



REFERENCE

Plant exports guide—specimen collection

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Purpose of this document

This document provides guidance to authorised officers (AO) on collecting pest and disease specimens during plant and plant product inspections. The correct collection and storage of specimens will help to ensure effective and timely identification.

When is pest identification required?

A pest is defined as any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products.

If a live pest is detected during inspection, formal identification may be required.

Note: The Guideline: *Inspection of horticulture for export* describes when formal identification is required.

What equipment is used for collection?

For information and images of equipment used in the collection of specimens see the Reference: [Plant exports guide—equipment](#).

Determining if the pest is alive

It is important that before a specimen is collected the AO determines if it is alive or not.

For insects, they can be put on a white piece of paper with a circle drawn around them. A light can be placed over the top of the insect to warm it up particularly if the consignment has been stored in a cold room. The AO will be able to see if the insect moves within the circle.

To ensure the insect does not escape, the AO can place a clear container over the top to keep it secure.

Collecting specimens

Specimens should be collected as per the:

- Work Instruction: *Inspecting horticulture for export using end-point sampling*
- Work Instruction: *Inspecting horticulture for export using in-line sampling*.

Recognising invertebrate pests

Invertebrates are animals without backbones. Commonly encountered invertebrate pests include insects and arachnids.

- **Insects** are small invertebrate animals with six legs and typically one or two pairs of wings and include, for example, flies, beetles and butterflies.
- **Arachnids** are small wingless invertebrates with eight legs and two body segments and include, for example, spiders and mites.

Life stages of insects and arachnids

The following table defines the different life stages of insects and arachnids.

Term	Definition
Egg	A round or oval structure containing an embryo. Most insects and arachnids lay eggs.
Larva (insect only)	The newly hatched, wingless, worm-like form of many insects that undergo metamorphosis and look very different to the adult stage.
Nymph (insect only)	The immature form of many insects. Similar in structure to the adult form but smaller and lacking fully-developed wings.

Term	Definition
Immature	An organism that is not fully grown or developed. For example, juvenile stages of spiders.
Pupa (insect only)	The non-feeding stage between the larva and adult in the metamorphosis of insects, during which the larva typically undergoes complete transformation within a protective cocoon or hardened case.
Adult	A fully grown, mature and reproductively viable organism.

Collecting invertebrate specimens

When collecting invertebrate specimens, AOs should:

- collect the specimen using a probe, forceps or fine paint brush
 - a small paintbrush wet with 80 per cent ethanol can be used to pick up live insects on the tip of the brush, using a rolling action. The insect can then be transferred to the sample vial
 - moths and other flying insects can be collected by placing the wet brush over the wings to prevent flight. Often a moth will struggle from beneath the brush. Once it is wet, however, it may be readily picked up
- collect specimens of different life stages if available
 - for suspected eggs, pupae and larvae inside the commodity, a slicing action can be used to slowly expose the inside of the product or a conical cut can be made around the symptomatic area to avoid damaging the specimen
- take care not to damage the specimen during collection
- try to keep substrate (plant material, soil) out of the vial as this material can break down, making it difficult to locate and identify specimens.

Collection methods for different invertebrate specimens

The best collection method will vary based on the type of invertebrate found. The following table provides useful information on collecting different types of invertebrate specimens.

Invertebrate specimen	Collection method
Live adult moths, butterflies or mosquitoes	<ul style="list-style-type: none"> • The specimen should be killed with knock-down spray or frozen. • The specimen should be placed in a clean, dry vial with tissue to ensure scales on the wings remain intact.
Colony-forming insects such as bees, ants or wasps	Specimens should be placed in a vial of 80 per cent ethanol.
Live mealybugs and scale insects	<ul style="list-style-type: none"> • These insects are best collected on the substrate. AOs should remove enough of the substrate (for example leaves, stem, fruit) around the insect using a sharp knife or secateurs. • The entire section should be placed into a dry vial or into 80 per cent ethanol.
Slug or snail	Specimens should be placed in a vial of 80 per cent ethanol.

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Invertebrate specimen	Collection method
Maggots and other soft-bodied larvae	Specimens should be placed under boiling water for a couple of minutes then drained and transferred to vial of 80 per cent ethanol.
Caterpillars, fleshy beetle larvae or grubs	Specimens should be placed in a vial of 80 per cent ethanol.
Eggs	<ul style="list-style-type: none"> • If possible, eggs should be removed from the substrate and secured in a clean dry vial. • If the eggs are inserted into the substrate, then the AO should remove the substrate around the eggs using a knife or secateurs, and secure the substrate in a clean, dry vial.

Recognising vertebrate pests

Vertebrates are animals with backbones including mice and birds.

Collecting vertebrate specimens

When collecting small vertebrate specimens, AOs should place live specimens in a hard, ventilated, container suitable for transport, and place dead specimens in a vial of 80 per cent ethanol.

Recognising pathogens

Plant pathogens include fungi, bacteria, phytoplasmas, viruses and nematodes. Symptoms of pathogens can include soft spots, light brown lesions, rots and decay, mould and fungal growth in a variety of colours, rusts and mottling.

Collecting disease specimens

What should be collected for diagnosis?

Where possible, the entire plant should be collected. If it is not possible to collect the entire plant, the AO should collect samples:

- representing the full extent of symptoms observed
- of both healthy and diseased tissue
- at the margin of infection between infected and healthy tissue
- from all parts of the plant (for example, fruit, stem and leaves if available) where symptoms occur
- that are representative of all stages of the disease; that is, if on one plant there is slight yellowing, while on another there are fungal pustules on the leaves and stem, samples from both plants should be collected.

How should samples be taken?

- Samples should be taken using secateurs or a sharp knife that has been disinfected with 80 per cent ethanol or other suitable disinfectants between samples.
- Used cutting implements should be disinfected by wiping generously with 80 per cent ethanol and then by washing it under a tap.

Are heavily-diseased samples suitable?

Heavily-diseased samples are normally not suitable for disease isolation. The diagnostician may require samples with less advanced stages of symptoms for diagnosis.

How should the sample be packed?

The following outlines the steps recommended for collecting disease specimens:

- The plant material or specimen should be double-bagged in snap-lock bags. If the sample is wet, it can be wrapped in dry paper towel before bagging.
- The bag should be large enough to enclose the sample without causing any damage.

Collecting nematode specimens

When nematodes are suspected, it is important to collect symptomatic material as the presence of galls, swelling, lesions or cysts could assist in identifying the nematode.

The following outlines recommendations for collecting free-living nematodes and not free-living nematodes present in a consignment.

Free-living

- The nematodes should be collected from the substrate using a dissecting needle or probe.
- The dissecting needle or probe should be gently dipped into a vial of cold tap water to dislodge specimens and the vial shut screwed.
- The symptomatic material should be collected and double-bagged in snap-lock bags.

Not free-living and cannot be easily isolated

- The symptomatic material should be collected and double-bagged with the suspected nematode.

Collecting photographic evidence

Where possible, an AO should take photographic evidence of the specimen before it is sent for identification. The images may also help with the identification process.

Some tips for taking photographic evidence include:

- for disease specimens, a photograph of healthy plants should be taken in addition to the specimen photographs
- a number of photographs should be taken while the specimens are available and in good condition—both close up and further out to provide perspective
- appropriate magnification should be used—un-zoomed, with the specimen filling most of the centre of the screen
- a scale should be used, such as a small ruler or coin in the corner of the photograph to show the size and scale of the specimen
- the specimen should be in focus with adequate lighting.

Labelling samples

Samples should be labelled using multi-purpose self-adhesive labels, in pencil (as ink can easily smudge), so that they may be readily identified.

Vials can be placed inside a snap lock bag and the label applied to the bag.

The required label information is detailed in:

- Work Instruction: *Inspecting horticulture for export using end-point sampling*
- Work Instruction: *Inspecting horticulture for export using in-line sampling*

Storage and transport

Clients should put samples in the fridge (not the freezer) if there is a delay in getting them identified.

If transport to the diagnostician will take longer than a day the sample should be transported with an ice pack.

Related material

- *Export Control Act 1982*
- Export Control (Prescribed Goods – General) Order 2005
- Export Control (Plants and Plant Products) Order 2011
- [Manual of Importing Country Requirements \(MICO – Plants\)](#)
- [Plant Exports Operations Manual \(PEOM\)](#)
 - Guideline: *Inspection of horticulture for export*
 - Work Instruction: *Inspecting horticulture for export using end-point sampling*
 - Work Instruction: *Inspecting horticulture for export using in-line sampling*
 - Reference: *Plant exports guide—equipment*
 - Reference: *Plant exports guide—horticulture inspection techniques*
 - Reference: *Plant exports guide—sampling horticulture*

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