Collection of suspect Emergency Plant Pests

Document revision history

Version	Date issued	Amendment details	
		Section(s)	Details
1.0	5 Dec 2013	All	Reformatted from Appendix 3 of PLANTPLAN (V1 Nov 2011). Original document separated into two SOPs. Internal references to Appendices in PLANTPLAN removed.
2.0	17 Dec 2014	All	Guideline developed from *Collection and transport of Emergency Plant Pests* SOP (V1 Dec 2013) by the Subcommittee on Plant Health Diagnostic Standards (SPHDS). Original SOP separated into two guideline documents. Approved by SPHDS October 2014. Endorsed by Parties November 2014.

Contents

1. Introduction

The purpose of these guidelines is to assist plant health staff/field officers when collecting suspect Emergency Plant Pest (EPP) samples for submission to diagnostic laboratories.

2. Critical issues

Correct sample collection is central to ensuring an accurate diagnosis. Critical issues include the following:

- availability of collection tools (see Appendix 1 - Resource equipment)
- the sample is fit-for-purpose (integrity intact, adequate numbers, and all necessary components sampled)
- the sample is appropriately contained, labelled and stored

- chain of evidence is followed at all times (see *Chain of evidence* standard operating procedure; SOP)
- a sample submission form is completed at the time of sampling capturing all pertinent details
- hygiene and disinfestation protocols are followed (see *Disinfection and decontamination* guidelines)
- safety of staff is considered at all times and all relevant Work, Health and Safety legislative requirements are followed.

3. Collecting and packaging samples

If necessary, consult with the laboratory to ensure the most appropriate sample is collected.

3.1 General

- Complete a sample submission form at the time of sampling (include details such as host, plant parts affected, location (GPS coordinates), sampling date, collector, property owner, contact details and any other relevant information).
- The collection point MUST be clearly marked in the field (e.g. with brightly coloured ribbon and/or GPS coordinates).
- Disinfect implements (e.g. with 80% v/v ethanol or 0.5% v/v available chlorine solution, as appropriate) prior to and after each sampling.
- For suspected root pests/diseases, include soil and crown (lower stem) tissues with root samples.
- Labelling of sample vials/bags must be clear and legible using an alcohol-proof and water-proof marker.
- Minimise the time between sampling and dispatch to the receiving laboratory, and store in a portable cool-box during this time.
- Use best practice biosecurity measures before entering a suspect site to avoid the potential of spread between sites.
- If possible, sample from perceived area of minimal damage to perceived area of high damage within a field/orchard and on the individual plant.
- Follow appropriate hygiene protocols for collecting samples and disinfecting hands, footwear and clothing (refer to *Disinfection and decontamination* guidelines).
- Follow *Transport of suspect Emergency Plant Pest* guidelines to send samples to the designated laboratory, including advising the laboratory of the expected arrival.

3.2 Insect samples (use pest specific protocols where available)

i. Where possible **insect samples should be sent dead**[1], and preserved in the manner required by the laboratory (see Appendix 2 - Insect sampling procedures).

ii. Where present collect a reasonable number of samples (commensurate with their size) of all life stages and variants, that are complete and in good condition.

[1] Note: In exceptional circumstances, the diagnostic laboratory may require live material. For example, if only immature stages are available and the diagnostic lab needs to rear material through to adult (in secure facilities). In such cases, special arrangements must be made, ensuring secure transportation, prompt collection of samples from airports etc. These circumstances will be decided by the State Coordination Centre (SCC) and/or the Chief Plant Health Manager (CPHM).

iii. Place the samples in a plastic vial, a crush-proof box, or similar, containing material to prevent damage.

iv. Secure the lid, label clearly, and triple-bag using tamper-proof bags, disinfecting between layers, and include the completed sample submission form in the outer bag - according to chain of evidence requirements (refer to *Chain of evidence* SOP).

3.3 Pathogen samples (use pest specific protocols where available)

i. Select a fresh and generous sample representing the full range of symptoms including both diseased and healthy sections, and place in a labelled zip-lock plastic bag (see also Appendix 3 - Preferred packaging methods for pathogen samples).

ii. Inspect for insects and, if present, ensure this is recorded on the outside of the bag and the submission form so correct containment can be used.

iii. Triple-bag using tamper-proof bags, disinfecting between layers, and include the completed sample submission form in the outer bag according to chain of evidence requirements (refer to *Chain of evidence* SOP).

iv. Store samples in a cool-box until dispatched unless the suspected pathogen is likely to survive better at room temperature.

3.4 Nematode samples (use pest specific protocols where available)

Soil, plant or root samples affected should be collected and packaged as per the plant samples above.

3.5 Seed samples

Seed sampling should be undertaken by an accredited seed sampler to ensure that a representative sample is taken incorporating chain of evidence procedures (refer to *Chain of evidence* SOP).

4. Appendix 1 - Resource equipment

Standard kit

The Standard kit includes equipment that may be required for the investigation of a suspected emergency plant pest.

- portable cooler or sturdy, sealable plastic crate with freezer blocks for keeping samples cool
- press seal bags of suitable sizes for samples
- sample jars or vials of varying sizes (e.g. 20 ml and 50 ml)
- disposable gloves
- disposable overalls
- fresh bleach or other suitable disinfectant (e.g. domestic use with 4 - 5% available Chlorine (Cl))
- a sturdy leak proof plastic bin for a footbath
- 65% ethyl-alcohol 35% water solution (for insect sample storage)
- trowel
- spade or coring tube for sampling soil
- sealable plastic bags of suitable micron thickness for disposing of personal protective equipment
- large, strong plastic bags for sealing contaminated equipment such as boots or spades (strong garbage bags are acceptable for this)
- washable boots i.e. rubber boots
- adhesive labels (either pre-prepared with bar code/unique ID or handwritten in field)
- evidence tape (tamper proof)
- permanent markers
- pencils/pens
- book or sample sheets for recording details of site and samples
- soap
- paper towels
- water (sufficient for washing hands)
- baby wipes for cleansing hands and face
- brightly coloured ribbon
- a mobile phone[2]
- camera[2]
- sample submission forms from lab
- list of contacts (laboratories/courier/diagnosticians/CPHM for the state)
- blank key list of samples
- 80% ethanol in spray bottle
- bucket (for disinfecting tools)
- plastic containers for sample storage (e.g. lunch boxes)
- trays/crates (for disinfecting equipment)
- quarantine tape or similar available
- GPS unit

[2] Any equipment which may be damaged by decontamination, such as cameras or mobile phones should be protected with a sealable plastic bag or similar.

- water (sufficient to wash hands and equipment)
- magnifying glass
- masks and other PPE if dealing with chemicals
- overnight express post packs

Additional basic equipment for collecting insect samples

- variety of vials with internal seals e.g. 20mm, 70mm
- insect net (or aspirator)
- McCartney bottles
- soft tweezers
- rigid tweezers
- fine scissors
- secateurs
- alcohol 75%
- very fine brush e.g. size 0 – 000
- larger plastic jars
- paper bags
- fine forceps
- pocket knife

It is suggested that all the above items could be stored in a large toolbox for easy access.

5. Appendix 2 - Insect sampling procedures

Table 1: Insect sampling and packaging procedures

Insect type	Kill method	Live insect sampling
Moths, butterflies	Freeze for 24 hours or place in an airtight container with a tissue or cotton wool that has been soaked in nail polish remover.	
Plant feeders (e.g. scarab larvae, scale)	Do not remove mealy bugs or scale insects from the leaves or stems on which they are feeding as this will damage their mouth parts and make identification difficult. Instead, cut out leaf tissue around the insect and place this in 70% ethanol.	Plant feeders with strong jaws should be sent with a handful of soil or leaves as they may otherwise damage each other in transit
Hard bodied insects (e.g. grasshoppers, beetles)	Freeze for 24 hours.	Carefully fold sample in tissue paper and place in crush-proof plastic tube or container with several holes in the lid for ventilation. For beetles with strong jaws, send with a handful of soil or leaves as they may otherwise damage each other in transit.
Soft bodied insects (e.g. caterpillars)	Place in hot water for 10 minutes. Then preserve in 70% ethanol.	Leave insect larvae (grubs, caterpillars or maggots) in grain or other seed or fruit as this will help to preserve them.
Ants	Spray with fly spray, then stick to **clear** sticky tape. Stick this to a piece of paper which also records the location where caught and the collector's name and contact details. Alternatively collect with a small paint brush into 70% ethanol.	
Small and/or soft bodied insects (e.g. thrips, aphids, mites and larvae)	Place sample in 70% ethanol (use methylated spirits) and completely fill the container. NOTE: A limited amount of ethanol is permitted to be posted by Australia Post under the International Air Transport Association's "Dangerous Goods Regulations".	

6. Appendix 3 - Preferred packaging methods for pathogen samples

Table 2: Preferred packaging methods for pathogen samples
All samples should be placed in a minimum of two sealed plastic bags before the final packaging (refer to Plant Biosecurity Cooperative Research Centre brochure available from http://plantbiosecuritydiagnostics.net.au/resource-hub/documents/pbcrc-packaging-project-2010/)

Plant material	Preferred packaging method
Fruit and vegetables	Wrap in paper towel or tissue, place in a sealed plastic bag and place in a hard sided box or screw top plastic vial with extra paper to prevent crushing or movement.
Above ground plant material (e.g. leaves, stems, or whole plants with no soil)	Wrap in dry paper towel or newspaper and seal in a plastic bag.
Roots – no soil	Wrap in moist paper towel and seal in a plastic bag with air removed.
Soil (with or without roots)	Seal in a plastic bag with air removed. Do not fill bag more than ½ full and keep to less than 1kg samples.
Seed	Seal in a plastic bag with air removed, or a material bag with secure fastening. Do not fill bag more than ½ full.