NATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

NSPM:

Detection and Monitoring Techniques for Quarantine Pests

2014

Table of Contents

Annexes

1. INTRODUCTION

1.1 Scope

The standard provides details for the plant quarantine and field officers with the detection and monitoring techniques for the pests of quarantine significance and its association with the pathway. This standard mentions attractant traps, signs and symptoms, detection and monitoring techniques and associated pathways to minimize the introduction and/ or spread of quarantine pests.

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.

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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

Detection methods are the first tools to be used by National plant protection organization (NPPO) and inspection techniques in order to find incursions of quarantine pests (Q-pests) at land and air border entry points. 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 NPPOs in the entry points and field with inspection services to enable early detection of Quarantine or exotic pests.

The problem of plant pathogens and pests slipping through undetected in consignments is a serious one. If introduced, this could cause serious economic and ecological consequences to the National biodiversity.

With the increased trade of agricultural products internationally, and modern and rapid transport system available, the chances of introducing exotic pests into new areas are steadily increasing. The traded commodity products can be a pathway for the new pests thus the NPPO should identify and target high-risk pathways of entry for pests on the basis of NSPM 32 (*Categorization of commodities according to their pest risk)* to minimize the phytosanitay risk of pest introduction and establishment into the country.

It obviously is of value to detect organisms new to an agricultural system shortly after introduction, when the populations are still localized and at low levels, so that appropriate containment and control actions can be mobilized by the NPPO.

NPPO of Nepal has notified the quarantine pest lists that may cause considerable economic and societal damage within agriculture, horticulture, forestry and natural ecosystems. So it is particular importance to develop detection methods and techniques and implement effective entry and border control procedures and monitoring techniques for detecting targeted quarantine pests and diseases. For this, all actions should be taken through surveillance as mentioned in 'Good Surveillance Practice' according to ISPM No. 6 (*Guidelines of surveillance*).

For early detection of Quarantine pest, surveillance is also included among the ways to contribute to the prevention of entry and spread of quarantine pests. This information is provided on National Standard on Phytosanitary Measures, "Guidelines for detection, monitoring and delimiting survey of plant pests in agriculture ecosystem"

In the simplest sense, if an inspector is looking for suspected pest using a detection method – the observation of symptoms. Upon finding a suspect sample the inspectors may send it to a laboratory to confirm the presence of a specific regulated pest. If confirmed, the inspector then return to the sites and conduct the detection / monitor/ delimiting survey to locate the spread of the pest.

Moreover, for effective implementation of phytosanitary standards skilled inspectors are required, thus adequate training needs to be provided by NPPO for personnel involved in phytosanitary surveillance, identification/ detection of pests, sampling methodology, data management, record-keeping, and preservation and transportation of samples.

This standard provides most commonly used methods to detect some plant pests of quarantine value belonging to groups of invasive organisms with high economic relevance, including Coleoptera, Diptera, Hemoptera, Lepidoptera, Thysanoptera, Acarina, Nematode, Fungi, Bacteria, Virus and Weed plant. Most of the quarantine pests referred in this standard is based on the list provided by NPPO.

2. DETECTION TECHNIQUES FOR PESTS OF QUARANTINE SIGNIFICANCE THROUGH SIGNS & SYMPTOMS IN PLANTS

Pests and diseases can cause harm to plants by damaging the leaves, flowers, fruit, seeds, bark and roots of plants. The plant shows different signs and symptoms if it is infected with a disease or being attacked by a insect that provides helpful hints in detecting the pests. Some of the signs and symptoms are; discoloration of leaves, holes in bark and leaves, moulds and mildews, spots, wilting and curled leaves etc. For detail information see Annex 1.

3. DETECTING AND MONITORING TOOLS

There are various trapping tools to detect and monitoring the insect pests that are used to attract the insects through the use of poisoned baits or light or insect lures. These traps are positioned in the field at pre-determined sites after taking into account the insect habitat and crop canopy. The inspector should visit the trapping sites to collect the insect pests for identification and recording of trapping data. Using traps/or traps baited with pheromone placed in and around cargo facilities where infested material may be stored, entry points and around nurseries or plantations with plants susceptible to attack by pests helps to monitor the target pests.

3.1 Physical Attractants

3.1.1 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 widely used to survey nocturnal moths. Light traps can attract flying and terrestrial insects, and lights can be combined with other traps as described below.

3.1.2 Pitfall traps are used for ground-foraging and flightless arthropods such as Carabid beetles and spiders. Pitfall traps consist of a bucket or container buried in soil or other substrate so that its lip is flush with the substrate.

3.1.3 Pan traps (also called water pan traps) are simple shallow dishes filled with soapy water or a preservative and killing agent. Pan traps are used to monitor aphids and some other small insects.

3.1.4 Sticky traps may be simple flat panels or enclosed structures, often baited, that ensnare insects with an adhesive substance. Sticky traps are widely used in agricultural monitoring to monitor pest populations.

3.1.5 Bucket traps and bottle traps, often supplemented with a funnel, are inexpensive versions that use a bait or attractant to lure insects into a bucket or bottle filled with soapy water or antifreeze. Many types of moth traps are bucket-type traps. Bottle traps are widely-used, often used to sample wasp or pest beetle populations.

3.1.6 Flight interception traps are net-like or transparent structures that impede flying insects and funnel them into collecting.

3.1.7 Barrier traps consist of a simple vertical sheet or wall that channels insects down into collection containers.

3.1.8 The Malaise trap, a more complex type, is a mesh tent-like trap that captures insects that tend to fly up rather than down when impeded.

3.2 Chemical Attractants

Chemicals attractants are volatile compounds. When released into the air, they can be detected by certain insects (those receptive to a specific compound) a few inches to hundreds of yards away. For use in monitoring, chemical attractants usually are impregnated or encased in a rubber or plastic lure that slowly releases the active components over a period of several days or weeks.

Traps containing these lures are constructed of paper, plastic, or other materials Most traps use an adhesive-coated surface or a funnel-shaped entrance to capture the target insect. Traps for some pests (such as the apple maggot) are coated with an adhesive that also contains the chemical attractant.

Chemical Attractant-baited traps are used to capture pest insects that are present at too low densities and to detect foreign or "exotic" pests as soon as they enter an area so that control measures can be initiated immediately. Chemical attractants capture only one species or a narrow range of species. This specificity simplifies the identification and counting of target pests. Sensitivity and specificity make attractant-baited traps efficient, labor-saving tools. Attractant-baited traps are used in monitoring programs for at least three purposes:

(a) to detect the presence of an exotic pest (an immigrant pest not previously known to inhabit a state or region);

(b) to estimate the relative density of a pest population at a given site; and

(c) to indicate the first emergence or peak flight activity of a pest species in a given area, often to time an insecticide application or to signal the need for additional scouting.

3.2.1 Pheromone trap is a type of insect trap that uses pheromones to lure insects. Pheromone rarely consists of a single chemical compound, but is generally combinations of several compounds. Sex pheromones that attract a mate and aggregating pheromones that call others to a suitable food or nesting site are the most common types used. A pheromone-impregnated lure is encased in a conventional trap such as a Delta trap, water-pan trap, or funnel trap.

Pheromone traps are ideally suited for monitoring populations as it is very sensitive, meaning they attract insects present at very low densities. They are often used to detect presence of exotic pests, or for sampling, monitoring, or to determine the first appearance of a pest in an area. Pheromone traps are highly species-specific can also be an advantage, and they tend to be inexpensive and easy to implement.

3.2.3. Para-pheromones usually designate powerful attractants of plant origin or from chemical synthesis which are active over variable but generally long distances. Para-pheromones have been extensively used in fruit flies and some compounds (methyl eugenol, cue-lure and trimedlure) have been found to be powerful male lures. Para-pheromones can replace pheromones when these are costly to prepare, unstable or ineffective under field conditions.

3.3 Using Colored Objects to Attract Insects

Specific colors are attractive to some day-flying insects. For example, yellow objects attract many insects and are often used in traps designed to capture winged aphids and adult whiteflies. Red spheres and yellow cards attract apple maggot flies. Like other attractants, colored objects can be used in traps for monitoring or mass trapping. Yellow plastic tubs filled with water, for example, are used to monitor the flights of aphids in crops where these insects are important vectors of plant viruses. Aphids attracted to the yellow tub land on the water and are unable to escape. Yellow, sticky-coated cards or plastic cups are widely used in mass trapping programs to help control whiteflies in greenhouses.

4. IMPORTANT ORDERS OF CLASS INSECTA REPRESENTING QUARANTINE PESTS INCLUDE:

4.1 Order: Coleoptera (beetles & weevils):

4.1.1 Borer and ambrosia beetles (Coleoptera: Curculionidae, Scolytidae),

Bark and ambrosia beetles pose a major threat to agriculture and forest resources globally and are known to be often associated with phytopathogenic fungi. A tiny bark beetle, the coffee berry borer, *Hypothenemus hampei* & H. obscurus is a major pest on coffee plantations around the world. The scolytid beetle feeds on the cotyledons and has been known to attack 100% of berries in a heavy infestation. Crop losses can be very severe and coffee quality from damaged berries is poor. The beetle spends most of its life cycle inside the coffee berry, thus it is particularly difficult to control with chemical or biological inputs.

The black twig borer, Xylosandrus morigerus, a coffee quarantine pest for Nepal must be considered, as the risk of introduction is most probably from the imported twigs and small branches. Once established, such species are difficult to eradicate, and are likely to spread with the movement of infested plants, as well as by normal dispersal of the adults. Attacked plants may show signs of wilting, branch die-back, shoot breakage, chronic debilitation, sun-scorch or a general decline in vigour. The direct risk of establishment of species of Xylosandrus in tropical and subtropical areas should be considered extremely serious.

Detection and monitoring

Trapping systems

The early detection of scolytid species may be achieved in two ways: trapping of flying adults or sampling of infested materials. Scolytids are very hard to detect and identify by sampling. However, scolytids are easily trapped using Red Brocap traps containing either a 1:1 or 3:1 ratio of methanol: ethanol, with Hercon Vaportapes as the killing agent, can capture hundreds up to thousands of adult female coffee berry borers per day.

Visual inspection of suspected infested material is required to detect the presence of ambrosia beetles. Infestations are most easily detected in living plants by the presence of wilting shoots or shoot dieback. Entry holes made by the attacking beetles, and the presence of frass produced during gallery construction, are additional indicators. When Xylosandrus species are detected in plant material, it is necessary to immediately destroy all of the infested material. When they are detected in traps, plant material in the vicinity of the trap should be actively inspected.

The use of traps in high-risk areas, such as land frontiers and airports, may give information useful in early detection, quarantine operations, timing of control measures and monitoring of possible insect dispersal.

Traps baited with chemical attractants commonly are used to capture ambrosia beetles for purposes of monitoring, studying population dynamics, predicting outbreaks, and mass trapping to reduce damage.

Pathways and commodities

The infested seedlings, saplings or cut branches and fruits are the associated pathways for transportation.

4.1.2 Weevils (Coleoptera: Curculionidae)

Weevils are a heterogeneous group, and feed mainly on leaves, fruits, seeds and roots. They are important pests of herbaceous crops, but there are numerous species that are harmful to woody plants in orchards, plantations and forests. The damage can be caused by larvae as well as adults. Many weevils are cryptic and nocturnally active, and collecting them can be difficult. As a result, exotic pest weevils entering an area may remain undetected for many years until their population builds up to economically important levels.

The cocoa weevil, *Araecerus fasciculatus* (DeGeer) is a cosmopolitan pest of many plants or plant products including coffee, cocoa, nutmegs, corn, peanuts, various nut crops, spices, grains, potato tuber and dried fruit. It is one of the major pests of stored coffee in India and Central America and of stored cacao in West Africa. It also attacks a variety of other stored products including cassava, sweet potatoes, dried plantain and various seeds and fruits.

Diaprepes abbreviatus, a sugarcane rootstalk borer weevil besides sugarcane its larvae destroy root systems of seedlings and mature citrus trees, while adult weevils feeding on new foliage may significantly reduce growth and productivity of trees.

Adults of the pepper weevil, Anthonomus eugenii feed on leaves and blossoms and bore into fruits. Early signs are small holes in immature fruits and small circular or oval holes (2-5 mm) in leaves.. Larvae feed on seeds and other tissue in the developing fruits

Visual inspection of suspected infested material is required to detect the presence of weevils.

Detection and monitoring

Trapping systems

Tedders traps captured the most weevils and most frequently weevil species. An inverted funnel trap hung in trees has proven somewhat successful in trapping adults. Recent advances in detecting adults as they emerge from pupating in the soil have been made using a modified version of the Tedders' trap.

Males of the pepper weevil *Anthonomus eugenii* produce an aggregation pheromone that attracts both sexes (Capinera, 2005).

Adult population of A. eugenii estimates are best obtained by visual examination and yellow sticky traps (Segarra-Carmona and Pantoja 1988a). Traps should be placed 10 to 60 cm above the soil; one 375 sq cm trap captures as many weevils as are detected by inspecting 50 buds (Riley and Schuster 1994). If visual monitoring is preferred, terminal bud sampling is effective.

Traps baited with the synthetic pheromone or attractant are used extensively to detect and monitor populations and sticky traps for monitoring *A.eugenii*, the pepper weevil.

Pathways and commodities

The pathway is related to the life-cycle of the species: many weevil beetles species are transported between countries inside fruits or seed containing the larvae or pupae and plants for planting. Another important pathway for some species is in the accompanying soil, which may contain larvae or pupae.

4.1.3 Leaf beetles (Coleoptera: Chrysomelidae)

Leaf beetles (Coleoptera: Chrysomelidae) are important pests in agriculture. Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the most important defoliator of potatoes world-wide and has developed resistance to all known classes of insecticides is a serious pest of solanaceous plants. The vector of the bean pod mottle virus is associated with the bean leaf beetle *Cerotoma trifurcata* (Forster). The virus may enter soybean plants through insect feeding. Potato Andean latent tymovirus is transmitted by the potato flea beetles *Epitrix* spp. at high population densities.

Detection and monitoring

Sticky traps of various types associated with pheromones appear to be the best methods to detect leaf beetles. If no lures are available, traditional sampling (e.g. emergence traps, vacuum, sweeping) is a reliable alternative, as well as trap crops for the Colorado beetle *L. decemlineata*.

Pathways and commodities

Leaf beetles are generally associated with the soil pathway, weed-contaminated seed and with the plants for planting.

4.2 Order Diptera (Fruit fly and Bulb Flies)

4.2.1 Fruit flies (Diptera: Tephritidae)

Fruit flies are a significant phytosanitary threat to horticulture, and many species in this family are listed as quarantine pests.

The damage caused by larval feeding makes fruit unfit for human consumption. In addition, the presence of an established population would cause a severe economic impact via restrictions/prohibitions on the export of fresh fruit both domestically and internationally.

Ceratitis capitata (Medfly), *Bactrocera carambolae* (carambola fruit fly), B. *caryae* is a quarantine pest for Nepal . The pattern of grey flecks in the basal wing cells distinguishes *Ceratitis* spp. from most other genera of tephritids. It is highly polyphagous and causes damage to a very wide range of unrelated fruit crops. It may also transmit fruit-rotting fungi (Cayol et al., 1994).

B. *caryae* is a much darker coloured species than B. dorsalis

Detection and monitoring

Fruits (locally grown or samples of fruit imports) should be inspected for puncture marks and any associated necrosis. Suspect fruits should be cut open and checked for larvae.

Monitoring is largely carried out by traps set in areas of infestation. A grid of methyl eugenol and cue lure traps, at least in high-risk areas (ports and airports) modelled on the Steiner trap (White and Elson-Harris, 1994) is used. Host fruit surveys should also be considered as part of the monitoring process.

Trapping systems

Fruit fly are monitored by traps baited with male lures. Capitata males are attracted to trimedlure (t-butyl-4(or 5)-chloro-2-methyl cyclohexane carboxylate) and terpinyl acetate, but not methyl eugenol. According to IAEA (2003), trimedlure, protein baits (nulure, torula, buminal, *etc*.), and a mixture of ammonium acetate, putrescine, and trimethylamine, can be used as attractants for *C. capitat*a. Ceralure is also a new potent and persistent attractant for *C. capitata* (CABI, 2004).

Traps are usually placed in fruit trees at a height of about 2 m above ground and should be regularly emptied, as it is possible to catch hundreds of flies in a single trap left for just a few days; however, a lure remains effective for a few weeks. Trapping efficiency may also be enhanced by the use of fluorescent colors, particularly light green (CABI, 2004)

The males of B. caryeae and B. carambolae are attracted to methyl eugenol (4-allyl-1,2-dimethoxybenzene) in very large numbers.

Areas surveyed and trap density

Traps are set up in areas considered to be at high risk: ports of entry, populous regions and commercial production areas in climatically suitable areas. As recommended by the IAEA (2003), urban areas and points of entry are considered to pose a higher risk than rural residential areas and host orchards.

The trap densities vary country to country. The densities of traps are double placed at points of entry and urban areas compared to rural residential areas. Based on three risk levels (1) international ports of entry, (2) areas presenting high risk of illegal fruit introduction, and (3) private houses, businesses or locations situated close to host production areas, the trap density may vary between 2 and 5 traps per mi^2 (square mile) in urban areas, and between 1 and 4 traps per mi² in residential areas, but, depending on the risk level, the trapping system and the target species, the density may vary from 1 trap per 6 mi² to 22 traps / mi² (Hoffman 2010).

The number of flies caught or the presence/absence of larvae are important parameters to consider, and may vary according to each specific programme and target species.

Pathways and commodities

The most common pathways consists of the import of fruit containing larvae, either as part of cargo, or through the smuggling of fruit in airline passenger baggage or mail. The second pathway is of considerable importance.

4.2.2 Bulb Fly (DIPTERA: ANTHOMYIIDAE)

The larvae of *Delia antiqua* Meigen (Onion fly) damage bulb onions, garlic, chives, shallots, leeks and the bulbs of flowering plants. Seedlings of onion and leek can be severely affected as can thinned-out onions and shallots. When plants are attacked, the leaves start to turn yellow and the bulbs rot quickly, especially in damp conditions.

Detection and monitoring

Yellow water pan traps and yellow sticky traps are used to collect *Delia* species. Sticky traps, however, remained significantly better than pan traps for trapping all *Delia* spp. males, as well as females. A combination of sticky traps and pan traps should be used over an entire growing season when conducting initial surveys for *Delia* species. In routine monitoring programs for *Delia* spp., sticky traps would be more efficacious early in the growing season and more practical than pan traps (James 1997).

Pathways and commodities

Bulb, seedlings and soil are of quarantine concern.

4.3 Order Hemiptera

4.3.1 Aphids (Hemiptera: Aphididae)

Aphids have mouthparts specially adapted for piercing and feed by sucking sap from plants. Aphids often transmit plant viruses and these viruses can sometimes kill the plants. Besides, damage by sap depletion, saliva toxicity and sooty mould growth which develop on honeydew is a serious. *Cerataphis lataniae* is listed as a quarantine pest of Nepal for banana commodity.

Detection and monitoring

Detection at borders is based mostly on direct visual inspection. Suspected host plants of being infested should be carefully examined for nymphs and adult aphids. Sticky traps and yellow pan traps (a colored cup filled with liquid) have both been used for monitoring flight activity of aphids,. However, sticky traps are attractive to many insects and must be replaced frequently. Furthermore, aphids caught in such traps require special solvents to remove them, and this process may make morphological identification difficult. Pan traps yield specimens in better condition, but also need to be emptied on a regular basis and are prone to flooding during periods of heavy rain.

Pathways and commodities

Most aphids introduce with their host plants. Aphids travel long distances with air currents so most species are cosmopolitan and are not included in quarantine lists. The pathways for introduction are known only in a very small number of cases.

4.3.2 Scale insects and Mealybugs (Diaspididae, Coccidae)

Scale insects and mealy bugs are important pest of horticulture and ornamental plants because as it attacks such a wide variety of species and damaging populations can build up rapidly. They can occur on foliage, stems and fruit of the host plant. The appearance of the plant is ruined, not only because of the presence of large numbers of conspicuous waxy scales or powdery 'mealy'-like wax , but also because of the sooty mould which grows on the copious quantities of honeydew excreted by the insects. Sooty mould blocks light and air from the leaves and impairs photosynthesis and lowers the market value of plants and plant products.

tection and monitoring

In quarantine procedures, fruits, plant parts and seedlings of suspect host plants should be thoroughly inspected, if necessary, under a hand lens.

No simple, alternative techniques are available rather than visual examination on field. In the field, scale insects and mealy bugs are detected and monitored by thoroughly inspecting its normal habitats such as fruits, growing plant tips, shoots and roots..

Pathways and commodities

ll life stages may be carried on consignments of plant material and products. Propagation material, nurseries and young plants, human activities contribute to spread the pests.

4.3.3 Whiteflies (Aleyrodoidea)

Whiteflies are polyphagous pests that occur in a wide range of ornamental and horticulture crops. Apart from direct feeding damage to plants, whiteflies transmit a large number of plant viruses. Among important genera *Aleurodicus*, *Aleyrodes*, the *Bemisia* whiteflies are of interest from a quarantine point of view principally due to the quarantine viruses they can transmit. Whiteflies feed on the phloem of plants, and during feeding they can take up and transmit plant viruses. Some examples of such viruses are (a) Bean golden mosaic virus, (b) Cowpea mild mottle virus, (c) Lettuce infectious yellows virus, (d) Pepper mild tigré virus, (e) Squash leaf curl virus, (f) Euphorbia mosaic virus, (g) Florida tomato virus. *Aleurodicus disperses* is the quarantine pests for Nepal.

Detection and monitoring

Whiteflies can be detected by plant sampling as both adult and immature stages are present on the above-ground plant parts. Alternatively, yellow sticky traps are being used as the yellow colour is attractive to the adult flies.

Pathways and commodities

Whiteflies are associated with a wide range of ornamental and vegetable commodities.

4.4 Order Lepidoptera

4.4.1 Moths and butterflies

The majority of species feed as larvae on plant tissues, mainly defoliators but some feed on stems, buds, flowers and fruits. Several of these species now pose a serious threat to agricultural and horticultural crops.

Detection and monitoring

Quarantine species of Lepidoptera are usually detected as flying adults by light or by pheromone traps. Sometimes larvae or emerging females can be trapped with sticky bands around tree trunks.

For monitoring and early detection, light traps are a more efficient general method for collecting nocturnal insects.

Sticky glue traps are often used for trapping, although this can sometimes make it difficult to identify the sample.

Pheromone traps or synergistic traps seem to be best for Lepidoptera. Where lures are not available, traditional trapping and detection methods should be used

Pathways and commodities

The main pathway for invasion of Lepidoptera is via plants transported by the horticultural industry. A few species are transported with grains and seeds. International transport of fruits via containers and packaging equipment (e.g. crates) and vehicles is also an important pathway. In many cases, as on arrival of commodities or before transport, early instars, mainly larvae or their mines, need to be detected visually on plants that are transported by the horticultural industry.

4.5 Order Thysanoptera

4.5.1 Thrips

Thrips are key pests of agriculture crops because of their ability to damage plants directly through feeding, and indirectly through transmission of plant viruses

Detection and monitoring

Detection of thrips can be done by plant sampling, since both adults and immature stages are present on the above-ground parts of the plant. Alternatively, blue (or yellow) sticky traps or water traps are used. The colour is attractive to the adult insects and will attract adults over a short distance. Monitoring of thrips is done mainly by using blue sticky traps (or water traps) and by looking at symptoms. Typical thrips symptoms are silvery feeding scars on leaves and fruits of plants. Heavy infestations may lead to scars, stunting and malformation of shoots and fruits.

Pathways and commodities

Most thrips species have a broad host plant range and can be associated with many different ornamental, vegetable and fruit crops. They have limited natural spread, but can be transported over long distances with plant material.

4.6 Phylum Arthropoda

4.6.1 Mites (Family Tetranychidae)

The spider mite *Tetranychus urticae* is a cosmopolitan agricultural pest with an extensive host plant range and an extreme record of pesticide resistance.

It poses a threat to host plants by sucking cell contents from the leaves cell by leaving tiny pale spots or scars where the green epidermal cells have been destroyed. Although the individual lesions are very small, attack by hundreds or thousands of spider mites can cause thousands of lesions and thus can significantly reduce the photosynthetic capability of plants.

Brevipalpus phoenicis (Geijskes) (false spider mite) has an extensive host range and may cause economic damage and is a serious pest to such crops as citrus, tea, papaya, guava and coffee, and can heavily damage numerous other crops. Apart from the physical damage this species is also a vector of both citrus leprosis and the coffee ringspot virus.

Detection and monitoring

Detection at border is mostly based on direct visual inspection of host plants. . The most common methods to monitor the insect population in and near crops is

visual and by shaking branches over collection trays to capture falling insects.

Pathways and commodities

Associated with many different ornamental, vegetable and fruit crops.

5. PLANT PARASITIC NEMATODES (PHYLUM: NEMATODA)

Introduction

Plant-parasitic nematodes are microscopic unsegmented worms, commonly described as filliform or thread – like multicellular invertebrates. They live in host tissue to feed upon to grow and reproduce. Plant parasitic nematodes inhabit all parts of plant, including developing flower, buds, leaves, stems, and roots, and they have a great variety of feeding habits. Often nematodes withdraw the contents of plant cells, killing them. When this type of feeding occurs, large lesions are formed in the plant tissue. Direct damage to plant tissues by shoot-feeding nematodes includes reduced vigor, distortion of plant parts, and death of infected tissues depending upon the nematode species.

Root- knot nematode, *Meleidogyne,* cyst nematodes, *Heterodera, Globodera, Cactodera* , gall forming nematodes *Rotylenchulus reniformis, Ditylenchus,*and *Aphelenchoides* are some of quarantine pests of concern for Nepal.

Detection and monitoring

The washing test combined with sieving is used for the detection of ecto-parasitic nematodes.

Baerman funnel technique is used for the detection of seed-borne nematodes and or endo-parasitic nematodes in stem and root tissues.

Cyst nematodes are extracted from infested soil using a Fenwick can. Monitoring in the infested field is done observing visually.

Pathways and commodities

The nematodes are dispersed through root material, soil debris, and poorly sanitized bare root propagative material. Infested plant materials including root, soil and surface water for irrigation are the main pathways including transport containing plant debris.

6. SPECIFIC GROUPS OF PATHOGENS

6.1 Bacteria

Plant-pathogenic bacteria cause the development of almost as many kinds of symptoms on the plants they infect as do fungi (see Annex 1.D).

6.1.1 Citrus canker *Xanthomonas campestris pv. aurantifolii*

Citrus canker, *Xanthomonas campestris* pv. *aurantifolii* is by far the most serious disease of commercial citrus varieties and citrus relatives. Its presence in a particular area can cause devastating socioeconomic and political impacts. Yield losses due to citrus canker may result from defoliation, premature fruit drop and blemished fruits, but losses are primarily due to a loss of export markets.

Detection and monitoring

Detection of the causal agent must be confirmed by inoculation tests to susceptible host plants or by molecular methods.

Since *X. campestris* can infect any green part of the plant, including leaves, twigs and fruits of host plants, these plant parts should be closely examined during monitoring.

Pathways and commodities

Infested fruits, saplings, seedlings are the main commodities

6.1.2 African greening *Candidatus liberibacter africanus*

Globally, greening has been regarded as one of the most important threats to commercial and sustainable citrus production. The disease is spread by vegetative propagation (grafting), and by two phloem-feeding psyllid vectors the Asian citrus psyllid, *Diaphorina citri* Kuwayama, and the African citrus psyllid, *Trioza erytreae* (del Guercio), Affected leaves are blotchily mottled, pale yellow, or have the appearance of foliage affected by zinc and other nutrient deficiencies. As the disease progresses, trees turn chlorotic, develop twig dieback, and rapidly decline to a nonproductive state.

Detection and monitoring

Greening is commonly detected in the field by foliage and fruit symptoms. A yellowing of the tree canopy, blotchy mottled leaves and small lopsided fruits with aborted seeds provide the best indication of citrus greening monitoring. Further diagnosis requires indexing on susceptible citrus seedlings by graft inoculation or confirmation through the use of DNA hybridization or polymerase chain reaction (PCR) to identify bacterium.

6.2 Fungi

In general, fungi cause local or general necrosis or killing of plant tissues, hypotrophy and hypoplasia (stunting) of plant organs or entire plants, and hyperplasia (excessive growth) of plant parts or whole plants. (Annex 1. C) for symptoms)

6.2.1 Sweet orange scab *Elsinoe australis*

Elsinoe australis, the sweet orange scab pathogen, is economically important because it attacks citrus species grown for the fresh-fruit market (oranges and mandarins). It is also important because the pathogen can be carried on fruits in

international trade.

Scab pustules on fruits are slightly raised and pink to light brown. Later, they become warty, cracked, yellow-brown, and eventually dark grey.

Detection and monitoring

As for many other fungal diseases, visual inspection, symptomatic plant tissue sampling and using molecular markers are the first steps for pathogen detection and identification.

In order to develop a fast and reliable diagnostic test independently of the presence of disease symptoms, Schweigkofler *et al*. (2004) presented a novel trapping approach using filter paper in combination with a rapid molecular method to detect the presence of inoculum and to quantify it in the air. The test can be used directly on trapped spores, without the need for spores to be germinated.

Pathways and commodities

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

6.2.2 Citrus mal secco, *Phoma tracheiphila*

The fungus appear in spring as leaf (veinal) and shoot chlorosis; as the disease progresses, the leaves wilt, dry up, fall and dieback occurs. The disease may develop so suddenly that the leaves dry up on the tree.

Infected fruit are of such poor quality that they are unlikely to be marketed or exported.

Detection and monitoring

The growth of sprouts from the base of the affected branches and suckers from the rootstock are a common response of the host to the disease

To detect and monitoring Mal Secco in the field, look for leaf and shoot chlorosis, wilt, and defoliation, followed by dieback of twigs and branches. On live twigs and branches, make diagonal cuts and look for the diagnostic orange-yellow to pink-salmon discoloration that develops in newly infected twigs and branches.

When submitting material for identification or confirmation of *P. tracheiphila*, discolored twigs and branches, or bark with pycnidia, will be most useful for identification purposes. Suspect plant material should be dried, labeled, and

placed in sealed double containers.

Pathways and commodities

Imported fruits and nursery stock are the main pathways. Movement of infected plant parts of *Citrus spp.* could introduce the pathogen into new areas. Infected propagative material would be the most likely means of spread. This material could easily serve as a source of inoculum due to conidium production in and on infected plant parts. The likelihood of infected fruit serving as a source of disease spread is very low.

6.3 Viruses

Plant viruses affect many plants and cause a wide range of discolorations and distortions in leaves, shoots, stems and flowers, but rarely kill the plant. Plant viruses is transmitted by a vector, most often sap-sucking insects such as aphids, thrips, whiteflies, or by leaf-feeding beetles, plant-feeding mites, soil-inhabiting nematodes and fungal pathogens can also transmit viruses.

Plant viruses cause various types of plant diseases, but the diseases do not typically result in plant death. They do however; produce symptoms such as ring spots, mosaic pattern development, leaf yellowing and distortion, as well as deformed growth (Annex 1.E).

6.3.1 Citrus leprosis virus

Citrus leprosis virus is transmitted by mites *Brevipalpus phoenicis*) *phoenicis, B. californicus*, and *B. obovatus*.

On leaves, lesions are usually round, with dark-brown central spots. On green fruits, the lesions are initially yellowish, later brownish or blackish (10 to

20 mm in diameter), sometimes depressed, and occasionally with gum exudation. On stems, lesions appear as grey or brown cortical bark scaling. Lesions may coalesce and cause the death of the twig.

Detection and monitoring

Traditionally, citrus leprosis has been detected by the examination of localized lesions on leaves, fruits, twigs, or the presence of mites (*Brevipalpus)* on lesions. Monitoring for viral pathogens is performed almost always by visual means by direct observation of symptoms in the field.

Current advances in molecular technology, such as the RT-PCR technique,

have allowed the development of specific primers for CiLV detection (Locali, et al., 2003).

Pathways and commodities

Infested plants, planting materials along with importation of vectors are the main source of introduction.

7. PARASITIC PLANTS/WEED SPECIES

The weed species broadly divided into two categories: parasitic weeds (phanerogams) and non-parasitic weed species. The parasitic weed species further sub categorized into total parasitic (e.g. *Cuscuta* sp (Doder) and *Cassytha*) and partial parasitic (*Orabanche, Loranthus, Striga*). The non-parasitic weed species categorized into noxious (poisonous or deleterous weeds), invasive and non-invasive species.

The examples of poisonous or deleterious weed species include: *Calotraphis*, *Datura stramonium; Argemone mexicana* (Mexican poppy);

The parasitic weed is capable of significantly reducing yields, in some cases wiping out the entire crop.

Detection and Monitoring

It is important to detect new invasive or aggressive weed species while the infestation is still localized and possible to eradicate. For detection of weed species see Annex 1. F.

For detection, using the sieve of different mesh size is useful for capturing the seeds of the weed in imported agriculture and forest seed, thus giving a quick indication of the presence of weed seeds in the sample.

Early detection and rapid response are necessary to avoid weed reproduction. Weeds are monitored in the field to assess current or potential threats to crop production, and to determine best methods and timing for control measures.

Pathways and commodities

Contaminated seeds, soil and plant debris in the transport are capable of spreading the weed.

8. 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.

The left over portion of used sample (fungal/bacterial specimens) should be collected in a safe disposal bin and treated with 4% formalin overnight before final disposal.

In most instances insect specimens should be sent dead and preserved in a manner required by the laboratory to ensure that economically and environmentally significant plant pests do not inadvertently escape into the environment.

It is essential that the time between sampling and dispatch of the sample for identification be kept to a minimum.

For collection and handling of pest samples see NSPM… (Standard Technical Protocols for collection and handling of disease samples) and NSPM… (Standard Technical Protocols for collection and handling of insect samples) .

9. PEST RECORD KEEPING

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.

Annex 1. Detection of Signs and Symptoms caused by Pests

A. Insects/Mites

- Boring/tunneling─ characterized by the presence of borer holes into stem (trunk/shoot/twigs), bark, flower buds, fruits & seeds (including nuts) including underground parts (e.g., stem borers, wood borers, bark borers, fruit borers, seed weevils)..

Bronzing ─ characterized by reddening or bronzing of leaves (e.g. mites)

- Dead hearts─ characterized by dead shoots (e.g. rice stem borer)
- Galls ─ Infested plant parts such as leaves and stems will have raised gall like structures (e.g. mango gall midge)
- Honey dew─ characterized by sugary secretion caused by aphid attack, which usually attracts ants.
- Leaf curling/puckering/cupping ─ characterized by downward or upward curling of leaves or puckering or cupping of leaves (e.g. mealy bugs).
- Leaf mining─ characterized by mining into leaf epidermis (e.g. serpentine leaf miner)
- Leaf Scraping─ characterized by epidermal scraping of leaves (e.g., thrips)
- Leaf webbing─ characterized by webbing of leaves and tender shoots (e.g. leaf webber)
- Shot hole ─ characterized by the presence of shot-holes in affected plant parts such as twigs, branches, trunk, bark and wood (e.g., shot hole borer) and subsequent debilitation of plant due to entry and infection by wood rotting fungi.
- Silver leaf ─ characterized by silvering appearance of leaf surface (e.g. white flies)

B. Nematodes

- Bulb rot─ characterized by rotting of underground bulbs (e.g. stem & bulb nematode)
- Bloating─ characterized by water-soaked lesions or raised swellings on the outer scale leaves of bulbs such as onion due to the presence of nematode (e.g. stem & bulb nematode)
- Galls─ characterized by hard gall like structures caused due to nematode infestation of floral parts (ears) (e.g. ear cockle of wheat)
- Necrosis─ characterized by death of plant tissue of affected plant parts (e.g. stubborn nematode).
- Root lesions discolored areas of the root (e.g. root lesion nematode)
- Root knots─ characterized by knots on the affected roots (e.g. root knot nematode)
- Stunting and yellows─ characterized by shortening of growth of plants and yellowing of leaves (e.g. potato cyst nematode)
- White tip─ characterized by blotching of leaves at the tip (e.g. white tip nematode)

C. Symptoms caused by Fungi

- Anthracnose─ characterized by necrotic lesions characterized by depressed dark necrotic centre with clearly defined pale margin
- Bud rot─ characterized by rotting of apical growth of stem (e.g., bud rot of palms)
- Cankers─ characterized by raised corky lesions or outgrowths resulting often gummy exudation from cankerous lesion
- Club root─ characterized by club-shaped roots (e.g., club root of cabbage)
- Damping-off─ characterized by sudden collapse of seedlings at the ground level
- Dieback─ characterized by withering and dieing of twigs from tip down words resulting in death of whole plant slowly (e.g., citrus dieback)
- Discoloration─ characterized by change in coloration of affected plant parts (e.g. red rot of sugarcane)
- Downey mildew─ characterized by white downy growth usually on undersurface of the leaves (e.g., downy mildew of sunflower)
- Dry root rot─ characterized by dieing of roots due to necrosis (e.g. texas root rot of cotton)
- Galls─ characterized by the presence hardy out growths in the form of galls usually on affected branches or hypertrophied seed (e.g. Protomyces galls in Coriander seed)

- Gummosis─ characterized by exudation of gummy like substance from wounded surface (e.g. sugarcane gummosis
- Leaf blight─ characterized by extensive blighting of leaves due to coalescing of spots (e.g. Alternaria blight of wheat)
- Leaf blotching─ characterized by extensive blotching of leaves
- Leaf shredding─ characterized by shredding of affected leaf tissue
- Leaf spots─ characterized by necrotic spots of varying shape & size with distinct margin (e.g. leaf spots).
- Malformation─ characterized by proliferation and transformation of auxiliary buds into malformed green leafy-like structures as well as inflorescence (e.g. mango malformation; green ear of pearl millet).
- Powdery mildew─ characterized by white powdery growth usually on upper surface of leaves (e.g., powdery mildew of cucurbits)
- Pustules─ characterized by rupturing of epidermal tissue of leaves, leaf sheath and stem giving a roughened surface (e.g. wheat stem rust).
- Scab─ characterized by raised spots usually on the surface of leaves, fruits and stem (e.g. citrus scab)
- Seedling rot/collar rots─ characterized by rotting of affected seedling before and or after emergency of seedlings. Collar rot is characterized by the rotting of seedling at collar level (e.g. Sclerotium rot)
- Sooty mould─ characterized sooty growth on the surface of affected plant growth due to sugar secretions
- Smuts & bunts─ characterized by usually transforming of whole grain into black powdery mass of spores (loose smut of wheat). Some times it is partial (e.g., Karnal bunt of wheat). Also smutting of leaves & growing shoots (e.g. sugarcane smut)
- Wart─ characterized by cauliflower like fluffy out growths (warts) (e.g. potato wart).
- Wet rot─ characterized by watery oozing from rotting tissue (e.g. Sclerotinia rot)
- Wilting─ characterized by sudden withering of plants
- Witches' broom─ characterized by proliferation of auxiliary buds and/or stipules, hypertrophied petioles often with smaller laminae, hypertrophy of the main axis in the cortex and secondary phloem and an increase in the number of nodes and/or the shortening of internodes (e.g., witches' broom of cocoa).

D. Symptoms caused by bacteria

- Angular leaf spots ─ characterized by dark-green, water-soaked spots, initially more clearly visible on the underside of the leaf lamina; the spots are angular in shape, being delimited by the smaller veins. Older spots become dark-brown or black and are visible on the upper surface of the leaves (e.g. angular leaf spot of cotton).

- Canker ─ characterized by raised, spongy eruptions on the surface of leaves, twigs and fruits. As the lesions enlarge, the spongy eruptions begin to collapse, and brown depressions appear in their central portion, forming a crater-like appearance. However the edges of the lesions remain raised above the surface of host tissue and are characterized by having a greasy appearance (e.g. citrus canker).

- Crown gall ─ characterized by galls (tumor-like swellings) on roots and stems below ground or at the crown of the plant (e.g. crown gall & hairy root of apple).

- Gummosis─ characteristic gum pockets are formed and the slime exudes from the cut surface of the stalks or stem (e.g. sugarcane gumming disease).

- Halo blight─ characterized by discolored necrotic leaf spots characterized by yellow haloes around the spot (e.g. halo blight of bean

- Hairy root─ characterized by excessive development of thin hairy roots (e.g. crown gall & hairy root of apple).

- Leaf blight─ characterized by extensive blighting of leaves with water-soaked margin (e.g. bacterial leaf blight of rice)

- Leaf streak─ characterized narrow necrotic leaf spots in the form of streaks with water soaked margin (e.g. bacterial leaf streak of rice)

- Ring rot─ characterized by discoloration of the vascular tissue at the stolon end and is most readily observed in tuber cross-sections. Discoloration varies from creamy-yellow to brown zones encompassing all or only a portion of the vascular ring. When pressure is applied to a cut tuber, creamy odorless ooze may be expressed from the tissue. Distinctive corky-brown tissue sometimes surrounds hollows that develop in the vascular ring (e.g., ring rot of potato).

- Scab─ characterized by roughly circular, raised, tan to brown, corky lesions of size ranging between 5-10 mm in diameter (e.g. common scab of potato)

- Soft rot─ characterized by wet, cream to brown with a soft, slightly granular consistency, and are easily washed away. The margin of the necrosis is often discolored (brown or black (e.g., black leg of potato).

- Wilt─ characterized by withering of affected plant parts and the affected stems when cut across reveal oozing out of bacteria from cut ends (e.g. bacterial wilt of tomato and potato).

E. Symptoms caused by viruses

- Bunchy top─ characterized by small erect choked leaves at the top (e.g., banana bunchy top virus)

- Enations─ characterized by swellings or outgrowths from veins (

- Flower break─ characterized by variegation in flower colour (e.g. tulip flower break virus)
- Fruit blotching─ characterized by discolored lesions or spots on the fruit surface (e.g., water melon mosaic virus)
- Leaf chlorosis─ characterized by pale or yellow colouration of leaves (e.g. bean yellow mosaic virus)
- Leaf curling─ characterized by downward curling of leaves (e.g., tomato leaf curl virus, papaya leaf curl virus
- Leaf flecking ─ characterized by clearing of veins at intermittent areas (e.g., citrus tristeza virus)
- Leaf mottling─ characterized by alternating light and dark green areas, which is best visualized under shade
- Leaf streak ─ characterized by the presence of chlorotic streaks in the affected leaves (e.g., sugarcane chlorotic streak virus)
- Leaf stripe ─ characterized by presence of chlorotic stripes in the affected leaves (e.g. barley stripe virus)
- Leaf puckering ─ characterized by raised blisters in the affected leaves (e.g., papaya ring spot virus)
- Leaf rolling ─ characterized by upward rolling of leaves (e.g., potato leaf roll virus)
- Little leaf ─ characterized by extreme reduction in leaf size caused by phytoplasmas (e.g., little leaf of brinjal, sandal spike)
- Mosaic ─ characterized by alternating light and dark green areas giving mosaic appearance (e.g., tobacco mosaic virus)
- Necrosis ─ characterized by dark brown necrotic areas in the form of specks, lesions, ring spots on the leaves, stem, buds etc (e.g., ground nut bud necrosis virus; tobacco ring spot virus).
- Oak leaf pattern ─ Interveinal chlorosis characterised by oak leaf appearance.
- Phyllody ─ characterized by inflorescence transformed into green leaf like structures caused by phytoplasmas (e.g., aster yellows, sesamum phyllody),
- Rosetting ─ characterized by progressively smaller, chlorotic, twisted and distorted leaflets with shortened internodes and stems thickened and plants are severely stunted giving rosette appearance (e.g., ground nut rosette virus).
- Shoe string effect ─ characterized in the form of extreme reduction of leaf margin giving shoe-string appearance (e.g., papaya ring spot virus)
- Stem pitting ─ characterized by many small conical pits (honeycombing) corresponding to bristle-like protuberances from the wood (e.g., citrus tristeza virus)

- Spindle tuber ─ tuber tapered into spindle shape (e.g. potato spindle tuber viroid)
- Stunting/dwarfing ─ characterized by shortened growth of plants (e.g., groundnut stunt virus)
- Swollen shoot─ characterized by stem swellings may develop at the nodes, internodes or shoot tips (e.g. cocoa swollen shoot virus).
- Vein clearing─ characterized by pale green colouring of veins (e.g., ground nut mottle virus)
- Vein banding ─ characterized by dark green banding of veins (e.g., potato virus X)
- Witches' broom ─ characterized by proliferation of axillary buds and/or stipules, hypertrophied petioles often with smaller laminae, hypertrophy of the main axis in the cortex and secondary phloem and an increase in the number of nodes and/or the shortening of internodes caused by phytoplasmas(potato witches' broom)
- Wilting─ characterized by sudden withering of plants (e.g., tomato spotted wilt virus)
- Yellows ─ characterized by extreme yellowing of leaves caused by phytoplasmas (e.g., aster yellows)

F. Weed Species Detection

- Weeds with thorns, spines and sharp prickles
- On fruit
- On stem at leaf base
- On leaves, stem, flower head, fruit
- Weeds with square stems
- Weeds with whorled leaves
- Weeds with exude milky sap from fresh roots, stems, foliage
- Weeds with ocrea (papery sheath that encloses the stem at the node)
- Weeds with palmately compound leaves
- Weeds with dissected leaves