency under certain conditions all contribute to sampling errors. Data generated using this protocol will be useful for

- conservation planning and strategy development
- local, regional and national assessments of species richness; and
- evaluations of population trends in different habitat types.

3.4.2 Sample selection and size

- The surveyor should obtain the correct sample number(s) from the appropriate sampling plan. All sample numbers have a unique identification name and code that identifies whether or not the sample is intended for monitoring, surveillance, compliance, or other inspection activities defined by the program. The size of the

sample is indicated in the specifications for each sampling plan and depends on the laboratory tests to be performed.

- Light traps: Set up light traps @ 1 trap/acre (0.4 ha or 8 Ropani) for monitoring and mass trapping insects. Light traps with exit option for natural enemies of smaller size should be installed and operate around the dusk time (6 pm to 10 pm). Use the light trap from evening until early morning.
- Total number of flies / trap/day should be recorded. The trapped flies should be destroyed and removed after each recording.
- Count and record the number of adults of rhizome fly present (Trapping method can be used to count fly).
- Check the traps once a week. If no flies are caught by the second week, move the trap to another location or remove it. If the traps catch only a few flies, the infestation is very small and can probably be controlled by the traps alone. In this case, leave the traps in place until no additional flies have been caught for a week. If 20 or more flies are caught per trap in a week, this probably indicates a more serious infestation, and time must be devoted to finding the source of the infestation. Every day flies are collected from the trap and total numbers are recorded. Insects are sent to Entomology Division of NARC or responsible relevant organization/institution for the identification.

3.4. 3 Frequency of observation

- Daily collection of pests trapped in the light trap
- Fly observation throughout the year but especially in peak activity of this pest.

3.5 Trapping records

The following information should be included in order to keep proper trapping records as they provide confidence in the survey results: trap location, plant where the trap is placed, trap type, servicing and inspection dates, and target fly capture.

Up-to-date records should be maintained and made available to the NPPO of the exporting country and, where justified, also to the NPPO of the importing country. Records should be maintained as determined by the NPPO of the exporting country. Records should include date, name and signature of the person who carried out the task or prepared the document.

3.6 Routine diagnostic method

- Flies should be dry preserved
- Card point mounting: The tip of the card point attaches with glue to the specimen near the middle right leg
- The fly mounted card is pinned properly with the help of insect pin (no. 4) and pinning block
- Care to handle specimen so that fly remains intact on card
- In case, maggots are encountered inside rhizome, they should be reared in its natural food until flies emerge

- Observe specimen body through dissecting microscope for ascertaining morphological characters as detailed in the identification
- Important diagnosing morphological characters are
 - ❖ body structure
 - ❖ shape, size and color of legs and antenna
 - ❖ abdomen structure
- Always maintain a record keeping

3.7 Rhizome sampling procedures

Each sample that plant protection officer submits for laboratory analysis is considered to be an official sample. Rhizome sampling may be used as a surveillance method in combination with trapping where trapping is less effective. All samples should be representative of the lot. Appropriate measures should be taken to ensure that samples are not compromised during its handling, storage and transport to the laboratory. If samples are improperly collected, mishandled, transported and/or are not representative of the sampled lot, the laboratory results may be inaccurate and inconclusive.

Stage of sampling

- Usually most injurious stage counted (maggots)
- Sometimes egg masses counted

Plant parts used for observation

- Rhizomes

3.8 Procedure for observation

Monitoring survey on pest occurrence at the main field should commence soon after crop establishment and at weekly intervals thereafter. In each of the fields, select five spots randomly as shown (four in the corners, at least 5 feet inside of the field borders, and one in the center). Select five random plants at each spot for recording counts of insects as per procedure finalized for individual insects.

3.9 Data collection procedures (field, lab)/ Data preparation and collection

Only trained persons should collect samples, otherwise be under the supervision of or be assisted by a trained person. Sampling should be carried out in such a way that the samples represent the lot. Each sample that surveyor submits for laboratory analysis is considered to be an official sample. Appropriate measures should be taken to ensure that samples are not compromised during its handling, storage and transport to the laboratory. If samples are improperly collected, mishandled, transported and/or are not representative of the sampled lot, the laboratory results may be inaccurate and inconclusive. See Table 1-3 and Annex I and II for recording data/information.

If you send a sample specimen for identification, we suggest you keep another for your own reference, because samples are not always returned. Along with the sample, you should send

records of potentially important information about the situation or problem surrounding the specimen. Keep a copy of this information for yourself. We suggest you follow this format:

- date the specimen was collected
- place or address where the specimen was collected
- previous pest control efforts
- host plant, if the insect was found on a plant
- weather, if it seems relevant
- time, if it seems relevant

3.10 Handling of samples

- Samples should be placed in strong polythene bags and immediately labeled by means of a pencil-written paper or plastic label placed inside the bag.
- Samples should be kept cool
 - do not leave in the sun or in a closed vehicle left in the sun
 - should be treated with care and processed or dispatched for analysis as soon as possible.
- If immediate dispatch or processing is impossible then samples should be stored in a refrigerator at 4–8°C for several days without severe deterioration
- For collection and handling of samples follow Appendix 3

3.11 Evaluating Trap Counts

- Count and record the number of adults of rhizome fly present (Trapping method can be used to count fly).
- Traps with the highest numbers of flies indicate a nearby harborage, and this is where management efforts should be concentrated.
- Traps with few or no flies should be moved to other locations until all main harborage areas are pinpointed.
- Large numbers of adults in the traps can indicate a population explosion.

4 Supervision activities

Supervision of trapping activities includes assessing the quality of the materials used and reviewing the effectiveness of the use of these materials and trapping procedures. The effectiveness of trapping should be officially reviewed periodically by individuals not directly involved in conducting trapping activities. The timing of review will vary by programme, but it is recommended to occur at least twice a year in programmes that run for six months or longer. Aspects of a review include quality of trapping materials, record-keeping, layout of the trapping network, trap mapping, trap placement, trap condition, trap servicing, trap inspection frequency and capability for fly identification.

5 Field survey checklists

Prior to starting field survey work, field survey teams should have following:

- Ensure that the survey team has all items on the check-list before departure
- Pre-printed field survey forms (sufficient number plus spares)
- Sample collection envelopes, paper (sufficient number plus spares)
- Pencils (at least 3 per team member)
- Permanent marker pen
- Scissors

6 Record Keeping

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Besides, records of all procedures (such as surveys, detections, occurrences or outbreaks and results of other operational procedures) undertaken should be retained for as long as possible.

The Log Book shall contain the following items:

- A copy of the Pest Management Plan and Service Schedule
- A copy of the current label and PRMD registration number for each pesticide used in the field
- Pest monitoring data sheets which record, in a systematic fashion, the number of pests or other indicators of pest population levels revealed by the monitoring program Annex 1 and 2)
- The location of all traps and trapping devices around the field. This information can be in either tabular or in list format, and should be accompanied by a map for pest.
- The surveyor shall be responsible for maintaining a complete and accurate Pest Management Log Book and maintained on each visit by the surveyor.

NPPO should document and keep appropriate records of new or exotic or quarantine species derived from monitoring of pests. Information kept should be appropriate for the intended purpose, for example support of specific pest risk analyses, establishment of pest free areas and preparation of pest lists. Voucher specimens should be deposited, where appropriate.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

7 Reporting

There are two types of reports that are outlined in the survey protocol of rhizome fly: progress reports and a final report. Progress reports summarizing the survey completed with general findings are typically brief. Final reports provide conclusions based on more comprehensive data analyses, and are the main focus of reporting described in the survey protocol.

- Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.
- Reporting schedule: Quarterly

8 Surveyors (Organization/Personal)

8.1 Date of collection and name of collector-As designated by DADO

8.2 Date of identification and name of identifier- Trained PPO

Arrangements should be made prior to the start of sampling as to who will identify your pests.

8.3 Date of verification and name of verifier- Designated by NPPO

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List of Tables

Table 1: Field Record Sheet for Rhizome flies

Name of fly monitoring:				Day/Week:			
				From:		To:	
Trap No.	Location of trap	Date inspection	Host	Light trap type	No of flies/trap	Species identified	Date recharge

Table 2: Specimen Identification Report

To

.....

.....

(Name and Address of Field Inspector)

Light Trap	Location	Name of the fly spp. identified	No. of flies

Signature and Date:

Name of Reference Entomologist:

Address for Communication:

Tel.:

Cell No:

Fax:

E-mail:

Table 3: Relative abundance of the flies recovered by light trap catches

Month	Fly population per trap per day	Fly population per trap per day
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
S. Error		
LSD Value*		

* Least Significant Differences (LSD): Least significant difference is used to compare means of different treatments that have an equal number of replications

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

2 Survey Protocol for White grubs ((*Phyllophaga spp.*) in Ginger

1. Introduction

1.1 Background of the Study

Several dozens of pest involve in crop damages. Some of them have gained nationally important pests notably, white grubs (many species of Coleopteran beetles within the Scarabaeidae family), depending on the season and locality. Field reports from various sources indicate that white grubs are among the most difficult soil pest insects to control in cash crops (e.g. vegetable, potato or ginger) and food crops (e.g. maize, millet, etc.) grown in upland areas. Monitoring surveys, applied to verify the characteristics of the pest population of white grubs. This paper highlights the importance of continuously collecting data on white grubs. An improvement in field surveys based on the use of such data will result in better pest and crop management.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger

1.3 Survey area detail

Fill in the location (latitude and longitude) and elevation details with other information

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

White grubs (WG) are the larval stage of insects commonly known as May or June beetles (or Junebugs). Only the larvae are known as "white grubs." These insects are generally referred to as white grubs. They are "C-shaped body, 25-50 mm in length and, as their name implies, white in color with a brown headcapsule. White grubs are the larvae of scarab beetles. Scarabaeid beetle larvae are common soil pests. White grubs are the larvae of those scarabaeid beetles which feed below ground on plants roots. Although white grubs belong to the family Scarabaeidae, not all scarabaeids are white grubs, other groups such as dung beetles are also included in the family (G. P. Bhawane, A. B. Mamlayya, S. R. Wagh and A. K. Chaugule. 2012).

White grubs are increasingly important pests of cereal and cash crops in Nepal. The situation is also aggravated by the fact that they are one of the most difficult insect pests to manage due to high levels of insecticide resistance. In recent years white grubs gained increasing awareness in Nepal and are considered as one of most important pest problems. The situation is also aggravated by the fact that, they are one of the most difficult pest insect to manage due to high levels of insecticide resistance. The severest damages are found in arable crops like maize, millet, potato and ginger in mid-hill and high-hill areas.

1.4.1. Pest name including scientific name of pest and family/order

Scientific name of pest: May and June beetles (*Phyllophaga spp.*)

Family: Scarabaeidae

Order: Coleoptera

1.4.2. Life cycle of the pest

Adults lay eggs in summer, grubs hatch and feed on roots in the fall, overwinter deep in the soil, and feed again in the spring. They generally pupate from May and June, hatch as adults to mate in June. But this timing varies across the northeast and can be weather-dependent.

May or June beetles (multi-year white grubs) have life cycles ranging from 1 to 3 years, depending on species and location. Adults emerge from the soil, some species as early as early April and others extending into mid September. Adults of most species are most common in May and June, hence their common name. After mating, females enter the soil and deposit about 50 eggs in small, earthen cells over a period of one to three weeks. Larvae hatch three to four weeks later and begin feeding on dead organic matter, later moving to the roots of plants. In fall, larvae move deeper into the soil for the winter. The following spring they move back to the root zone to feed; most of their damage occurs during this time. At maturity, they move deeper into the soil to pupate.



White Grubs

White grub larva feeding on grass roots



Adult white grubs, often called May or June beetles, are commonly attracted to lights at night

1.4.3 Damage

White grubs are the larvae of those scarabaeid beetles which feed below ground on plants root. Larvae feed on roots, while adults feed on foliage. The grubs feed on roots of almost all the crops, like potato, maize, wheat, barley, jowar, bajra, groundnut, sesame, sunflower, chilies, cotton, sugarcane, tobacco, brinjal, cucurbit, and lady's finger including turf, meadows, lawns and forest trees (Oya, 1995; Fujiie and Yokoyama, 1996; Arita et al., 1993; Potter et al., 1992). The larvae prefer corn, groundnut, potatoes and strawberries but dislike legumes (Matheson, 1985), sweet clover (Metcalf and Flint, 1975) and lucerne (Keller et al., 2000). Second and third stage larvae feed on and damage roots, causing retarded growth and occasionally total crop failure; moreover, crops with severely damaged roots lodge during strong winds. Most damage usually takes place in late summer or early fall when voracious second- and third-instar larvae are actively feeding and summer temperatures are at their peak. Damage may be severe in September and October when grubs are reaching maturity. During drought, damage caused by the insect pest is higher.

In particular, larvae of *Phyllophaga* spp. can be devastating agricultural pests by feeding on crop roots which often results in plant death. Second and third stage larvae feed on and damage roots, causing retarded growth and occasionally total crop failure; moreover, crops with severely damaged roots lodge during strong winds. Heavily infested grass pulls up easily.

White grubs are sensitive to differences in soil moisture and texture. Since these factors are not uniform throughout any given field, a white grub infestation, likewise, is not uniform. Therefore, within the same field, some areas may be completely destroyed while others are undamaged.

1.4.4 Damage Thresholds of White grub

Once you have made observations and collected general information about the condition of your field in terms of pest population, you can use the following sections to correct crop and pest problems:

If there are more than 8 grubs per square foot, treatment may be necessary. Populations greater than this have the potential to cause dieback of the turf (sod), resulting in economic damage.

1.4.5 Target host/alternate host

White grubs feed beneath the soil surface on the roots of grasses and other plants. White grubs are usually considered polyphagous. Local crops such as maize, millet and sorghum may be vulnerable, and wild grasses are likely host. Also found in soils of potatoes, and fruits. They also infest various pasture grasses, lawns, and nursery plantings. The adults, who are strongly attracted to fragrant flowers and ripe fruits, feed on the foliage of forest, shade and fruit trees.

1.4.6 Pest distribution

Asiatic garden beetle (AGB) was introduced from Japan in the 1920's. It is most common in northeastern United States from New England to Ohio and down into South Carolina.

1.4.7 Pathways for introduction (possible pathways of spread)

Serious crop damage generally occurs when the crop has followed pasture, as the ovipositing beetles prefer weedy fields. Ovipositing generally occurs within 90m of trees in which adult beetles feed. Immature larvae prefer plants with shallow, matted root systems. Sandy loam and clay loam soils with a moisture content of 15-25% moisture content favor larvae and egg development. Adults disperse/spread to uninfected areas by flight or through wind currents.

1.4.8 Diagnostic characteristics

- Damage to plant
- Fed-upon roots or root loss
- Abnormal plant height
- Color of the plant
- Orange-yellow leaves
- Wilting

1.4.9 Factors favoring insect pest development

- Plants with fibrous root system
- Soil moisture requirements

1.4.10 Conditions Favoring Outbreaks

Planting into old sod, pasture, hay or set aside acreage; planting into double crop or full season soybean stubble, especially fields with heavy grass infestations the previous season

2. Purpose of survey

- To monitor the pest population in the field and monitor the incidence of larvae by soil sampling to determine whether the larval population has exceeded the economic threshold.
- To teach proper grub sampling

3. Survey Methodology

3.1 White grub Scouting and Monitoring

Scouting turf is very important to determine what species of grubs are present, their stage of development, infestation level and distribution within the field. To determine if a grub problem is imminent a homeowner/surveyor can utilize a variety of techniques that may indicate a potential

for white grub infestations. Records can be kept of locations with a past history of problems. Grub damage is usually most severe on field/lawns with south facing slopes and in full sunlight. Monitoring can be performed for both the adult and larval forms of the beetles. Proper sampling, monitoring, identification, and use of thresholds are fundamental to best management programs for such pests. Monitoring for white grubs can begin in mid-August and continue through mid-October, resuming again in the following April and May.

3.2 Sampling-collecting repeated systematic data of an organism in its environment over a specified time

For general observations such as pest population or density, it is important to be sure that the sampling points where you collect your data should be representative of the entire field. Generally, the data collected in an individual field are averaged to describe that field. If the field has a lot of variability, you may want to make separate sets of measurements in the different sections of the field. While the number of data collection locations per field generally depends on the type of data needed, it is often advisable to select at least 10 separate locations.

3.2.1 Sample selection and size

Begin sampling in August when grubs are easily seen and actively feeding, but before signs of injury are visible. Mid-August, when the grubs are primarily 2nd instars, is the time to monitor for potentially damaging WG populations in the upper 3 to 4" of soil under the turf. To save effort, sampling can be concentrated on suspected infestation areas, high-risk or low tolerance areas, or areas with a history of grub infestations. If historical information is not available and/or a more accurate idea of grub distributions is necessary, map and survey.

Walk in a zigzag pattern across the field, taking samples at 10–20 foot intervals from at least 10 locations or mark sampling spots at 10 to 20' apart in a grid pattern. It is a good idea to sample multiple locations in your field. Grubs may be present in one area while absent in another. A shovel is used to assess white grub populations by looking at a one square foot area 6 to 12 inches in length with a depth of approximately 3 inches at a number of locations (at least 10 locations) in the field. Take square foot samples using a small shovel to cut through the turf and thatch (excess grass cuttings and debris) on three sides of a square. It is a good idea to sample multiple locations. Grubs may be present in one area while absent in another. To find the grubs, shake the sample, and probe through the soil and roots with a pocket knife or screwdriver. Count the number and species of grubs found at each sampling site and record these on a map of the area (Table 1). Five to ten grubs per square foot would be the baseline for threshold levels. Replace the sod after sampling and irrigate thoroughly (See Annex 1: Procedures for Shovel Samples for White Grub)

Procedures for Shovel Samples for White Grub

- From August to May monitor for grubs by digging in the root zone.
- Divide into equal groups of 3 or more.
- Take square foot samples using a small shovel to cut through the turf and thatch on three sides of a square. If there is a suspected turfgrass infestation cut away a 1 sq. foot section of soil to assess grub populations. With a spade cut three sides of a square one foot deep.

Peel this piece back and look for larvae, pupae, and adults. This should be done in August when grubs are large enough to be seen easily. When finished place the grass back in its place and water

- Sample several areas and determine the average number of grubs per square foot. Each group should take three- five samples of soil about 6-12 inches deep and one foot square and more if the average number of grubs is close to the action threshold (AT). Thresholds: The fall AT is 1.6 or whiter grubs per Compact Method (CM) soil sample. The spring AT is 1.04 or more white grubs per CM soil sample
- Peel back the turf and inspect the thatch and upper 2–3 inches of soil.
- Take each soil sample and place it into an enamel tray.
- Break the soil apart and sort through it to look for white grub. To find the grubs, shake the sample, and probe through the soil and roots with a pocket knife or screwdriver.
- Place the insects from the two separate samples into jars.
- These samples will be used to estimate the white grub density in the field.
- After collecting the samples, all groups should gather at a central location to identify white grub larvae.
- Count the number and species of grubs found at each sampling site and record these on a map of the area
- Replace the sod after sampling and irrigate thoroughly.
- A quicker method is to use a golf course cup cutter. This cuts a round core of about 1/10 square foot. Multiply the average grubs per core by 10 to get the approximate number of grubs per square foot.
- During June and July monitor for adults using a black light

3.2.2 Monitoring of Adults

Begin looking for adults in late June through early August. Often adult beetles can be seen flying at dusk or seen on trees and shrubs. During monitoring activities of scarab beetles, light trap with 15 Watt electric bulb can be operated in farmer's field. Insects attracted to the light traps are collected into a nylon mesh through a funnel trap fitted just beneath the electric light. Trapped insects are separated into scarab beetles and others; scarab beetles only are counted and preserved for taxonomical identification. Scarab beetles are kept in a vial of 15 cm height x 7 cm diameter size. A cotton swab with ethyl acetate moistened placed inside the vial is used as a killing agent. Collected beetles are pinned properly, stored in an insect collection box and bring to the Entomology Division (NARC), Plant Protection Directorate/ Regional Plant Protection Laboratory for identification based on reference insects maintained there. Trap counts can be plotted on a graph of numbers versus date. When the trap capture has decreased for ten days, mark the date of peak activity.

During **June and July** monitor for adults using a black light or by looking for adult emergence holes from pupal cells on the side of the berm. During summer, they are often seen flying around lights at night. Populations of most grub species tend to be highest in older plantings of sod, or in soils high in decomposing organic matter.

3.2.3 Time frame and frequency of survey

- Season long monitoring light trap

3.2.4 Field Observations on Population Density

The distribution of white grub larvae is very patchy, and the correlation between larval density and visible damage is not obvious in casual observations. To test this more objectively a scoring method for damage is prepared, so that larval densities in soil samples could be taken from stools different categories of damage (Table 1-2)

A damage index can be drawn up as follows:

- 1 = all tillers are healthy;
- 2 = up to 1/4 of tillers are yellow and stunted;
- 3 = 1/4 to all tillers is yellow and stunted;
- 4 = all of stool stunted, some dead or dying tillers; and
- 5 = stools either completely dead or only a few green tillers which are small and unhealthy

3.2.5 Field survey checklist

Prior to starting field survey work, field survey teams should have following:

- Ensure that the survey team has all items on the check-list before departure
- Pre-printed field survey forms (sufficient number plus spares)
- Pencils (at least 3 per team member)
- Permanent marker pen
- Collecting net, forceps,
- Vials containing 70% alcohol as preserving media,
- Plastic containers for temporary storage and transportation.
- Polythene bags for storing plant material,
- Rearing material, or other samples.
- Several digging tools are used to collect soil dwelling insects includes long handled spade, ground breakers and sickles.

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method

- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8:1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

There are two types of reports that are outlined in the survey protocol of white grubs: progress reports and a final report. Progress reports summarizing the survey completed with general findings are typically brief. Final reports provide conclusions based on more comprehensive data analyses, and are the main focus of reporting described in the survey protocol.

- Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.
- Reporting schedule: Quarterly

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

Arrangements should be made prior to the start of sampling as to who will identify your pests.

6.3 Date of verification and name of verifier- Designated by NPPO

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List of Tables

Table 1: White Grub Monitoring

Shovel Samples

Sample Number	White Grubs(Nos.)	Remarks
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
Total		

Table 2: Field Observations on Population Density

Number of third instars recorded in standard $0.5 \times 0.5 \times 0.5$ m quadrats in areas of location assessed with damage index of 1 ± 5 (1 = all tillers are healthy; 2 = up to 1/4 of tillers are yellow and stunted; 3 = 1/4 to all tillers are yellow and stunted; 4 = all of stool stunted, some dead or

dying tillers; and 5 = stools either completely dead or only a few green tillers which are small and unhealthy).

Location	Damage Index					Mean
	1	2	3	4	6	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
Mean						

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

3 Survey Protocol for Ginger Borer (*Conogethes punctiferalis*)

1 Introduction

1.1 Background of the Study

Specifically it is

- to predict and evaluate potential pest problems and non-target effects and provide info for designing appropriate control actions
- to evaluates effectiveness of management practice and establishe a pest history for the specified period and commodity.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger

1.3 Survey area detail

The information with details of site such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil type etc), Meterological (tempeture, rainfall, hailstone etc) should be recorded.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1 Pest name including scientific name of pest and family/order

Scientific Nmae: *Conogethes punctiferalis* (Guenée)

Order: Lepidoptera

Family: Crambidae

Common names

Castor capsule borer/ peach moth/yellow peach moth/castor borer/shoot borer/castor seed caterpillar/peach pyralid moth/corn moth/ginger shoot borer

1.4.2 Life cycle of the pest

Most *C. punctiferalis* adults emerge at night, particularly between 20.00 and 22.00 h. They are active until about 0.500 h, when they hide and remain still on the back of host leaves during the day. The longevity of *C. punctiferalis* adults varies between different generations with the

longest-lived recorded in the first generation with an average lifespan of 10.4 days for the female and 6.1 days for the male in China.

Both female and male adult *C. punctiferalis* feed on the nectar of the host and surrounding plants. The ratio of females to males is generally more than 1:1. Females release sex pheromones to attract males and mate a day after emerging. Two to three days after mating; females start to lay eggs on the surface of fruits, maize ear silk and tassels. Each female lays 20-30 eggs. Eggs hatch in the early morning, 5-8 days after oviposition. The hatching rate may reach 100%.

Newly hatched larvae crawl rapidly on the fruit surface and bore into the fruit within several hours. On maize, first-instar larvae feed on pollen and ear silk and bore into the stem and ear. The duration of the larval stage is about 15-18 days. *C. punctiferalis* overwinter as mature (fifth-instar) larvae in host stems or fruit or under the bark of fruit trees. They form cocoons and pupate during the following June and show about 10% lower mortality in winter. In southern China, *C. punctiferalis* was found to have five generations per year (Wang and Cai, 1997).

Eggs

C. punctiferalis eggs are elliptical, about 2-2.5 mm with fine circular sculpturing on the surface. Newly laid eggs are milk-white, later becoming light-yellow or tangerine.

Larvae

Mature *C. punctiferalis* larvae (which are the largest fruit borers in China) are about 22 mm long. The head, protergum and epiproct are brown and the dorsal body is dark-red. Meso, metathorax and abdominal sections I-VIII generally bear eight hair-scales.



Larva

Pupae

The pupae are brown, 13 mm long and 4 mm wide. The maxilla, mid-legs and antennae are beyond the middle of fifth abdominal segment. The end of the abdomen bears six rolling thorns.

Adults

C. punctiferalis adults are yellow and 12 mm long. There are many black spots on the wings, thorax and abdomen; 25-30 spots on the forewing, about 15 spots on hindwing, 3-4 spots on the abdominal dorsum (except the second segment) and two spots on the eighth segment. The number and distribution of black spots, however, differs between individuals fed on different host plants.

In China and Japan, the morphological differences between adults from larvae fed on fruit and Pinaceae have been described. On *C. punctiferalis* adults fed on Pinaceae, the black spots on wings were bigger than those fed on fruits (Chai and He, 1987). The labial palpi were black and there is a clump of black hairs between the tibia and the tarsus on the male hind leg. On the adults from larvae fed on fruit, the labial palpi are yellow but the dorsum on the second segment is black and no hairs are found between the tibia and tarsus of the male hind leg. It is likely that a complex of species is involved and until taxonomic studies are carried out it will not be possible to separate the different taxa and relate them to their biology.

1.4.3 Damage

Symptoms by affected plant part

- Fruits/pods: internal feeding; lesions; premature drop; discoloration.
- Growing points: internal feeding; boring; external feeding; dead heart; mycelium present.
- Leaves: internal feeding; webbing.
- Stems: internal feeding.

Images of pest



Symptoms

The shoot borer (*Conogethes punctiferalis*) is the most serious pest of ginger. The larvae bore into pseudostems and feed on internal tissues resulting in yellowing and drying of leaves of infested pseudostems. The presence of a bore-hole on the pseudo stem through which frass is extruded and the withered and yellow central shoot is a characteristic symptom of pest infestation. The adult is a medium sized moth with a wingspan of about 20 mm; the wings are orange-yellow with minute black spots. Fully grown larvae are light brown with sparse hairs. The pest population is higher in the field during September-October.

C. punctiferalis larvae bore into corn ears to feed on seeds. An early symptom of *C. punctiferalis* attacks on the whorls of maize and sorghum seedlings is the appearance of a pattern of small, irregularly spaced holes, which are different from the orderly row holes in whorls attacked by *Ostrinia furnacalis*.

Larvae newly hatched from eggs laid in corn tassels feed on the pollen in the flowers, resulting in a decreased number of flowers and the postponement of flowering.

In the later stages of an attack, larvae bore in the growing points and kill them, producing characteristic dead hearts.

1.4.2 Dispersal mode

Adults have wings and can fly, although their natural dispersal capability is not known. As the larvae are highly polyphagous, they are unlikely to be limited by host availability, and, if moved in trade, would be capable of establishment if sufficient numbers could form a breeding population.

1.4.4 Target host/alternate host

C. punctiferalis is a polyphagous insect which has a wide host range. Its primary hosts are peaches, sorghum, sunflowers and masson pine. Maize has been recorded as a primary host in Zhejiang, China (Lu et al., 1995). *C. punctiferalis* can feed on over 20 fruit and pinaceae plants, including cardamoms (Kadoi and Kaneda, 1990; Sarma et al., 1992), ginger (Kadoi and Kaneda, 1990; Koya et al., 1991), soapnuts (Rao, 1992), grapes (Gour and Sriramulu, 1992), Chinese hawthorn (Sun et al., 1992) and turmeric (Koya et al., 1991). In China, *D. punctiferalis* mainly feeds on fruits and maize.

Affected Plant Stages: Flowering stage, post-harvest, seedling stage and vegetative growing stage. Incidence of this pest is found severe in July-August (Shrawan-Bhadra) in Nepal.

Affected Plant Parts: Fruits/pods, growing points, leaves and stems.

List of hosts plants

Major hosts

Averrhoa carambola (carambola), Carica papaya (papaw), Elettaria cardamomum (cardamom), Gossypium (cotton), Helianthus annuus (sunflower), Macadamia ternifolia (Queensland nut), Morus alba (mora), Nephelium lappaceum (rambutan), Prunus persica (peach), Psidium guajava (guava), Sorghum bicolor (sorghum), Zea mays (maize), Zingiber officinale (ginger)

Minor hosts

Castanea mollissima (hairy chestnut), Citrus nobilis (tangor), Cryptomeria japonica (Japanese cedar), Curcuma longa (turmeric), Diospyros (malabar ebony), Eriobotrya japonica (loquat), Ficus carica (fig), Gossypium herbaceum (short staple cotton), Malus domestica (apple), Piper nigrum (black pepper), Prunus japonica (Japanese bush cherry tree), Punica granatum (pomegranate), Ricinus communis (castor bean), Vitis vinifera (grapevine)

1.4.5 Pest distribution

Conogethes punctiferalis is found in Asia (from India eastwards) and Australasia. The full list of countries the *C. punctiferalis* species complex has been recorded from are: Australia, Brunei Darussalam, Burma, Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, North Korea, Papua New Guinea, Philippines, South Korea, Sri Lanka, Taiwan, Thailand and Vietnam (CABI, 2011). Interceptions of the pest in fruit from Pakistan have been made by both the Plant Health and Seeds Inspectorate (PHSI) of England and Wales (Fera unpublished data) and the Plant Protection Service in the Netherlands (M. van der Straten, pers. comm.). While much of the

species' distribution is in the subtropics, *C. punctiferalis* has also been recorded from Hokkaido prefecture, north Japan (Inoue & Yamanaka, 2006), and northern China (CABI, 2011).

1.4.6 Pathways for introduction (possible pathways of spread)

Conogethes punctiferalis is a highly polyphagous pest, the larvae boring into fruit, seeds and stems of plants in many different families. Larvae feed hidden inside the fruit, and although they have been detected on tropical produce because the holes (with or without secondary rot) are visible, they may remain undetected by inspectors and the fruit will be discarded before or after sale. The wide host range increases the potential for emerging adults to find suitable hosts.

2 . Purpose of survey

- Estimation of changes in insect pest distribution and abundance
- Information about insect pests and life history
- Influence of biotic and abiotic factors on pest population

3. Survey Methodology

3.1 Collecting insect samples

A good insect sample consists of a range of the organism's life stages if possible presented in well preserved conditions. Following are general guidelines for collecting insect samples.

- Avoid touching insects with fingers
- Collect different life stages of the insect
- Collect multiple specimens

3.2 Detection and Inspection

C. punctiferalis larvae bore into host-plant stems and feed on the inner core, resulting in the formation of dead hearts.

Peaches attacked by *C. punctiferalis* appear discoloured, scorched, and are covered with brown excretion mass around holes. The fruits fall or rot with an unpleasant smell.

C. punctiferalis overwinter as fifth-instar larvae in cocoons in the bark of fruit trees or in the feeding place. They may be discovered by scraping off the bark.

3.3 Pheromonal Control

Sex pheromones which attract *C. punctiferalis* males have been applied as a control (Cai and Mu, 1993; Liu et al., 1994). In Korea the best attraction of males to various synthetic sex pheromone blends was obtained at the 7:3 ratios of E10-hexadecenal and Z10-hexadecenal and at the 80:20 ratios for the flying upwind response (Jung JinKyo et al., 2000).

Observation: Visual observation and lab analysis of suspected samples

- 3.4 Collecting plant samples** Examine the entire plant for symptoms - Examine all of the main plant organs for disease symptoms: roots, stems, leaves, and blossoms.

- Collect several plant specimens
- Do not collect dead plants or plant organs
- Use plastic bags - For most samples including leaves, stems and roots, use plastic bags to prevent plant samples from drying out during transport.

Time of Survey – When the crop stand in the field

3.5 Area to be surveyed

- Parts to be observed: Pseudostem
- Ways to collecting samples :
- Frequencies of observations :
- Sample dispatch :
- Sample verification :
- Preservation of collected specimens

3.6 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

- 5.1 Date of collection and name of collector-As designated by DADO
- 5.2 Date of identification and name of identifier- Trained PPO
- 5.3 Date of verification and name of verifier- Designated by NPPO

7 References

<http://agropedia.iitk.ac.in/content/shoot-borer-pest-ginger> - retrieved on 9 march 2015

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See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

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Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

4 Survey Protocols for the Oriental Army Ant (*Dorylus sp.*) in Ginger

1 Introduction

1.1 Background information

Very little information is available about the Oriental armyant damage type, intensity of damage in ginger crop. Ginger is highly potential crop for export. In some districts of Far Western Development Region especially during dry period some cases of ants' damage in ginger have been observed (Personal communication). Thus to have information on its distribution pattern, and behavior and rate of spread, for designing control strategies, this protocol has been designed.

1.2 Target commodity detail:(See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger)

1.3 The survey area and site selection

In the first year of carryout the survey selecting three districts from each development region and in each district in coordination with the DADO select 10 commercial sites. In the selected 10 commercial sites conduct awareness meeting with the farmers of the survey sites and get permission to visit sites take required samples. Farmers should agree should allow the technician to visit the site, dig the hole, take the sample and estimate the damage. While carrying out these activities some plants may have to be uprooted. So provision for compensation is made. Review the district program on ginger and then based on the production areas with, collect short topographical description of the survey area.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1 General information

Ants live in colony and form 15–25% of the terrestrial animal biomass. Ant's eusociality is well known because of their cooperative brood care (including brood care of offspring from other individuals), overlapping generations within a colony of adults, and a division of labour into reproductive and non-reproductive groups. Army ant is a name that people use for ants that move in a line killing every insect and small animal in their path. Some species of this genus seasonally, when food supplies become short, they leave the hill and form marching columns of up to 50,000,000 ants. The soldier ants march at the side of the column to defend the queen. Ants release specific pheromones along with tactile cues (tapping each other's antennae) to elicit a specific response from fellow workers. They use a blend of chemicals for recruitment of nest mates for collecting food; or to alert the entire colony when attacked by intruders. Moreover, a unique colony odor, released by the queen ants present in each ant's nest. Information on pest

life cycle (*Dorylus* spp.) is very much lacking. Army ants foraging workers are of different sizes, 2.4 to 5.6 mm in length. Male ants with two pairs of wings occasionally come out of ant colony being attracted in light during June.

Many published doc on *Dorylus* damaging potato and other tuber crop in Bhutan, Bangladesh and Nepal and India and elsewhere are found, however there no reference available specifically done in ginger. Similarly effort was made to identify and assess the repelling status of locally available materials against the red ants in soil biology studies conducted during the autumn and winter season of the 1995/96 at LARC (1675 m asl) revealed that few of the local materials viz. Titepati (*Artemisia vulgaris*) and banamara (*Eupatorium adenophorum*) have given encouraging results at par with the standard check chloropyriphos to control red ants in potatoes. Study done else where also show *Dorylus* appears to be an effective enemy for *Eupatorium*. (NIU YanFen^{1,2}, FENG YuLong¹, XIE JianLei³ & LUO FuCheng College of Animal Science and Technology, Yunnan Agricultural University, Kunming 650201, China, July 23, 2010

1.4.2 Classification



Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hymenoptera
Family: Formicidae
Sub-family: Dorylinae
Genus: Dorylus
Species: orientalis
Sc.name: Dorylus orientalis Westwood

1.4.3 Behavior



Dorylus sp. consuming a grasshopper

Most ant species send individual scouts to find food sources and later recruit others from the colony to help; however, army ants dispatch a cooperative, leaderless group of foragers to detect and overwhelm the prey at once. The constant traveling is due to the need to hunt large amounts of prey to feed its enormous colony population. These ants have **nomadic and stationary phase of activity** that constantly cycle, and can be found throughout all army ants species. The nomadic phase begins around 10 days after the queen lays her eggs. This phase will last approximately 15 days to let the larvae develop. The ants move during the day, capturing insects, spiders, and small vertebrates to feed their brood. At dusk, they will form their nests or bivouac, which they change almost daily.

The stationary phase, which lasts about two to three weeks, begins when the larvae pupate. From this point on, the prey that was previously fed to the larvae are now fed exclusively to the queen. The abdomen (gaster) of the queen swells significantly, and she lays her eggs. At the end of the stationary phase, both the pupae emerge from their cocoons (eclosion) and the next generation of eggs hatch so the colony has a new group of workers and larvae. After this, the ants resume the nomadic phase. Male larvae have a larger body size relative to workers, the larvae become too large to be transported so colonies with a sexual brood must remain in a nest for a period of 41–56 days as compared to non-reproductive colonies that remain in the nest an average of 17 days.

1.4.4 Foraging

Ants are responsible for destroying more vegetation than any other group of animals. Leaf cutters are known to be able to strip an entire citrus tree of its leaves less than 24 hours. Underground species prey primarily on ground-dwelling arthropods seeds tuber, rhizome. When army ants forage, the trails that are formed can be over 20m wide and over 100m long. They stay on the path through the use of a concentration gradient of pheromones.

1.4.5 Nesting

Army ants build a living nest with their bodies, known as a bivouac. Bivouacs tend to be found in tree trunks or in burrows dug by the ants. The members of the bivouac hold onto each other's legs and so build a sort of ball, which may look unstructured to a layman's eyes, but is actually a well-organized structure. The older female workers are located on the exterior; in the interior are the younger female workers. At the smallest disturbance, soldiers gather on the top surface of the bivouac, ready to defend the nest with powerful pincers/stingers. The interior of the nest is filled with numerous passages and contains many chambers with food, the queen, the larvae, and the eggs.

1.4.6 Dispersal modes

Adult male ants dispersed being air-borne while the colony including worker ants and queen/s shifts one locality to another being subterranean. In some occasions, when it is cloudy worker ants found foraging on the soil surface within the coverage of grasses.

1.4.7 Distribution

D. orientalis Westwood however has long been known as an important pest of potato and is distributed in Nepal, India, Bangladesh, Sri Lanka, Burma, Thailand, China. In Nepal it is reported from the plains of Terai and is severe problems in the hills. It is however not found above 2800m as per the survey conducted in Regional Agricultural Research Center, Lumle. The seasonal changes in their distribution and abundance are closely associated with the seasonal variations in ambient atmospheric temperature and relative humidity.

1.4.8 Crop hosts

Red ant has been found damaging over 30 species of crops and is one of the major production constrain in potato and in several vegetable such as cauliflower, cabbage, broccoli, turnip, BLM (*Brassica juncea*), radish, rape. Other solanaceous crops such as peeper, tomato, tree tomato (*Cyphomandra betacea*) are also attacked. Similarly, red ant also attack carrot, common beans, onion and garlic. Some forest tree such as utis (*Alnus nepalensis*) and ornamental plants such as *Dahlia* spp and *Helichrysen* spp are also severely damage.

1.4.9 Nature of damage

The workers have strong mandibles and they feed by scraping the host tissue. Hundreds of workers attacks a single plant or its parts such as the rhizome, tubers or bark of the root or stem either around the collar or on undersurface of the branches that touch the ground. While feeding on above ground parts they construct shelter cover of friable soil to avoid sunlight but continue to feed their even after the soil is washed off by rain. Though overall yield loss has not been estimated in Nepal but in potato up to 100% loss has been observed in some plots. At harvest, the damage ranged from 70.0 to 90.0 percent.

Variety reaction have been reported by Chaubey and Tyagi (1994) from India, where the greatest extent of the loss (42%) has been reported in Kufri Badshah and Kufri Chandramukhi. The maximum tuber damage was recorded on potato cv Kufri Chandramukhi and the minimum on hybrid JN-2626. Ants' attack at later stage causes various symptoms such as partial wilting, retarded growth of leaves and stems.

2 Purpose of the Survey

- To gather information on *Dolynus* sp occurrence, abundance and distribution pattern, to determine the nature and extent of damage in ginger to support phytosanitary certification
- To ascertain its potential damage and explore effective control measure to minimize the economic loss of commercial ginger crop production

3 Survey Methodology

3.1 For selecting Survey sites

Review the district program on ginger and then based on the production areas with, short topographical description select 10 production sites and each plot should be about 100 m away from each other. From each plot take 5 samples.

3.2 Sampling sites and sampling points

- The total area in each district is sub grouped into 10 commercial plots and in each commercial production mark an area of 1-2 ha. Then within that area 5 sites at a row to row distance of 1 meter are identified for carrying out the study in ginger examining the rhizome.

1 Sample point.....X.....
2.....
3.....
4.....
5.....

- Remember during that period of initial observation especially in hill districts the ant's movement may be slowed down.
- While selecting plots /area consider the soil components, soil temperature and vegetation also, Sand, clay and stony. Soil temperature also needs to be measured using a digital thermometer with the probe placed approximately 3 cm below the soil surface. Baits can be placed at 15–30 cm deep holes into the soil, to take the specimen (See different baits)
- Each location should be divided into five areas (each 5m x 1m) and from each site take 5 samples (plant damage sample, rhizome, and soil samples).
- Number of samples per site: Randomly selected 5 developing rhizomes in each point.

Different Ant baits

Food preferences vary among ant species but may include fruits, seeds, nuts, fatty substances, dead or live insects, dead animals, and sweets.

Common Ant Bait Products (For reference)

Active Ingredient	Example product name	Formulation	application	bait
avermectin B (abamectin)	Enforcer AntMax Bait Stations	solid	bait station	protein
	Raid Ant Baits III	solid	bait station	protein

borate-based products²	Drax SF Ant Gel ³			
	Grants Kills Ants	gel		sugar
	Liquid Ant Bait	liquid	cracks	sugar
	Terro Ant Killer II	liquid	bait station	sugar
	Liquid Ant Baits	liquid	bait containers	sucrose solution
fipronil	Gourmet Liquid Ant Bait	refillable dispensers		
	Combat Ant Killing Gel			
	Combat Quick Kill	gel	cracks	protein
	Formula 3	solid	bait discs	protein
	Maxforce FC Ant Killer Bait Gel ³	gel	cracks	sugar
hydramethylnon	Combat Source Kill		bait discs	
	Amdro Kills Ants	solid	ant stake bait	protein
	Killing Bait	dry	stations	protein

¹ Effectiveness varies according to ant species and product.

² Borate products may include the following active ingredients: borax (sodium tetraborate decahydrate), disodium octaborate tetrahydrate, or orthoboric acid.

³ May be available for professional use only.

2.3 Timing of survey

- The frequency and the time of monitoring of *army ants* should coincide with pre planting to harvesting time of ginger i.e from January until November (May, June, July specifically for estimation of the extent of damage and February to December for phenological information).
- Observation Frequency: Once in Two-week. Important data to collect include probable nest location, damage estimation, and monthly occurrence.

3.4 Sampling tool

- A new method employing palm oil as bait has been developed for studying the distribution and ecology of subterranean Army ants in both forest and open environments but it needs to be continued for the whole cropping period. The oil-method is easy to detect and monitor populations of army ants of the genus *Dorylus* in cultivated habitats.
- Small paraffin lined pill box or cans can also be used baited with sugar solution or syrups, or meat attracts ants. The ants can be destroyed by dropping the can in to boiling water.
- Can also test using locally made ant bait. Mix 2 tablespoons of peanut butter, 2 tablespoons of honey, and about ½ teaspoon of boric acid powder. Protein-loving ants respond best to a bait made of both protein and sugar. (see also Annex-1)

3.5 Sampling technique

- For sample collection check 4–6 cm above portion of the stem also along with rhizome, search the surrounding soil, ant hills, and plants. Generally, the feeding behavior of the ant in general in plants of woody stem the ants first eat the roots in the uppermost 3–5 cm of topsoil, piling up soil around the plant stems, and then to eat the stems under the ant hill. The ants move from one plant to another through a tunnel located 5–10 cm below the soil surface.
- Sampling point need to be sprinkled with 80 ml of palm oil and in plot. A additionally with 40 ml should be added (every fortnightly), to prevent a decrease in attractiveness of older sampling points.
- At the beginning of the investigation monitored the ant numbers at the sampling point every day by digging a hole 30 cm deep at each of them. Afterwards carefully put back the oily earth into the hole. Additionally place pieces of toast soaked with oil on the ground surface.
- In addition use of attractant trapping, whole plant sampling, visual inspection, sample collection and laboratory analysis and diagnostic and reporting procedures should also be done.
- Monitoring of the ants in the selected district will be carried out prior crop planting, rhizome initiation, and bulking and maturation. For monitoring, palm oil trap and pitfall traps as well as soil sampling can be used.
- Pitfall trap: The ground-dwelling insects will be monitored using pitfall traps constructed from round plastic containers (1 liter capacity with an opening 11 cm in diameter, 12 cm depth). 3-5 traps per selected survey area are installed for the growing season. Trap adjusted to the ground surface
- After selection of the district and commercial production areas Location details, Crop information, sampling units, Data collection procedures (field, lab)/ Data preparation and collection is prepared (Table 1-4 and Annex I-III).

3.6 Data Management and Analysis

- Data entry, verification, and editing. Immediately after collection of data from the sample sites, the data need to be entered and weekly verification be done.
- Adjust statistical analysis methods when where felt necessary

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method

- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and, where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

- Report content need to be designed from the beginning considering the ISPM 6 and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 Reference

A.Weissflog¹, E. Sternheim¹, W.H.O. Dorow², S. Berghoff¹ and U. Maschwitz¹:How to study subterranean army ants: a novel method for locating and monitoring field populations of the South East Asian army ant *Dorylus (Dichthadia) laevigatus* Smith, 1857 (Formicidae, Dorylinae) with observations on their ecology.2000.

C. K. Dash^{1,*}, K. Hassan¹, M.E.A. Pramanik², M. H. Rashid³ Development of Management Strategies Against Red Ant (*Dorylus Orientalis* Westwood) of Potato GC. Y. D. and Keller, Siegfried (2002). Association of fungal pathogens with White grubs. In: Neupane, F. P. (ed.) 2003. Integrated Pest Management in Nepal. Proceedings of a National Seminar, Kathmandu, Nepal, 25-26 September 2003. Himalayan Resources Institute, New Baneshwar, Kathmandu, Nepal, 37-41 Pp.

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www.pestcontrolmanagement.org/...r-own-ant-baits.html

List of Tables

Table1: Data collection format

District	Locality	Sampling area (Infestation percentage)				
		1	2	3	4	5
Illam	Samalbung					
.....						

Table 2: Specimen Identification Report

To

.....

.....

(Name and Address of Field Inspector)

Trap	Location	Name of the Army ant . identified	No. of ants.

Signature and Date:

Name of Reference Entomologist:

Address for Communication:

Tel.:.....

Cell No:.....

Fax:.....

E-mail:.....

Table 3: Relative abundance of ant by light trap catches and from soil sample

Month	Ant population per trap per day	Ant per soil sample /site
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		

Table 4: Monitoring of Red ant

Monitoring of Red Ant					Day/Week From: To:			
Trap No	Location of Trap	Date inspection	Pitfall trap type		No of Male ant/trap	Spp identified	Ants/soil sample	Date recharge

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

5 Survey Protocol for *Meloidogyne javanica* in Ginger

1. Introduction

1.1 Background of the Study

This survey protocol is designed for estimating the prevalence and incidence of *Meloidogyne javanica* in the ginger crop. *Meloidogyne javanica*, a plant parasitic nematode has international significance due to its economic importance in terms of causing enormous crop loss to ginger and other crops. Ginger, being a potential commodity export for Nepal, the developed survey protocol should be continuously run by NPPO to check the distribution of nematode in ginger crop and to assess the effectiveness of the eradication program.

This protocol must be endorsed by NPPO for the implementation by the districts.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger

1.3 Survey area detail

Ginger pocket sites of Nepal (as per the requirement of NPPO)

The location and unique identifying details of each site should be recorded. The information such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district), topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded (See appendix No.: 3).

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1 Pest name including scientific name of pest and family/order

Order: Tylenchida, Family: Meloidogynidae, Genus: *Meloidogyne*, Species: *javanica*

1.4.2 Life cycle of the pest

Meloidogyne javanica pass through an embryonic stage, four juvenile stages and an adult stage. Mature females in roots lay eggs into a gelatinous egg sac, which may or may not protrude from the root and which completely envelops the eggs and acts as a barrier to water loss. Reproduction is probably always parthenogenesis. On hatching, infective second-stage juveniles move towards roots and accumulate at root tips, minor wounds or regions of previous penetration. Within a day of penetration of the host there is a rapid loss of mobility and infectivity.

The length of the life cycle is temperature/moisture-dependent. Generally, nematodes complete their life cycle within three to six weeks during the growing season. *M. javanica*, development occurs between 13 and 34 °C, with optimal development at about 29 °C. Extreme moisture and temperatures will kill some species of nematodes.

1.4.3 Dispersal mode

Besides their normal mode of transmission, human activities and conveyances by land and air increase the chances of dispersal of plant nematodes. The nematodes are brought in with exotic plants, imported rhizomes with roots and in soil attached to packing material, machinery, tiers of motor vehicles, tools and even shoes.

1.4.4 Target host/alternate host/host range

Ginger/ brinjal, tomato, okra, potato, tobacco

1.4.5 Pest distribution

M. javanica is widely distributed in Central region, Chitwan

1.4.6 Pathways for introduction (possible pathways of spread)

Adults; eggs; juveniles are introduced through bulbs, tubers, corms, rhizomes and growing media accompanying plants, roots, seedlings, micro propagated plants

2 Purpose of Survey

The focus of the survey is to determine the distribution of M. javanica through monitoring survey for pest record documentation and also for planning pest management programs.

3 Survey Methodology

3.1 Sample design of the study

To monitor M. javanica populations in the field, soil samples are important as this nematode live in the roots. Symptoms of damage by M. javanica include poor growth of crop with stunting and chlorosis of the aerial parts and a reduced and galled root system.

For nematode sampling, ten ginger growing sites representing the district, distributed randomly should be selected and statistical sampling designs must be used to obtain quick and reliable data on nematode prevalence. In each site select fivespots randomly four in the corners, at least 5 feet inside the borders and one in the centre. At each spot select ten random infested plants for sampling of soil and roots.

Samples should be kept out of direct sunlight and in a cool place during transportation to the diagnostic lab.

3.2 Sampling unit, sample frame, and population

Each sampling unit should be relatively uniform. This means that parts of the field which show different patterns of crop growth or have different soil moisture, soil texture or drainage characteristics, are sampled separately.

3.3 Sample selection and size

When sampling soil from crops during the growing season, it is very important to get the feeder roots of the crop in the soil sample, since this is where many nematodes live.

- Observations of the crop. If there is variability in crop growth, areas of poor or uneven growth patches should be sampled separately from healthy areas.
- Collect both roots and soil from poor growing patch.
- For root sample carefully dug up with a little of the surrounding soil.
- Collect soil samples from within the root zone (0-15 cm depth)
- Collect a composite sample of soil no fewer than 8 to 10 soil cores from unhealthy plants and also in healthy plants to diagnose nematode problem during the growing season
- Soil sample should be taken when soil is moist not too wet
- Samples should be collected at specific growth stages. For example, samples should be collected during active growing season and at crop harvest.
- If there is no visible damage, use a zigzag, W-, or X- shaped pattern to sample the field.

A useful approach is to collect the soil cores in a clean bucket, mix the soil thoroughly and take a 500g sub-sample for lab processing. Be careful to mix the soil gently as nematodes can be destroyed by abrasion, particularly if soil is dry. However, thorough mixing is essential, as poor sub-sampling procedures can introduce extra errors into the estimate.

3.4 Time frame and frequency of survey

Soil populations of most plant parasitic nematodes tend to be highest in September and October after crops have senesced and died. This is the best time of year to sample for nematodes. Sampling in the spring prior to planting a crop is also being reliable.

Fields with a history of nematode problems may be sampled routinely to determine if the nematode population is approaching or has exceeded an economic threshold.

Periodic large-scale surveys are essential to assess and monitor nematode populations. They should be repeated every two to four years to determine the relative abundance of species and new introductions.

Monitoring survey of nematode in the absence of crop (pre-cropping survey), growing season of ginger (cropping survey) and infected root after crop harvesting should be carried out.

3.5 Data collection procedures (field, lab)/ Data preparation and collection

The root and soil samples should be forwarded for detailed laboratory testing at diagnostic lab. The field data of soil and root survey should be recorded as per the format prescribed in Table-1 and 2.

For collection and handling of nematode samples follow NSPM... (Standard Technical Protocols for collection and handling of disease samples)

3.6 Field survey checklist

A soil sampler or augur, shovels, garden trowels, digging forks, rubber gloves, plastic bags, a bucket, water-resistant marker pen, record forms .

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6 and also the objective of the survey requirements.

6. Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

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Sampling Soil and Roots for Plant Parasitic Nematodes (2012)-Michael Celetti; John Potter, Agriculture and Agri-Food Canada

List of Tables

Table-1 Field Data Recording Form (Soil Survey)

S. No.	Date	Name of the Farm	Field Area (ha)	Previous crop	Standing crop (days)	Qty of soil sampled (gm)	Sign of Surveyor

Table-2 Field Data Recording Form (Root Survey)

S. No.	Date	Location	Variety	Previous crop	Standing Crop	No of root sampled	Sign of Recorder

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

6 Survey Protocol for *Culvularia* spp. in Ginger

1 Introduction

1.1. Background of the Study

This survey protocol is prepared to monitor the disease prevalence and also to determine and maintain pest-free status and for pest record documentation. The protocols must be endorsed by NPPO for the implementation by the districts.

Culvularia spp. has been known to cause diseases in many crop plants with symptoms like leaf spots, black kernel, and sheath blight. The fungus belongs to Ascomycota phylum from the Euascomycetes class.

Nepal is the world's third largest producer of ginger after India and China, according to the statistics of FAO. Ginger, being a potential commodity export for Nepal, the developed survey protocol should be continuously run by NPPO to check the distribution of yellow disease in ginger crop and to assess the effectiveness of the management program. This protocol should be endorsed by NPPO for the implementation by the districts.

1.2 Target commodity detail: (See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger)

1.3 Survey area detail: Ginger pocket areas: Terai/ inner terai/mid-hill (as per the requirement of NPPO)

The information with details of site such as a GPS reading (latitude, longitude and altitude), location (Village, ward no, VDC, district), topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded (See Appendix No.: 2).

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

Curvularia was first described by Boedijn in 1933. Its teleomorphs are included in the ascomycete genus *Cochliobolus*. The genus *Curvularia* contains approximately 30 species. *Curvularia* is a hyphomycete (mold) fungus which is a facultative pathogen of many plant species and of the soil.

1.4.1 Pest /Disease name including scientific name of pest and family/order

Scientific name of pathogen: *Culvularia* spp.

Phylum: Ascomycota

Class: Euascomycetes

Order: Pleosporales

Family: Pleosporaceae

Genus: *Curvularia*

Common Name: Leaf spot of Ginger

1.4.2 Life cycle of the pest

Curvularia spp., are darkly pigmented fungi with spores (conidia) efficiently adapted for aerial dissemination. It has very distinctive and beautiful spores, which can be easily identified in both spore traps and direct exams. The shape of the spore is so distinct that its name came from its shape. Microscopically, the spore appears boomerang shaped to more or less spindle shaped and bent. The spores are smooth walled with 3-4 true transverse septa (cross walls), resulting in 4 and 5 celled spores. In the 4 septate (5- celled) spores, the central cell is swollen, distinctively enlarged and darker than the other cells. In the 3 septate (4-celled) spores, the central crosswalk is truly meridional (centrally located) and darker than the other two. Macroscopically, colonies on agar media appear black, hairy and expanding.

Curvularia lunata (Wakker) Boedijn Colony of *Curvularia lunata* is brown, gray, or black, cottony, hairy, or cushion-like and spreads loosely. Conidia 3-5 celled with middle cell enlarged, dark and curved. Hyphae of *Curvularia lunata* are branched and septate. Conidiophores are erected unbranched and septate.

Pathogen and disease characteristics: Conidia are $29-42 \times 13-20 \mu\text{m}$ at broadest part, triseptate, clavate, and almost always slightly curved at the third cell from the base, which is larger than the others. Cell at each end are subhyaline or pale, intermediate cells are brown, third cell from the base is usually more pigmented and darker.

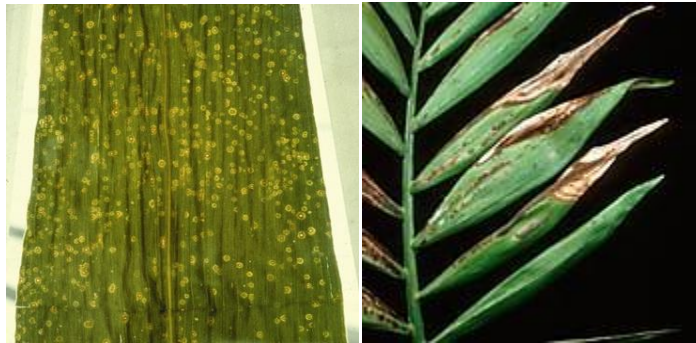
Symptoms:

- Leaf spots are caused by: *Curvularia* spp.; more or less circular brown spots, often with a yellow halo
- Infection usually starts on the older leaves and then spreads up to the newer leaves. The initial symptoms may appear as a yellow to greenish splotchy appearance
- Small circular yellow spots/ haloes appear, expands rapidly and gradually becomes light brown on leaves
- The lesions develop near the leaf tips and margins, and later coalesce to form large necrotic areas. Lesions are more common on leaf margins.
- Centre of the spots dries up leaving a sunken impression
- Signs: Lesions are about 0.5 cm in diameter when fully developed.

Nature of damage

- These fungi produce small necrotic or chlorotic spots with a light colored halo. They can cause significant damage to the crop.

Images of diseases



1.4.3 Dispersal mode

The disease is mainly air-borne in nature also is carried with infected plant material.

1.4.4 Target host/alternate host

One of the hosts is ginger. *Curvularia* leaf spot diseases, caused by different *Curvularia* species, have been previously found on many grass species worldwide

Other Hosts range: *Oryza*, *Pennisetum*, *Sorghum*, *Triticum*.

1.4.5 Pest Distribution

- Geographic distribution: Australia, India, Indonesia, Malawi, Nepal, Nigeria, Pakistan, continental United States, Hawaii (*C. lunata*, Raabe et al. 1981), Zimbabwe.
- Widely distributed in warm, tropical and sub-tropical climates of the Ginger growing areas

1.4.6 Pathways for introduction (Possible pathways of spread)

The main pathway of introduction is infected planting material. The disease could be introduced through contaminated seed. The spores spread via air and are the common cause of disease in plants. The pathogen of *Curvularia* leaf spot (*Curvularia lunata*) is seed borne and survives in plant debris.

2 Purpose of survey

The purpose of the survey is to determine the distribution of pest through monitoring survey for pest record documentation, maintaining pest free areas and also for planning pest management programs.

3 Survey Methodology

3.1 Sample Design of the study

In each selected site, select five spots randomly such that four are from four corners and one from the center of the field. Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a ginger field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations.

3.2 Sample unit, sample frame and population

For plant sampling, minimum fifty plants taken at random for each site, consisting of ginger roots and stems and rhizomes should be collected. Five to ten tissue pieces per sample, derived from the lower stem, root rhizomes should be sampled. Samples should also be carried out on 100 rhizomes per sampling unit harvested from infected crops for disease incidence.

Data collection in field is better at maturation stage of crop. Samples should be initially collected over a representative area of the infected crop to determine the disease distribution. While taking samples the following parameters should be considered.

- Samples should be collected that represent a range of symptoms observed in the infected crop
- Plants should be assessed for yellowing, stunting, wilt and plant death.
- Lower stems should be broken or sliced to reveal vascular discolouration in case plants not showing external symptoms.
- Suspect stunted and wilted plants should be carefully removed from the soil so that the roots can be checked for reduced growth without external fungal growth.
- Field soil (5Kg.) from affected crops should also be collected
- Samples should be processed as quickly as possible after sampling from the field. Once removed from the field, fresh plant samples can deteriorate and become contaminated by other mould fungi and bacteria.
- Samples should be placed into polythene bags and clearly labeled to indicate location, sample number and date of collection.
- Samples should be treated in a manner that allows them to arrive at the laboratory in a fresh, well-preserved state.

- Follow up samples should be forwarded to the diagnostic laboratories for processing
- The field data of plants, soil and seed survey should be recorded as per the format prescribed in Table-1.
- For collection and handling of diseased samples see NSPM... (Standard Technical Protocols for collection and handling of disease samples)

Disease Ratings

Disease severity was initially scored 2-3 weeks after inoculation, corresponding to 7-14 days after appearance of visible symptoms, by visually estimating the percentage of infected leaf area. The second scoring was conducted one month after the first scoring.

Disease incidence was calculated based on the following equation:

$$\text{Disease incidence (\%)} = \frac{x_1}{x_2} \times 100$$

Where, x_1 is the total number of each isolate of fungi and x_2 is the total number of collected plants which showed leaf blight and leaf spot symptoms.

3.3 Time frame and frequency of survey

The recording of observations on the diseases in the selected fields should be done mainly in following crop stage:

1. Reproductive stage
2. Physiological maturity stage

3.4 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- A clipboard.
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing soil samples

It is believed that such a list will be of great value to pathologists and others interested in plant diseases and their control, as it will provide a means of determining quickly and easily what diseases have been reported on our important economic plants and their present known distribution, with the name of the causal organism in the case of parasitic diseases and the common name of the disease. It is hoped also that the list will be helpful in bringing about greater uniformity and certainty in the use of both common and scientific names. The data on which this list is based have come chiefly from four sources: (1) Reports of the Plant-Disease Survey collaborators in the differ

ent districts, (2) specimens preserved in the Pathological Collections, (3) the literature on plant pathology and mycology, and (4) the investigations of many specialists who have supplied information. It will be noted that the information concerning the geographic distribution of many diseases is obviously incomplete. It is hoped, however, that this preliminary list may stimulate more interest in the subject and lead all who have an opportunity to add to our information to do so. It is particularly important to have plant-disease material and specimens to verify the occurrence of new and little-known diseases from different localities.

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and, where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

- Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 References

Bridges CH (1957) Maduromycotic mycetomas in animals; *Curvularia geniculata* as an etiologic agent. Am J Pathol 33(3):411-427

Elad D et al (1991) Eumycetoma caused by *Curvularia lunata* in a dog. Mycopathologia 116(2):113-118

Herráez P et al (2001) Invasive phaeohyphomycosis caused by *Curvularia* species in a dog. Vet Pathol 38(4):456-459

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

Table 1. Field Data Recording Form (Lentil wilt Incidence)

Date	Area/ Location	Stage of crop	Plants part effected	No of plants sampled	Soil samples size (kg)	Seed samples size (kg)	Surveyor name & sign

See list of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

7 Survey Protocol for *Fusarium oxysporum* in Ginger

1 Introduction

1.1 Background of the Study

This survey protocol is prepared to monitor the disease prevalence and also to determine and maintain pest-free status and for pest record documentation. The protocols must be endorsed by NPPO for the implementation by the districts.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger

1.3 Survey area detail

Ginger production areas: Terai/ inner terai /mid-hills (as per the requirement of NPPO)

The information with details of site such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district), topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1. Pest name including scientific name of pest and family/order

Order: Hypocreales, *Family:* Nectriaceae, *Genus:* *Fusarium*, *Species:* *oxysporum*

1.4.2. Life cycle of the pest

Entry is either direct, through wounds, or opportunistic at the point of formation of lateral roots. The root tips of healthy plants growing in contaminated soil are penetrated by the germ tube of spores or fungal mycelium. The mycelium takes an intercellular path through the cortex, and enters xylem vessels through the pits. The pathogen is primarily confined to the xylem vessels in which the mycelium branches and produces microconidia. The microconidia detach and are carried upward in the vascular system until movement is stopped, at which point they germinate and the mycelium penetrates the wall of the adjacent vessel. Lateral movement between vessels is through the pits. It then spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. The water economy of infected plants is eventually severely compromised by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, often followed by death of the whole plant. The fungus then invades all tissues of the plant, to reach the surface where it sporulates profusely. Spores may then be dispersed by wind and water or movement of soil or plant debris. *F. oxysporum* can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and

stem tissue buried in the soil for more than 6 years. Chlamydospores can survive in soil either in dormant form or saprophytically for without a suitable host. The disease is favoured by warm and dry soil conditions with an optimal temperature of 22-25°C.

Adult wilt symptoms usually appear from flowering to late pod-filling stage and are characterized by sudden drooping of top leaflets of the affected plant, leaflet closure without premature shedding, dull green foliage followed by wilting of the whole plant or of individual branches. Seeds from plants affected in mid-pod-fill to late pod-fill are often shrivelled.

Images of pest



1.4.3. Dispersal mode

The movement of infected seed plays an important role in the long distance dispersal and transmission of fusarium wilt diseases into new areas.

Transmission of the fungus occurs primarily through plant debris and soil contamination, where it infects the plant through the root system.

1.4.4. Target host/alternate host/host range

lentil/ *Vicia montbretii* (vetch)

1.4.5. Pest distribution

Widely distributed in warm and tropical climates of the country

1.4.6. Pathways for introduction (possible pathways of spread)

Although the fungus may be introduced by several pathways (seedlings, insect vectors), the most important risk of introduction is by seed

2 Purpose of Survey

The focus of the survey is to determine the distribution of pest through monitoring survey for pest record documentation, maintaining pest free areas and also for planning pest management programs.

3 Survey Methodology

3.1 Sample design of the study

In each selected site, select five spots randomly such that four are from four corners and one from the center of the field. Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a lentil field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations.

3.2. Sampling unit, sample frame, and population

For plant sampling, minimum fifty plants taken at random for each site, consisting of lentil roots and stems, should be collected. Seven to eight tissue pieces per sample, derived from the lower stem and a variety of root sizes should be sampled. Samples should also be carried out on 100 seeds per sampling unit harvested from infected crops for disease incidence.

3.3 Sample selection and size

- Random selection
- 3-10 sampling point per sample site in field
- 10 plant per sampling point

3.4 Data collection procedures

At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations. Data collection in field is better at last reproductive stage to maturation stage of crop. Samples should be initially collected over a representative area of the infected crop to determine the disease distribution. The disease may appear as hotspots or patches within the crop or may have developed along fence lines or drainage lines. The following points should be taken into due consideration while collecting samples from the field.

- Samples should be collected that represent a range of symptoms observed in the infected crop
- Field soil (5Kg.) from affected crops should also be collected as this will also harbour *F. oxysporum*.
- Samples should be processed as quickly as possible after sampling from the field. Once removed from the field, fresh plant samples can deteriorate and become contaminated by other mould fungi and bacteria.

- Samples should be treated in a manner that allows them to arrive at the laboratory in a fresh, well-preserved state. Samples should be wrapped in damp newspaper, bundled into a plastic bag and clearly labeled to indicate location, sample number and date of collection.
- For collection and handling of diseased samples see Appendix 4 (Standard Technical Protocols for collection and handling of disease samples)
- Follow up samples should be forwarded to the diagnostic laboratories for processing

3.5 Time frame and frequency of survey

The recording of observations on the diseases in the selected fields should be done mainly in following crop stage:

3. Reproductive stage
4. Physiological maturity stage

3.6 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- A clipboard.
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing soil samples

See the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 References

Fusarium wilt (of chickpea, lentil & lupin) *Fusarium oxysporum* f. sp. *ciceris*, *F. oxysporum* f. sp. *lentis*, *F. oxysporum* f. sp. *lupini* (2009) - Prepared by Kurt Lindbeck and Plant Health Australia

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

List of commodities having National pest status record of Nepal (2005) - PPD, NPQP, Nepal

Table 1. Field Data Recording Form (Lentil wilt Incidence)

Date	Area/ Location	Stage of crop	Plants part effected	No of plants sampled	Soil samples size (kg)	Seed samples size (kg)	Surveyor name & sign

See list of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

8 Survey Protocol for *Phyllosticta zingiberi* in ginger

1. Introduction

1.1 Background

The major constraints that limit production of ginger are soft rot, rhizome rot complex, leaf spot and storage rots. Leaf spot caused by *Phyllosticta zingiberi* Ramakr. is one of the major foliar diseases of ginger. For the expansion of the export market it is essential to monitor the pest distribution. Surveillance is carried out to gather information that provides the basis for pest listing, documentation of pest status, pest occurrence or absence supporting phytosanitary certification and the designing the future pest management strategies and enabling Pest Surveillance is primary objective of designing the survey protocol. This activity also support to collect information required for maintaining pest records. Present protocol is designed based on review on published materials both at national and international research periodicals. This document outlines recommendations for general protocols and procedures for surveillance emphasizing on factors such as host, pest biology and epidemiology, potential impact to the ginger commercialization, pathway assessment.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger

1.3 Survey area detail

There are usually different step for survey area selection. There is no single best method for site selection. The main point is transparently document the choice and reason for such choice. The method can then be considered and discussed with the basis of the choice, given the circumstances.

- Select site according to development region, districts, VDCs, field site, sampling site and sampling point.
- Use one or a combination of random sampling, systematic sampling, stratification sampling, targeted sampling for site selection
- The location and unique identifying details of each site should be recorded. Describing the sampling site would include information such as a GPS reading(latitude, longitude, altitude), location(Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil tye etc), Meterological (tempeture, rainfall, hailstone etc)
- Survey area will be based on need and survey plan of institutions.
- Whole Nepal especially gingers growing area of Nepal mostly of hill and inner terai.
- Ginger growing pocket area or districts
- As per need of survey institutions.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

Leaf spot disease of ginger leads to heavy reduction in rhizome yield through the destruction of chlorophyllous tissue (Ramakrishnan, 1942). During the wet weather conditions, the disease causes severe leaf blight and results in significant reduction in the number and size of rhizomes thereby resulting 13 to 66 percent yield losses (Sarma et al., 1994). Leaf spot disease of ginger is noticed on the leaves from July to October. The incidence of the disease is severe in ginger grown under exposed conditions. Wound inoculations were successful on ginger and turmeric. Soon after isolation, cultures on ginger leaves are able to infect unwounded ginger leaves. This fungus does not agree with the description of *Coniothyrium zingiberi*. The spores are smaller and never coloured. Hence it is given the name of *Phyllosticta zingiberi*.

1.4.1 Preferred scientific name and Taxonomic position

Preferred scientific name: *Phyllosticta zingiberi*

Taxonomic position

Domain: Eukaryota

Kingdom: Fungi

Phylum: Anamorphic fungi

1.4.2 Other scientific names

Phyllosticta zingiber

1.4.3. Common names

Leaf spot of ginger

1.4.4 Life cycle of the pest

The fungus overwinters in infected plant debris and seed rhizomes. The disease begins to appear towards the end of June when temperature varies from 23.4 to 29.6 and RH is in between 83.3 to 90.2 per cent. Later, in July when rainy days and total rainfall increases, the disease aggravates and spread fast. Higher intensity of rain, accompanied by wind, exert greater impact on target leaves and fungus is splashed to a greater distance resulting in increased disease incidence.

Two types of spores of this fungus germinate when moisture is present: conidia and ascospores. Conidia can quickly be carried from diseased plants to healthy ones by splashing rainwater, sprinklers or watering. In addition, the ascospores are discharged into the air and can travel between plants on a breeze or current. If they land on a moist leaf, ascospores germinate, infect the host, and begin the cycle anew.

1.4.5 Symptoms

The disease starts as water soaked spot and later turns as a white spot surrounded by dark brown margins and yellow halo. The lesions enlarge and adjacent lesions coalesce to form necrotic areas. The disease starts as a water soaked spot and later turns as a white spot surrounded by dark

brown margins and yellow halo and in these pycnidia of the fungus are formed. The lesions enlarge and adjacent lesions coalesce to form necrotic areas.

1.4.6 Favorable Environment and disease development

From June to September due to heavy rainfall accompanied by high humidity and moderate temperature ginger foliage are attacked by number of leaf spots. Small spindle to oval or elongated spots appear on younger leaves. In case of severe attack the entire leaf dries up.

1.4.7. Target host/alternate host

Target host: *Zingiber officinale* (ginger)

Affected Plant Stages: Vegetative stage.

Affected Plant Parts: leaf

1.4.8. Pest distribution

South East Asia, Central America and Caribbean, Saint Lucia

1.4.9. Pathways for introduction (possible pathways of spread)

- Rain splashes
- Air
- Plant residue

2. Purpose of Survey

Purpose of survey should be identified first. The reason for surveys identifies the purpose.

Purpose may be

1. Monitor and detect the target pest
2. Determination of pest status
3. Estimation of changes in insect distribution and abundance
4. Obtain data to support PFAs, PFPP or PFPS
5. Requirements for the establishment of areas of low pest prevalence.

3. Survey Methodology

3.1 Sample design of the study

3.1.1 Plant sampling

Plant sampling is depends upon the purpose of survey. In each selected field, select five spots randomly such that four are from four corners and one from the center of the field. Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random. At each spot select 5 plants adjacent to each other for observations relating to all and whole plant observations.

3.1.2 Sample collection for laboratory diagnosis

Samples should be kept on open and shade condition for 24 hrs to remove excess moisture if not reached sample in laboratory timely. Use mailing or A4 size nepali/paper envelope to dispatch collected samples to be reached in diagnosis laboratory as soon as possible but not later than one week. Examine the entire plant for symptoms - Examine all of the main plant organs for disease symptoms: roots, stems, leaves, and blossoms. Collect several plant specimens. Do not collect dead plants or plant organs.

3.1.3 Sample selection and size

- Random selection
- Minimum 3-5 sampling point per sample site
- 5-10 plant per sampling point

3.2 Time frame and frequency of survey

The recording of observations on the diseases in the selected fields should be initiated with the sowing of the crop and continued till the end of the season(s) and throughout the surveillance period. Survey will be done mainly in following crop stage:

- Early seedling stage
- Vegetative stage
- Rhizome formation stage
- Physiological maturity stage

3.3 Disease scoring

Disease scoring is done according to 1-9 scale by eye estimation.

Rating	Description of severity
1	No disease
2	1-5 % leaf area of lower leaves affected
3	6-10 % leaf area of lower and middle leaves affected
4	11-20 % leaf area of lower and middle leaves affected
5	21-30% leaf area of all lower and middle leaves affected
6	31-40% leaf area of all lower and middle leaves affected
7	41-60% leaf area of lower and middle leaves affected
8	61-80% damage to lower and middle leaves
9	81-100% leaf area affected, almost all leaves withered and bare stem seen

3.4 Field Survey Checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils
- Permanent marker pen

- Newspapers for wrapping plant samples
- Polythene bags for storing samples

See the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements

6 Surveyors (Organization/Personal)

- 6.1 Date of collection and name of collector as designated by DADO
- 6.2 Date of identification and name of identifier Trained PPO
- 6.3 Date of verification and name of verifier Designated by NPPO

7 References

A.K. SINGH, SHASHANK SINGH and S. EDISON* Effect of shading on the Phyllosticta leaf spot, sun burn of leaves and yield of ginger, Indian Phytopath. 57 (2) : 197-199 (2004)

CPC 2007

<http://pip.coleacp.org/files/documents/GBPP-gingembre%2010-2009-08-1-UK.pdf>

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

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NICRA team of Groundnut Pest Surveillance, 2011. Manual for Groundnut Pest Surveillance. Jointly published by National Centre for Integrated Pest Management, New Delhi, Central Research Institute for Dryland Agriculture, Hyderabad and Directorate of Groundnut Research, Gujarat. 29 pp.

RUCHI SOOD and N.P. DOHROO. Epidemiology and management of leaf spot of ginger in Himachal Pradesh Indian Phytopath. 58 (3) : 282-288 (2005)

See list of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

9 Survey Protocol for Rhizom Rot (*Pythium aphanidermatum*) in Ginger

1.Introduction

1.1 Background of the Study

Damping-off diseases of seedlings occur worldwide in valleys and forest soils, in tropical and temperate climates, and in every greenhouse. The disease affects seeds, seedlings, and roots of all plants. In all cases, however, the greatest damage is done to the seed and seedling roots during germination either before or after emergence. Losses vary considerably with soil moisture, temperature, and other factors. Quite frequently, seedlings in seedbeds are completely destroyed by damping-off or they die soon after they are transplanted.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger

1.3 Survey area detail

- Pocket areas: as per the requirement of NPPO
- The information with details of site such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

Pythium aphanidermatum is a soil born plant pathogen. *Pythium* is a genus in the class Oomycetes, which are also known as water molds. Oomycetes are not true fungi, as their cell walls are made of cellulose instead of chitin, they are diploid in their vegetative state, and they contain coenocytic hyphae (lacking crosswalls), called a protist. Also, they reproduce asexually with motile biflagellate zoospores that require water to move towards and infect a host. Sexually, they reproduce with structures called antheridia, oogonia, and oospores.

1 .4.1. Pest name including scientific name of pest and family/order

Common name: Pythium seed rot, Damping-off, Root rot, and Soft rot

Casual Organism – *Pythium aphanidermatum*

Parts affected - Stems, leaves, whole plant, Storage organs (bulbs, tubers, corms, rhizomes)

Scientific Classification

Order - Peronosporales

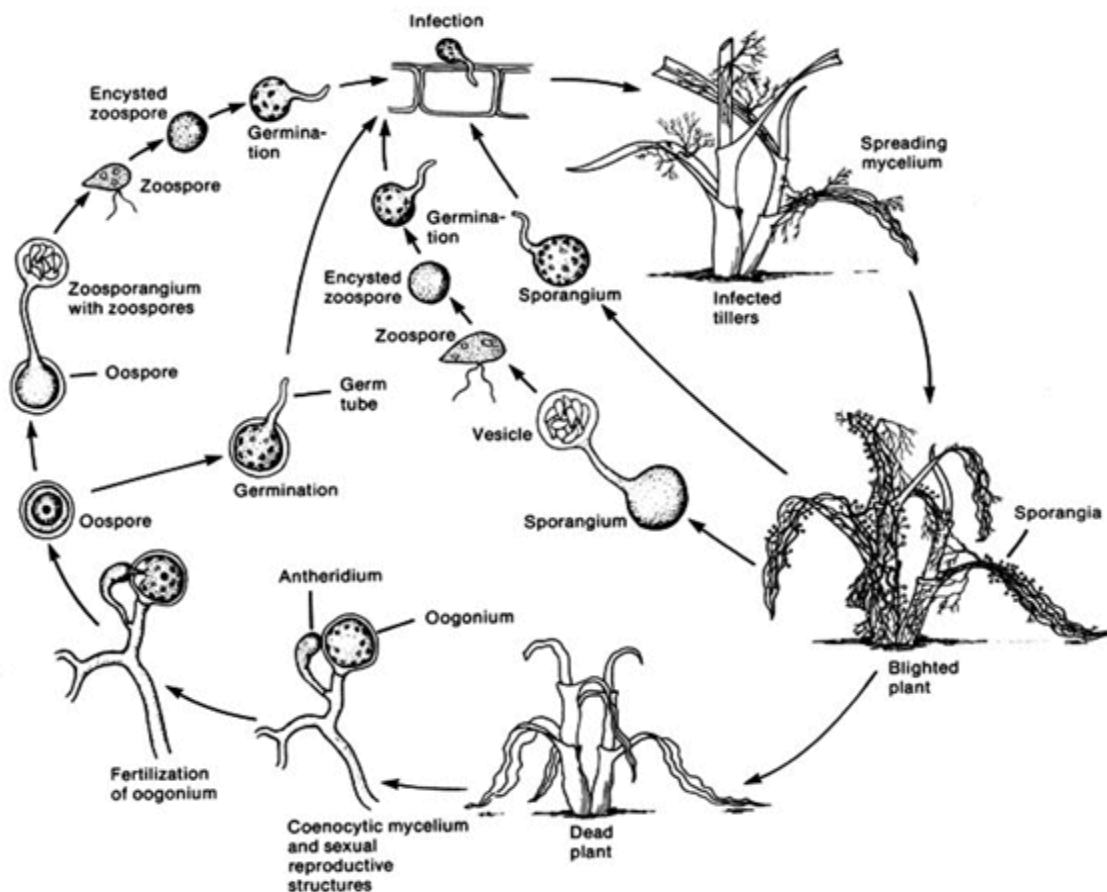
Family - Pythiaceae

Genus – *Pythium*

Species - *aphanidermatum*

1.4.2. Life cycle of the disease

Pythium aphanidermatum overwinters in the soil as oospores, hyphae and/or sporangia. Oospores can produce a germ tube and infect the plant directly, or, if the environment is favorable (that is an adequate amount of water is present), the oospore may produce sporangia, which in turn produce motile, biflagellate zoospores that swim to the host plant, encyst, and germinate. This infection can occur on seeds, which can rot, or produce a weak seedling. If it infects the roots of a seedling, the mycelium will grow throughout the plant tissue, releasing digestive enzymes which break down plant cell walls allowing the pathogen to absorb the nutrients, effectively killing the plant overtime. *P. aphanidermatum* is a polycyclic disease A polycyclic plant pathogen has several life cycles during a season, meaning it can reinfect the host plant, or travel to another plant. After infection, several things can happen that spreads the infection: 1) More asexual structures form, including sporangiophores and sporangia, which release more zoospores which can reinfect the host plant, or move to other plants. 2) There can also be sexual reproduction when two different mating hyphal types meet, creating an oogonium (female structure), and an antheridium (male structure). This results in genetic recombination and exists as an oospore-the original overwintering stage of the pathogen.



(Courtesy R.W. Smiley, P.H. Dernoeden, and B.B. Clarke), Compendium of Turfgrass diseases, 2nd edition, Page 47.)

1.4.3 Disease symptom

The disease symptoms included brown spots on ginger rhizomes followed by soft rot, stems and leaves above ground becoming withered and yellow, and water soaking on the collar region. The soft rot did not produce offensive odors, which is different from bacterial rots. Disease symptom may be seen in leaves, stem and rhizome. In Leaves reddish brown lesions are seen in lower leaves. The plant become yellow and fall down. The rhizome is rotten but not bad odours as in bacterial rot.

1.4.4 Diagnosis

Almost all plant pathogenic fungi spend part of their lives on their host plants and part in the soil or in plant debris on the soil. One first studies under a microscope the morphology of its mycelium, fruiting structures, and spores. The fungus can then be identified and checked in an appropriate book of mycology or plant pathology to see whether it has been reported to be pathogenic, especially on the plant on which it was found. If the symptoms of the plant correspond to those listed in the book as caused by that particular fungus, then the diagnosis of the disease is, in most cases, considered complete.

1.4.5 Target host/alternate host

Pythium aphanidermatum has a wide host range, and can have economic impact on the cultivation of beets, peppers, chrysanthemum, cucurbits, cotton and turf-grasses.

1.4.6 Pest distribution

Pythium aphanidermatum is cosmopolitan in greenhouses and field systems. Globally, this pathogen is present in tropical and subtropical regions and is expected to be present in greenhouses that produce poinsettia. Ginger *Pythium* soft rot caused by *P. myriotylum* is reported in Taiwan.

1.4.8 Pathways for introduction (possible pathways of spread)

Pythium survives in the soil as oospores, hyphae and sporangia. The fungus can survive unfavorable soil moisture and temperatures for several years as oospores. Oospores may form a germ tube directly to infect the plant or they may form sporangia. The sporangia produce zoospores, which are the motile form of the fungus. The zoospores swim around briefly before encysting and forming a germ tube, which can cause infection. Sporangia that have developed on plant tissue can germinate in a similar manner as the oospores, by either germinating directly or by forming zoospores. The pathogen is dispersed when infected debris is transported to uninfested areas and when the soil moisture is enough to allow the zoospores to swim freely.

2. Purpose of survey

- To conduct quantitative survey-useful for enumeration of pests
- To monitor and assess the pest population
- To study changing pest status (minor to major)

- To predict pest outbreak which needs control measures

3. Survey Methodology

3.1 Disease survey

Field observation – Field observation based on diseased symptom. If disease is suspected, suggest going for lab analysis for confirmation of the disease (Annex III)

Lab analysis- Fungus observation - Fungi – Determine the exact nature after making microscopic studies – morphology of mycelium, fruit bodies, spores etc

If no pathogen is detected, cultured or transmitted from a diseased plant, the disease should be presumed to be due to abiotic agent

3.2 Collecting plant samples Examine the entire plant for symptoms - Examine all of the main plant organs for disease symptoms: roots, stems, leaves, and blossoms.

- Collect several plant specimens
- Do not collect dead plants or plant organs
- Use plastic bags - For most samples including leaves, stems and roots, use plastic bags to prevent plant samples from drying out during transport.

3.3 Time of Survey – When the crop stand in the field

3.4 Area to be surveyed –

Parts to be observed: Stems, leaves, whole plant, root

3.5 Ways to collecting samples

- Frequencies of observations :
- Sample dispatch :
- Sample verification :
- Preservation of collected specimens :

3.6 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

See the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)

- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier-Trained PPO

6.3 Date of verification and name of verifier-Designated by NPPO

7 References

Agrios GN 2005. Plant Pathology 5th edition. Elsevier Academic Press. Oxford

CPC 2007. CABI.

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See lists of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

10 Survey Protocol for *Trichothecium roseum* Rosc of Ginger

Introduction

1.1 Background of the Study

Very little information on this disease on ginger is known. This survey protocol is designed for estimating the prevalence and incidence of *Trichothecium roseum* Rosc in the ginger crop. Ginger, being a potential commodity export for Nepal, the developed survey protocol should be continuously run by NPPO to check the distribution of the pest in ginger crop and to assess the effectiveness of the eradication program. This protocol must be endorsed by NPPO for the implementation by the districts.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Rhizome Fly of Ginger

1.3 Survey area detail

There are usually different step for survey area selection. There is no single best method for site selection. The main point is transparently document the choice and reason for such choice. The method can then be considered and discussed with the basis of the choice, given the circumstances. The location and unique identifying details of each site should be recorded. Describing the sampling site would include information such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district), topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) Survey area will be based on need and survey plan of institutions.

- Whole Nepal especially gingers growing area of Nepal mostly of hill and inner terai.
- Ginger growing pocket area or districts as per need of survey institutions.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1. Pest name including scientific name of pest and family/order

Preferred scientific name: *Trichothecium roseum* Link

Taxonomic position

Domain: Eukaryota

Kingdom: Fungi

Phylum: Anamorphic fungi

Other scientific names

Cephalothecium roseum CORDA

Common Names:

- Pink mould rot

- fruit rot of tomato
- pink rot of apple
- soft rot of mango
- pink mould rot of cucurbits

1.4.2. Life cycle of the pest

Little information is available on this disease. The optimal growth temperature for the fungus lies between 20⁰ C and 25⁰C and decay is very slow at temperature below 10⁰C (Snowdown, 2010).

Fruiting body:

Anamorph producing small, low isolated mounds which can remain somewhat separate, or more often growing together forming various sized, continuous, and extensive resupinate patches on bark, up to 3 mm above the substrate, bright pink when fresh and remaining so on drying.

Microstructures:

Basal mycelium 3–7 µm wide, hyaline, thinwalled, septate, smooth, either parasitic, saprophytic or both. Conidiophores long and thin, hyaline, smooth, unbranched and septate, arising from the basal mycelium. Conidiophores with intact conidia difficult to find in mounts, because they've all fallen off, but naked conidiophores and conidia are abundant in all mounts. Conidia didymospores 17–33 × 9–16.5 µm, irregularly ellipsoidal to pyriforme, quite variable in form, hyaline, thickwalled, smooth, often constricted at the septa between cells, pink in mass, but appearing hyaline in microscopic mounts of 3% ammonium hydroxide or 3% KOH; basal cell often (but not always) slightly longer and narrower than the apical cell, narrowed to where formed and attached on the conidiophore; apical cell rounded above.

1.4.3. Dispersal mode

The fungus is a soil inhabitant, and its spores (conidia) are probably dispersed by air currents, irrigation water and insects.

1.4.4. Target host/alternate host

- Target host: *Zingiber officinale* (ginger)
- Affected Plant Stages: Vegetative stage.
- Affected Plant Parts: Rhizomes

1.4.5. Pest distribution

Czechoslovakia (former) ;Hungary ; Poland; Romania, Russian Federation; Shanxi (China); India; Japan; South Africa; Canada ; USA.

1.4.6. Pathways for introduction (possible pathways of spread)

- Air, water, soil
- Plant residue
- planting materials

2 Purpose of Survey

- Detection of pest
- Determination of pest status
- Obtain data to support PFAs, PFPP or PFPS
- Guideline for pest eradication
- Requirements for the establishment of areas of low pest prevalence.

3. Survey Methodology

3.1 Sample design of the study

3.2.1 Plant sampling

Plant sampling is depends upon the purpose of survey. In each selected field, select five spots randomly such that four are from four corners and one from the center of the field. Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots that would be sampled continuously. At each spot select 5 plants adjacent to each other for observations relating to all and whole plant observations at rhizome formation stage, maturity and harvesting.

3.2.2 Sample collection for laboratory diagnosis

Samples should be kept on open and shade condition for 24 hrs to remove excess moisture if not reached sample in laboratory timely. Use mailing or A4 size Nepal/paper envelope to dispatch collected samples to be reached in diagnosis laboratory as soon as possible but not later than one week. Examine the entire plant for symptoms - Examine all of the main plant organs for disease symptoms: roots, stems, leaves, and blossoms. Collect several plant specimens. Do not collect dead plants or plant organs.

3.2.3 Sample selection and size

- Random selection
- Minimum 3 sampling point per sample site
- 5-10 plant per sampling point

3.2 Time frame and frequency of survey

The recording of observations on the diseases in the selected fields should be initiated with the sowing of the crop and continued till the end of the season(s) and throughout the surveillance period. Survey will be done mainly in following crop stage:

- Rhizome formation stage
- Physiological maturity stage
- Harvesting stage

3.3 Field Survey Checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

See the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6 and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

- 6.1 Date of collection and name of collector- As designated by DADO
- 6.2 Date of identification and name of identifier- Trained PPO
- 6.3 Date of verification and name of verifier- Designated by NPPO

7. References

CABI. 2007. Crop protection compendium. CAB International. Wallingford, UK.

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

Snowdon, A.L. 2010. A color atlas of post-harvest diseases and disorders of fruits and vegetables (volume 2: Vegetables). Ebook available at [www. mansonpublishing.com](http://www.mansonpublishing.com)

See list of Annexes

Annex I: General Pest Record

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Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

11 Survey Protocol for Cowpea Aphid (*Aphis craccivora*) in Lentil

1. Introduction

1.1 Background of the Study

The study will be carried out to predict and evaluate potential pest problems and non-target effects and provide info for designing appropriate control actions and also to evaluate effectiveness of management practice and establish a pest history for the specified period and commodity. Production of lentil is affected by many biotic and abiotic stresses which hamper the realization of actual field potential. Pest's adaptation ability, wide range of host, ongoing the changes in climate, economic loss in lentil production can be serious, so regular monitoring surveys need to be done in all commercial lentil growing areas.

1.2 Target commodity detail

Lentil (*Lens culinaris* ssp. *culinaris*.) one of the oldest and popular food legumes was first grown in South West Asia about 7000 B.C. It is widely cultivated in temperate and subtropical regions and in the tropics at higher altitudes, especially in the Indian Subcontinent. This crop is occupying 1.8 million hectares in the world of which 60% is in the South Asia. It provides a valuable and balanced protein source. Split lentil (dhal) is an important source of dietary protein in the Mediterranean and South Asia regions. It is also used as a meat substitute in gluten free diabetic low salt, low calorie, low cholesterol and high fiber contents due to its high protein contents and quality. Total area under Lentil in Nepal, is 206512 ha and the production is about 226923mt and the productivity is 1099 kg /ha (MOAD 2013). In area and production, Nepal occupies 6th in rank in lentil production. Lentil is one of the most exportable agriculture commodities of Nepal. In Nepal it is a winter season crop and is sown after rice harvest in most places. It is sown during the post-rainy season, (October- early Nov) when temperatures start getting cool and humidity is low.

Under conducive conditions, the diseases caused by fungi, bacteria, viruses and nematodes singly, or collectively can cause a significant reduction in both grain yield and quality. This crop is occupying 1.8 million hectares in the world of which 60% is in the South Asia. It provides a valuable and balanced protein source. Split lentil (dhal) is an important source of dietary protein in the Mediterranean and South Asia regions. It is also used as a meat substitute in gluten free diabetic low salt, low calorie, low cholesterol and high fiber contents due to its high protein contents and quality. Production of lentil is affected by many biotic and abiotic stresses which hamper the realization of actual field potential.

Preferred scientific name: *Lens culinaris* ssp. *culinaris*

1.3 Survey area detail

Survey area will be based on need and survey plan of institutions.

- Whole Nepal especially lentil growing area of Nepal of terai and inner terai.
- Lentil growing pocket area or districts
- As per need of survey institutions

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1 Pest name including scientific name of pest and family/order

Order : Hemiptera

Family : Aphididae

Scientific Name: *Aphis craccivora* Koch.

1.4.2. Life cycle of the pest

The aphid, is autoecious holocyclic (not host alternating, but with a sexual generation) in colder regions, and anholocyclic (parthenogenetic reproduction throughout the year) where the winter climate is mild. But cowpea aphid is anholocyclic almost everywhere. In tropics it is exclusively anholocyclic, with only females (winged and wingless) normally encountered, and parthenogenetic reproduction occurring throughout the year (CABI, 2007). The aphid is ovoviviparous, with females retaining eggs inside their bodies and giving birth to small larvae(nymph) (CABI, 2007). *Aphis craccivora* Koch (L.) is a relatively cold-hardy species, although mortality of anholocyclic populations during winter is an important factor in colder regions and take overwintering may be as egg or hibernation. Males are alate and sexual forms also have been recorded from Germany. Young colonies of this small aphid was found concentrating on growing points of plants and regularly tended by ants for honeydew.

There are four nymphal instars prior to the adult stage. The pest is capable of rapid population development. Singh and Jackai (1985) revealed that the adults lived from 5 to 15 days, and had a fecundity of over 100, and daily progeny production varied 2 to 20, with four nymphal instars on cowpeas in the laboratory. The total nymphal periods averaged 5.6, 5.1, 5.15 and 4.86 days in May-June, August-September, October-November and March- April respectively, and the duration of the total life cycle during the corresponding periods were 11.07, 11.15, 10.79 and 10.42 days (Patel and Srivastava, 1989).

1.4.3. Dispersal mode

The main means of *A. craccivora* dispersal is wind-borne dispersal of the winged forms, with dispersal on plant material being of only minor importance. However, sanitary measures are important within crops to prevent the spread of viruses for which *A. craccivora* is a vector. Virus-infected planting material offers an easy means of transmission for the virus (CABI, 2007).

1.4.4. Target host/alternate host

Cowpea aphid has a broad host range with a marked preference for Leguminosae, but is found on plants in several plant families including weed and crop. Major hosts are *Arachis hypogaea* L., *Vigna unguiculata*(L) Walp, *Cajanus cajan*(L.) Millsp., *Medicago sativa* L., *Vigna radiate* (L.) Wiczek, and other more than 20 leguminous and nonleguminous crops including cotton and citrus (CABI,2007).

1.4.5. Pest distribution

A. craccivora now is virtually worldwide. It is particularly well distributed in the tropics, where it is one of the most common aphid species. This disease has been reported to many countries of Europe, Asia, Africa, Central America & Caribbean, North America, South America, and Oceania (CPC 2007). This insect is commonly found in many leguminasease crop of Nepal.

1.4.6. Pathways for introduction (possible pathways of spread)

Salivary secretions of aphids have a vital role in plant-aphid interactions. The aphid stylet punctures the plant tissue and injects aphid salivary secretions into the host. Aphids ingest phloem sap from their hosts through narrow piercing-sucking mouthparts and use the phloem as a feeding site mainly because this tissue has the greatest capacity for maintaining a supply of nutrient sap under pressure (Mittler 1957). During probing, the stylets of aphids transiently puncture epidermal, mesophyll, and parenchyma cells, and this mechanical damage may influence plant responses to infestation.

2. Purpose of Survey

- To gather information on pest occurrence, abundance and distribution pattern, to determine the nature and extent of damage in lentil to support phytosanitary certification
- To ascertain its potential damage and explore effective control measure to minimize the economic loss of commercial lentil crop production

3. Survey Methodology

3.1 Sample design of the study

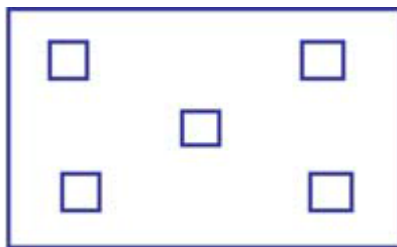
Two fields in ten selected villages of the lentil growing pockets are to be fixed for pest surveillance. Fixed fields are those grown with lentil, that once selected would be continuously monitored year round on different growth stages of crop for this using the specified data sheet formats. In addition, a random survey covering eight fields at the rate of one per village among villages other than the ten selected villages for fixed surveillance has also to be done in similar manner.

3.2 Selection of fixed fields

Two fields per village at ten villages representative of the agro ecology of the identified region in the same or and neighboring district, distributed randomly should be selected. In each village, two fields grown with lentil crop of near to two ropani each are to be fixed (designated as Fixed 1 and Fixed 2) for observations (NICARA, 2011). Assigning fields of farmers as Fixed 1 and Fixed 2 should be made at the time of field and village selection and same should be maintained all through the surveillance period. In case of field destruction due to any reason, almost similar field nearby in the same village has to be used for continuing pest surveillance. Care should be taken to select farmers growing common lentil varieties of the pocket area or region.

3.3 General information for fixed fields

The geographical, cropping system and agronomical details relating to each of the Lentil fixed fields designated as Fixed 1 and Fixed 2 should be collected using (Annex V) once in the beginning of the season.



3.4 Specific guidelines for observations in fixed fields

The recording of observations on the diseases in the selected fields should be initiated with the sowing of the crop and continued till the end of the season(s) and throughout the surveillance period. Fill in the details of, village or location name of the field being sampled along with the designated field type and appropriate field number as Fixed 1 and Fixed 2 in the format (Annex V). In each selected field, select five spots randomly such that four are from four corners and one from the center of the field (refer figure). Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a lentil field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations.

3.5 Random field survey

During the survey fields located in the villages having large area under lentil crop, will be selected randomly. Around 7-8 km distance can be maintained between two villages for survey and in each village the observation is to be made in one lentil field using the same data sheet as per the guidelines given earlier for fixed fields.

3.6 Guidelines for pest observations

All relevant details of random survey viz., date of survey, GPS coordinates, village name and area of the field sampled should be recorded and the pest observations are to be carried out as per the Annex VI for Fixed plots. Eight individual data sheets (VII) should be used for the eight fields across eight villages during random survey. Additional details such as plant protection sprays, fertilizer management, and weather events of the week need not be done for fields of random survey. The general information relating to Annex V need not be collected for random fields.

3.7 Pest observations and frequency

Number of plants infested: Count the number of aphid infested plants out of 10 plants in the selected spot and record. Severity of infestation: Assess the severity of infestation by selecting one of the maximum infested plants among the 10 observed plants in the spot on a 0-3 scale based on the approximate number of aphids present on the plant.

Pest Scaling:

Scale

Number of aphids

0

Nil

1	1-25
2	26-50
3	>5

Survey will be done in following crop stage:

- 1 Early seedling stage (VE to V1)
- 2 Late vegetative stage (V5 the first multifoliate leaf has unfolded at the fifth node)
- 3 Early bloom to full bloom (R1 to R2)
- 4 Pod cavity formation stage (R3 to R4)
- 5 Seed formation stage (R5 to R6)
- 6 Physiological maturity stage (R7 to R8)

3.8 Monitoring through yellow pan trap

The appearance and dispersal of alate aphids will be studied during crop growing season through yellow pan trap. One Yellow pan water traps having size of 46 cm x 26cm x 8 cm with painted white outside and yellow inside will be placed in each fixed plots at the height of crop above the ground level and filled 2/3 level with water. The trapped aphids will be collected from each tray at weekly interval with the help of camel hair brush and then transferred into the plastic vials containing 70% ethanol. These collections will be brought to the laboratory for examination under the binocular microscope to confirm species and count number of alate *A. craccivora*(Annex IV).

3.9 Sample collection for laboratory diagnosis

Collected insects should be placed inside the screw capped 70% alcohol contained vials with collection data printed with pencil; wrap each vials with tissue paper to ensure their physical damage during transportation;for ensuring extra safety, the left over internal space inside the box should be filled up with thermocol specks; packing box cover must have sender and receiver's addresses; the box should be wrapped with suitable and durable material until it reaches to addressee (see NSPM#...Standard Technical Protocols for Collection and Handling of Insect Samples)

3.10 Field survey checklist

Prior to starting field survey work, field survey teams should have following:

- Ensure that the survey team has all items on the check-list before departure
- Pre-printed field survey forms (sufficient number plus spares)
- Pencils (at least 3 per team member)
- Permanent marker pen
- Collecting net, forceps,
- Vials containing 70% alcohol as preserving media,
- Plastic containers for temporary storage and transportation.
- Polythene bags for storing plant material,
- Rearing material, or other samples.

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

- 6.1 Date of collection and name of collector-As designated by DADO
- 6.2 Date of identification and name of identifier- Trained PPO
- 6.3 Date of verification and name of verifier- Designated by NPPO

7 References

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Table 1: Pest Data Record Sheet for *A. craccivora* in Yellow Pan Trap

Date of monitoring:

Weather:

Trap No.	Location of trap	Host other than lentil	No. of <i>A. craccivora</i>	No of other aphids	Remarks

Signature and Date:

Name of Reference Entomologist:

Address for Communication:

Tel.:

Cell No:

Fax:

E-mail:

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

Annex V : General information of survey of fixed plots

Annex VI : Data Sheet for observation in fixed and random fields

Annex VII: Data Sheet for observation for random fields

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

Colour plates





Courtesy: <http://www.nbair.res.in/Aphids/Aphis-craccivora.php>

12 Survey Protocol for Lentil Cutworm (*Agrotis segetum*) in Lentil

1. Introduction

1.1 Background of the Study

Lentil is export potential crop and so details pest information to assure the importing countries that the commodity is of high quality and free of pest is essential. Surveillance need to be carried out to gather technical basis for pest listing, documentation of pest status, pest occurrence or absence supporting phytosanitary certification and the designing the future pest management strategies. Present protocol is designed based on review on published materials both at national and international research periodicals and meet the requirements to implement lentil cutworm monitoring survey. This document outlines recommendations for general protocols and procedures for surveillance emphasizing on factors such as host, pest biology and epidemiology, potential impact to the lentil commercialization, pathway assessment.

1.2 Target commodity detail :

(See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production)

1.3 Survey area detail

- In view of pest's adaptation ability, wide range of host and effect of ongoing the changes in climate, there is high likelihood that it can easily establish causing economic damage in lentil. So a regular monitoring survey needs to be done in all commercial lentil growing areas.
- District growing more than 500 ha lentil area include Udaipur, Jhapa Morang Sunsari, Saptari, Siraha, Sindhupalchwoke, Sindhuli, Dhanusa Mahottari, Rathut, Kailali, Sarlahi, Bara, Parsa, Chitwon Nawalparasi, Rupendhim, Kapilvastu, Salyan, Jajarkot, Dang, Banke, Bardia, Surkhet, Bhajang, Doti and except Manag and Mustang all other remaining districts also grow lentil in less than 500ha area.
- Thus the monitoring survey is recommended to be conducted in first phase in 15 districts having more than 500 ha as listed above representing Hills and Terai and Mountain.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1. Pest name including scientific name of pest and family/order

Scientific name: *Agrotis segetum* Denis & Schiffermüller 1775.

Common names: Turnip moth, black cutworm, common cutworm, cutworm, dark moth, dart moth, tobacco cutworm, Turnip dart moth

Similar Species

There are several species of cutworms that look very similar. These species include the black cutworm (*Agrotis ipsilon*), the brown cutworm (*Agrotis longidentifera*), the common cutworm (*Agrotis segetum*) and the grey cutworm (*Agrotis subalba*). The adult moths can be distinguished on the basis of wing shape and colour but this would require taxonomic expertise.

Phylum: Arthropoda; Class: Hexapoda (Insecta); Order: Lepidoptera; Family: Noctuidae; Genus: *Agrotis*

1.4.2. Life cycle of the pest

Adult moths are nocturnal in nature and begin egg laying 5-11 days after emergence. The larvae hatch after 3 (at +29-30°C) to 24 days (at +10-12°C), and the young cutworms feed on vegetation near the ground. After 10-14 days they avoid daylight and become nocturnal feeders, remaining in the soil during the daylight hours. Larvae develop in 24-40 (sometimes 90-100) days, reaching 40-52 mm in length at the last 6th instar, depending on available food. When fully fed, the cutworms rest in the soil for about a week before pupating about 40 mm below soil level. The pupae stage lasts two to three weeks but may take up to two months depending on temperature. Depending on the species and latitude, one to three or four generations may occur in a year. It is the larva feeding on plants.

Eggs

The eggs are pearly white, rounded, about 0.5mm in diameter. They are laid singly or in clusters of up to 2,000 eggs but generally between 600 and 800 eggs. Eggs are laid on vegetation, on moist ground around plants, or in cracks in the soil.

Larvae

Larvae plump, smooth caterpillars, greasy in appearance and can range in colour from grey to brown or black depending on the species. They may be up to 40mm long. Larvae generally have two spotted yellow stripes running down the back. The larvae curl into a C shape and when disturbed and remain motionless for a short period. They commonly hide just under the soil or litter by day or may be found on the soil surface adjacent to severed vegetation.

The larvae are indiscriminate feeders, causing damage to a variety of crops, mainly when the crops are young. Larger larvae may completely cut through stalks, which can cause plants to wilt and die.

Adults

Adults are dark grey, black or brown coloured moths with markings on the front wings, which vary according to species. They have a wingspan of 40-50mm and are 20-30mm long. Females are darker than males but otherwise similar.

1.4.3. Dispersal mode

The adult moths are strong fliers and can be carried long distances on the wind and Human aided dispersal will also contribute to their rapid spread and do external feeding on leaves by the first-instar and second-instar larvae of *A. segetum*

1.4.4. Target host/alternate host

Cutworms are indiscriminate feeders, causing damage to a variety of crops when the plants are at seedlings and young plants stage (maize, tobacco, potatoes, young coffee, cotton, asparagus, bean, beet, cabbage, castor bean, grape, lettuce, onions, cabbages, peanut, pepper, potato, radish, spinach, squash, strawberry, rice, rye and tomato, lentil). They attack mostly stems, leaves in very young plants

1.4.5. Pest distribution

Different species of cutworm are found worldwide however documented detail information wrt Nepalese condition could not be found. It has been reported mostly in maize, chilies, vegetables, potato..... more than 25 common crops grown in Nepal .



World distribution Scenario

1.4.6 Damage

Cutworms usually feed at night or during overcast days. Newly hatched larvae feed on weeds, and/or young crops plants if present, leaving small irregular holes in the leaves.

- Larger larvae may completely cut through stems, which can cause plants to wilt and die. They sometimes drag cut plants under soil clods or into small holes in the soil to continue their feeding during the daylight hours.
- In crops like potatoes and root vegetables, damage can take the form of unsightly holes in the subterranean tubers, which may allow the penetration of secondary fungi. Cutworms can destroy as much as 75% of a crop

Damage Symptoms

- The adult moths are strong fliers and can be carried long distances on the wind and Human aided dispersal will also contribute to their rapid spread and do external feeding on leaves by the first-instar and second-instar larvae of *A. segetum* and result

in the presence of very small round 'windowpanes' where the larvae have eaten away the upper epidermis and the parenchymal tissue, but left the lower epidermis. External feeding on leaves stalks and stems results in falling leaves, small holes in the stems or cut stems. External feeding on tubers and roots results in a variety of holes.

- The activity of the third-instar and fourth-instar larvae is easier to recognise because whole leaves may fall off the plant after being cut through at the base of the stalk. Alternatively, holes may be found on the stems and roots at the soil surface.
- A further sign of activity is the presence of leaf pieces partly pulled down into the soil.
- Feeding by fifth and sixth instars is very obvious. Whole plants fall over, and on root crops deep holes become visible at and above the soil surface.
- Damage to underground tubers (e.g., potatoes) may be difficult to recognise before harvest. Damage is far more severe under very dry conditions (Esbjerg 1990) and occurs deeper below the surface.
- If crops are attacked by larvae later in their development, then far fewer plants are killed by *Agrotis* sp. and the presence of larvae may be difficult to detect (see Hopkins 1987; Michael 1988, 1994). Adults do not feed, except on water and nectar.
- An important complicating factor regarding lentil cutworms is that some other species of *Agrotis* are already present and are pests of some important crops that *A. segetum* would also attack. This makes the potential for an incursion to be identified potentially complicated by the possibility of misdiagnosis or confusion of any symptoms on host plants that are colonised by several of *Agrotis* species as not being due to *A. segetum*.

2. Purpose of Survey

- To regularly observe, measure, and record information on pest populations and infestation levels within a given area
- To assess the extent of the damage due to the pest in lentil production and support to design appropriate pest management strategies to minimize the economic loss.
- To gather technical information to analyze the potential impact of this pest in commercial lentil production.

3. Survey Methodology

3.1 Survey area

- In view of pest's adaptation ability, wide range of host and effect of ongoing the changes in climate, there is high likelihood that it can easily establish causing economic damage in lentil. So a regular monitoring survey needs to be done in all commercial lentil growing areas.
- District growing more than 500 ha lentil area include Udaipur, Jhapa, Morang, Sunsari, Saptari, Siraha, Sindhupalchok, Sindhuli, Dhanusa, Mahottari, Rautahat, Kailali, Sarlahi, Bara, Parsa, Chitwan, Nawalparasi, Rupendehi, Kapilvastu, Salyan, Jajarkot, Dang, Banke, Bardia, Surkhet, Bhajang, Doti and except Manang and Mustang all other remaining districts also grow lentil in less than 500ha area.

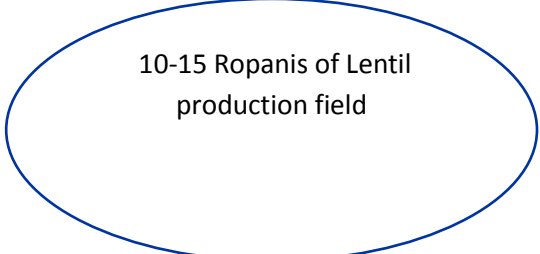
- Thus the monitoring survey is recommended to be conducted in first phase in 15 districts having more than 500 ha as listed above representing Hills and Terai and Mountain.
- Not many published information on cutworm in lentil is available. Cutworm in lentil till date has not been observed as serious, as in other tuber crops, but in view of the nature of the pest it is suspected that if the farmers and researcher are not watchful then it can cause serious economic loss

3.2 Direct diagnosis of moth, eggs and larvae

- Taxonomic methods using keys and descriptions are adequate for identification of undamaged *Agrotis segetum* adults. Although the forewing markings provide an indication of species, identification with a degree of certainty is by examining the genitalia, with genitalial dissection required.
- Eggs are oviposited in soil, are small (0.5mm diameter, 0.4mm height), have 32-35 straight ridges, are white though become grey before larval emergence.
- Larvae are difficult to identify and, if possible, should be collected live and reared with quarantine facility by trained entomologists. Larvae have convex granules on the abdominal body segments, the mentum and submentum is heavily sclerotized and shiny black in color (<http://www.padil.gov.au/>)

3.3 Diagnosis by symptoms of damage to host plants

- Eggs are oviposited in soil, are small (0.5mm diameter, 0.4mm height), have 32-35 straight ridges, are white though become grey before larval emergence.
- Fifth and sixth instar damage leads to whole plants falling over, while deep holes are visible at the soil surface in root crops.
- Suspected contaminated soil would be expected to provide some risk of containing eggs and larvae; hence destruction would need to cater for this material while taking samples. Similar action needed adults trapped in pheromone or other non-destructive traps.
- Consider to have support from expert for diagnosing immature life stages, plus laboratory experience in preparation of specimens for microscopic genitalial examination
- If delimiting, and monitoring surveillance is to be conducted active flight makes design and operation of surveys difficult, thus need to take into account wind direction and speed, overwintering potential. For further information (see PHA website)
- To monitor pest populations in commercial lentil growing sites sample sites representing lentil growing area under District's Agriculture Service, should be randomly selected and statistical sampling designs be used to obtain quick and reliable data on pest prevalence. Identify 10 commercial production site of about 10-15 ropanis area and within that area select fivespots randomly for trapping adults, taking plant and soil sample for other stages of the insect and damage plants.



10-15 Ropanis of Lentil
production field

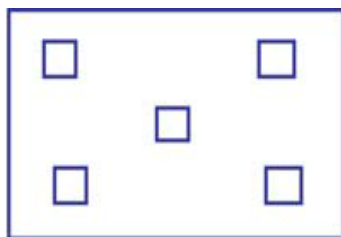
3.4 Sampling method

The choice of any sampling method is best guided by knowledge of the biology of moth in combination with accepted detection methods,

- The use of pheromone or light traps would be suitable to detect the presence of adults. Some hand sampling of soil (pupae or larvae) or host plants for signs of feeding damage would also be suitable.
- Eggs and larvae can be examined soil samples taken from surveyed areas suspected of potentially containing eggs or larvae.
- Survey the field and get agronomic as well as metrological information of the production area considering soil moisture, soil texture or drainage characteristics, varieties, topography etc. Daily records of weather data from the observatory of the research station or any other nearest location should be collected.
- A standard procedure is to distribute pheromone traps charged with dispensers loaded with a synthetic female sex pheromone blend, at a density of about 1-2 traps per field of about 10-15 ropanis. Traps need to be monitored regularly, during the crop season.

Trap Spacing: When trapping for more than one species of moth, separate traps for different moth species by at least 20 meters .Refer lisa.d.jackson@aphis.usda.gov for further information

- Place traps over the crop for good ventilation, and put them up as the crop grows.
- Monitored Traps **weekly**, to collect and note down the number of insects captured and verify that traps are still in the correct position.
- Once Adults moths detected the first catches is advisable to make a visual estimation by direct sampling in the plants. Then evaluate the situation on the ground and determine the measures to be taken in each case.



In each selected field, select five spots randomly such that four are from four corners and one from the center of the field (see figure). Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during.

- The geographical, cropping system and agronomical details relating to the lentil should be collected using the format as given in Annex-I in the beginning of season

Number of specimen to be collected

- Ideally, it is best to collect multiple specimens from as many life stages as can be found. Adults will in most cases be the easiest to find and collect and are desirable as the adult life stage is the easiest to use for identification.

- Record the host plant, location distance and direction to identifiable landmarks, and other host crops or plants where the specimen has been located.

Collecting plant samples

- Where adult moths are unable to be collected, it is possible that plant material showing feeding damage or with larvae attached may be collected. The likely plant parts to be collected will be leaves where larvae are feeding on these, though they also prefer to bore at the base of plants and into tubers or underground parts.

Preserving plant samples

- Plant samples with immature life stages associated or attached should be packed between dry paper sheets, or (moistened paper for leaves), and sealed in plastic bags. Double bagging is recommended with additional paper also placed in outer bags. Bags should be placed in crush resistant containers for transport.

Management of insect samples

- Adults should be killed by standard methods – freezing, ethyl acetate. Varying concentrations of ethanol for preserving Lepidopteran specimens have been recommended, ranging from 70-95%. Ethanol should be used where morphological identification of adult moths is planned and for future analysis should be stored in absolute ethanol, though acetone has been recommended (Mandrioli *et al.*, 2006). Acetone has the additional advantage of being effective at preserving morphological features of Lepidopteran specimens at room temperature.
- Where taxonomic expertise is readily available and identification can be carried out quickly it may be practical to keep adult moths alive or kill and relax the insect immediately prior to transport. Live insects (any life stage) should not be transported unless it is considered essential, and then such that containers are only opened in containment facilities.

Transporting insect samples

- Vials containing the samples in a preservative should be sealed to avoid leakage and packed in a manner to minimize shock to the vials (i.e. with cushioning material in a strong box). It is important to ensure that vials are filled with preservative so as to remove excess air that through movement of the vial will allow agitation of the preservative and quickly degrade the specimen.

3.6 Time frame and frequency of survey

- Collect a composite sample of plant, soil no fewer than 8 to 10 soil samples from unhealthy plants and also in healthy plants to see the larval damage problem during the growing season
- Samples should be collected during active growing season especially vegetative growth period, flowering and pod formation period.

3.7 Data collection procedures (field, lab)/ Data preparation and collection (Refer Annex1-6)

- The plant and soil samples should be forwarded for detailed laboratory testing at diagnostic lab.
- For collection and handling of samples follow NSPM... (Standard Technical Protocols for collection and handling of insect samples)

3.8 Detection

Cutworms can be difficult to detect because they are nocturnal. However, signs of cutworm damage such as holes in seedlings or plant parts just below the soil surface indicate their presence. Pheromone traps can be used to monitor cutworm adults. Dig in the soil around damaged or adjacent plants in the row and then find and take necessary observations. If there is sunflower, a favored host, around the perimeter of the sample area it is also helpful to find out their presence.

3.9 Field survey checklist

A pheromone traps, pheromones , garden trowels, digging forks, rubber gloves, plastic bags, a bucket, water-resistant marker pen, record forms, plastic bag, killing bottles,

4 Record keeping

When data are collected as part of targeted surveillance programs, the following information should be recorded:

- Scientific name of the pest
- Common name of the host
- Types of sampling method used e.g. trap, soil sample, sweep net
- Location (e.g. address including specific details such as paddock or glasshouse identifier, GPS or map coordinates)
- Collection details (i.e. date of collection and name of collector)

Additional information (e.g. first date of detection of the moth , level of infestation, growth stage of plant, symptoms of pest damage, Where samples are collected and identified as part of monitoring survey, details of the identification (e.g. date and name of personnel undertaking initial identification) and verification (e.g. date and name of personnel from verification laboratories) should also be recorded

- Moths are relatively easy to see and are reasonably able to be distinguished
- In addition to pheromone and light traps, sweep nets can be used for collecting adults in host crops.
- Larvae may be collected from oil sample. The likely plant parts to be collected will be lower

- Where larvae are suspected observations should be of low stem and soil areas, looking for typical cutworm symptoms, including damage to any tubers

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 References

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List of Tables

Table 1: Field Record Sheet for cutworm

Name of cut worm moth monitoring:				Day/Week:			
				From:		To:	
Trap No.	Location of trap	Date inspection	Host	Light trap type	No of moths /trap	Species identified	Date recharge

--	--	--	--	--	--	--	--

Table 2: Specimen Identification Report

To

.....

.....

(Name and Address of Field Inspector)

Light Trap	Location	Name of the cutworm spp. identified	No. of flies

Signature and Date:

Name of Reference Entomologist:

Address for Communication:

Tel.:.....

Cell No:.....

Fax:.....

E-mail:.....

Table 3: Relative abundance of the cutworm both moth and larvae by light trap catches and from soil sample taken

Month	Cutworm moth population per trap per day	Larvae per soil sample /site
January		
February		
March		
April		

May		
June		
July		
August		
September		
October		
November		
December		

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

13 Survey Protocol for *Callosobruchus chinensis* in Lentil

1. Introduction

1.1 Background of the Study

This survey protocol for *Callosobruchus chinensis* is developed to monitor the prevalence of pest in the storage condition of lentil crop. This survey protocol also can be used to determine pest-free status and as well as for pest record documentation. This protocol must be endorsed by NPPO for the implementation by the districts. Lentil is the Nepal's third largest exportable commodity during the year 2009-10 (TEPC 2011). *C. chinensis* is one of the most damaging crop pests to the stored legume industry. Infestation starts in the pods before harvest and carries over into storage where substantial losses may occur. Due to invasion of the beetle, deterioration in quality and quantity of lentil occurs, being unfit for the export and consumption.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. In Commercial Lentil Production

1.3 Survey area detail

Lentil pocket areas: Terai/ inner terai (as per the requirement of NPPO)

The information with details of site such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district), topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded (See appendix No.: 3).

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1. Pest name including scientific name of pest and family/order

Scientific name: *Callosobruchus chinensis* (Linnaeus, 1758)

Order: Coleoptera, Family: Bruchidae, Genus: *Callosobruchus*, Species: *Callosobruchus chinensis*

1.4.2. Life cycle of the pest

The legume seed weevil females lay their eggs directly on the surface of the legume singly. They can lay as many as 90 eggs after a single fertilization. The eggs usually hatch after 3–5 days and the new larvae will burrow into the bean for the rest of development. The larvae chew tunnels through the bean until it is ready to pupate. Mature adults emerge from the bean, biting a neat circular exit from the pod as soon as 25 days after hatching. The adult beetles live up to two

weeks after emerging from the pupa. Infestation commonly begins in the field, where eggs are laid on maturing pods and after threshing carry over into storage where substantial losses may occur. In the early stages of attack the only symptoms are the presence of eggs cemented to the surface of the pulses. As development occurs entirely within the seed, the immature stages are not normally seen.

1.4.3. Dispersal mode

The chances of dispersal of *C. chinensis* are from seed and lentil for consumption.

1.4.4. Target host/alternate host/host range

Lentil/ Chickpea, Green Gram, Cowpea, Pigeon pea, Broad beans, Soybean

1.4.5. Pest distribution

C. chinensis in storage is widely distributed in the country

1.4.6. Pathways for introduction (possible pathways of spread)

The infested pod, seed and the whole lentil are the pathways for introduction

2 Purpose of Survey

The focus of the survey is to determine the incidence of *C. chinensis* through monitoring survey for pest record documentation and also for planning pest management programs.

3 Survey Methodology

3.1 Sample design of the study

For sampling, ten sites representing the district, distributed randomly should be selected and statistical sampling designs must be used to obtain quick and reliable data on pest prevalence. In each site select three points randomly to collect the samples with the help of probe. While collecting the samples, the temperature and humidity of the site from where the samples were collected should be recorded.

For plant sampling, from randomly selected ten lentils growing sites select five spots randomly four in the corners, at least 5 feet inside the borders and one in the center. At each spot select ten plants for collecting the mature pods.

3.2 Sampling unit, sample frame, and population

To determine the pest status of *C. chinensis* samples on storage should be collected from different sources (the farmers, the villagers and commercial storage).

Sampling unit should be a minimum of 10 primary samples. In store lentil, samples should be drawn at random taken from different parts to make composite sample.

The population density of pests in the field should be done by harvesting the mature pods from randomly selected minimum 50 mature plants/site.

3.3 Sample selection and size

- Random selection
- 3-10 sampling point per sample site in field
- 10 plant per sampling point

Bin/sacks Sample

- Sample should be taken in the store houses from a bulk or from stored in bin/sacks. The samples should be drawn at random from bags with the help of a slotted tube sampler.
- Collect samples from various depths and avoid the center and the corners to get representative samples. From each store 2 samples should be taken, one from top and one from bottom. One sample should consist of 10 sub sample taken from different parts of the storage (Table-1).
- Each of the primary samples drawn shall be thoroughly mixed and balanced to constitute a homogeneous composite sample. From the composite sample a sample of 1 kg. is drawn and submitted in sealed polyethylene covers with appropriate label for detailed laboratory testing.
- In case of bulk pulses, sample from as many as possible representative parts within the bulk with a bucket and mix it for making composite sample.

Mature pod sample

- Sampling should be performed when physiological maturity of plants begins. The character is leaves start yellowing and 70% - 90% of the pods turn yellow and golden-brown.
- Sample should be taken by harvesting 50 mature plants randomly in the field (Table-2)
- Collect all the pods, make composite sample and mix thoroughly
- Take a sample of 1 kg. Keep in sealed polyethylene bag and send to the lab for pest incidence and infestation percentage.

3.4 Time frame and frequency of survey

The recording of observations on the pests in the selected fields should be initiated after seed formation stage to maturity stage throughout the surveillance period. Survey will be done in following crop stage:

- Seed formation stage
- Physiological maturity stage

In the storage condition survey will be done throughout the year monthly or fortnightly. Interval also depends upon the temperature and humidity of the storage.

3.5 Data collection procedures (field, lab)/ Data preparation and collection

The field data of pod survey should be recorded as per the format prescribed in Table 1 and 2. For collection and handling of insect samples follow NSPM... (Standard Technical Protocols for collection and handling of insect samples)

The samples should be forwarded for detailed laboratory testing at diagnostic lab as per Annex IV

3.6 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- A clipboard.
- Pencils (at least 3 per team member)
- Permanent marker pen
- Vials containing 70% alcohol as preserving media for adult/ larva
- Polythene bags for storing plant material

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6 and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 References

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

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TEPC (2011)- Trade and Export promotion center (TEPC). Republica, March 9, 2011.

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List of Tables

Table 1: Data Recording Form (Store Survey)

Date	Area/location	Name of farm/storage	Temperature	Humidity	No.of sacks/bin examined	samples size (kg)	Surveyor name

Table 2: Field Data Recording Form (Pod Survey)

S. No.	Date	Location	Variety	No of plants harvested	Pod samples size (kg)	Sign of Recorder

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

14 Survey Protocol for Cowpea weevil (*Callosobruchus maculatus*) in Lentil

1 Introduction

1.1 Background of the Study

Lentil is the Nepal's third largest exportable commodity during the year 2009-10 (TEPC 2011). *Callosobruchus maculatus* (Fabricius, 1775) is one of the most damaging important primary pests of lentil. Infestation may start in the pods before harvest and carry over into storage where substantial losses may occur. Due to invasion of the *Callosobruchus maculatus* beetle, deterioration in quality and quantity of lentil occurs, being unfit for the export and consumption.

For the expansion of the export market and making strategy of pest management it is essential to monitor the pest distribution. Surveillance is carried out to gather information that provides the basis for pest listing, documentation of pest status, pest occurrence or absence supporting phytosanitary certification and the designing the future pest management strategies and enabling Pest Surveillance is primary objective of designing the survey protocol. This activity also support to collect information required for maintaining pest records. Present protocol is designed based on review on published materials both at national and international research periodicals. This document outlines recommendations for general protocols and procedures for surveillance emphasizing on factors such as host, pest biology and epidemiology, potential impact to the lentil commercialization, pathway assessment.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production

1.3 Survey area detail

There are usually different step for survey area selection. There is no single best method for site selection. The main point is transparently document the choice and reason for such choice. The method can then be considered and discussed with the basis of the choice, given the circumstances.

- Select site according to development region, districts, VDCs, field site, sampling site and sampling point.
- Use one or a combination of random sampling, systematic sampling, stratification sampling, targeted sampling for site selection
- The location and unique identifying details of each site should be recorded. Describing the sampling site would include information such as a GPS reading(latitude, longitude, altitude), location(Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil tye etc), Meterological (tempeture, rainfall, hailstone etc)

Survey area will be based on need and survey plan of institutions.

- Whole Nepal especially lentil growing area of Nepal of terai and inner terai.

- Select geographic area where rainfall is <400 mm and average temperature during growing period is around 18-20°C.
- Lentil growing pocket area or districts
- As per need of survey institutions.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

Bruchid is a general name of insects of family Bruchidae. Family Bruchidae composes over 1300 species of insects. Bruchids have strong specificity to legumes plants 84% of their hosts are leguminous plants. Bruchids can develop and reproduce on leguminous plants. They not only infest leguminous plant in field, but also infest stored gram legume.

1.4.1 Preferred scientific name and Taxonomic position

Preferred scientific name: *Callosobruchus maculatus* (Fabricius, 1775)

Order: Coleoptera

Family: Bruchidae

1.4.2 Common names

Cowpea weevil, spotted cowpea, bruchid, southern cowpea weevil, four-spotted bean weevil, Cowpea seed beetle

1.4.3 Life Cycle

Callosobruchus maculatus lay eggs on the pods of legume hosts as they approach maturity in the field but emergence usually occurs after harvest (Booker 1967). In storage, *C. maculatus* lay eggs on the seeds. Larval development and pupation are completed entirely within a single seed. *C. maculatus* females and males live an average of 7 days under laboratory conditions and very few survive more than 2 weeks at 25°C. The beetles have a short generation time (22-28 days at 28°C) (Messina 1993, Fox 1993).

Cowpea bruchid adults are small, slightly elongate (3-mm long) orange-brown beetles with dark markings, and body that tapers towards the head. Cowpea (and other) bruchids do not have the elongated snout of true weevils, which infest cereal grains. Their wing covers (elytra) are slightly shorter than their abdomen. They are not true weevils and do not have the long rostrum of true weevils. The eggs are small, flat and white and, despite being only 0.6 mm long, are readily visible on the surface of seeds. The C-shaped larvae look like short cream-coloured maggots, and are rarely seen as they feed within seeds. The first instar in the family Bruchidae is different from the 3 subsequent larval instars in possessing well formed thoracic legs, an H-shaped plate on the prothorax and a hatching spine on each lateral side of the 1st abdominal segment (Pajni 1987). Prior to pupation larvae excavate a pupal chamber just below the seed surface, visible as a small transparent window in the seed coat.

1.4.4 Favorable Environment

As seed is a good insulator, harvested seed in bulk storage maintains its at-harvest temperature for a long time, even during winter. The development of bruchids in seeds in silos is therefore much faster than if they were exposed to the outside air temperatures. Seed moisture content below 10% impairs normal storage insect activity.

1.4.5 Target host/alternate host

Affected Plant Stages: Fruiting stage and post-harvest.

Affected Plant Parts: Seeds.

Target host: *Lens culinaris* ssp. *culinaris* (lentil)

Major hosts: *Acacia nilotica* (gum arabic tree), *Cajanus cajan* (pigeon pea), Fabaceae (leguminous plants), *Glycine max* (soyabean), *Phaseolus* (beans), stored products (dried stored products), *Vigna radiata* (mung bean), *Vigna unguiculata* (cowpea), *Voandzeia subterranea* (bambara groundnut)

Minor hosts: *Astragalus cicer* (Cicer milkvetch), *Cicer arietinum* (chickpea), *Lablab purpureus* (hyacinth bean), *Lathyrus sativus* (grasspea), *Lupinus albus* (white lupine), *Phaseolus acutifolius* (tepany bean), *Pisum sativum* (pea), *Vigna* (cowpea), *Vigna angularis* (adzuki bean), *Vigna mungo* (black gram), *Vigna umbellata* (Rice- bean), *Vigna vexillata* (wild sweetpea)

1.4.6 Pest distribution

The two most widespread species of bruchid beetle are *C. maculatus* and *C. chinensis*, which are distributed throughout the tropics and sub-tropics. *C. maculatus* originated in Africa where it remains dominant. Africa, Australia, Central and South America, Europe, northern Asia, Mediterranean, South and South-east Asia, USA and Canada.

1.4.7 Pathways for introduction (possible pathways of spread)

Callosobruchus maculatus adults show an imaginal polymorphism with differences in morphological, behavioural and reproductive characteristics. There are two phenotypic morphs; a sedentary morph is short-lived and highly fecund whereas a dispersing morph displays migratory behaviour and delayed reproduction. This polymorphism permits use of host legumes at both the developing pod stage (attacked by the dispersing morph) and the dried seed stage (attacked by the sedentary morph). Storage populations are usually founded by few individuals but expand rapidly on dried seeds (Messina 1993, Zannou *et al.* 2003). The main method of transmission is via infested seed, either from the paddock into storage, or in contaminated seed within and between storage facilities.

2 Purpose of Survey

Purpose of survey should be identified first. The reason for surveys identifies the purpose. Purpose may be

- a. Detection of pest
- b. Determination of pest status
- c. Obtain data to support PFAs, PFPP or PFPS

- d. Guideline for pest eradication
- e. Requirements for the establishment of areas of low pest prevalence.

3. Survey Methodology

3.1 Sample design of the study

3.1.1. Sample collection and preservation

Having handout material that can be used in the field may be critically important, particular if the pest has not been seen before by the surveillance team. Having a reference collection of pest 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. Insect preservation procedure should follow properly. Sample collection is necessary in field and storage.

3.1.2 Field sampling

Field sampling is depends upon the purpose of survey. In each selected field, select five spots randomly such that four are from four corners and one from the center of the field. Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a lentil field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations. Data collection in field is better at last reproductive stage to maturation stage of crop.

3. 1.3 Storage sampling

Storage sampling also depends upon the purpose of survey. If the purpose is only detection, no of sample unit should be 3 sampling unit per sample site. If the purpose is monitoring of pest then sample size should increase up to 10 sampling unit per sample site. In every sample data collection and population dynamic should analyze. Data collection in storage may be throughout the year but interval may be longer than field. Interval also depends upon the temperature and humidity of the storage.

Sample should be taken in the store houses from a bulk or from stored in sacks. The samples shall be drawn at random from bags with the help of a slotted tube.

Collect samples from various depths and avoid the center and the corners to get representative samples. From each store 2 samples should be taken, one from top and one from bottom. One sample should consist of 10 sub sample taken from different parts of the stored grain. Each of the primary samples drawn shall be thoroughly mixed and balanced to constitute a homogeneous composite sample. From the composite sample a sample of 1 kg should be drawn and submitted in sealed polyethylene cover with appropriate label for detailed laboratory testing. A minimum of 3 samples are drawn for commodity for detailed laboratory testing (See Annex VIII).

If the lot contains nine bags, then sample at least three bags. ($9=3$)

If the lot contains 100 bags, then sample at least 10 bags. ($100=10$).

3.1.4 Sample selection and size

- Random selection
- 3-10 sampling point per sample site in field
- 10 plant per sampling point
- 10 sampling points for storage

3.1.5 Time frame and frequency of survey

The recording of observations on the insects in the selected fields should be initiated after seed formation stage to maturity stage throughout the surveillance period. Survey will be done in following crop stage:

- Seed formation stage
- Physiological maturity stage
- In the storage condition survey will be done throughout the year monthly or fortnightly.

3.1.6 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- A clipboard.
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis

- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6 and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector as designated by DADO

6.2 Date of identification and name of identifier Trained PPO

6.3 Date of verification and name of verifier Designated by NPPO

7 References

Bandaara, K.A.N., Saxena, R.C. 1995. A technique for handling and sexing *Callosobruchus maculatus* (F.) adults. (Coleoptera: Bruchidae). *Journal of Stored Products Research*, **31**(1): 97-100.

CPC 2007

Diagnostic Methods for Cowpea weevil or cowpea bruchid *Callosobruchus maculatus*

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T8Y-3YMFW5B-1S&_user=139754&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&_view=c&_searchS trilId=1101648743&_rerunOrigin=google&_acct=C000011578&_version=1&_urlVersion=0&_userid=139754&md5=7e0b238bfb40d2514cc7704b3e7c3253

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

Mel LIUZhu. Proceedings of the 7th Internatwnal Working Conference on Stored-product Protection - Yolume 2 Assessment of role of quarantine in control of Bruchidae
NPQP, Technical guidelines for detection survey of plant pests in NEPAL, 2071

Table 1: Data Recording Form (Store Survey)

Date	Area/location	Name of farm/storage	Temperature	Humidity	No.of sacks/bin examined	samples size (kg)	Surveyor name

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

Annex VIII : e08f/Of 8f6f l;6

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

15 Survey Protocol for Lentil Pod Borer (*Helicoverpa armigera*) in Lentil

1 Introduction

1.1 Background of the study

- This protocol has been designed with technical information and managing requirements to implement lentil Pod borer monitoring survey.
- Monitoring survey for Assessment of lentil pod borer occurrence and population status.
- Pod borer is a major pest of Lentil and this pest's adaptation ability, wide range of host, ongoing the changes in climate, economic loss in lentil production can be serious and so a regular monitoring survey need to be done in all commercial lentil growing areas

1.2 Target commodity detail:

(See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production)

1.3 Survey area detail

- District growing more than 500 ha of lentil area include Udaipur, Jhapa Morang Sunsari, Saptari, Siraha, Sindhupalchhowe, Sindhuli, Dhanusa Mahottari, Rathut, Kailali, Sarlahi, Bara, Parsa, Chitwan Nawalparasi, Rupendhim, Kapilvastu, Salyan, Jajarkot, Dang, Banke, Bardia, Surkhet, Bhajang, Doti and all other remaining districts grow lentil in less than 500ha area except Manag and Mustang.
- Select 15 districts having more than 500 ha as listed above representing Hills and Terai and Mountain and based on the finding identify the sites. Based on statistical sampling designs principle, to obtain quick and reliable data on pest prevalence, fix the sample sites and monitor pest.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1. Pest name including scientific name of pest and family/order

Order: Lepidoptera
Family: Noctuidae
Genus: *Helicoverpa*
Species: *armigera*

Crop base name: lentil Pod borer

1.4.2. Life cycle of the pest

Understanding the life cycle and behaviour of *Helicoverpa* improves our ability to identify weak points in the pest's biology and ecology and manage it more sustainably. The pest has 3-4 annual generations, Nocturnal. Under favorable conditions, the eggs can hatch into larvae within three days and the whole life cycle can be completed in just over a month. Late instar *Helicoverpa* larvae can vary greatly in colour and markings



Larva

Pupa

Adults

Adult: Like many other Noctuidae, the adults of the *Helicoverpa* emerge after sunset, and are generally active during the night. Females may mate several times, but never more than once per night. The female can lay several hundred eggs, distributed on various parts of the plant. Moths feed on nectar and live for around 10 days. Females lay around 1000 eggs in singles or clusters on growing points, leaves, flower buds, flowers and developing fruits, and sometimes on stems. Moths tend to lay eggs on the top third of healthy plants and on vigorously growing terminals

Larvae: Under favorable conditions the larvae hatch 3-5 days after oviposition, and commence feeding. In summer within 2-3 weeks become up to 40 mm in length. The time required for embryonic development varies with temperature, varying from 3 days between 25 and 35 ° C up to 20 days at 13 ° C. Larvae prefer feeding on the parts of the plant with high concentrations of nitrogen. The fruit/seeds/pod / are the most affected part, but flowers, stems and leaves can also be damaged, resulting in optimal conditions for rot or attack of secondary pests

Pupae: After attaining their maximum size the larvae leave the host and enter the soil, where they pupate. Pupal development lasts a week in the summer season while in winter it may take more than 4 months. The pupae develop inside a silken cocoon over 10 to 15 days in soil at a depth of 4–10 centimeters (1.6–3.9 in)

1.4.3 Dispersal mode

They can disperse very far and can be aided by wind (100's km is possible)

1.4.4 Target host/alternate host

Survives in wide range of food, fiber, oil and fodder crops and a variety of wild plants, occasionally on perennial horticultural crops. Important crop hosts are tomato, cotton, pigeon

pea, chickpea, sorghum and cowpea including groundnut, okra, peas, field beans, soybeans, lucerne, *Phaseolus* spp., other *Leguminosae*, tobacco, potatoes, maize, flax, *Dianthus*, *Rosa*, *Pelargonium*, *Chrysanthemum*, *Lavandula angustifolia*.

1.4.5. Pest distribution

It is a cosmopolitan pest, migratory in nature species and is widespread in central and southern Europe, temperate Asia, Africa, Australia and Oceania, and some countries of South America and in Nepalese case sparse reporting shows its distribution through mid-hills and Terai region and often in lower belt of Mountain region. When ovipositing, females can move 5-10km (dependent on landscape and hosts available).

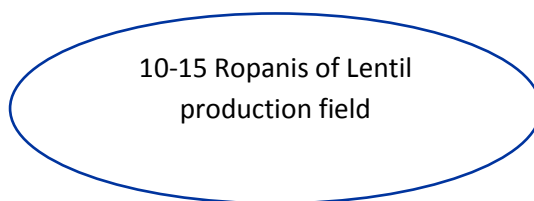
2. Purpose of Survey

- Lentil is export potential crop and detail pest information to declare the importing countries that the commodity is free of pest supporting phytosanitary certification..
- To assess the extent of the damage due to *Helicoverpa armigera* and support to design appropriate pest management strategies to minimize the economic loss in commercial lentil production.

3 Survey Methodology

3.1 Survey area and sampling procedure

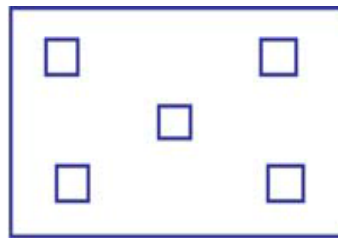
- District growing more than 500 ha area include Udaipur, Jhapa Morang Sunsari, Saptari, Siraha, Sindhupalchhoke, Sindhuli, Dhanusa Mahottari, Rathut, Kailali, Sarlahi, Bara, Parsa, Chitwan Nawalparasi, Rupendhim, Kapilvastu, Salyan, Jajarkot, Dang, Banke, Bardia, Surkhet, Bhajang, Doti and all other remaining districts also grow lentil in less than 500ha area except Manag and Mustang.
- Thus the monitoring survey is recommended to be conducted in first phase in 15 districts having more than 500 ha as listed above representing Hills and Terai and Mountain and based on the finding redesign the survey plan.
- To monitor pest populations in commercial lentil growing sites sample sites representing lentil growing area under District's Agriculture Service, should be randomly selected and statistical sampling designs be used to obtain quick and reliable data on pest prevalence. Identify 10 commercial production site of about 10-15 ropanis area and within that area select five spots randomly for trapping adults, taking plant and soil sample for other stages of the insect and damage plants.



3.2 Sampling method

The choice of any sampling method is best guided by knowledge of the biology of moth in combination with accepted detection methods,

- The use of pheromone or light traps would be suitable to detect the presence of adults. Some hand sampling of soil (pupae or larvae) or host plants for signs of feeding damage would also be suitable.
- Adults can fly considerable distances, especially when assisted by prevailing winds. Some reports of flight lengths of over 7 km, though much greater distances are possible when wind assisted. Need to consider this.
- Eggs and larvae can be difficult to detect in soil, so attention should be paid to finely inspecting any soil samples taken from surveyed areas suspected of potentially containing eggs or larvae, .
- Moths are relatively easy to see and are reasonably able to be distinguished
- Survey the field and get agronomic as well as metrological information of the production area considering soil moisture, soil texture or drainage characteristics, varieties, topography wind blowing pattern etc. Daily records of weather data from the observatory of the research station or any other nearest location should be collected.
- A standard procedure is to distribute pheromone traps charged with dispensers loaded with a synthetic female sex pheromone blend, at a density of about 2-3 traps per field of about 10-15ropanis. Traps are monitored regularly, four days after the first male is trapped, or during the crop season.
- Take necessary management approach, after 3-4 males are trapped in two consecutive checks. Using a monitoring tool viz; traps and pheromones with an insecticide, detect the presence or absence of adults.
- Place traps over the crop for good ventilation, and put them up as the crop grows.
- Monitored Traps **weekly**, to collect and note down the number of insects captured and verify that traps are still in the correct position.
- Attractants must be renewed every **6 weeks**.
- Once Adults moths detected the first catches is advisable to make a visual estimation by direct sampling in the plants, observing the fruits, and other parts of the plant. Then evaluate the situation on the ground and determine the measures to be taken in each case. It can complete the cycle on the natural vegetation.



In each selected field, select five spots randomly such that four are from four corners and one from the center of the field (refer figure). Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during

General information for farmers and Lentil field:

- The geographical, cropping system and agronomical details relating to the lentil should be collected using the format as given in Annex-1 in the beginning of season

Number of specimen to be collected

- Ideally, it is best to collect multiple specimens from as many life stages as can be found. Adults will in most cases be the easiest to find and collect and are desirable as the adult life stage is the easiest to use for identification.
- Record the host plant, location distance and direction to identifiable landmarks, and other host crops or plants where the specimen has been located.

Collecting plant samples

- Where adult moths are unable to be collected, it is possible that plant material showing feeding damage or with larvae attached may be collected. The likely plant parts to be collected will be leaves where larvae are feeding on these, though they also prefer to bore at the base of plants and into tubers or underground parts.

Preserving plant samples

- Plant samples with immature life stages associated or attached should be packed between dry paper sheets, or (moistened paper for leaves), and sealed in plastic bags. Double bagging is recommended with additional paper also placed in outer bags. Bags should be placed in crush resistant containers for transport.

Management of insect samples

- Adults should be killed by standard methods – freezing, ethyl acetate. Varying concentrations of ethanol for preserving Lepidopteran specimens have been recommended, ranging from 70-95%. Ethanol should be used where morphological identification of adult moths is planned and for future analysis should be stored in absolute ethanol, though acetone has been recommended (Mandrioli *et al.*, 2006). Acetone has the additional advantage of being effective at preserving morphological features of Lepidopteran specimens at room temperature.
- Where taxonomic expertise is readily available and identification can be carried out quickly it may be practical to keep adult moths alive or kill and relax the insect immediately prior to transport. Live insects (any life stage) should not be transported unless it is considered essential, and then such that containers are only opened in containment facilities.

Transporting insect samples

- Vials containing the samples in a preservative should be sealed to avoid leakage and packed in a manner to minimize shock to the vials (i.e. with cushioning material in a strong box). It is important to ensure that vials are filled with preservative so as to remove excess air that through movement of the vial will allow agitation of the preservative and quickly degrade the specimen.

3.3 Time frame and frequency of survey

- Collect a composite sample of plant ,soil no fewer than 8 to 10 soil cores from unhealthy plants and also in healthy plants to see the larval damage problem during the growing season
- Samples should be collected during active growing season especially vegetative growth period, flowering and pod formation period.

3.4 Data collection procedures (field, lab)/ Data preparation and collection (See Table 1-3 and Annex 1-3)

- The plant and soil samples should be forwarded for detailed laboratory testing at diagnostic lab.
- For collection and handling of samples follow NSPM... (Standard Technical Protocols for collection and handling of insect samples)

3.5 Field survey checklist

A pheromone traps, pheromones , garden trowels, digging forks, rubber gloves, plastic bags, a bucket, water-resistant marker pen, record forms, plastic bag, killing bottles.

4 Record keeping

The following information should be recorded:

- Scientific name of the pest
- Common name of the host
- Types of sampling method used e.g. trap, soil sample, sweep net
- Location
- Collection details (i.e. date of collection and name of collector)

Additional information (e.g. first date of detection of the moth , level of infestation, growth stage of plant, symptoms of pest damage, Where samples are collected and identified as part of monitoring survey, details of the identification (e.g. date and name of personnel undertaking initial identification) and verification (e.g. date and name of personnel from verification laboratories) should also be recorded

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 References

CAB International (2007). Crop Protection Compendium. Wallingford, UK. CAB International.

Insect Pests of Lentil and Their Management - ResearchGate. Available from:http://www.researchgate.net/publication/227249632_Insect_Pests_of_Lentil_and_Their_Management [accessed Mar 22, 2015].

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

A **diapause and emergence computer model** (available on the [CottASSIST website](#)) uses current local temperatures to calculate the rate of development of pupae in the soil, and predicts the timing of emergence of moths. This model can also be used to predict the beginning of diapause of *H. armigera*, which can then be used to identify at-risk fields for pupae busting in any given year

List of Tables

Table-1 Field Record Sheet Helocoverpa spp

Helicoverpa moth monitoring:				Day/Week:			
				From:		To:	
Trap No.	Location of trap	Date inspection	Host	Light trap type	No of moths /trap	Species identified	Date recharge

Table-2: Specimen Identification Report

To

.....

.....

(Name and Address of Field Inspector)

Light Trap	Location	Helocoverpa spp. identified	No of Moths

Signature and Date:

Name of Reference Entomologist:

Address for Communication:

Tel.:

Cell No:

Fax:

E-mail:

Table 3: Relative abundance of the Helicoverpa light trap catches and larvae from soil sample taken

Month	Adult population per trap per day	Larvae per soil sample /site
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		

See list of Annexes

Annex I: General Pest Record

Annex II: Monitoring Form at the field level

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Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

16 Survey Protocol for *Stemphyllum* blight (*Stemphyllum botryosum*) of Lentil

1 Introduction

1.1 Background of the Study

Stemphyllum blight disease of lentil is a serious disease of Nepal. It is serious when the environment is wet that is the humidity around the lentil growing is high. The losses caused by this disease could be from 10 to 100 percent. The disease has been reported from all over the world. This survey protocol is prepared to monitor the disease prevalence and also to determine and maintain pest-free status and for pest record documentation. The protocols must be endorsed by NPPO for the implementation by the districts.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production

1.3 Survey area detail

Survey area will be based on need and survey plan of institutions.

- Whole Nepal especially lentil growing area of Nepal of terai and inner terai (as per the requirement of NPPO)
- Lentil growing pocket area or district
- The information with details of site such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1 Pest name including scientific name of pest and family/order

Scientific name of the pathogen is *Stemphyllum botryosum* Wallr.

Order: Family:

English (Comon name): *Stemphyllum* blight of lentil

1.4.2 Life cycle of the pathogen

It survives on crop residue. Prefers warm temperatures (>25C) and a minimum of 8 hours of leaf wetness. High relative humidity (85%) is favorable for disease development. it can be seed transmitted. Researchers have generally agreed that infected plant debris is normally the main

source of primary inoculum and the pathogen can survive for long periods. Srivastava et al. (1996) reported that *S. vesicarium* remained viable for 4 months on diseased plant debris, for 3 months at soil depths of 2.5, 5.0 and 7.5 cm and for 2 months at soil depths of 10 and 15 cm. The burial of the infected plant debris reduced the disease severity in the coming season. However, *S. botryosum* should be able to survive for more than 8 months in Saskatchewan for successful infection in the following spring.

Symptom

Initial symptom on leaves consists of small lesion that enlarge and coalesce each and increase size of the lesion. Later blighting entire leaves and shoot. Thus the name Stemphyllium blight. Pedicels and flowers can also be infected resulting in flower abortion. Symptoms are prominent in the upper canopy, but entire plant can be blighted under favorable environmental condition.

A blighted dull yellow appearance is observed with infected foliage and branches. Defoliation occurs rapidly, leaving the branches with terminal leaves. The stems and branches also bend down, dry up and gradually turn ashy white, but the pods remain green. White mycelia growth can also be observed on the infected stems

The pathogen spreads by air-borne conidia that develop in successive generation on distinct conidiophore on leaf surface.

Images of disease





1.4.3. Dispersal mode

The conidia and ascospores produced on crop debris are responsible for the spread of stemphylium blight. As with other pathogens, the air-borne ascospores are discharged into the air from the protruding pseudothecia.

Plant parts liable to carry the pest in trade/transport as a general (CABI, 2007)

All aerial plant leaves, stems, flowers seeds can carry the pathogen. Seed is the main means of long distance transport from one area to other. Plant parts not known to carry the pest in trade/transport as a general

1.4.4. Target host/alternate host

The host range of the disease is very wide.

S. botryosum can infect many plant species at different environment condition. Host plant other than lentil is spinach, tomato, alfalfa, onion, clover and others. Many countries including neighbouring countries like Bangladesh and India have reported lentil crop devastation by the

pathogen. Therefore; the disease is one of the major constraints for lentil production. The pathogen can infect the lentil crop at any stage. Early infection may result in high yield reduction and loss in quality of seed.

Affected Plant Stages: Flowering stage, fruiting stage, post-harvest, pre-emergence, seedling stage and vegetative growing stage.

Affected Plant Parts: Aerial parts of plants

List of hosts plants

Stemphylium spp. is pathogenic to a wide range of crops in varying geographic regions under diverse environmental conditions. Some of the host crops include cotton (Intergrata et al., 1998), tomato (Diener, 1955), lucerne (Irwin et al., 1991), garlic (Boiteux et al., 1994), mango (Johnson et al., 1990), pear (Montesinos et al., 1995), oilseed rape (reviewed in Singh et al., 1999) and asparagus (Menzies et al., 1992).

1.4.5. Pest distribution

The disease has been reported from all lentils growing area, from cold country like Canada to Argentina, Australia, Bangladesh, Canada, Chile, Colombia, India, Myanmar, Nepal, Pakistan, Spain, Turkey and USA etc.

1.4.6. Transport pathways for long distance movement

- Infected seed and aerial dispersal of conidia in windy condition may carry conidia and infected part of plants to long distanced areas.

2. Purpose of Survey

- To gather information on pest, abundance and distribution pattern, to determine the nature and extent of damage in ginger to support phytosanitary certification
- To ascertain its potential damage and explore effective control measure to minimize the economic loss of commercial lentil crop production

3 Survey Methodology

3.1 Survey area detail

Survey area will be based on need and survey plan of institutions.

- Whole Nepal especially lentil growing area of Nepal of terai and inner terai.
- Select geographic area where rainfall is <400 mm and average temperature during growing period is around 18-20°C.
- Lentil growing pocket area or districts
- As per need of survey institutions.

3.2 Sample design of the study

Two fields in ten selected villages of the lentil growing pockets are to be fixed for pest surveillance. Fixed fields are those grown with lentil, that once selected would be continuously

monitored year round on different growth stages of crop for this using the specified data sheet formats. In addition, a random survey covering eight fields at the rate of one per village among villages other than the ten selected villages for fixed surveillance has also to be done in similar manner.

3.2.1. Selection of fixed fields

Two fields per village at ten village's representative of the agro ecology of the identified region in the same and neighboring district, distributed randomly should be selected. In each village, two fields grown with lentil crop of near to two ropani each are to be fixed (designated as Fixed 1 and Fixed 2) for observations (NICRA, 2011). Assigning fields of farmers as Fixed 1 and Fixed 2 should be made at the time of field and village selection and same should be maintained all through the surveillance period. In case of field destruction due to any reason, almost similar field nearby in the same village has to be used for continuing pest surveillance. Care should be taken to select farmers growing common lentil varieties of the pocket area or region.

3.2.2 General information for fixed fields

The geographical, cropping system and agronomical details relating to each of the Lentil fixed fields designated as Fixed 1 and Fixed 2 should be collected using (Annex IX) once in the beginning of the season.

3.2.3 Specific guidelines for observations in fixed fields

The recording of observations on the diseases in the selected fields should be initiated with the sowing of the crop and continued till the end of the season(s) and throughout the surveillance period. Fill in the details of, village or location name of the field being sampled along with the designated field type and appropriate field number as Fixed 1 and Fixed 2 in the format (Annex IX). In each selected field, select five spots randomly such that four are from four corners and one from the center of the field (refer figure). Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a lentil field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations.



3.2.4 Random field survey

During the survey fields located in the villages having large area under lentil crop, will be selected randomly. Around 7-8 km distance can be maintained between two villages for survey and in each village the observation is to be made in one lentil field using the same data sheet as per the guidelines given earlier for fixed fields.

3.2.5 Guidelines for pest observations

All relevant details of random survey viz., date of survey, GPS coordinates, village name and area of the field sampled should be recorded and the pest observations are to be carried out as per the Annex X for Fixed plots. Eight individual data sheets (Annex XI) should be used for the eight fields across eight villages during random survey. Additional details such as plant protection sprays, fertilizer management, and weather events of the week need not be done for fields of random survey. The general information relating to Annex IX need not be collected for random fields.

3.2.6 Pest observations and frequency

The disease scoring will be done using 1 -9, scale (Pande *et al*, 2006).

Rating Scale	Description (Take from Sarda)
1	No infection on any part of the plant
2	Minute water-soaked lesions on emerging tender leaves, usually not seen
3	Minute water-soaked lesions on 1–5% emerging and upper-most tender leaves, usually seen after careful examination
4	Water-soaked lesions on 6–10% upper-most tender leaves and tender shoots
5	Water-soaked lesions; soft rotting of 11–25% of tender leaves and shoots
6	Water-soaked lesions and soft rotting of 26–40% of top leaves and shoots
7	Soft rotting and fungal growth on 41–55% of the leaves and branches
8	Soft rotting, fungal growth on 56–70% of the leaves, branches, and stems
9	Extensive soft rotting, fungal growth on above 70% of the leaves, branches, and stems

Source: Pande *et al* (2006)

Survey will be done in following crop stage:

- 4 Early seedling stage (VE to V1)
- 5 Late vegetative stage (V5 the first multifoliate leaf has unfolded at the fifth node)
- 6 Early bloom to full bloom (R1 to R2)
- 7 Pod cavity formation stage (R3 to R4)
- 8 Seed formation stage (R5 to R6)

9 Physiological maturity stage (R7 to R8)

3.2.7 Sample collections for laboratory diagnosis

Samples should be kept on open and shade condition for 24 hrs to remove excess moisture if not reached sample in laboratory timely. Use mailing or A4 size nepali/paper envelope to dispatch Collected samples to be reached in diagnosis laboratory as soon as possible but not later than one week.

3.2.8 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

Also see the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 References

Anon, 1993. International rules for seed testing: rules 1993. Seed Science and Technology, 21 (Suppl.):214.

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See list of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

Annex IX: General information of survey of fixed plots

Annex X: Data Sheet for observation in fixed and random fields

Annex XI: Data Sheet for observation for random fields

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

17 Survey Protocol for Grey Mold (*Botrytis cinarea*) in Lentil

1 Introduction

1.1 Background of the Study

Meaning of Botrytis is derived from the Greek word, meaning a bunch of grapes, which describes the appearance of the conidiophore. It is more common where rainfall > 400 mm. Average temperature of 18-20° C and relative humidity of 85% has been reported to favour the development of the disease and is capable of causing serious yield losses in years when spring rainfall is high and/or there are prolonged wet periods. Botrytis grey mould (BGM) has also been reported as a serious problem through out the subcontinent including Bangladesh, Nepal and Pakistan. The optimum temperature for lentil grey mold development is 15-25°C. The protocol assesses the pest population/damage from randomly selected spots representing larger area and also provides information on pest level over large area.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production

1.3 Survey area detail

- Pocket areas: Terai/ inner terai (as per the requirement of NPPO)
- The information with details of site such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1 Pest name including scientific name of pest and family/order

Preferred scientific name: *Botrytis cinarea* Pers.: *Fr [anamorph]*

Order: Helotiales

Family: Sclerotiniaceae

English (Common name):

grey mould-rot; grey mould-rot of crucifers; grey rot; Botrytis rot; bunch rot; slipskin; grey mould, brownish-grey mildew

1.4.2. Life cycle of the pest

Pests overwinters as sclerotia in soil or infected plant tissues, as saprophytic mycelium in dead plant material, or passes the winter on different host plants. Four types of dispersal propagule are important in the epidemiology of the fungus: ascospores, conidia, mycelia and sclerotia.

Microconidia are believed to have only a sexual function and chlamydospores and oidia have unknown and probably unimportant roles.

The mechanism of discharge of ascospores may follow the general pattern of inoperculate discomycetes with 'puffing' and dispersal by air currents. The role of wind-blown and rain-splashed plant debris containing mycelia as dispersal propagules is probably underestimated. Infected petals and senescent flowers are dispersed by wind and rain and provide a large, saprophytically-based inoculum. Mycelia can also be important in latent infections in stored fruits and vegetables, as well as in bulbs and corms.

Sclerotia may germinate producing mycelium, conidiophores and conidia or apothecia. Several environmental conditions influence the differentiation of sclerotia; for example, temperature, nutrition, C:N ratio and light. Sclerotia of *B. cinerea* are not infective structures themselves, but they aid dispersal via the production of spores.

Primary infective structures of *Botrytis cinerea* are macroconidia, which are shortlived, and mycelium. Ascospores may also cause infection. Temperature affects the rate of sporulation. The optimum temperature for spore formation was found to be 15°C while it was 20°C for mycelial growth. At temperatures below 15°C, the rate of sporulation falls sharply and at 10°C it is only 10% of the sporulation at 15°C or even less. Optimum relative humidity for spore production is about 90%, and most spores are produced during the night, provided the temperature is sufficiently high. The excessive use of fertilizers, especially nitrogen, and excessively high canopy density increase the incidence of grey mould.

1.4.3. Dispersal mode

Plant parts liable to carry the pest in trade/transport as a general (CABI, 2007)

- Bulbs/Tubers/Corms/Rhizomes: Spores, Hyphae; borne internally; borne externally; visible to naked eye.
- Fruits (inc. Pods): Spores, Hyphae; borne internally; borne externally; invisible.
- Growing Medium Accompanying Plants: Sclerotia; borne internally; visible under light microscope.
- Flowers/Inflorescences/Cones/Calyx: Spores, Hyphae; borne internally; borne externally; visible to naked eye.
- Leaves: Spores, Hyphae; borne internally; borne externally; visible to naked eye.
- Stems (above Ground)/Shoots/Trunks/Branches: Spores, Hyphae; borne internally; borne externally; visible to naked eye.
- True Seeds (inc. Grain): Hyphae; borne internally; borne externally; invisible.

Plant parts not known to carry the pest in trade/transport as a general

- Bark
- Seedlings/Micropropagated Plants
- Wood.

1.4.4. Target host/alternate host

The host range of the disease is very wide. It is conceivable that *B. fuckeliana* parasitizes well over

200 hosts with no apparent specialization (CABI, 2007).

Affected Plant Stages: Flowering stage, fruiting stage, post-harvest, pre-emergence, seedling stage and vegetative growing stage.

Affected Plant Parts: Leaves and stems.

List of hosts plants

Botrytis cinerea is a non-specialised pathogen well known for its global distribution and extensive host range of more than 100 plant species from different genera including ornamental plants, vegetables, fruit, field and glasshouse crops, several weeds, and post-harvest produce. The host range includes species such as black gram, strawberry, grapevine, apple, cabbage, carrot, cucumber, eggplant, lettuce, lentil, mungbean, mustard, paddy, pea, pepper, pigeonpea, squash, tomato, chrysanthemum, dahlia, lily, rose, gladiolus, and tulip (Chand 1997).

1.4.5. Pest distribution

The disease has been reported from Argentina, Australia, Bangladesh, Canada, Chile, Colombia, India, Myanmar, Nepal, Pakistan, Spain, Turkey and USA etc. The anamorph stage *Botrytis cinerea* has a worldwide distribution. It has been reported in areas ranging from cool-temperate zones of Alaska, Canada and Greenland to subtropical areas such as Egypt, Cameroon and New Zealand. The worldwide distribution and high frequency of the anamorph *B. cinerea* is well established. This disease has been reported to many countries of Europe, Asia, Africa, North America, South America, and Oceania (CABI, 2007).

1.4.6. Transport pathways for long distance movement

- Soil, Gravel, Water, etc.: Sclerotia in agricultural soils.

2. Purpose of Survey

- To gather information on pest, abundance and distribution pattern, to determine the nature and extent of damage in ginger to support phytosanitary certification
- To ascertain its potential damage and explore effective control measure to minimize the economic loss of commercial lentil crop production

3 Survey Methodology

3.1 Survey area detail

Survey area will be based on need and survey plan of institutions.

- Whole Nepal especially lentil growing area of Nepal of terai and inner terai.
- Select geographic area where rainfall is <400 mm and average temperature during growing period is around 18-20⁰C.
- Lentil growing pocket area or districts
- As per need of survey institutions.

3.2 Sample design of the study

Two fields in ten selected villages of the lentil growing pockets are to be fixed for pest surveillance. Fixed fields are those grown with lentil, that once selected would be continuously monitored year round on different growth stages of crop for this using the specified data sheet formats. In addition, a random survey covering eight fields at the rate of one per village among villages other than the ten selected villages for fixed surveillance has also to be done in similar manner.

3.2.1. Selection of fixed fields

Two fields per village at ten village's representative of the agro ecology of the identified region in the same and neighbouring district, distributed randomly should be selected. In each village, two fields grown with lentil crop of near to two ropani each are to be fixed (designated as Fixed 1 and Fixed 2) for observations (NICRA, 2011). Assigning fields of farmers as Fixed 1 and Fixed 2 should be made at the time of field and village selection and same should be maintained all through the surveillance period. In case of field destruction due to any reason, almost similar field nearby in the same village has to be used for continuing pest surveillance. Care should be taken to select farmers growing common lentil varieties of the pocket area or region.

3.2.2 General information for fixed fields

The geographical, cropping system and agronomical details relating to each of the Lentil fixed fields designated as Fixed 1 and Fixed 2 should be collected using (Annexure IX) once in the beginning of the season.

3.2.3 Specific guidelines for observations in fixed fields

The recording of observations on the diseases in the selected fields should be initiated with the sowing of the crop and continued till the end of the season(s) and throughout the surveillance period. Fill in the details of, village or location name of the field being sampled along with the designated field type and appropriate field number as Fixed 1 and Fixed 2 in the format (Annex IX). In each selected field, select five spots randomly such that four are from four corners and one from the center of the field (refer figure). Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a lentil field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations.



3.2.4 Random field survey

During the survey fields located in the villages having large area under lentil crop, will be selected randomly. Around 7-8 km distance can be maintained between two villages for survey

and in each village the observation is to be made in one lentil field using the same data sheet as per the guidelines given earlier for fixed fields.

3.2.5 Guidelines for pest observations

All relevant details of random survey viz., date of survey, GPS coordinates, village name and area of the field sampled should be recorded and the pest observations are to be carried out as per the Annex X for Fixed plots. Eight individual data sheets (Annex XI) should be used for the eight fields across eight villages during random survey. Additional details such as plant protection sprays, fertilizer management, and weather events of the week need not be done for fields of random survey. The general information relating to Annex IX need not be collected for random fields.

3.2.6 Pest observations and frequency (Annex II and III)

The disease scoring will be done using 1 -9, scale (Pande *et al*, 2006).

Rating scale for Botrytis grey mould of Lentil

Rating Scale	Description
1	No infection on any part of the plant
2	Minute water-soaked lesions on emerging tender leaves, usually not seen
3	Minute water-soaked lesions on 1–5% emerging and upper-most tender leaves, usually seen after careful examination
4	Water-soaked lesions on 6–10% upper-most tender leaves and tender shoots
5	Water-soaked lesions; soft rotting of 11–25% of tender leaves and shoots
6	Water-soaked lesions and soft rotting of 26–40% of top leaves and shoots
7	Soft rotting and fungal growth on 41–55% of the leaves and branches
8	Soft rotting, fungal growth on 56–70% of the leaves, branches, and stems
9	Extensive soft rotting, fungal growth on above 70% of the leaves, branches, and stems

Source: Pande *et al* (2006)

Survey will be done in following crop stage:

- Early seedling stage (VE to V1)
- Late vegetative stage (V5 the first multifoliate leaf has unfolded at the fifth node)
- Early bloom to full bloom (R1 to R2)
- Pod cavity formation stage (R3 to R4)

- Seed formation stage (R5 to R6)
- Physiological maturity stage (R7 to R8)

3.2.7 Sample collections for laboratory diagnosis

Samples should be kept on open and shade condition for 24 hrs to remove excess moisture if not reached sample in laboratory timely. Use mailing or A4 size nepali/paper envelope to dispatch Collected samples to be reached in diagnosis laboratory as soon as possible but not later than one week.

3.2.8 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

Also see the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the

results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 References

- Anon, 1993. International rules for seed testing: rules 1993. Seed Science and Technology, 21 (Suppl.):214.
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See list of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

Annex IX: General information of survey of fixed plots

Annex X: Data Sheet for observation in fixed and random fields

Annex XI: Data Sheet for observation for random fields

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

Colour plates



Plate 1. BGM in stem



Plate 2. BGM in stem



Plate 3. BGM in pods

18 Survey Protocol for *Fusarium oxysporum* sp. *lentis* in Lentil

1 Introduction

1.1 Background of the Study

This survey protocol is prepared to monitor the disease prevalence and also to determine and maintain pest-free status and for pest record documentation. The protocols must be endorsed by NPPO for the implementation by the districts.

F. oxysporum f. sp. *lentis* only infects the cultivated crop of lentil (*Lens culinaris* spp. *Culinaris*).

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production

1.3 Survey area detail

Lentil pocket areas: Terai/ inner terai (as per the requirement of NPPO)

The information with details of site such as a GPS reading (latitude, longitude, and altitude), location (Village, ward no, VDC, district), topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded (See Appendix No.: 2).

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1.4.1. Pest name including scientific name of pest and family/order

Order: Hypocreales, *Family*: Nectriaceae, *Genus*: *Fusarium*, *Species*: *oxysporum*

1.4.2. Life cycle of the pest

Entry is either direct, through wounds, or opportunistic at the point of formation of lateral roots. The root tips of healthy plants growing in contaminated soil are penetrated by the germ tube of spores or fungal mycelium. The mycelium takes an intercellular path through the cortex, and enters xylem vessels through the pits. The pathogen is primarily confined to the xylem vessels in which the mycelium branches and produces microconidia. The microconidia detach and are carried upward in the vascular system until movement is stopped, at which point they germinate and the mycelium penetrates the wall of the adjacent vessel. Lateral movement between vessels is through the pits. It then spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed. The water economy of infected plants is eventually severely compromised by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, often followed by death of the whole plant. The fungus then invades all

tissues of the plant, to reach the surface where it sporulates profusely. Spores may then be dispersed by wind and water or movement of soil or plant debris. *F. oxysporum* can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for more than 6 years. Chlamydospores can survive in soil either in dormant form or saprophytically for without a suitable host. The disease is favoured by warm and dry soil conditions with an optimal temperature of 22-25°C.

Adult wilt symptoms usually appear from flowering to late pod-filling stage and are characterized by sudden drooping of top leaflets of the affected plant, leaflet closure without premature shedding, dull green foliage followed by wilting of the whole plant or of individual branches. Seeds from plants affected in mid-pod-fill to late pod-fill are often shrivelled.

1.4.3. Dispersal mode

The movement of infected seed plays an important role in the long distance dispersal and transmission of fusarium wilt diseases into new areas.

Transmission of the fungus occurs primarily through plant debris and soil contamination, where it infects the plant through the root system.

1.4.4. Target host/alternate host/host range

lentil/ *Vicia montbretii* (vetch)

1.4.5. Pest distribution

Widely distributed in warm and tropical climates of the country

1.4.6. Pathways for introduction (possible pathways of spread)

Although the fungus may be introduced by several pathways (seedlings, insect vectors), the most important risk of introduction is by seed

2 Purpose of Survey

The focus of the survey is to determine the distribution of pest through monitoring survey for pest record documentation, maintaining pest free areas and also for planning pest management programs.

3 Survey Methodology

3.1 Sample design of the study

In each selected site, select five spots randomly such that four are from four corners and one from the center of the field. Five feet distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a lentil field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations.

3.2. Sampling unit, sample frame, and population

For plant sampling, minimum fifty plants taken at random for each site, consisting of lentil roots and stems, should be collected. Seven to eight tissue pieces per sample, derived from the lower stem and a variety of root sizes should be sampled. Samples should also be carried out on 100 seeds per sampling unit harvested from infected crops for disease incidence.

3.3 Sample selection and size

- Random selection
- 3-10 sampling point per sample site in field
- 10 plant per sampling point

At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations. Data collection in field is better at last reproductive stage to maturation stage of crop.

Samples should be initially collected over a representative area of the infected crop to determine the disease distribution. The disease may appear as hotspots" or patches within the crop or may have developed along fence lines or drainage lines. Depending on the stage of infection the symptoms may appear as:

Small patches of yellowing (dying) plants scattered throughout the crop

Larger patches of yellowing and dying throughout the crop

Patches of yellowing and dying plants along fence or drainage lines from adjacent paddocks

- Samples should be collected that represent a range of symptoms observed in the infected crop
- Plants should be assessed for yellowing, stunting, wilt and plant death.
- Lower stems should be broken or sliced to reveal vascular discoloration in case plants not showing external symptoms.
- Suspect stunted and wilted plants should be carefully removed from the soil so that the roots can be checked for reduced growth without external fungal growth.
- Field soil (5Kg.) from affected crops should also be collected as this will also harbour *F. oxysporum*.
- Samples should be processed as quickly as possible after sampling from the field. Once removed from the field, fresh plant samples can deteriorate and become contaminated by other mould fungi and bacteria.
- Samples should be placed into polythene bags and appropriate labeled to indicate location, sample number and date of collection.

3.4 Time frame and frequency of survey

The recording of observations on the diseases in the selected fields should be done mainly in following crop stage:

- Reproductive stage

- Physiological maturity stage

3.5 Samples Collection

Samples should be treated in a manner that allows them to arrive at the laboratory in a fresh, well-preserved state. Samples should be wrapped in damp newspaper, bundled into a plastic bag and clearly labeled.

For collection and handling of diseased samples see Appendix 4 (Standard Technical Protocols for collection and handling of disease samples)

3.6. Data collection procedures (field, lab)/ Data preparation and collection

Follow up samples should be forwarded to the diagnostic laboratories for processing as per Annex IV. A pathogenicity test on lentil is necessary to confirm *F. oxysporum* f. sp. *lentis*.

The field data of plants, soil and seed survey should be recorded as per the format prescribed in Annex III

For collection and handling of diseased samples follow Appendix 4 (Standard Technical Protocols for collection and handling of disease samples)

3.7. Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils
- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

Also see the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

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

- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6 Surveyors (Organization/Personal)

6.1 Date of collection and name of collector-As designated by DADO

6.2 Date of identification and name of identifier- Trained PPO

6.3 Date of verification and name of verifier- Designated by NPPO

7 References

Fusarium wilt (of chickpea, lentil & lupin) *Fusarium oxysporum* f. sp. *ciceris*, *F. oxysporum* f. sp. *lentis*, *F. oxysporum* f. sp. *lupini* (2009) - Prepared by Kurt Lindbeck and Plant Health Australia

ISPM 27. 2006. Diagnostic Protocols for Regulated Pests. Rome, IPPC, FAO.

List of commodities having National pest status record of Nepal (2005) - PPD, NPQP, Nepal

Table 1. Field Data Recording Form (Lentil wilt Incidence)

Date	Area/ Location	Stage of crop	Plants part effected	No of plants sampled	Soil samples size (kg)	Seed samples size (kg)	Surveyor name & sign

See list of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

19 Survey Protocol for Stem rot (*Sclerotinia sclerotiorum*) in Lentil

1.Introduction

1.1 Background of the Study

Sclerotinia sclerotiorum is a plant pathogenic fungus and can cause a disease called white mold if conditions are correct. *S. sclerotiorum* can also be known as cottony rot, watery soft rot, stem rot, drop, crown rot and blossom blight (Agrios 546). A key characteristic of this pathogen is its ability to produce black resting structures known as sclerotia and white fuzzy growths of mycelium on the plant it infects. Sclerotia are hard, asexual, resting structures which can survive for years in soil. *Sclerotinia sclerotiorum* (Lib.) de Bary is a facultative parasitic Ascomycete fungus (Kirk et al., 2001), and can grow well even in an unfavorable environment and survive for up to 8 years in soil in the sclerotia form (Adams and Ayers, 1979). These sclerotia give rise to a fruiting body in the spring that produces spores in a sac which is why fungi in this class are called sac fungi Ascomycetes. When *S. sclerotiorum* is onset in the field by favorable environmental conditions, losses can be great and control measures should be considered. The protocol provides the steps on correct identification, monitoring, economic thresholds and choice of optimum pest control option.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production

1.3 Survey area detail

The information with details of site such as a GPS reading (latitude, longitude, altitude), location (Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil type etc), Meteorological (temperature, rainfall, hailstone etc) should be recorded.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

1 .4.1. Pest name including scientific name of pest and family/order

Common name: White blight of mustard/wilt/collar rot/white blight/stem rot of lentil

Casual Organism: *Sclerotinia sclerotiorum* (Lib.) de Ba

1.4.2. Life cycle of the pest

The lifecycle of *Sclerotinia sclerotiorum* can be described as monocyclic, as there are no secondary inoculums produced. Optimal temperatures for growth range from 15 to 21 degrees Celsius. Under wet conditions, *S. sclerotiorum* will produce an abundance of mycelium and Sclerotia. The fungus can survive in the soil mainly on the previous year's plant debris. During

late summer/ early fall the fungus will produce a survival structure called a sclerotium either on or inside the tissues of a host plant. The following spring the dormant sclerotia will germinate to produce fruiting bodies called apothecia, which are small, thin stalks ending with a cup-like structure about 5-15mm in diameter (similar in shape to a small mushroom). The cup of the apothecium is lined with asci, in which the ascospores are contained. *S. sclerotiorum* is capable of invading nearly all tissue types including stems, foliage, flowers, fruits, and roots. Eventually white, fluffy mycelium will begin to grow on the surface of the infected tissues. At the end of the growing season, *S. sclerotiorum* will once again produce sclerotia. The sclerotia will then remain on the surface of the ground or in the soil, on either living or dead plant parts until the next season. The lifecycle will then continue respectively. Like most fungi, *S. sclerotiorum* prefers darker, shadier conditions as opposed to direct exposure to sunlight.

1.4.3. Dispersal mode

When the ascospores are released from the asci, they are carried by the wind until they land on a suitable host. The ascospores will then germinate on the host and begin to invade the host's tissues via mycelium, causing infection

1.4.4. Target host/alternate host

S. sclerotiorum is a polyphagous pathogen. It has an extremely wide host range, including horticultural crops, field crops, cereals, forage crops, medicinal plants, fruit trees and weeds. Early records showed that the pathogen attacks higher plants in 64 families, 225 genera and 383 species (reviewed in Purdy, 1979). The host range record increased to 71 families and 396 species by 1982 (Wu, 1982), and to 75 families, 278 genera and 408 species by 1994 (Boland and Hall, 1994). In Taiwan, *S. sclerotiorum* was found on at least 111 plant species (Wu, 1982). Common hosts of white mold are herbaceous, succulent plants, particularly flowers and vegetables. It can also affect woody ornamentals occasionally, usually on juvenile tissue.

1.4 5 Symptoms Based Diagnosis

White mold can affect their hosts at any stage of growth, including seedlings, mature plants, and harvested products. It can usually be found on tissues with high water content and in close proximity to the soil. One of the first symptoms noticed is an obvious area of white, fluffy mycelial growth. Usually this is preceded by pale to dark brown lesions on the stem at the soil line. The mycelium then covers this necrotic area. Once the xylem is affected, other symptoms occur higher up in the plant. These can include chlorosis, wilting, leaf drop, and death quickly follows. On fruits, the initial dark lesions occur on the tissue that comes in contact with the soil. Next, white fungal mycelium covers the fruit and it decays. This can occur when the fruit is in the field or when in storage.

The fungus infected old leaves firstly and expanded rapidly through the petiole into the stem, causing water-soaked lesions. The upper leaves are subsequently infected until the whole plant finally died off. The most obvious symptoms of plants infected by *S. sclerotiorum* are necrotic tissues covered with patches of fluffy white mycelia, and sclerotia are produced after mycelial growth when the nutrition is not sufficient or other conditions are favourable for sclerotial development (Christias and Lockwood, 1973).

1.4.6 Pest distribution

S. sclerotiorum is geographically cosmopolitan and has a broad ecological distribution, though it is most common in temperate regions. It was originally believed to occur only in cool, moist areas, but is now known to occur in hot, dry areas as well.

1.4.7 Pathways for introduction (possible pathways of spread)

Air-borne ascospores are the most important means of spread. Mycelium from sclerotia can also cause infection, but usually this mode of infection stays within an area. Moving contaminated soil (on farm equipment, shoes, infected seedlings) and fertilizing with manure from animals fed infected plant debris, are two common ways of spreading sclerotia or mycelium from place to place. Irrigation also has been shown to be involved in the spread of *Sclerotia* spp. from field to field. Irrigation transported sclerotia remained viable for at least 10-21 days in flowing water. In addition, seed may be an infective source, either from contaminating sclerotia or internal mycelium. However, support for this method of transmission is questionable.

2. Purpose of Survey

Purpose of survey should be identified first. The reason for surveys identifies the purpose. Purpose may be

1. Monitor and detect the target pest
2. Determination of pest status
3. Estimation of changes in insect distribution and abundance
4. Obtain data to support PFAs, PFPP or PFPS
5. Requirements for the establishment of areas of low pest prevalence.

3. Survey Methodology

3.1 Collecting plant samples

Examine the entire plant for symptoms - Examine all of the main plant organs for disease symptoms: roots, stems, leaves, and blossoms.

- Collect several plant specimens
- Do not collect dead plants or plant organs
- Use plastic bags - For most samples including leaves, stems and roots, use plastic bags to prevent plant samples from drying out during transport.

3.2 Time of Survey – When the crop stand in the field

3.3 Area to be surveyed

- Parts to be observed: Stems, leaves, whole plant, root
- Ways to collecting samples:
- Frequencies of observations:

- Sample dispatch:
- Sample verification:
- Preservation of collected specimens:

3.4 Isolation of Pathogen

Isolating pathogens from any diseased plant tissue in contact with soil presents the additional problems of numerous saprophytic organisms invading the plant tissue after it has been killed by the pathogen. For this reason, the first step in isolating the pathogen is repeated thorough washing of such diseased tissues to remove all soil and most of the loose, decayed plant tissue in which most of the saprophytes are present. If the diseased root is small, once it is washed thoroughly, pathogens can be isolated from it. If isolation is attempted from fleshy roots or other fleshy tissues penetrated only slightly by the pathogen and showing only surface lesions, the tissue is washed free from adhering soil, and several bits of tissue from the margin of the lesions are placed in Clorox solution. The tissue sections are picked from the solution one by one, blotted or washed in sterile water, and placed on agar in petri plates.

To conduct pathogen identification and analysis, the fungus isolated from infected tissues of lentil and cultured on PDA medium. The fungus started to produce white masses when growing to the edge of the Petri dish. The size of mycelium masses became bigger and their colors became darker as time proceeded.

3.5 Fungal isolation and purification

Standard procedure by Fang (1998) is followed a for fungal isolation and purification. The infected tissues of sample are collected from fields, cut into small pieces, and then rinsed 3~4 times with diluted water after treated with 70% (v/v) ethanol for 2~3 s and with 0.1% (w/v) mercuric chloride solution for 3~5 min. The treated tissues are transferred to potato dextrose agar (PDA) medium and cultured at 25 °C. to confirm pathogenicity of the isolate, the pure culture is reinoculated onto host plants. When actively growing on PDA plates, mycelium-agar plugs are excised from the margin of the fungal colony and used to inoculate host leaves. Inoculated leaves are then incubated in a growth chamber at 23~25 °C with 100% relative humidity until observation.

3.6 Treatment of sclerotia at low temperature to induce apothecium development

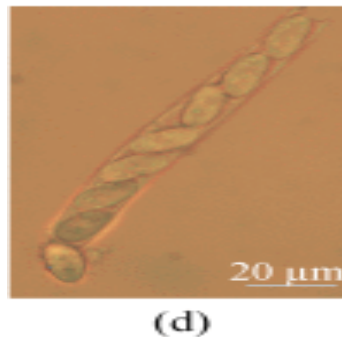
The sclerotia produced on PDA medium are collected, placed in moist sands evenly, and then cultured in 4 °C for 6~8 weeks and left in place at 15~20 °C under scattered light until the apothecia developed (Smith and Boland, 1989).

3.7 Microscopic observation of ascospore morphology

Apothecia are cut into thin slices and stained with trypan blue solution over night to observe the morphology of asci and ascospores, or stained with 0.5 µg/L ethidium bromide (EB) for 30 min to observe nuclei, or stained with 10 µg/ml Calcofluor white solution for 5 min to observe cell walls and septa between cells, then rinsed with sterile water, and photographed with visible, ultraviolet light and fluorescence under an Olympus microscope, respectively.

3.8 Morphology of asci and ascospores

Mycelia of *S. sclerotiorum* (Lib.) de Bary in host plants or in culture look hyaline, septate, branched and multinucleate, and their colors changed from white to dark as melanin accumulates. It cannot produce conidiophore during the asexual period. Hyphae tend to form sclerotia (Kirk et al., 2001). These are also the characteristics of isolate. Further observation on sclerotia germination and apothecia production confirms that there are three stages during the course of sclerotial development (Chet and Henis, 1975; Bolton et al., 2005). At initiation stage, hyphae aggregate to form a white mass, then further aggregate to increase the size of sclerotia; finally, surfaces of sclerotia are delimited, with melanin deposited in peripheral rind cells, and interiors of sclerotia become consolidating. Each sclerotium can produce one or more apothecia consisting of a stipe and a receptacle with a flat to convave hymenial layer (2~10 mm in diameter). Asci are cylindrical sac-like zygot cells and are rowed in the hymenial layer. Each ascus contains eight hyaline, ellipsoid binucleate ascospores [(4~6) μm ×(9~14) μm]. The morphology and development of our isolate were similar to those of *S. sclerotiorum* as described by Kohn (1979).



3.9 Observation: Visual observation and lab analysis of suspected samples

3.10 Field survey checklist

Prior to starting field survey work, field survey teams should have following:

- Ensure that the survey team has all items on the check-list before departure
- Pre-printed field survey forms (sufficient number plus spares)
- Pencils (at least 3 per team member)
- Permanent marker pen
- Plastic containers for temporary storage and transportation.
- Polythene bags for storing plant material,
- Record forms and killing bottles

Also see the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements

6 Surveyors (Organization/Personal)

6.1 Sample collector – As designated by DADO

6.2 Identifier – Trained PPO

6.3 Verifier – Designated by NPPO

6.4 Recording – RPPL, NARC Laboratory

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See list of Annexes

Annex I: General Pest Record

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Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

20 Survey Protocol for *Rhizoctonia solani* of lentil

1. Introduction

1.1 Background of the Study

Lentil is the Nepal's third largest exportable commodity during the year 2009-10 (TEPC 2011). *Rhizoctonia solani* (teleomorph = *Thanatephorus cucumeris* Frank (Donk)) is one of the most damaging important disease of lentil. The fungus is usually recovered from soils all over the world and is considered as a very destructive plant pathogen, with a broad host range, and causes diseases in a great variety of crops, including agronomical, ornamental and forestry species (Anderson, 1982; Sneh et al., 1991). For the good crop production and expansion of the export market it is essential to monitor the pest distribution. Surveillance is carried out to gather information that provides the basis for pest listing, documentation of pest status, pest occurrence or absence supporting phytosanitary certification and the designing the future pest management strategies and enabling Pest Surveillance is primary objective of designing the survey protocol. This activity also support to collect information required for maintaining pest records. Present protocol is designed based on review on published materials both at national and international research periodicals. This document outlines recommendations for general protocols and procedures for surveillance emphasizing on factors such as host, pest biology and epidemiology, potential impact to the lentil commercialization, pathway assessment.

1.2 Target commodity detail

See section 1.2: Target commodity detail of Survey Protocol for Cowpea Aphid, *Aphis craccivora* Koch. in Commercial Lentil Production

1.3 Survey area detail

There are usually different step for survey area selection. There is no single best method for site selection. The main point is transparently document the choice and reason for such choice. The method can then be considered and discussed with the basis of the choice, given the circumstances.

- Select site according to development region, districts, VDCs, field site, sampling site and sampling point.
- Use one or a combination of random sampling, systematic sampling, stratification sampling, targeted sampling for site selection
- The location and unique identifying details of each site should be recorded. Describing the sampling site would include information such as a GPS reading(latitude, longitude, altitude), location(Village, ward no, VDC, district) , topographical feature (facing, sloppiness, vegetation type, soil tye etc), Meterological (tempeture, rainfall, hailstone etc)

Survey area will be based on need and survey plan of institutions.

- Whole Nepal especially lentil growing area of Nepal of terai and inner terai.
- Select geographic area where rainfall is <400 mm and average temperature during growing period is around 18-20⁰C.
- Lentil growing pocket area or districts
- As per need of survey institutions.

1.4 Pest Information

Brief information is provided on the pest, including, where appropriate, taxonomic, its life cycle, morphology, host range (in general), geographical distribution (in general), mode of transmission and dissemination.

Rhizoctonia solani, the most widely recognized species of *Rhizoctonia* was originally described by Julius Kühn on potato in 1858. *Rhizoctonia solani* is a basidiomycete fungus that does not produce any asexual spores (called conidia) and only occasionally will the fungus produce sexual spores (basidiospores). In nature, *R. solani* reproduces asexually and exists primarily as vegetative mycelium and/or sclerotia. Unlike many basidiomycete fungi, the basidiospores are not enclosed in a fleshy, fruiting body or mushroom. The sexual fruiting structures and basidiospores (i.e. teleomorph) were first observed and described in detail by Prillieux and Delacroix in 1891. The sexual stage of *R. solani* has undergone several name changes since 1891, but is now known as *Thanatephorus cucumeris*.

1.4.1 Preferred scientific name

Preferred scientific name: *Thanatephorus cucumeris* (Frank) Donk [teleomorph]

Order: Ceratobasidiales

Family: Ceratobasidiaceae

Common names

There are many names of this disease depending on host. Like sheath blight of rice, web blight (legumes, ornamentals), rice sheath blight, rhizoctonia, , head rot, rhizoctonia leaf blight, damping-off, root rot, stem canker (potato, sweet potato), bare patch (tulips, cereals), crater disease (cereals), crown and root rot (sugar beet), collar rot, leaf blight (of rice), black scurf and stem rot (potato), fruit rot, bottom rot of lettuce, leaf blight and tuber soft rot (yam), areolate leaf spot, stem blight of rice sclerotial blight of rice, black speck of potato, bordered rice sheath spot, foot rot, sharp cereal eyespot, sore shin of tobacco, bulb rot (ornamentals), crown bud rot (alfalfa), stem rot, basal stem rot (soyabeans), seed rot (cotton, lupins, peanuts), leaf blight (alfalfa, soyabeans, rape, mustard), seedling blight (alfalfa, clover, vegetables), wirestem (cotton) spear tip (wheat), root canker (alfalfa), black leg of sugar beet, basal stem rot (soybeans) basal rot.

1.4.2 Life cycle of the pest

Rhizoctonia solani can survive for many years by producing small (1 to 3-mm diameter), irregular-shaped, brown to black structures (called sclerotia) in soil and on plant tissue. Certain pathogens of *R. solani*, have evolved the ability to produce sclerotia with a thick outer layer that allows them to float and survive in water. *R. solani* also survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity. Sclerotia and/or mycelium present in soil and/or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of food and fiber crops.

The fungus is attracted to the plant by chemical stimulants released by actively growing plant cells and/or decomposing plant residues. As the attraction process proceeds, the fungal hypha will come in contact with the plant and become attached to its external surface. After attachment,

the fungus continues to grow on the external surface of the plant and will causes disease by producing a specialized infection structure (either an appresorium or infection cushion) that penetrates the plant cell and releases nutrients for continued fungal growth and development. The infection process is promoted by the production of many different extracellular enzymes that degrade various components of plant cell walls (e.g. cellulose, cutin and pectin). As the fungus kills the plant cells, the hyphae continue to grow and colonize dead tissue, often forming sclerotia. New inoculum is produced on or in host tissue, and a new cycle is repeated when new substrates become available.

1.4.3 Favorable Environment

The pathogen is known to prefer warm wet weather, and outbreaks typically occur in the early summer months. Most symptoms of the pathogen do not occur until late summer. A combination of environmental factors has been link to the prevalence of the pathogen such as: presence of host plant, frequent rainfall/irrigation and increased temperatures in spring and summer. In addition, a reduction of drainage, create favorable environments for the pathogen.http://en.wikipedia.org/wiki/Rhizoctonia_solani - cite note-10

1.4.4 Dispersal mode

The pathogen is dispersed as sclerotia, and these sclerotia can travel by means of wind, water or soil movement between host plants.

1.4.5 Target host/alternate host

Affected Plant Stages: Flowering stage, fruiting stage, post-harvest, pre-emergence, seedling stage and vegetative growing stage.

Affected Plant Parts: Fruits/pods, growing points, inflorescence, leaves, roots, seeds, stems and whole plant.

Target host: *Lens culinaris* ssp. *culinaris*

Other Major hosts: *Acacia nilotica* , *Albizia lebbeck* , *Arachis hypogaea* , *Azadirachta indica*, *Beta vulgaris* var. *saccharifera*, *Brassica napus* var. *napus*, *Brassica oleracea* var. *botrytis*, *Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *gemmifera*, *Brassica oleracea* var. *italica*, *Brassica rapa* subsp. *rapa*, *Brassicaceae*, *Capsicum annuum*, *Chenopodiaceae* , *Citrus* , *Corchorus olitorius* , *Cucumis sativus*, *Cucurbitaceae* , *Daucus carota*, *Elaeis guineensis* , *Euphorbia pulcherrima* , *Glycine max*, *Gossypium*, *Hordeum vulgare*, *Lactuca sativa*, *Lupinus*, *Lycopersicon esculentum* , *Manihot esculenta*, *Medicago sativa* (lucerne), *Ocimum basilicum*, *Oryza sativa*, *Oxalis tuberosa* , *Phaseolus*, *Poaceae* (grasses), *Raphanus sativus*, *Solanum melongena*, *Solanum tuberosum*, *Sorghum bicolor*, *Trifolium*, *Triticum aestivum*, *Tulipa*, *Ullucus tuberosus*, *Vigna radiata*, *Vigna unguiculata*, *Zea mays*, *Ziziphus mauritiana*

Minor hosts: *Chenopodium quinoa* (quinoa), *Pelargonium* (pelargoniums), turfgrasses , Wild hosts, *Azolla pinnata* (mosquito fern), *Lolium multiflorum* (Italian ryegrass)

1.4.6 Pest distribution

T. cucumeris (as *Rhizoctonia solani*) is extremely widespread and probably occurs throughout the world. It is doubtful whether any country is totally free from this disease. It is probably

present in all arable soils and has been isolated from virgin soils. Little is known of the distribution of individual strains (Mordue, 1974). Note that within this Compendium the distribution of any plant in the database can be superimposed on that of *T. cucumeris*. Because this pathogen affects so many hosts and is so widespread, overlaying the distribution of a particular host with that of the pathogen may indicate the potential range of the disease.

1.4.7 Pathways for introduction (possible pathways of spread)

- Irrigation water
- Soil and plant debris
- Farm machinery and tools
- Seed and air

2 Purpose of Survey

- Detection of pest
- Determination of pest status
- Obtain data to support PFAs, PFPP or PFPS
- Guideline for pest eradication
- Requirements for the establishment of areas of low pest prevalence.

3 Survey Methodology

3.1 Sample design of the study

3.1.1 Soil sampling and isolation

Soil sampling is done from different locations of survey site in sufficient number. Samples (500 mL) were taken from the top 15 cm of non-tilled fields prior to planting. Each sample combined at least 30 soil cores taken randomly from across the field. Information recorded at sampling included the soil type, crop, previous crop and date of sampling, as well as the geo-coordinates of the location.

Rhizoctonia isolates were obtained from the soil by using a modified baiting method as described by Paulitz & Schroeder (2005). Dry soil samples were moistened with sterile water prior to starting with the isolation procedure. Autoclaved wheat and barley grains and wooden toothpicks were placed as bait into 14 cm diameter Petri dishes containing the soil samples and incubated in darkness at room temperature for 2 days. Cereal baits were chosen because the objective of this study was to detect *Rhizoctonia* relevant to cereals. After incubation, baits were transferred from the soil onto Petri dishes (9 cm diameter, without vents) with ANS-agar (50 mg L⁻¹ each of aureomycin, neomycin and streptomycin; Sigma-Aldrich) and incubated in darkness at room temperature in order to stimulate mycelial growth from the baits. After 24 h, single hyphae were cut from the agar as mycelial plugs under sterile conditions and transferred onto PDA (potato dextrose agar: 39 g L⁻¹ PDA in sterile demineralized water; Merck) for multiplication. *Rhizoctonia* was distinguished both macroscopically and microscopically from other fungi by the typical hyphal morphology.

3.1.2 Plant sampling

Plant sampling is depends upon the purpose of survey. In each selected field, select five spots randomly such that four are from four corners and one from the center of the field. Five feet

distance alongside of boundary of the field in all directions should be left out as buffer space during observations. The spot selection for pest observations during each visit should be random and it is not the fixed spots in a lentil field that would be sampled continuously. At each spot select 10 plants adjacent to each other for observations relating to all and whole plant observations.

3.1.3. Sample collection for laboratory diagnosis

Samples should be kept on open and shade condition for 24 hrs to remove excess moisture if not reached sample in laboratory timely. Use mailing or A4 size nepali/paper envelope to dispatch Collected samples to be reached in diagnosis laboratory as soon as possible but not later than one week. Examine the entire plant for symptoms - Examine all of the main plant organs for disease symptoms: roots, stems, leaves, and blossoms. Collect several plant specimens. Do not collect dead plants or plant organs.

3.1.4. Sample selection and size

- Random selection
- Minimum 3 sampling point per sample site
- 10 plant per sampling point

3.2. Time frame and frequency of survey

The recording of observations on the diseases in the selected fields should be initiated with the sowing of the crop and continued till the end of the season(s) and throughout the surveillance period. Survey will be done in following crop stage:

- 4 Early seedling stage
- 5 Late vegetative stage
- 6 Early bloom to full bloom
- 7 Pod cavity formation stage
- 8 Seed formation stage
- 9 Physiological maturity stage

3.3. Disease Scoring

Index value	Incidence
0	No incidence
1	< 1%
3	1 - 5%
5	6 - 25%
7	26 - 50%
9	51 - 100%

3.4 Field survey checklist

- Pre-printed field survey forms (sufficient number plus spares)
- Pencils

- Permanent marker pen
- Newspapers for wrapping plant samples
- Polythene bags for storing samples

Also see the Field Survey Checklist of Survey Protocol for *Culvularia* spp. in Ginger

4 Records

This section provides information on the records that should be kept:

- scientific name of pest identified
- code or reference number of the sample (for traceability)
- nature of the infested material including scientific name of host where applicable
- origin (including the geographic location if known) of the infested material, and location of interception or detection
- description of signs or symptoms (including photographs where relevant), or their absence
- methods used in the diagnosis and the results obtained with each method
- for morphological or morphometric methods, measurements, drawings or photographs of the diagnostic features (where relevant) and, if applicable, an indication of the developmental stage(s)
- where appropriate, the magnitude of any infestation (how many individual pests found, how much damaged tissue)
- the name of the laboratory and , where appropriate, the name of the person(s) responsible for and/or who performed the diagnosis
- dates of collection of the sample, and of detection and identification of the pest
- where appropriate, state of the pest, alive or dead, or viability of its development stages.

Evidence such as culture(s) of the pest, preserved/mounted specimens should be retained in particular in cases of non-compliance (ISPM 13 :2001) and where pests are found for the first time (ISPM17:2002). Additional items may be required under other ISPMs such as ISPM 8 :1998. The period for which records should be kept depends on the purpose for which a diagnosis is made. In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results of the diagnosis should be retained for at least one year.

5 Reporting

Report content need to be designed from the beginning considering the ISPM 6. Guidelines for Surveillance (1997) and also the objective of the survey requirements.

6. Surveyors (Organization/Personal)

- 6.1 Date of collection and name of collector as designated by DADO
- 6.2 Date of identification and name of identifier Trained PPO

6.3 Date of verification and name of verifier Designated by NPPO

7. References

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V. González García¹, M. A. Portal Onco² and V. Rubio Susan³ Review. Biology and Systematics of the form genus Rhizoctonia ,Spanish Journal of Agricultural Research (2006) 4(1), 55-79

Table 1:Field Data Recording Form (Soil Survey)

S. No.	Date	Name of the Farm	Field Area (ha)	standing crop	Qty of soil sampled (gm)	Sign of collector

See list of Annexes

Annex I: General Pest Record

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{

Annex IV: Specimen Forwarded for identification by Diagnostic/Referral Laboratory

See list of Appendix

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

21 List of Annexes

Annex I: General Pest Record

Reference Number	
Scientific name of pest <ul style="list-style-type: none"> • Common Name: • Species Name • Family • Order 	
Life stage of pest	Egg [] Larvae(Maggots) [] Pupae [] Adult [] For disease:
Scientific name of pest <ul style="list-style-type: none"> • Variety: • Common Name: • Species Name; • Family: 	
Plant parts affected	
Stage of crop	Early seedling stage [] Vegetative stage [] Physiological maturity stage []
Locality <ul style="list-style-type: none"> • Village • District 	
Date of Collection	
Name of the Collector	
Date of Identification	
Name of the Identifier	
Date of Verification	
Name of the Verifier	

Annex II: Monitoring Form at the field level

Surveyor name: _____

Country/Institution: _____

Date of survey (dd/mm/yy):

Location name:

Latitude (decimal degrees): **N S**

		.						
--	--	---	--	--	--	--	--	--

Longitude (decimal degrees): **E W**

		.						
--	--	---	--	--	--	--	--	--

Elevation: _____ meters

Survey site: Farmer field or others

Name of the Farm:

Growth stage:

Field area size: _____ ha

Variety: _____

S.N.	No inspected (Infected parts of the plant)	No of larvae/maggots	Sign of Recorder

Infestation Level	L	M	H	None
Incidence at field level				
Severity (on infected plants)				

L (low) = 1-20% M (moderate) = 20-40% H (high)= more than 40%

Annex III: ;e]{sf nflu ;fdfGo tYof+s ;+sng kmd{
lkmN8 8f6f l;6

!= c]nf]sg ;DjGwL ;fdfGo hfgsf/L		
s_ c]nf]sg ul/Psf] ldl		
v_ ;e]{÷lkmN8 Kn6 g+=, lf]=km=		
@= lhlkP; l/k m]G; g+=	clff+; M	
	b]zfGt/ M	
	prfO{ M	
#= :yfg	ufpFm / j8f g+=	
	uf= j=;	
	lhNnf	
\$= ;f] 7fp+sf] xfjfkfgL	cf}ift Go"gt d tfkqmd - ;]=__	
	cf}ift clwstd tfkqmd - ;]=__	
	jiff{ -ld=ld=__	
%= df6f]sf] lsl;d		
^=hldg – l;+lrt÷cl;+lrt		
&= jfnLsf] pd]/÷cj:yf		
*= dnvfb / /;folgs dnsf] k[of]u	5÷5}g olb 5 eg] M uf]7]dn jf sDkf]:6 M ===== o"l/ofsf] dfqf M ===== l8=P=lk= M ===== kf]6f; M =====	
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c_ /f]u /sL/f sf] k[sf]k	z'?sf] cj:yf < % Ü	

	dWod cj:yf %—@% Ü w]/} k efljt >@% Ü	
cf_ k sf]ksf] k'j{ Oltxf;		
O_ pkrf/sf lalw cjnDjg ul/Psf eP		
!!=j}slNks cf>obftf lj?jf÷ k hftL		
!@= cGo s]xL eP		
!#= ;+snssf] gfd		

Annex IV: 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:	<p>* <input type="checkbox"/> roots; <input type="checkbox"/> stems; <input type="checkbox"/> leaves; <input type="checkbox"/> inflorescence; <input type="checkbox"/> fruits; <input type="checkbox"/> seeds/nuts <input type="checkbox"/> others (_____)</p> <p>*tick out in appropriate box</p>
10. Category of pest specimen/organism submitted	<p>* <input type="checkbox"/> insects; <input type="checkbox"/> mites; <input type="checkbox"/> nematodes; <input type="checkbox"/> fungi; <input type="checkbox"/> bacteria; <input type="checkbox"/> virus; <input type="checkbox"/> others (_____)</p> <p>*tick out in appropriate box</p>
11. Life stage of the pest (Applicable to Insects)	<p>* <input type="checkbox"/> egg; <input type="checkbox"/> larvae; <input type="checkbox"/> pupae; <input type="checkbox"/> adult; <input type="checkbox"/> nymphs; <input type="checkbox"/> juveniles; <input type="checkbox"/> cysts; <input type="checkbox"/> others</p>

	(_____) *tick out in appropriate box
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 (_____) *tick out in appropriate box
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: _____ Date: _____ _____ <div style="text-align: right;">(Signature/Name/Designation of Identifier)</div>	
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 any to the sender of the specimen and duplicate copy will	

be retained by the identifier.

Annex V: General information of survey of fixed plots

Performa 1

Agroclimatic zone:

Region:

District:

VDC :

Wards:

Village:

Name of farmer:

Growing season:

For Fixed plots: Fixed 1 or Fixed 2

Spot code no:

Latitude:

Longitude:

Altitude:

Lowland/ upland:

Soil type:

Irrigation facility: Irrigated/ partial irrigation/ Rainfed

Area:

Agronomic details:

Variety:

Sole/Inter/ Relay crop:

Previous crop:

Seed rate:

Date of sowing:

Spacing:

Seed treatment: If yes dose and type of fungicide

Fertilizer application:

a) Manure

b) Chemical fertilizer

Crops in the adjacent fields:

Methods of irrigation:

Plant protection activities:

Others;

For Random field survey: As it differs in each sampling survey please note it data collection format Annex VI)

Annex VI: Data Sheet for observation in fixed and random fields

Region: District:
VDC : Wards: Village:
Name of farmer
Date: Weather on that day:
Variety:
Crops in the adjacent fields:
Plant protection activities if done in that period :
Information on survey day:
Crop growth stage:
General crop health:

Aphid severity

Sample plant	Scale of aphid severity (0-3)					Remarks
	Spot1	spot 2	Spot 3	Spot 4	Spot 5	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Remarks:

Annex VII: Data Sheet for observation for random fields

Region: District:
 VDC : Wards: Village:
 Name of farmer (not necessary):
 Date: Weather on that day:
 General information:
 Latitude: Longitude: Alitude: Lowland/ upland: Soil type:
 Irrigation facility: Irrigated/ partial irrigation/ Rainfed
 Total lentil Area:
 Sole/Inter/ Relay crop:
 Crops in the adjacent fields:
 Plant protection activities:
 Information on survey day:
 Crop growth stage: General crop health:

Aphid severity

Sample plant	Scale of aphid severity (0-3)					Remarks
	Spot1	spot 2	Spot 3	Spot 4	Spot 5	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Remarks:

Annex VIII: e08f/0f 8f6f l;6

e08f/0f 8f6f l;6		
!= c]nf]sg ;DjGwL ;fdfGo hfgsf/L		
s_ c]nf]sg ul/Psf] ldl		
v_ ;e]{÷e08f/0f g+= =		
@= lhlkP; l/k m]G; g+=	clff+; M	
	b]zfGt/ M	
	prfO{ M	
#= :yfg	ufpFm / j8f g+=	
	uf=lj=;=	
	lhNnf	
\$= ;f] 7fp+sf] xfjfkfgL	cf}ift Go"gtD tfkqmd - ;]=_	
	cf}ift clwstd tfkqmd - ;]=_	
	jiff{ -ld=ld=_	
%= e08f/0f cj:yf	Tfkqmd	
	Cfbtf	
^= e08f/0f ul/Psf] ;do		
&= e08f/0f ul/Psf] jfnLsf]	lr:ofg	
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(_ pkrf/sf lalw c]nDjg ul/Psf eP		
!)= cGo s]xL eP		
!!!= ;+snssf] gfd		

Annex IX: General information of survey of fixed plots

Performa 1

Agroclimatic zone:

Region:

District:

VDC :

Wards:

Village:

Name of farmer:

Growing season:

For Fixed plots: Fixed 1 or Fixed 2

Spot code no:

Latitude: Longitude: Altitude: Lowland/ upland: Soil type:

Irrigation facility: Irrigated/ partial irrigation/ Rainfed

Area:

Agronomic details:

Variety: Sole/Inter/ Relay crop: Previous crop:

Seed rate: Date of sowing: Spacing:

Seed treatment: If yes dose and type of fungicide

Fertilizer application:

a) Manure

b) Chemical fertilizer

Crops in the adjacent fields:

Methods of irrigation:

Plant protection activities:

Others:

Annex X: Data Sheet for observation in fixed and random fields

Region: District:

VDC : Wards: Village:

Name of farmer

Date: Weather on that day:

Variety:

Crops in the adjacent fields:

Plant protection activities if done in that period:

Information on survey day:

Crop growth stage:

General crop health:

Disease scoring (1-9) table

Sample plant	Disease scoring in 1-9					Remarkss
	Spot1	spot 2	Spot 3	Spot 4	Spot 5	
1						

2						
3						
4						
5						
6						
7						
8						
9						
10						

Remarks:

Annex XI: Data Sheet for observation for random fields

Region:

District:

VDC :

Wards:

Village:

Name of farmer (not necessary):

Date:

Weather on that day:

General information:

Latitude:

Longitude:

Alitude:

Lowland/ upland:

Soil type:

Irrigation facility: Irrigated/ partial irrigation/ Rainfed

Total lentil Area:

Sole/Inter/ Relay crop:

Crops in the adjacent fields:

Plant protection activities:

Information on survey day:

Crop growth stage:

General crop health:

Disease scoring (1-9) table

Sample plant	Disease scoring in 1-9					Remarks
	Spot1	spot 2	Spot 3	Spot 4	Spot 5	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Remarks:

22 List of Appendixes

Appendix 1: Light traps for Phototropic Insects

Effective insect pest management relies on the early detection of insect pests, hopefully before they become established and cause damage. Trapping helps indicate the best possible time to spray for maximum effectiveness. There are long-established trapping systems that have been used to survey fly populations. Trapping conditions may vary depending on, for example, the target rhizome fly and environmental conditions. Fly monitoring by light traps is a cheap, simple and efficient method.

Different light sources can be used, ranging from simple oil lamps to different types of electric bulbs. There are a variety of light traps on the market. Different light sources can be used, ranging from simple oil lamps to different types of electric bulbs. In general, cone-type traps are more useful for long-term (many weeks) trapping because it takes longer for the flies to find their way out of the trap. Light traps with or without ultraviolet light, attract certain insects. Light sources may include fluorescent lamps, mercury-vapor lamps, or black lights. Designs differ according to the behavior of the insects. Light traps are mainly used for attracting moths & other night flying insects which are attracted towards the light. The insects are actively caught or encouraged to enter a trap. Light traps are widely used to survey nocturnal moths. Light traps are used to monitor the activity of a range of insect species. Light traps are the only option for monitoring the activity of species for which there are no known (or commercially available) pheromones. Light traps are easy to construct and deploy, and suitable for very recognizable species that are not easily damaged in the trap (e.g. beetles).

The major downside to light traps is that they are not selective and numerous species of moths, beetles, flies, and other insects, most of which are not pests, are attracted to the light. Sorting collections is extremely time consuming and requires a high degree of competency in insect identification. Individual insects (particularly moths) are often damaged by beetles in the traps, making identification even more difficult. Deploying light traps for a short period of time (e.g. a few hours around dusk), killing the insects as they are collected with insecticide or directly into alcohol will make the task of sorting considerably easier.

Light trap is one of the effective tools of management of the insect pests as it mass-traps both the sexes of insect pests and also substantially reduces the carryover pest population. Since long, the researchers and farmers are using ordinary light trap to mass trap and sample insect pests of crops. Light traps are also used to determine seasonal patterns of insects' density in the cropped areas. It also provides information related to insect distribution, abundance, flight patterns and helps to decide the timing of the application of chemical pesticides, biopesticides or the release of biocontrol agents. While the light source attracts the insects, the traps need another device to kill or capture them. Often this is a container with water that is placed under the light source. The moths that circle around the light will drop in the water and can be counted the next morning. It is recommended to add a bit of soap to the water. Instead of water, it is also possible to use sticky plates.

Fly traps can be used to reduce adult fly populations, capture specimens for identification, and monitor the effectiveness of control programs. Fly traps are not toxic and are more selective than using insecticide. Traps need to be serviced regularly, appropriately placed, and repaired or replaced when damaged.

Flies per trap per day

Flies per trap per day (FTD) is a population index that indicates the average number of flies of the target species captured per trap per day during a specified period in which the trap was exposed in the field. The function of this population index is to have a comparative measure of the size of the adult pest population in a given space and time. It is used as baseline information to compare the size of the population before, during and after the application of a fly control programme. The FTD should be used in all reports of trapping. The formula is as follows:

The FTD should be used in all reports of trapping surveys. FTD is obtained by dividing the total number of captured flies by the product obtained from multiplying the total number of inspected traps by the average number of days the traps were exposed. The formula is as follows:

$$\text{FTD} = \text{Flies/Trap/Day} = \frac{F}{T \times D}$$

Where,

F = Total number of flies trapped

T= Number of serviced traps

D= Average number of days traps were exposed in the field/Number of trapping days

In cases where traps are regularly inspected on a day basis, the parameter may be “flies per trap per day”. It estimates the number of flies captured by one trap in one day.

Trapping density

Trap density(number of traps per unit area) is critical for fly surveys. The densities need to be adjusted based on many factors including: trap efficiency and location regarding altitude, type and presence of host, climate, topography, program phase and type of fly species. The density of trapping would be higher for those areas with high probability of introduction.

Decision Making

When traps are used for monitoring, this is often done to predict outbreaks of a pest species and to assist the farmer in making decisions. But care should be taken in the interpretation of results. A sudden increase in insects caught in a trap does not always mean that an outbreak will occur. Farmers will have to use data from traps in combination with other observations to make crop management decisions. Light traps make use of a light source to attract night-flying insects. They are especially useful to monitor certain species of moths. Catching moths in the light trap will tell the farmer that it is time to start searching for eggs or caterpillars in the field. When pest level crosses ETL, control measure has to be taken to prevent pest from reducing EIL.

To get the most from your traps

- Check traps regularly (at least weekly)
- Identify (correctly) pests and other insects
- Keep accurate records
- Remove trapped insects to avoid confusion during subsequent counts
- Service (change sticky boards or water) or change traps regularly

Appendix 2: Percent Infestation

Maggot's counts

The protocol on intensity of infestation below describes how to do this

- Randomly collect 50-100 rhizome from the field
- Dissect and count the number of maggots
- Any rhizome, even with one maggot in it is to be considered infested

The percentage Fly infestation for crop at the time of sampling is calculated as

$$\frac{\text{Number of rhizome with Fly maggot}}{\text{Total number of rhizome dissected/observed}} \times 100$$

Appendix-3: Standard Technical Protocols for Collection and Handling of Insect Samples

INTRODUCTION

1.1 Scope

This standard describes the technical protocols for the collection and handling the insect samples to be used by the quarantine staff or field staff. It focuses on preservation, mounting, labeling and packaging of insect samples for sending specimen to diagnostic lab 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

IPPC. 1997. *International Plant Protection Convention*. Rome, IPPC, FAO.

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1.3 Definitions

Definitions of phytosanitary terms used in this standard are as in ISPM 5 (*Glossary of 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 for the inspection of 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. There is also a certain probability that a pest which is present in a consignment or lot may not be detected. In such cases or pests if also have been detected by signs may require specialized analysis in a laboratory or by a specialist before a determination can be made on the phytosanitary risk of the pest.

Precaution also has to be taken for handling the Quarantine pests and/ or new organisms detected for sending the materials for diagnosis purpose in the referral laboratories. The accurate diagnoses depends upon the acquisition of appropriate sampling, packaging and timely delivery of specimen materials which are carefully handled so as to retain, as much as possible their key diagnostic features. In such cases 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.

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.

2. BACKGROUND

Proper pest identification is crucial for the appropriate application of phytosanitary measures. The correct identification also is required to determine if pests should be regulated and the strength of phytosanitary measures to be taken against them. New organisms detected during consignment sampling or detected during general and specific surveys needs identification in a

laboratory to justify the phytosanitary action and also to include in the National pest data information sheet.

The collection and transport of insect samples to the diagnostic laboratory with proper handling and without damaging is very essential for proper identification. Special care also needs to prevent from a potential risk of escape of exotic pests to the environment.

Thus the provided protocols must be followed in order to get correct identification through proper collection, preservation and proper handling of insect samples.

3. GENERAL REQUIREMENT

The following general provisions apply to the protocol related to sampling 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.
- Chemicals or equipment in this protocol 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 insect sample should have knowledge and skill of plant pest 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. In service training should be organized to update new development in the subject regularly. Refresher training and Orientation on lab equipment and Handling should be conducted on regular basis.

4. SPECIFIC REQUIREMENTS

4.1 Basic equipment for Insect Collection and Handling

The following items are required for the collector:

- a) Forceps: Soft-touch or "Feather-touch" forceps are best for the collection of specimens without damage
- b) Vials of "Screw-cap" types containing alcohol or other preservatives
- c) Killing bottles of various sizes clearly and prominently labeled "POISON"
- d) Small boxes or containers for storing specimens after their removal from killing bottles. These may be made of cardboard, plastic, or metal and should be partly filled with soft tissue or cloth to keep specimens from rolling about. Cotton should not be used because specimens become entangled in the fibers and may damage.

- e) Small envelopes for temporary storage of delicate specimens and/or gel caps for tiny specimens.
- f) Aspirators: An apparatus to trap the minute sized live insects.
- g) Sweeping Nets: used for catching flying insects
- h) Absorbent tissue for use in killing bottles and aspirators.
- i) Relaxing bottle: An apparatus used to keep the insect soft for further handling
- j) Absorbent tissue for use in killing bottles and aspirators.
- k) A strong knife for opening galls, seed pods, twigs, etc and a pair of scissors for cutting labels.
- l) A small, fine brush (camel's hair is best) for picking up minute specimens.
- m) A hand lens.
- n) Notebook and writing equipment for noting down notes and labeling.

This list may be modified according to the special kinds of insects.

4.2 Chemicals and Materials used for processing the Insect Specimens (see Annex 1)

5. INSECT COLLECTION TECHNIQUES

- Insects can be collected from various sources as: from the growing field, different types of attractant traps such as light traps, pheromone traps etc. and from the drawn samples of consignments. Hand-collecting or sweep nets, aspirators may be used for collecting the insects.
- Collected insects should be clean and in good condition, i.e. complete with appendages such as antennae, wings and legs. Collect the mature insects for accurate identification.
- It is advisable to collect a two or more specimens, one to keep for future reference and one to send for identification.
- The collected insect samples need correct handling, preservation, labeling, packaging techniques and timely delivery of samples to an appropriate diagnostician for proper identification.

5.1 Handling of samples

Samples should be placed in strong polythene bags and immediately labeled by means of a pencil-written paper or plastic label placed inside the bag.

Samples should be kept cool

- do not leave in the sun or in a closed vehicle left in the sun
- should be treated with care and processed or dispatched for analysis as soon as possible.

If immediate dispatch or processing is impossible then samples should be stored in a refrigerator at 4–8°C for several days without severe deterioration

6. SPECIMEN PRESERVATION

6.1 Killing Methods

Collected insects should be killed before pinning and mounting. The insect to be preserved should be well handled throughout the procedures so as not to damage their parts. The most widely used method for killing the collected specimens is the killing bottle. In the field work, if not possible to take/get killing bottles, a heavy scale plastic can also be used with collected insects and a small piece of cotton pad soaked with liquid killing agent inside. Most widely used liquid killing agent is Ethyl acetate. Then tie up the plastic and carry to the laboratory.

Large, hard-bodied insects such as beetles die slowly and may damage other insects placed in the same killing jar, so more than one jar is needed. Pinching the thorax of large butterflies or moths before placing them in the killing jar will prevent them from flopping around and damaging the wings. Squeeze them for 20-30 seconds between thumb and index finger and put them in the killing jar. Remove butterflies and moths from the killing jar fairly soon so their wings do not become soaked with killing agent.

The most often used solid killing agents in killing jars are the cyanides. All the cyanides have to be handled with extreme care because they are very dangerous, rapid-acting poisons. But nowadays, the cyanide is very difficult to get at regular market.

All killing agents are to some extent hazardous to human health. So all killing jars or bottles should be clearly labeled "POISON" and should be kept away from children or persons who may be unaware of their potential danger.

6.2 Preserving Insects

Insects and mites after killing should be preserved in liquid agents, but it is first necessary to look for insect types.

Soft-Bodied Insects - Many types of insects, including aphids, springtails, thrips, mayflies or silverfish, are soft-bodied and cannot be pinned successfully. These are preserved in glass vial with ethyl alcohol, usually of about 70 percent concentration (70% alcohol, 30% water). Stronger alcohol solutions can cause colors of specimens to darken and bodies to shrivel. Isopropyl alcohol and formaldehyde solution can also be used in different concentration, but alcohol is common and easily available.

For immature insects such as caterpillars, beetle and wasp larvae and others should be collected in alcohol and subsequently killed in boiling water for 1-5 minutes to fix the insect tissues and color. For smaller specimens, simply dip them in boiling water for about 30 seconds and then transfer them to vial with 70 percent alcohol. For permanent collection mites, aphids, thrips whiteflies are mounted on microscope slides. Larvae are usually kept permanently in alcohol.

Hard-Bodied Insects - Insects have a hard shell or exoskeleton and all of the soft parts are on the inside, they can be kept well by drying, even for long periods of time. Hard bodied insects

such as beetles can be mounted directly after removal from alcohol. Specimens must be fresh or flexible but if left for some period, they must be well relaxed for some time.

6.3 Cleaning Specimens

Cleaning the specimens is important for further preservation process. Cleaning can be done in different ways as per quantity of dirt, size and type of specimen. A fine camel-hair brush can work for dirt, pollen dust type materials. Similar dirt can be removed in water to which synthetic detergents has been added. Specimens can be picked by fine forceps and drop at the dish containing clean water or mixed with detergents for some time. Greasy materials in the specimen have to be cleaned with the help of organic solvent. Specimen covered with mold or fungus can be removed by fine brush dipped in carbolic acid. Beetles and other hard insects can be made clean by gently scrubbing with water or weak alcohol. After cleaning specimen it can be returned to the relaxing jar.

7. TEMPORARY SAMPLE STORAGE OF SPECIMENS

After specimens have been collected and brought to laboratory, it is not often possible to prepare them for permanent storage. Some of the methods for keeping them in good condition are such:

- a. Refrigeration and freezing: Generally medium to large specimens are placed in tightly closed bottles for several days and left in a refrigerator. Keep absorbent paper between the insects in the jar. At the time of removal for preservation, place them immediately on absorbent paper.
- b. Dry preservation: The specimens is kept dried in small boxes, paper envelopes for some time. But do not store soft-bodied insects in this method as they become shriveled and damage. Keep the insects in tightly closed any kind of container with absorbent paper between the specimens. Care should be taken to avoid mold to develop.
- c. Papering: Papering has been used long time for larger specimens of Lepidoptera, Trichoptera, Neuroptera, Odonata and some other groups. This is a traditional way of storing un-mounted insects and still been used. At first envelope are made using some glassine papers, where the specimens are placed with the wings folded together dorsally. These paper triangles are stapled and piled up in dry condition. Care should be taken to avoid mold to develop.
- d. Liquid preservation: Small, soft bodied insects are kept at vial with alcohol in it. Generally alcohol with 70 percent concentration is used. These vials are further kept inside wide-mouthed jars. Do not expose them in direct sunlight.

8. MOUNTING SPECIMEN

Specimens are mounted so that they may be handled and examined with the greatest convenience and with the least possible damage.

8.1 Preparing Dry Specimens for Mounting

Any dry insect that is to be pinned must be relaxed, that is to soften so that it will not break when the pin is inserted.

8.1.1 Relaxing Method

It is always not possible to pin the specimens collected at the same day. So before pinning these specimens left in the killing jar too long, to restore their flexibility, it is necessary to "relax" them. Relaxing is done by placing the insect specimen in a relaxing jar for certain time. The relaxing jar is simple equipment and can be made by any glass material. Some moist sand are placed at the bottom and covered with blotting paper where the insect specimens will be placed.

Check the jar, if the insects are flexible, mount them immediately. If the specimens are still too stiff, keep them in the relaxer for a few more days, but watch them carefully. Insects will mold and decompose if held in the relaxer too long.

8.2 Preparing Liquid-Preserved Specimens

Most specimens preserved in fluid must be removed from the liquid so that they will dry. Specimens which have been in fluid for some time should generally be washed with clean solution before drying. Only specimens with hard exoskeletons, such as beetles and some bugs (Pentatomidae, Cydnidae) may be mounted without special treatment when removed directly from the preserving fluid into pins or points.

8.3 Pinning

Insect Pins

Special pins are needed to pin insects. Pin widths are numerically-designated, with standard sizes 0-3 increasing in thickness with increase in numerical value. Extremely narrow pins are of size 00, and extremely thick pins are of sizes 4-7 but are longer. Most insects large enough to be pinned may be pinned on sizes 2 or 3. These pins are longer, stronger, and thinner than an average pin.

8.3.1 Direct Pinning

Different groups of insects require different pin placement. With most Orders, the pin should pass through the middle of the thorax such that the specimen appears balanced in relation to the placement of the pin. The insect's body should be perpendicular to the pin. In bees, wasps and flies (Hymenoptera and Diptera) the pin should pass between the bases of the forewings, just to the right of the middle. In beetles (Coleoptera) the pin should pass down through the right outer wing. In the Hemiptera and Homoptera, the pin should pass through the triangular scutellum slightly to the right. With grasshoppers and crickets (Orthoptera) pin through the thorax to the right of the middle line. In butterflies, moths, and dragonflies (Lepidoptera and Odonata), the pin goes through the center of the thorax and the wings should be spread properly. Consider visibility, breakage, and space when positioning legs and antennae. Once insects are mounted, it should be allowed to dry as they are very fragile.

8.3.2 Double Mounts

Insects that are too small to be pinned directly on standard pins and yet should be preserved dry may be pinned as double mounts. The insect is mounted on a minuten or card point, which in turn is mounted or attached to a standard insect pin. Minutens are available from supply houses in 10 and 15 mm lengths and in two or three thicknesses. They are finely pointed at one end, headless on the other, and generally of stainless steel. Double mounts are assembled by inserting the minuten into a small cube of soft, pithy

material such as fine cork, balsa wood, fine-textured plastic, or polyporus, which is a pure white material. Minutens should be handled with forceps.

Many entomologists prefer to mount insects is the card mount. These are small rectangular cards that can be purchased commercially or can be make byself. In this method the specimen is mounted laterally at about a 45 degree angle. If done properly, this technique allows viewing of all structures on the insect and the surrounding card provides greater protection for small and fragile specimens. However, caution must be used to ensure that the specimens' mouthparts, wings, etc. do not become embedded in the mounting media. With some practice, this method provides very good results. Ordinary white (casein) glue, clear acetate cement, or fingernail polish is used commonly as adhesive.

8.3.3 Spreading Board

The spreading board is usually made of soft wood/fine cork with the center groove to receive insect pins. Large moths and butterflies and some other winged insects are mounted with wings neatly spread on a spreading board to display the taxonomic characters used for insect identification.

8.3.4 Slides –Some insect specimens are best mounted on microscope slides.

These include very small and soft-bodied insects as well as dissections of insect parts. The most permanent mountant available is Canada balsam. Some insects (scale insects, aphids and lice, tiny “parasitic” Hymenoptera, and many insect larvae) are typically prepared by clearing and differential staining before being mounted on microscope slides.

9. ATTACHING LABELS TO SPECIMENS

Labeling must be done as soon as possible after collecting, pinning and mounting otherwise the information may be lost.

Labeling paper: The paper used for making labels should be firm and heavy enough so that the labels remain flat and do not rotate loosely on the pin.

Writing ink: The ink should be a good grade of ink (India ink or permanent ink), which will be permanent. Be sure the ink is completely dry before placing the label in the liquid.

Lead pencil (HB, B or 2B) also is used for alcoholic liquid preserved specimen.

Printing labels: Though computer printed labels is easy to use, hand written labels using technical pens with very fine points are still widely used at many institutions. If typewritten or computer generated labels are to be used, take care of the proper size. The letter size must be very small (about 6 point on most fonts) but readable. Labels with larger font (10 or 12) can be used in vials with alcohol.

Label Data: The information on label must answer the questions of where, when, and who in that order. This kind of data should be given as follows:

- **Locality:** The collection locality should be given in such a manner that it could be found on any good map.
- **Date:** Write day, month, and year in that order
- **Collector:** Write the last name of the collector or collectors, using initials for given names if space permits.
- **Other data:** It is often useful to have additional information, like host plant, found under rock, trapping methods etc. in separate label called as ecological label.

Information on labels

Labels for pinned insects should be placed beneath specimens, aligned parallel with the insect's body.

More than one label can be placed according to the information collected. The top label should have the locality from which the insect was collected, the collection date and the name of the collector. The lower label should show the taxonomic information of the insect. Place another label beneath the collection label which gives the host plant, habitat or other pertinent information called as ecological label.

For labeling the vials, Special care should be taken with labels placed in alcohol. The paper should be high quality. Always place labels inside the vial because if it is fixed at the outer surface of the vial, it will be difficult to observe the specimens inside. The writing needs to be visible from outside the specimen container so the label needs to be facing outwards. A single label large enough to include all data should accompany material in fluid.

10. PACKING AND TRANSPORTING INSECT SPECIMENS

Most insects and mites submitted to experts for identification should not be shipped alive. The insects to be sent have to be categorized and packed accordingly. The small, soft bodied insects have to be sent in liquid preserved materials, while large insects have to be sent in dry pinned condition.

10.1 Procedures for packing dried pinned specimen:

Generally medium to large insects are sent in dry pinned process. For packing, various types of boxes made by wood, cardboard paper, metal etc are used. Cork, thermocol or similar material is cut to the size of the postal boxes and placed tight at its bottom. The dried pinned specimens are kept accordingly and the pins must be pushed deep into the cork. The insects should be prevented from rotating on their pins by extra pins just touching each side of the body. The postal boxes should be well packed by sticking packaging tape at all sides. Each and every specimen kept inside the boxes should be numbered and well labeled with all the information.

10.2 Procedures for packing liquid preserved specimen:

Generally small, soft bodied insects are sent in liquid preserved materials. Place the specimens in vials and pour the vials with liquid preservatives (70 % alcohol). The vials have to be wrapped individually in cotton-wool, tissue paper, or polystyrene sheet to avoid glass surface touching each other. The vials then are placed in postal boxes.

10.3 Procedures to be followed

- Notify the diagnostician that a suspect emergency plant pest is being sent to the laboratory and the estimated time of arrival. The diagnostician should be informed of
- any additional precautions that may be required on arrival e.g. open sample in
- appropriate containment facilities
- Choose the most reliable and fastest method of dispatching the sample.
- If a delay is expected of more than 2 days in sending samples, store sample under
- appropriate conditions prior to sending.
- Ensure samples are sent directly to the chosen laboratory.
- Attach consignment notice to outside of package if sending abroad.

Samples must either be dispatched to the diagnostic facility by a courier provider which ensures overnight or same day delivery of package. On arrival signature receipt is required.

Alternately, the sample can be hand delivered to the diagnostic facility. Remember to keep samples cool and out of direct sunlight.

11. PLACE AND PERSON/S FOR INSECT SPECIMEN DIAGNOSIS

The collected specimens have to be correctly identified for different purposes: to prepare country pest list, conducting pest risk analysis to determine whether it is quarantine pests and the management options of the insect pests. Identification is not only possible at field or local level, so it has to be sent at national institutes or sometimes abroad also.

National Institutes

- Entomology Division, NARI, NARC, Khumaltar, Lalitpur.
- Natural History Museum, Swayambhu, Kathmandu.

- Regional Plant Protection Labs

International Institutes

- British Museum (Natural History), Cromwell Rd, London SW7 5BD, United Kingdom
- Commonwealth Agriculture Bureau International, Nosworthy Way, Wallingford, Oxfordshire OX10 8DE, UK

National institutions having insect museums in Nepal are:

- Entomology Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur
- Department of Entomology, Institute of Agriculture and Animal Science, Tribhuvan University, Rampur, Chitawan
- Natural History Museum, Tribhuvan University, Swoyambhu, Kathmandu
- Regional Plant Protection Lab
- Department of Entomology, Himalayan College of Agricultural Science and Technology, Ghattaghar, Bhaktapur
- Prithvi Narayan Campus, Tribhuvan University, Pokhara, Kaski
- Annapurna Conservation Area, Kaski and some others

Before sending the specimen's prior approval from the concerned institution is very necessary. The concerned personnel must be notified, so that necessary arrangements may be made.

12. HANDLING/DISPOSAL OF QUARANTINE SAMPLES

The quarantine samples received at the laboratory should be clearly marked and stored separately from the domestic samples. If it is not possible to store in separate refrigeration units, at least they should be stored in a separate container to avoid mixing of samples and held under the custody of laboratory supervisor..

If any live insect specimens are received at the diagnostic laboratory, they should immediately be killed and preserved to prevent their escape before proceeding with pest identification.

13. PEST RECORD KEEPING

The diagnostic labs and quarantine office has to keep appropriate records derived from lab reports. Voucher specimens should be deposited, where appropriate. Information kept should be appropriate for the intended purpose, for example support of specific pest risk analyses, establishment of pest free areas and preparation of pest lists.

Annex 1. Chemicals And Materials used in Processing Insect Specimen

Chemicals Used For Insect Killing

Liquid Killing Agents

- Ethyl Acetate
- Carbon Tetra Chloride
- Ether (diethyl ether, trichloro-ethylene)
- Diethyl Ether
- Ammonia Water

Solid killing agents

- Potassium cyanide
- sodium cyanide
- calcium cyanide

Chemicals Used As Preservatives

- Ethyl Alcohol
- Formaldehyde
- Glacial Acetic Acid

Chemicals Used for Cleaning

- Carbolic Acid
- Benzene

Chemicals For Slide Preparation

- Xylene- xylene bath will clear the specimen by removing fats and oils
- Glycerin- for sealing the slides
- Potassium Hydroxide –remove clear the soft tissues
- Canada Balsom- fixing the specimen (mounting cement)

Mounting Specimen materials

Insect pins	Minute pins	Pinning block
Spreading board	Stiff good quality paper	Scissor
Forceps	Glue	Specimen box

Appendix-4: Standard Technical Protocols for Collection and Handling of Disease Samples

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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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 (*Glossary of 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 pest have been detected by signs may 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 for 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 disease diagnosis 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 detected during 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 phytosanitary measure may be of limited use. Sample collection can be a difficult, time-consuming process, and there is no guarantee that the diagnosis problem 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 to sampling 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, Packaging and 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 placed in a paper bag or an unsealed plastic bag.

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

4.1.6 Provide background and 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 reviewed as 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 observations 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 hypochloride, 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 Samples should 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 start 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 -I : 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

Spirit -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:					Forwarded to:			
_____					-----			
Date					Name of diagnostic Laboratory and address			

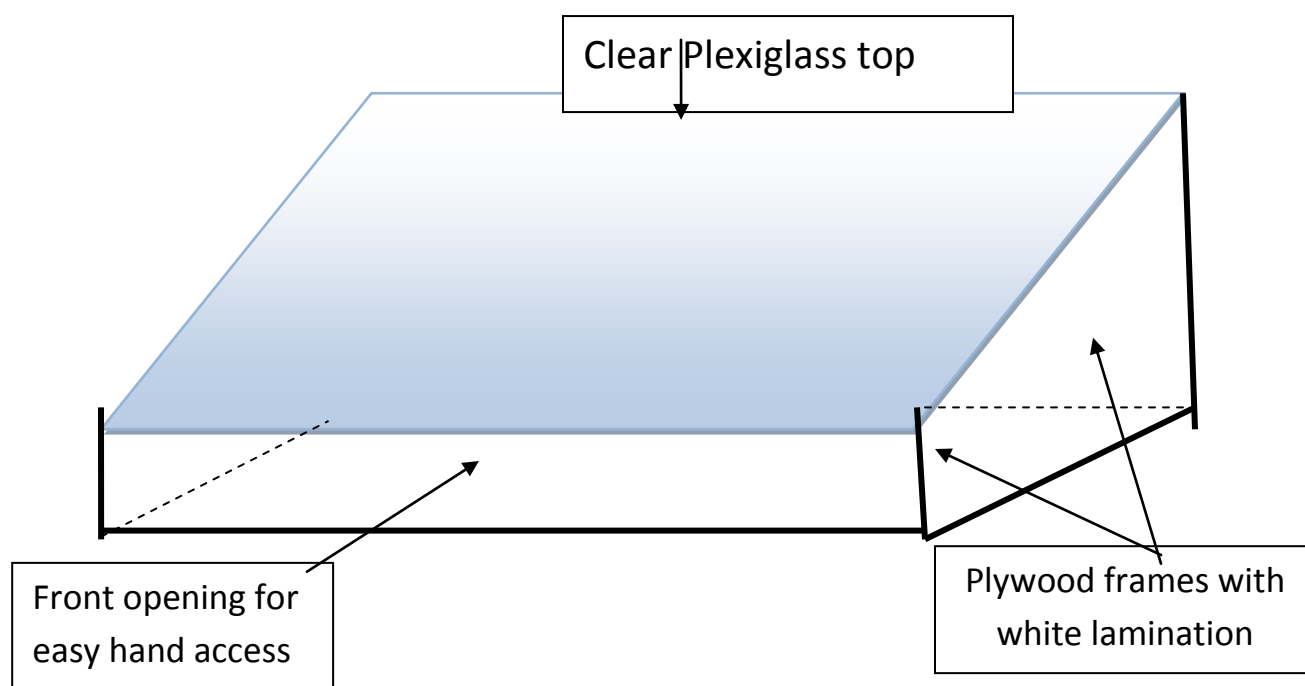


Fig.1. Portable isolation chamber

