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EPPO STANDARD ON PHYTOSANITARY PROCEDURES

PM 3/98 (1) Inspection of growing media associated with consignments of plants for planting

Specific scope: This Standard describes the inspection and sampling of growing medium (including soil) associated with consignments of plants for planting to ensure the growing media is free from pests. The Standard does not cover inspection of plants for planting in the consignment. Growing media moved as a separate commodity or contaminating a commodity is also not considered in this Standard. The Standard includes relevant EPPO A1 and A2 pests[1] recommended for regulation. This Standard provides guidance that may be relevant to inspections for export.
Specific approval: This Standard was first approved in 2024–09.

1 | INTRODUCTION

Many plants for planting are imported or traded within the EPPO region with growing media. ISPM 5 Glossary of phytosanitary terms (IPPC, 2019) defines growing medium as 'Any material in which plant roots are growing or intended for that purpose'. Soil is included in this definition of growing media and consequently this Standard will refer to growing media without re-specifying that growing media includes soil. Plant pests such as bacteria, nematodes, molluscs, insects and fungi can all be associated with growing media. Growing media can provide a substrate for pests to survive and possibly reproduce on the host or in the soil. In addition, pests in soil have the potential to be introduced into a suitable habitat as the plants may be replanted, even in outdoor situations. As a result, growing media attached to plants is considered as a high-risk pathway for the introduction or spread of quarantine pests (ISPM 40 *International movement of growing media in association with plants for planting*, IPPC, 2017a). EPPO has long recognized the risk of movement of soil with plants for planting and in 2016 an EPPO Council declaration was published where the Council reiterated that the intercontinental movement of soil with plants for planting is a high risk for plant health (https://www.eppo.int/RESOURCES/position_papers/council_soil_movement).

Growing media acts to protect the root system and enables the plant to sustain vitality and survival while being moved. Some plants such as bonsai are mainly imported with growing media attached.

Many countries in the EPPO region have restrictions on the import of growing media attached to plants. The pest risk of growing media depends on a number of different factors such as the type of media, its origin, production mode, treatment, storage, and the way the plants for planting have been produced (ISPM 40: IPPC, 2017a). Soil attached to plants is mostly prohibited from import into the EPPO region and only certain types of growing media are allowed for import and these must have been stored and/or treated to ensure freedom from pests.

Inspection and testing is performed to verify that growing media attached to or associated with plants for planting does not represent a risk.

1.1 | Types of growing media and their respective risk

Annex 1 of ISPM 40 (IPPC, 2017a) lists common components of growing media along with their relative pest risk.

The following section lists the types of growing media relevant to this Standard that may facilitate pest survival.

Soil: Natural untreated soil presents a high risk irrespective of where the material was collected. Some soil-borne pests can persist in soil for a few years, whereas others can remain viable for decades. A wide variety of pests can be found in soil which may use the substrate for part or all of their lifecycle.
Compost: Commercial compost is processed from plant-based material and can include peat, coconut or rice husks, and added nutrients and non-plant-based media. Depending on the origin of the organic material, the processing method and how it is stored, compost may contain a variety of pests, including plant pathogens (see EPPO, 2022).

[1]This Standard does not include inspection for invasive alien plants. These pests are covered in the PM 3/97 (1) *Inspection of consignments of plants for planting for invasive alien plants* (EPPO, 2024).

Manure: This is an organic material formed from animal faeces that is used as a fertilizer. It may be combined with straw. The risk associated with manure will depend on different factors including the place of origin, the type of animal, animal feeds and the processing method (EFSA, 2015). The import of unprocessed manure is prohibited in many EPPO countries.

Peat: Harvested from bogs and fens, peat is considered to be free from the majority of plant pathogens (EFSA, 2015). The pest risk is considered lower when the origin of peat is from certified bogs (ISPM 40: IPPC, 2017a).

Water: Can be associated with imported plants for planting, especially with aquatic plants which may be encased in containers with water. Some life stages of different pathogens can be associated with water, e.g. zoospores of fungal, oomycete or bacterial pathogens (EFSA, 2015) and the pest risk depends on the source of the water and whether it has been treated (ISPM 40: IPPC, 2017a).

Other plant based growing media: Types of such media can include paper, coconut fibres, sawdust and wood shavings, cork, mosses (such as sphagnum), bark, tree fern slabs. These growing media can present risks depending on the origin, the level of processing and how the material has been sourced and stored. The size of wood pieces, e.g. for wood shavings and bark can affect the type of pests present.

Other non-plant based growing media: Sand, and synthetic media (e.g. polystyrene, vermiculite) have a very low risk as they will not support pest survival.

Plants for planting may be associated with one type of growing media or different types of growing media may be mixed.

2 | PHYTOSANITARY INSPECTIONS

ISPM 5 *Glossary of phytosanitary terms* (IPPC, 2019) defines inspection as Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to verify conformity with phytosanitary requirements.

The procedures described in this Standard mainly concern the inspection of consignments at a point of entry in an EPPO importing country, but they may also be applicable for export inspection to check compliance with the phytosanitary requirements of the importing country.

The phytosanitary certificate that is accompanying a consignment should be examined before initiating the visual examination. It can provide useful information such as the country of origin, place of production and compliance with phytosanitary measures (e.g. level of processing, treatment of the growing media).

Inspections at import (including checking documents and identity checks) aim to verify compliance with phytosanitary import requirements such as the absence of regulated pests. Inspections may also be carried out for the detection of pests for which the phytosanitary risk has not yet been determined. When an unfamiliar pest is detected, the procedures specified in EPPO Standard PM 5/2: *Pest risk analysis on detection of a pest in an imported consignment* (EPPO, 2002), should be followed to allow the NPPO to decide the phytosanitary action to take.

Inspection of growing media attached or associated with plants in the importing country can be carried out at the approved point of entry or places of destination, depending on the inspection premises, the possibilities of carrying out efficient inspections and keeping the plants under official control until the result of the inspection is known.

When applying risk based inspection, the following factors should be taken into account:

- Types of growing media (see Section 1.1),
- Origin of the plants for planting,
- Processing and treatment of growing media,
- Plant and growing conditions (for example the plant species, how plants were grown: indoors or outdoors, in a field or in pots, how long the plant has been in the growing media).

Other factors may be taken into account when planning a risk based inspection.

Visual examination alone is inadequate to detect all regulated pests. If pests cannot be detected visually, inspection methods should be based on a combination of visual examination, sampling and laboratory testing.

The inspection method should focus on testing a representative sample of growing media for pests.

The general background for carrying out import inspections is included in ISPM 20 *Guidelines for a phytosanitary import regulatory system* (IPPC, 2017b) and ISPM 23 *Guidelines for inspection* (IPPC, 2016a).

General background information on inspection of consignments is given in the EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production*, *area-wide surveillance*, *inspection of consignments and lot identification* (EPPO, 2009).

Specific information regarding the movement of growing media in association with plants for planting can be found in *ISPM 40* (IPPC, 2017a).

3 | COMMODITIES CONCERNED

Plants for planting imported with growing media are usually traded in large lots and transported by sea or land in

palletized boxes, containers, or (in often smaller lots) by air freight. These plants are either intended for direct sale (in garden centres or other outlets) or the plants will be further grown on in nurseries and sold at a later date.

Examples of commodities of plants for planting associated with growing media covered by this Standard are: plants for planting (including plants with roots, corms, bulbs, tubers and rhizomes) in pots or where the roots are contained in another substance, such as root balls of large shrubs or trees wrapped in a hessian fabric (e.g. burlap).

Plants for planting may have been grown outdoors in natural soil and potted with other growing media before export. These plants may have natural soil attached to their roots and present a high risk for the introduction of pests. Alternatively, plants may have been grown permanently in growing media which presents a lower risk depending on production conditions of the plants.

4 | PESTS OF CONCERN FOR THE EPPO REGION

This Standard covers mainly pests that are found in growing media and are listed in the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests (see Tables 1 and 2). The phytosanitary procedures described in the Standard are primarily aimed at preventing the introduction of these pests into the EPPO region via growing media associated with plants for planting.

Details of the pests concerned (biology, geographical distribution, and host plants) can be found in the EPPO Global Database (EPPO, 2023a), in EPPO Standards regarding the specific pests, in EFSA Plant Pest Survey Cards and in the relevant scientific literature. Inspectors can consult the EPPO Global Database for pests specific to the hosts detailed in Tables 1 and 2. It should be noted that pests may be present in growing media associated with species other than known hosts.

The EPPO Lists of A1 and A2 pests are subject to additions and deletions. The present list may need to be revised when new quarantine pests are identified and added to the lists.

The pests detailed in Tables 1 and 2 are not exhaustive and other pests may be present within growing media which are EPPO A1 or A2 pests, or pests which are regulated by specific EPPO countries.

5 | LOT IDENTIFICATION

General background information on lot identification is given in the EPPO Standard PM 3/72(2) *Elements common to inspection of places of production*, *area-wide surveillance*, *inspection of consignments and lot identification*.

According to ISPM 5, a lot is "a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment" (IPPC, 2024).

A consignment may be composed of one or more lots of plants for planting with growing media.

Lots identified on the phytosanitary certificate should be the starting point for planning the inspection. Inspection of associated growing media may not be based on individual lots of the commodity but on combined lots which are homogenous firstly in origin and secondly in type of growing media.

6 | INSPECTION AND SAMPLING

The number of individual plants with associated growing media that should be selected for inspection to detect a specified level of infestation in a specified lot size is indicated in tables 1, 3 and 4 of ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2016b).[2]

The NPPO should determine the sample size. For example, if 448 potted plants with growing media are inspected from a lot of 10000 plants, this would provide a 99% confidence of detecting symptoms present in 1% of the plants, provided the symptoms are visible and are uniformly distributed and the plants are randomly selected. To reach the same level of confidence for small lots (fewer than 1000 plants), all plants should be inspected.

Inspectors should take all necessary precautions during inspection and sampling, such as wearing protective clothes: coat, overshoes, gloves etc. to avoid pest spread. Good hygiene procedures when collecting samples should be followed by decontaminating tools and hands.

Inspection should be combined where appropriate with sampling followed by laboratory testing.

6.1 | Sampling for inspection

The place where the inspection is conducted should be well lit and equipped with an inspection table.

The visual examination should begin with an overall examination of the consignment. Visual examination of the container, packaging and means of conveyance can provide indications of adverse conditions during transport (e.g. adverse temperatures or signs of damp or wetness) which may affect the physical condition of the consignment.

Sampling the units for inspection is generally random but could be targeted based on observations during the overall examination.

If there is an abnormal die-off of plants in a consignment or lot, or there are other anomalies within the plants present (e.g., abnormal growth, differences

[2]ISPM 31 provides information on the number of units to be sampled, which is considered useful to determine sample sizes for both consignments and places of production.

TABLE 1 EPPO A1 pests recommended for regulation (absent from the EPPO region) associated with growing media.

A1 pest (absent from the EPPO region)	Hosts (major hosts according to EPPO global database)
Fungi	
Phymatotrichopsis omnivora	Polyphagous. Major hosts include *Carya illinoinensis*, *Gossypium barbadense*, *Gossypium herbaceum*, *Gossypium hirsutum*, *Malus domestica*, *Medicago sativa*, *Prunus persica*, *Vitis vinifera*
Insects	
Anastrepha fraterculus	Polyphagous. Major hosts include *Acca sellowiana*, *Annona cherimola*, *Eriobotrya japonica*, *Eugenia uniflora*, *Malus domestica*, *Prunus persica*, *Psidium cattleyanum*, *P. guajava*, *P. guineense*, *Pyrus communis*, *Syzygium jambos*
Anastrepha ludens	*Citrus* × *aurantiifolia*, *C.* × *aurantium*, *C.* × *aurantium* var. *paradisi*, *C. reticulata*, *C.* × *aurantium* var. *sinensis*, *Mangifera indica*
Anastrepha obliqua	*M. indica*
Anastrepha suspensa	Polyphagous. Major hosts include *Eriobotrya japonica*, *Eugenia uniflora*, *Psidium cattleyanum*, *P. guajava*, *Syzygium jambos*, *S. malaccense*, *S. samarangense*
Bactrocera dorsalis	Highly polyphagous
Bactrocera minax	*Citrus maxima*, *C. reticulata*, *C.* × *aurantium* var. *sinensis*
Bactrocera tryoni	Polyphagous. Major hosts include *Anacardium occidentale*, *Annona glabra*, *A. squamosa*, *A.* × *atemoya*, *Averrhoa carambola*, *Capsicum annuum*, *Carica papaya*, *Casimiroa edulis*, *Chrysophyllum cainito*, *Citrus reticulata*, *C.* × *aurantium* var. *paradisi*, *C.* × *aurantium* var. *sinensis*, *Coffea arabica*, *Eriobotrya japonica*, *Eugenia uniflora*, *Fortunella japonica*, *Malus sylvestris*, *Mangifera indica*, *Manilkara zapota*, *Morus nigra*, *Nauclea orientalis*, *Passiflora edulis*, *P. suberosa*, *Prunus persica*, *P. persica var. nucipersica*, *Psidium cattleyanum*, *P. guajava*, *Solanum lycopersicum*, *Syzygium aqueum*, *S. forte*, *S. jambos*, *S. malaccense*, *S. suborbiculare*, *S. tierneyanum*, *Terminalia arenicola*, *T. aridicola*, *T. catappa*, *T. muelleri*, *T. platyphylla*, *T. sericocarpa*, *T. subacroptera*
Bactrocera tsuneonis	*Citrus reticulata*
Ceratitis rosa	*Citrus reticulata*, *C.* × *aurantium* var. *sinensis*
Conotrachelus nenuphar[a]	*Amelanchier arborea*, *A. canadensis*, *Cydonia oblonga*, *Malus domestica*, *Prunus armeniaca*, *P. avium*, *P. cerasus*, *P. domestica*, *P. mume*, *P. persica*, *P. persica var. nucipersica*, *P. salicina*, *P. serotina*, *Pyrus communis*, *Vaccinium corymbosum*, *V. stamineum*, *Vitis rotundifolia*
Diabrotica barberi	*Zea mays*
Diabrotica speciosa	*Phaseolus vulgaris*, *Solanum tuberosum*, *Triticum aestivum*, *Zea mays*
Diabrotica undecimpunctata howardi	*Arachis hypogaea*, *Citrullus lanatus*, *Cucumis melo*, *C. sativus*, *Cucurbita maxima*, *C. moschata*, *C. pepo*, *Zea mays*
Diabrotica undecimpunctata undecimpunctata	*Cucumis melo*, *C. sativus*, *Phaseolus vulgaris*, *Zea mays*
Epitrix tuberis	*Solanum tuberosum*
Exomala orientalis	*Ananas comosus*, *Saccharum officinarum*, *Zea mays*
Gonipterus gibberus	*Eucalyptus camaldulensis*, *E. globulus*, *E. globulus subsp. maidenii*, *E. punctata*, *E. robusta*, *E. smithii*, *E. viminalis*
Gymnandrosoma aurantianum	*Citrus reticulata*, *C.* × *aurantium* var. *paradisi*, *C.* × *aurantium* var. *sinensis*, *Macadamia integrifolia*, *Plukenetia volubilis*, *Theobroma cacao*
Heteronychus arator	*Lolium perenne*, *Solanum tuberosum*, *Zea mays*
Margarodes prieskaensis	*Vitis vinifera*
Margarodes vitis	*Vitis vinifera*
Margarodes vredendalensis	*Vitis vinifera*
Melanotus communis	*Ipomoea batatas*, *Saccharum officinarum*, *Solanum tuberosum*, *Zea mays*
Naupactus leucoloma	*Medicago sativa*, *Phaseolus vulgaris*
Naupactus xanthographus	*Erythrina crista-galli*, *Vitis vinifera*
Pheletes californicus	*Avena sativa*, *Beta vulgaris*, *Hordeum vulgare*, *Solanum tuberosum*, *Triticum aestivum*
Rhagoletis fausta	*Prunus avium*
Rhagoletis indifferens	*Prunus avium*
Rhagoletis mendax	*Vaccinium angustifolium*, *V. corymbosum*
Rhagoletis pomonella	*Malus domestica*

TABLE 1 (Continued)

A1 pest (absent from the EPPO region)	Hosts (major hosts according to EPPO global database)
Nematodes	
Nacobbus aberrans sensu lato	*Beta vulgaris*, *Capsicum annuum*, *Phaseolus vulgaris*, *Solanum lycopersicum*, *Solanum tuberosum*, *Taraxacum officinale*
Radopholus similis citrus race	*Citrus* × *aurantium* var. *paradisi*, *C.* × *aurantium* var. *sinensis*
Xiphinema americanum sensu stricto[a]	*Prunus persica*, *Rosa* spp., *Vitis vinifera*, *Zoysia* spp.
Xiphinema californicum[a]	*Canna* spp., *Citrus* spp., *Cocos nucifera*, *Ipomoea batatas*, *Malus domestica*, *Medicago sativa*, *Olea europaea*, *Persea americana*, *Prunus cerasifera*, *Prunus mahaleb*, *Prunus persica*, *Rosa* spp., *Sorghum* spp., *Vasconcellea pubescens*, *Vitis vinifera*, *Zea mays*

[a]No major hosts listed in EPPO Global Database, only hosts.

TABLE 2 EPPO A2 pests recommended for regulation (present in the EPPO region) associated with growing media.

A2 pest (present in the EPPO region)	Hosts (major hosts according to EPPO global database)
Fungi (and fungus like organisms)	
Phytophthora kernoviae	*Fagus sylvatica*, *Rhododendron ponticum*
Phytophthora lateralis	*Chamaecyparis lawsoniana*
Phytophthora ramorum	*Kalmia latifolia*, *Larix decidua*, *L. kaempferi*, *Notholithocarpus densiflorus*, *Pieris*, *Quercus agrifolia*, *Rhododendron*, *Syringa vulgaris*, *Viburnum*
Synchytrium endobioticum	*Solanum tuberosum*
Insects	
Bactrocera zonata	*Mangifera indica*, *Prunus persica*, *Psidium guajava*
Carposina sasakii	*Malus domestica*, *Prunus persica*, *Pyrus communis*, *P. pyrifolia*
Ceratitis capitata	Highly polyphagous
Diabrotica virgifera virgifera	*Zea mays*
Epitrix cucumeris	*Solanum tuberosum*
Gonipterus scutellatus species complex[a]	*Eucalyptus* spp.
Grapholita inopinata	*Malus baccata*, *Malus mandshurica*
Popillia japonica	*Corylus avellana*, *Glycine max*, *Malus domestica*, *Phaseolus vulgaris*, *Prunus armeniaca*, *P. avium*, *P. domestica*, *P. persica*, *P. spinosa*, *Rosa*, *Vitis vinifera*, *Wisteria*, *Zea mays*
Rhagoletis cingulata	*Prunus avium*, *P. salicina*
Strobilomyia viaria	*Larix gmelinii*, *L. gmelinii var. olgensis*, *L. laricina*, *L.* × *lubarskii*
Tecia solanivora	*Solanum tuberosum*
Nematodes	
Globodera pallida	*Solanum tuberosum*
Globodera rostochiensis	*Solanum tuberosum*
Heterodera glycines	*Glycine max*
Meloidogyne chitwoodi	*Solanum lycopersicum*, *S. tuberosum*
Meloidogyne enterolobii	*Coffea arabica*, *Glycine max*, *Ipomoea batatas*, *Nicotiana tabacum*, *Psidium guajava*, *Solanum lycopersicum*, *S. melongena*
Meloidogyne fallax	*Beta vulgaris*, *Daucus carota* subsp. *sativus*, *Fragaria* × *ananassa*, *Hordeum vulgare*, *Lactuca sativa*, *Lolium multiflorum*, *Medicago sativa*, *Solanum lycopersicum*, *S. tuberosum*, *Trifolium repens*
Meloidogyne mali	*Malus domestica*, *Morus alba*, *M. bombycis*, *M. latifolia*, *Ulmus chenmoui*, *U. glabra*
Radopholus similis	*Goeppertia insignis*, *G. makoyana*, *Musa* × *paradisiaca*
Xiphinema rivesi[a]	*Acer negundo*, *Allium sativum*, *Celtis* spp., *Citrus* × *aurantium* var. *sinensis*, *Diospyros kaki*, *Juglans* spp., *Juniperus* spp., *Liquidambar styraciflua*, *Malus domestica*, *Mangifera indica*, *Medicago sativa*, *Populus* spp., *Prunus avium*, *P. persica*, *Quercus* spp., *Rubus idaeus*, *Rubus* spp., *Solanum tuberosum*, *Sorghum bicolor*, *Vitis vinifera*

[a]No major hosts listed in EPPO Global Database, only hosts.

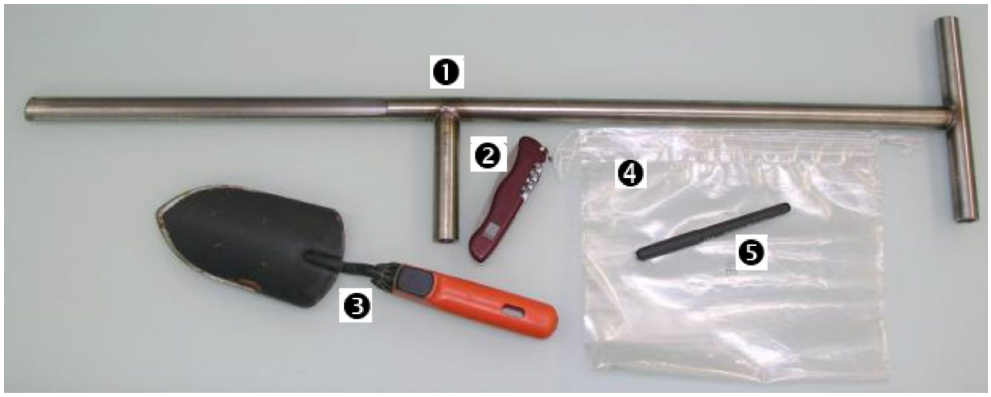

FIGURE 1 Equipment for growing medium sampling: Sampling probe (for large plants) (❶), knife (❷), planting shovel (❸), plastic bag (❹), permanent marker (❺).

in colour), these lots should be inspected with specific attention.

Any wrapping surrounding the plant should be removed. The inspector should examine the surface of the media to check for any sign of pests. Scraping the surface of the media may also reveal contamination just below the surface.

For potted plants the pot should be removed if it is possible to do so, and the growing media can be removed from the roots and spread out on a white tray to inspect for pests. A brush can assist in dislodging the growing media from plant roots.

Appendix 2 provides a short procedure for inspectors.

6.2 | Sampling for laboratory testing

Sampling for laboratory testing is required to detect and identify certain pests (e.g. nematodes). This section contains guidance on sampling for laboratory testing of growing medium.

It is necessary to use adequate equipment to sample growing medium (Figure 1).

6.2.1 | Visible pests and/or symptoms

Samples should be taken from the growing media where pests are visible, or where signs of pests are present (e.g. larval cases, frass, disturbance) and cannot be immediately identified by the inspector. In these cases, the sample consists of the plant(s) and associated growing media.

Samples of adult insects, larvae, pupae and to a lesser extent eggs should be put in a pot with screw cap. Living organisms should be sent to the laboratory together with plant material (roots with growing media) of the host plant in a suitable container. Insects may also be collected in small tubes with ethanol.

For further details on sampling for identification of invertebrate pests in growing media see Appendix 1.

6.2.2 | Non-visible pests

When no pests are visible, the presence of plant pests in growing media can only be detected and identified by laboratory testing. For parasitic nematodes different amounts of soil may be required depending on the extraction method (e.g. 250–1000 mL) (EPPO, 2013). When it is not possible to sample the required quantity of growing media, the sample should be as big as possible. In this case, the roots and growing media can be sampled with a knife.

For large plants, trees or shrubs with big root balls, sampling should be conducted using a soil sampling probe. After removal of the top 5 cm of growing media, a sample should be taken from the next 25 cm where nematodes are more active. If this layer can be reached more easily from the side of the root ball, the probe can be entered from the side. It is important to avoid taking samples from only the outer part and surface of the root ball.

For the presence of other non-visible pests (e.g. fungi), growing media and/or root samples should be sent to the laboratory.

Growing media samples should be placed in a labelled and sealed plastic bag. Samples should be tested in a laboratory as quickly as possible.

If a pest found during inspection is suspected to be a quarantine pest, the suspected lot(s) should be detained under official control pending a test result.

All other lots potentially at risk of infestation should also be detained under official control.

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The Panel on Phytosanitary Inspections reviewed the Standard.

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APPENDIX 1 - SAMPLING FOR IDENTIFICATION OF INVERTEBRATE QUARANTINE PESTS IN GROWING MEDIA

For each of the pests mentioned below basic information on host range, biology, detection and identification can be found in EPPO Global Database (https://gd.eppo.int/), as well as in EPPO Datasheets, EPPO Diagnostic Standards, and EFSA Plant Pest Survey Cards. Illustrations are available on the EPPO Global Database and the EFSA Plant Pest Survey Cards (link). When an EPPO Diagnostic Standard exists, it is mentioned in the text. The fact that there is no EPPO Diagnostic Standard does not mean that no method for diagnosis is available in the scientific literature.

Appendix 1 includes all EPPO A1 and A2 invertebrate pests listed in Tables 1 and 2.

(A) Coleoptera

Conotrachelus nenuphar (Curculionidae: EPPO A1 list)

Life stages associated with soil

Larvae, pupae and adult stages are associated with soil (Figure A1). The larvae develop in fallen and rotting fruits and when mature 3–5 weeks later they eat their way out into the soil where they pupate at a depth of 10–15 cm. Larvae cannot survive in dry soil. First-generation adults emerge in the summer months (EPPO, 2023b).

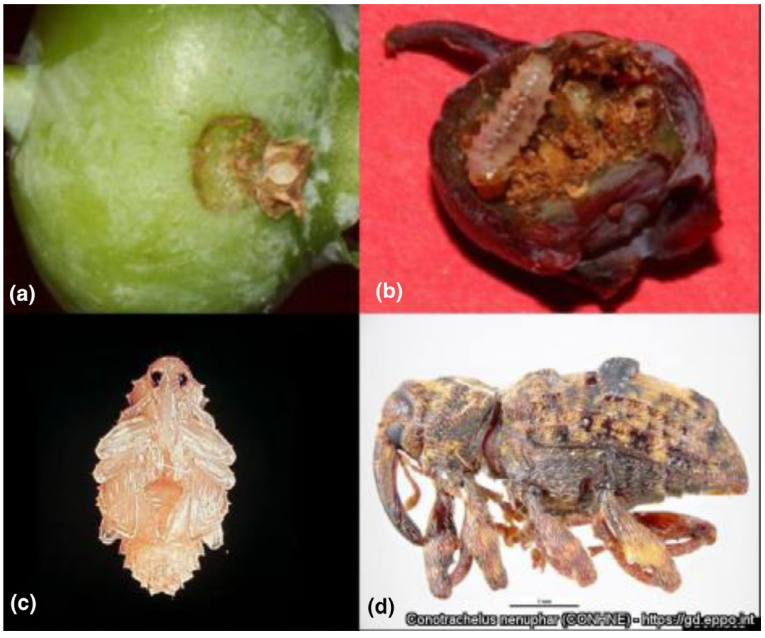

FIGURE A1 *Conotrachelus nenuphar* (a) egg, (b) larva, (c) pupae, and (d) adult. EPPO Global Database Courtesy: Dean Polk, Dean Polk, J. F. Walgenbach, and Pest and Diseases Image Library, Bugwood.org, respectively.

Sampling and identification

EPPO (2023b) provides the following information: larvae are cylindrical, whitish and legless, usually bent in a semi-circle, and possessing a small brown head. There are four larval instars that can be distinguished by head capsule width. Fully-grown larvae measure 6–9 mm long. The pupa is yellowish-white with dark spots in the position of the eyes and measures 5–7 mm long. The adult is 0.7 cm long with a typical rostrum. The postmedian band of the elytra consists of reddish-brown or reddish-yellow and white recumbent setae; small areas of the elytra are intensely black with humps. Identification of *C. nenuphar* is based on the morphological identification of the adult (IPPC, 2018). Infested samples of growing media should be collected and placed in a labelled plastic zip lock bag together with a piece of slightly damp absorbent paper, kept in cool conditions and sent to a diagnostic laboratory as soon as possible.

***Diabrotica barberi* (Chrysomelidae: EPPO A1 list), *D. speciosa* (EPPO A1 list), *D. undecimpunctata howardi* (EPPO A1 list), *D. undecimpunctata undecimpunctata* (EPPO A1 list), *D. virgifera virgifera* (EPPO A2 list)**

Life stages associated with soil

Eggs, larvae, pupae and adult stages are associated with soil (see Figure A2a,b). Eggs are laid in the soil at the base of host plants. Larvae develop in the soil feeding on the rootlets and roots. Pupation occurs in the soil and adults emerge from the soil to mate above ground. Adults can be found overwintering on the soil surface and underneath plant debris.

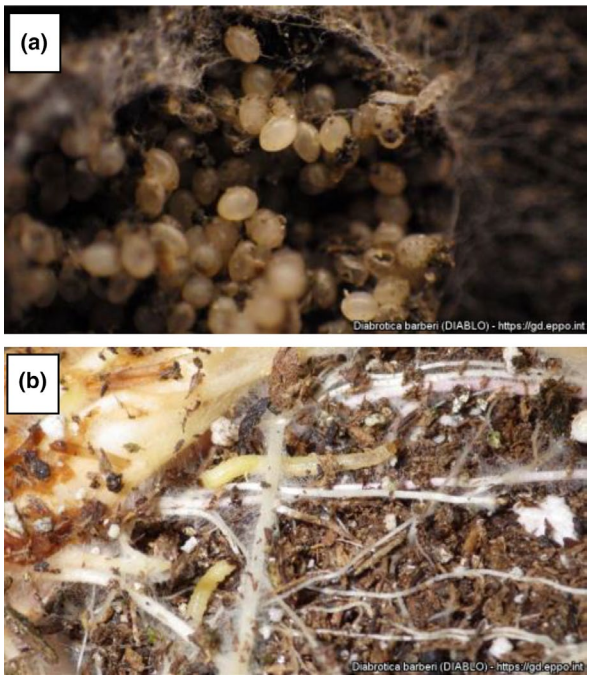

FIGURE A2 (a) *Diabrotica barberi* eggs (b) *Diabrotica barberi* larvae. EPPO Global Database Courtesy: V. Calles-Torrez, North Dakota State University, Fargo, US.

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

Generally, the eggs of *Diabrotica* species are oval in shape and light coloured, white or light yellow, and 0.7–0.5 mm in length. Larvae can vary in colour and are generally yellowish white, with a wrinkled body, 12–19 mm long, with six very small legs, and a greyish-brown head. Larvae are typically elongate and cylindrical. The pupa is small generally white in colour and becoming yellow with age.

***Epitrix tuberis* (Chrysomelidae: EPPO A1 list), *E. cucumeris* (EPPO A2 list)**

Life stages associated with soil

Eggs, larvae, pupae and adult stages are associated with soil (Figure A3). Adult beetles overwinter in the soil and this is also where eggs are laid. Larvae feed on roots and tubers and pupate in the soil.

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

Epitrix tuberis adults are small, dull black beetles with rows of short white hairs across the elytra, 1.6–2.0–mm long, with brownish-yellow antennae. *Epitrix cucumeris* adults are small shiny black beetles, 1.56–1.92 mm long, with rows of punctures along the elytra arranged into striae and one row of white setae between elytral striae (Deczynski, 2016; EPPO, 2017). Eggs of *Epitrix* species are minute, whiteish and approximately elliptical (0.5 mm long). Larvae are white, slender and cylindrical. Mature larvae are about 5 mm long with a brown head. Pupae are white, about 2.5 mm long and 1.5 mm wide across the mesothorax. For further details see EPPO (2023c, 2023d).

***Exomala orientalis* (Scarabaeidae: EPPO A1 list)**

Life stages associated with soil

Eggs, larvae, pupae and adult stages are associated with soil (Figure A4). Female adults can be found at varying soil depths from summer to autumn. During this period, females deposit eggs in the soil. Larvae have three instars and develop in the soil feeding on roots and humus and can descend to up to 40 cm depending on the moisture in the soil. Larvae overwinter in an inactive state. In spring larvae move up below the soil surface and feed and eventually pupate. The

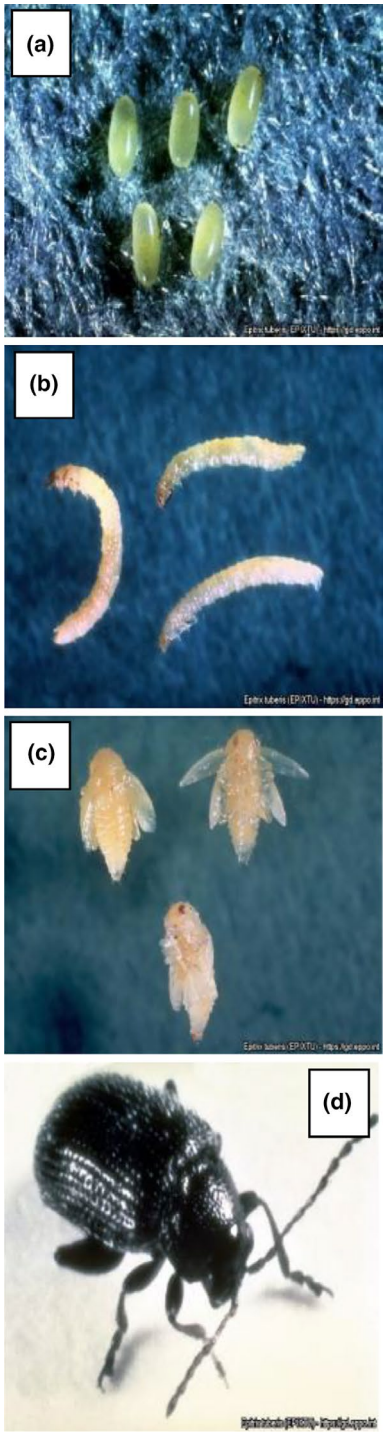

FIGURE A3 *Epitrix tuberis* (a) eggs, (b) larvae, (c) pupae and (d) adult. EPPO Global Database Courtesy: Agriculture Canada, Ottawa (CA).

pupae can be found in the soil during the summer months.

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

EPPO (1997a) states that eggs are approximately 1 mm in diameter. The newly hatched larva is 1.5 mm

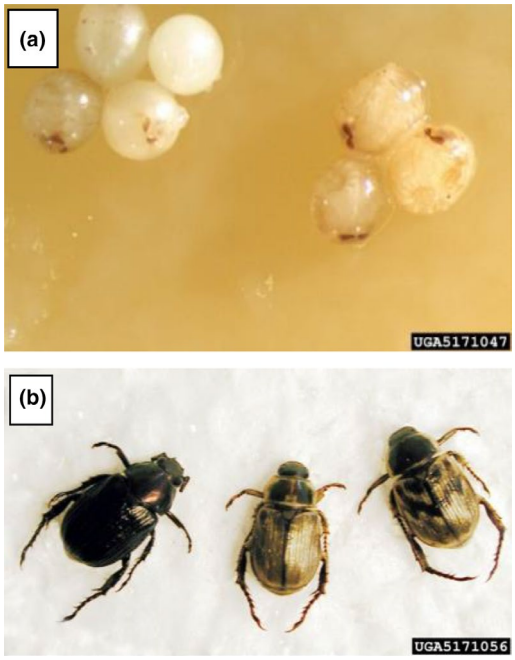

FIGURE A4 *Exomala orientalis* (a) eggs and (b) adults. Invasives.org. Courtesy: Michael Reding USDA Agriculture Research Station, US.

in length and when fully grown it can reach 25 mm. The larva has two longitudinal rows of pointed spines (11–15 in each row) on the underside of the last segment and can be distinguished from other white grubs (Melolonthinae) by the smaller size and transverse, rather than V- or Y-shaped anal opening. Pupa are found in the cast and have a yellowish colour. Adults are 8–11 × 4.5–6 mm, typically straw-coloured with dark markings, but may be entirely straw coloured or entirely black. A light, median line may divide the thorax into two black areas. The wing covers usually have one or two U-shaped bands and a black spot at the inner basal angle of each.

Gonipterus gibberus (Curculionidae: EPPO A1 list), *G scutellatus* species complex (EPPO A2 list)

Life stages associated with soil

Larvae, pupae and adults can be associated with soil (Figure A5a,b). Eggs are laid on the plant and larvae and adults feed on plants. The mature larvae drop to the ground and pupation occurs in cells about 5 cm deep in the soil. Pupation takes 30–40 days to complete.

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

The head of the larvae of *G. scutellatus* species complex is black and the body is yellowish-green with black shield like spots on each segment and

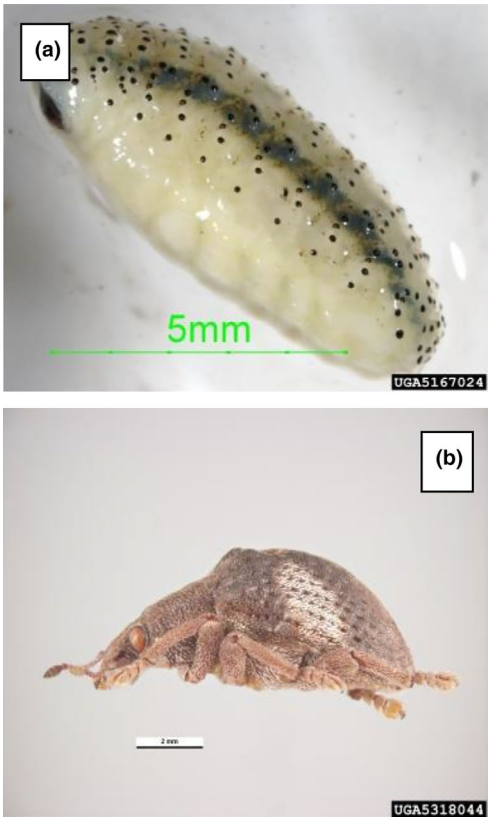

FIGURE A5 (a) *Gonipterus gibberus* larva Invasives.org. Courtesy: Pest and Diseases Image Library and (b) *Gonipterus scutellatus* adult Invasives.org. Courtesy: Charles Olsen USDA APHIS PPQ.

dark lateral stripes. Larvae are covered in a sticky slime (EFSA, 2018). *G. gibberus* larvae are yellowish-green with black marks, 14 mm long. *G. scutellatus* species complex adults are 7.5–9.4 mm grey-brown, with a black x-shaped marking on the elytra and a white scutellar stripe, often extending to the head (EFSA, 2018). These markings may fade with the age of the beetle and vary between species. Identification up to genus level can be done using morphological characteristics. *Gonipterus gibberus* adults are 12–14 mm grey-brown weevil, with a light, transverse band on the elytra. The morphology of the male genitalia and DNA barcoding techniques are used for species identification (Mapondera et al., 2012).

Heteronychus arator (Scarabaeidae: EPPO A1 list)

Life stages associated with soil

All life stages can be found in the soil (EPPO, 1997b) There is one generation per year. Adults generally overwinter in free-draining soil. Adults (Figure A6) lay eggs singly in soil at a depth of about 10 mm. Egg-laying continues until early summer. Depending upon soil temperature, eggs hatch within 6 weeks. Young larvae feed on soil organic matter, while more mature larvae attack

FIGURE A6 *Heteronychus arator*. Courtesy: Pest and Diseases Image Library Invasives.org.

plant roots. There are three larval instars. The final instar burrows to a depth of 10 cm to pupate. In Australia, development from egg-laying to adult emergence takes about 3 months. Temperatures above 15°C are most favourable for development and survival of *H. arator*, with optimum larval development occurring at 20–25°C (King et al., 1981).

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

Eggs are white, oval and about 2 mm in length. The larva are white or bluish-white with a brown head capsule and orange spiracles along the sides of the thorax and abdomen. Fully developed larvae are 25 mm long and 6 mm in diameter. Smith et al. (1995) provided detailed illustrated descriptions and a laboratory and field key to third-instar larvae. Pupa are yellow to light brown turning reddish-brown before adult emergence; 12–15 mm long. Adults are 12–15 mm long, approximately oval in shape and shiny black. Males have a thickened tarsus on each foreleg.

Melanotus communis (Elateridae: EPPO A1 list)

Life stages associated with soil

All life stages can be found in the soil. Overwintered adults become active in early summer, mate and lay individual eggs in the soil. First-instar larvae feed on roots and tubers and overwinter in the soil as second-instar larvae. Most immatures continue to develop over the next 5 years moulting once or twice each year, but some develop fully in 3 years. Mature larvae construct oval earthen cells 15–30 cm deep in the soil and pupate. Adults emerge 7–39 days later (Fenton, 1926).

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

Eggs are pearly white and shiny, spherical to oval, 0.3 mm in diameter. The main body of the larva is pale

FIGURE A7 *Melanotus communis* Courtesy: Judy Gallagher https://www.flickr.com/photos/52450054@N04/14155144828/, CC BY 2.0, https://commons.wikimedia.org/w/index.php?curid=55246515.

yellow to reddish brown while the head is brown. The body is slender, cylindrical, hard-bodied and jointed, 21–25 mm long (Figure A7). Riley et al. (1974) provide a key to larvae for nine *Melanotus* species from the northern central USA. Pupa are white soft bodied and oval, 13 mm long. Adults are brown to black, 13 mm in length.

Naupactus leucoloma (Curculionidae: EPPO A1 list)

Life stages associated with soil

All life stages can be found in the soil. However, eggs can be laid on plants near the soil surface. The entire larval stage is spent in the soil usually at a depth of 1–15 cm, but some may burrow deeper. Larvae enter the soil and feed on roots of host plants and pupate in oval chambers early in the summer months. Adults (Figure A8) can emerge from pupae after 2 or 3 weeks or overwinter in the soil (EPPO, 2023e).

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

Eggs are oval, approximately 0.9 mm long and 0.6 mm wide. When freshly laid they are milky white, but after 4–5 days, they change to a dull light yellow. Eggs are laid in clusters of 20–60, usually within crevices on the stems or on the ground in plant litter. Fully grown larvae are about 13 mm long and 6 mm wide with a small, round pale-brown head, which is

FIGURE A8 *Naupactus leucoloma* adult. EPPO Global Database Courtesy: María Guadalupe del Río.

tucked back into the prothorax with only the black mandibles protruding. The pupa is about 10–12 mm long and changes colour from white to brown as the body appendages darken before transformation to the adult. The adult female is 8–12 mm in length and 4 mm wide across the abdomen. It has a short snout, with subcylindrical prothorax and oval shape abdomen, completely concealed by the elytra. Molecular identification methods are available (del Río et al., 2018; Lin et al., 2008).

Naupactus xanthographus (Curculionidae: EPPO A1 list)

Life stages associated with soil

All life stages except the eggs can be found in the soil. Newly hatched larvae fall to the ground, enter the soil and start to feed on the rootlets of the host plants. The larvae generally live in the soil at depths of 20–60 cm, and rarely below that (Ripa, 1992). The development of the larval stage can take on average 9 months with five instars in total. Pupation occurs in the soil in a pupal cell which is lined with body secretions. The development of the pupal cell and pupation occur at depths of 20–30 cm below the surface (Vicchi, 2014). The pupal stage requires about 30 days before the adult starts to emerge. Humidity is very important for survival of eggs and larvae, and is essential for the emergence of adults (they usually emerge after rain or irrigation) (EPPO, 2023f).

Sampling and identification

Larvae are legless, white in colour with brown head capsule and mandibles (Figure A9a). First-instar larvae measure approximately 1.5 mm and mature larvae can reach up to 20 mm. The last abdominal segment

FIGURE A9 *Naupactus xanthographus* (a) larva and (b) adult male EPPO Global Database Courtesy: Renato Ripa (BIOCEA Ltda, Argentina).

has four thin dark bands. Morphological identification of larvae is difficult and therefore larvae should be reared to adults to allow identification. Pupae are creamy white (or slightly yellowish), changing to brown before eclosion, and are 11–22 mm in length (Loiácono & Díaz, 1992), although 22 mm is an outlier and a more common maximum would be 15 mm. Younger adults are brown or grey-brown with white or white/yellow stripes on pronotum and elytra (Figure A9b). They can be dark brown when they age. Morphological characters of *N. xanthographus* adults are given in Lanteri and del Río (2017).

Pheletes californicus (Elateridae: EPPO A1 list)

Life stages associated with soil

All life stages can be found in the soil (EPPO, 1997c). Overwintering adult beetles emerge from the soil in the late spring. Once mated, females lay eggs in the soil. During early summer, individual female beetles lay 200–1400 eggs in loose or cracked soil and under lumps of soil just below the surface or up to depths of 15 cm. The larvae feed on roots or germinating host plants. Older larvae are usually found feeding in the top 15 cm of soil. Larval activity is affected by soil temperature and moisture. Cool wet weather brings the larvae nearer the surface; hot dry weather forces them deeper into the soil. Pupation occurs during summer, at depths of 5–10 cm in the soil. However, adults do not emerge until the following spring.

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

Eggs are less than 1 mm in length, oval and pearly white. The larvae are white when they first hatch, but change to shiny yellow and then to brown as they mature. They are slender, cylindrical, jointed and hard-bodied, up to 25 mm long. They have three pairs of legs behind the head. The last abdominal segment is flattened and elongated with short, stout appendages on the end.

Popillia japonica (Scarabaeidae: EPPO A2 list)

Life stages associated with soil

All life stages can be found in the soil. 40–60 eggs per female (Campbell et al., 1989), are usually laid in moist grassland in the summer singly or in small clusters. Sometimes, females form a burrow in the upper 10–cm of soil to deposit the eggs (Metcalf & Metcalf, 1993). The third larval instar burrows deeper and overwinters at depths of 10–20 cm to avoid cooler temperatures. All larvae stages feed on roots of host plants. In the spring, as the soil warms, larvae rise to shallower depths in the soil where they form a chamber in which they pupate (EPPO, 2023g).

Sampling and identification

For sampling see *Conotrachelus nenuphar*.

Eggs are laid in soil cavities. Newly deposited eggs are variable in size and shape: they may be round, elliptical or nearly cylindrical, with a length of about 1.5 mm. They can be translucent to creamy white and the external surface is marked with hexagonal areas. The larvae (Figure A10a) can be distinguished from other scarab larvae by the characteristic V-shaped arrangement of two medial rows of 67 spines on the 10th abdominal segment (EPPO, 2016). The pupae are 14 mm long and 7 mm wide, and resemble adults, but the wings, legs and antennae are held close to the body and are functionless (Figure A10b). The colour changes from cream to tan and eventually the metallic green observed in the adult. The larvae are highly cryptic (living in the soil) and could easily be accidently moved with rooted plants. Adults are brightly coloured with a metallic green thorax and head and coppery bronze wing cases (elytra), oval in shape and vary from 8 to 11 mm in length, and 5 to 7 mm in width (Figure A10c). Along each lateral side of the wing cases are five tufts of white setae present and two dorsal spots of white setae on the last abdominal segment (pygidium) (Malumphy et al., 2016).

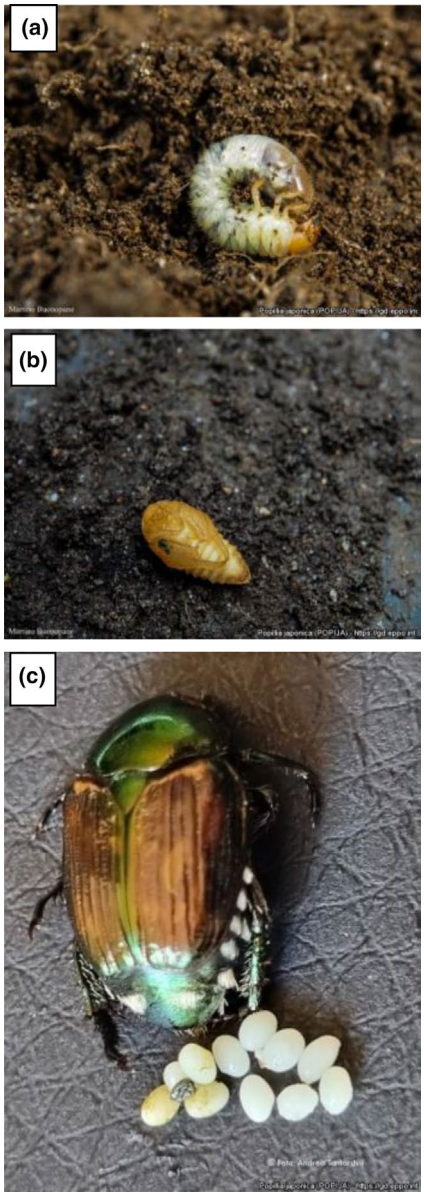

FIGURE A10 *Popillia japonica*. (a) larva, (b) pupa and (c) adult and eggs. EPPO Global Database Courtesy: Martino Buonopane (Plant Protection Service, Lombardia, IT).

(B) Diptera

Strobilomyia viaria (Anthomyiidae: EPPO A2 list)

Life stages associated with soil

Late instar larvae, pupae and adults are associated with soil. Larvae initially develop in the cones of larch species. The 3rd instar larvae leave the cone and fall to the ground. When on the ground, larvae build a puparium in the upper soil layer where they overwinter. Some adults may emerge the following year but a variable part of the population extends the winter diapause for an additional 1–3 year period (Skuhravá & Roques, 2000).

Sampling and identification

The species is difficult to separate from other cone flies by external examination of adults, and genitalia dissection must be systematically used for accurate identification, especially of trapped flies. The larva resembles that of other larch cone flies with an elongated, legless body but some specific features have been described. Fully developed larvae are 4–6 mm long (Skuhravá & Roques, 2000). The puparium is reddish-brown, nearly ovoid, 3.0–6.0 mm by 1.3–1.5 mm.

Tephritidae: *Anastrepha fraterculus* (Tephritidae: EPPO A1 list), *A. ludens* (EPPO A1 list), *A. obliqua* (EPPO A1 list), *A. suspensa* (EPPO A1 list), *Bactrocera dorsalis* (Tephritidae: EPPO A1 list), *B. minax* (EPPO A1 List), *B. tryoni* (EPPO A1 list), *B. tsuneonis* (EPPO A1 list), *B zonata* (EPPO A2 list), *Ceratitis capitata* (Tephritidae: EPPO A2 list), *C. rosa* (EPPO A1 list), *Rhagoletis cingulata* (Tephritidae: EPPO A2 list), *R. fausta* (EPPO A1 list), *R. indifferens* (EPPO A1 list), *R. mendax* (EPPO A1 list), *R. pomonella* (EPPO A1 list)

Life stages associated with soil

Larvae and pupae can be associated with the soil. Some species pupate in a puparium. Mature larvae exit host fruit and enter the soil to pupate.

Sampling and identification

Visual examination does not allow identification to the species level and laboratory diagnostics is necessary (EPPO, 2013, 2011, 2020). Larvae (Figure A11) can be sent alive in an airtight, secure container, except if the phytosanitary risk is high. If collected larvae are to be preserved, they should be killed in boiling water for a few seconds (until they become immobile) and then transferred to 70% ethanol (if a molecular test is to be carried out subsequently, 95%–100% ethanol is recommended). Adults can be sent for identification in

FIGURE A11 Tephritidae: (a) *Bactrocera dorsalis* larva.

a hermetic tube or container in 70% ethanol. Placing the adults live in a hermetic tube allows for the colour and pattern of the body and wings to appear, which can aid identification. It is recommended to send several adults for identification, ideally at least one male and one female.

(C) Hemiptera

Margarodes prieskaensis (Margarodidae: EPPO A1 list), *M. vitis* (EPPO A1 list), *M. vredendalensis* (EPPO A1 list)

Life stages associated with soil

All life stages can be found in the soil. Nymphs of the species attach themselves to roots and feed on them. Once feeding is complete, the nymphs are capable of secreting a protective waxy covering to form pearl-like cysts. These usually live at depths of 20–60 cm, but can occur at depths of up to 120 cm. Infested grapevine plants show a progressive decline where shoots become thinner and shorter and leaves smaller. One or more of the branches of the vine may die, followed in severe infestations by the eventual death of the whole plant.

Sampling and identification

For the collection of cysts, soil and root samples may be washed with water through a sequence of sieves. Live cysts sink into the water, dead cysts float. Live cysts of various sizes can be collected with small brush-pencils and placed on moist filter paper in plastic boxes and females gathered on emergence. Further details on identification are available in EPPO Standard PM 7/82 (1) *Margarodes prieskaensis*, *Margarodes vitis*, *Margarodes vredendalensis* (EPPO, 2007).

(D) Lepidoptera

Carposina sasakii (Carposinidae: EPPO A2 List)

Life stages associated with soil

Larvae and pupae are associated with the soil. *Carposina sasakii* can overwinter as hibernating larvae in cocoons in the soil.

Sampling and identification

Larvae are orange-red when newly hatched, changing to milky-white and then back to orange-red at maturity (Figure A12). Mature larva are up to 13 mm long. The setation is illustrated by Wu and Hwang (1955) and Lee et al. (2013). The pupae are reddish-brown in the cocoon.

FIGURE A12 Larvae of *Carposina sasakii* EPPO Global Database Courtesy: Zhiwei Zhang (College of Forestry, Shanxi Agricultural University, China).

Grapholita inopinata (Tortricidae: EPPO A2 list)

Life stages associated with soil

Larvae and pupae can be associated with the soil. *G. inopinata* overwinters as pronymph in silk cocoons in the soil or among dead leaves (Danilevsky & Kuznetsov, 1968) though it can also overwinter under the bark close to the bases of the main trunk (Gibanov & Sanin, 1971). Pupation occurs during the following spring and moths start emerging about one month later.

Sampling and identification

Matured larvae reach 10 mm length. They are pinkish with one red stripe on each segment dorsally and with red spots laterally (Figure A13) (often missing in pupating larvae and in ethanol-preserved specimens); the head is brownish (Akulov & Kirichenko, 2014). The main morphological features of *G. inopinata* larvae are (1) a short seta on the mid-abdominal segments (the seta length is not longer than the distance from the stigma to the base of this seta), (2) the location of setae on the abdominal segments on separate shields, (3) the abdominal legs with 20–30 hooks, (4) the anal crest with 4–5 teeth. The young pupa is light yellow, and darkens while maturing; the fully developed pupa is black (Lopatina, 1978). Adults have a wingspan of 10–11 mm (Akulov & Kirichenko, 2014). Colour variously described as dark-brown with metallic lead-blue lines on the forewing (Danilevsky & Kuznetsov, 1968) or dark-grey with some purple lustre (Takizawa, 1936); top of the forewings with black dot, the outer edge of the forewings with unclear speculum with 3-4 black dots inside it; the hindwings are greyish-brown, somewhat paler than forewings (Akulov & Kirichenko, 2014).

FIGURE A13 Larvae of *Grapholita inopinata*. EPPO Global Database Courtesy: Evgeny Akulov.

Gymnandrosoma aurantianum (Tortricidae: EPPO A1 list)

Life stages associated with soil

Larvae and pupae can be associated with the soil (EPPO, 2023h). The larvae can pupate in the soil though depending on the crop, pupation can occur in other places such as in the fruit (Parra et al., 2004; White & Tuck, 1993). In laboratory experiments, *G. aurantianum* was found to pupate between 0 and 1.5 cm depth, in both humid and dry substrates (Bento, 2008).

Sampling and identification

Larvae of *G. aurantianum* are eruciform, 5 mm long (newly hatched larvae) to ca. 15–19 mm long (mature larvae) (Figure A14). The body is pale yellow and the head pale yellow to pale orange with a red-brown patch (Adamski & Brown, 2001). Pupae are fusiform, 9–12 mm long and 2.5–3 mm wide, rounded at both extremities (Adamski & Brown, 2001). Pupae are first pale yellow, becoming brown (Blanco-Metzler, 1994). Pupae of different species of *Gymnandrosoma* cannot be distinguished (Adamski & Brown, 2001).

FIGURE A14 Larvae of *Gymnandrosoma aurantianum*. EPPO Global Database Courtesy: Nadège Villette – BCP Roissy CDG Airport (FR).

For molecular identification, barcodes are available for several specimens from various countries (http://v4.boldsystems.org/).

APPENDIX 2 - SHORT PROCEDURE FOR INSPECTORS

HYGIENE MEASURES

Following good hygiene procedures is important when inspecting and collecting samples for the laboratory. In order not to spread and increase infestations, adequate precautions should be taken during inspection and sampling, such as wearing protective clothes (coat, overshoes, gloves, etc.). Gloves should be changed between different lots. All equipment for sampling must be decontaminated between different samples. Samples should be sent to the laboratory as soon as possible after collection.

LOT IDENTIFICATION

Lots identified on the phytosanitary certificate should be the starting point for planning the inspection. Inspection of associated growing media may not be based on individual lots of the commodity but on combined lots which are homogenous firstly in origin and secondly in type of growing media.

INSPECTION

It is up to the NPPO to set the sample size. For example, from a lot of 10 000 plants, 3 689 plants need to be inspected to provide a 99% confidence of detecting symptoms present in 0.1% of the plants, provided the symptoms are uniformly distributed and the plants are randomly selected. To detect symptoms present in 1% of the plants, with a 99% confidence, 448 randomly selected plants should be inspected.

For small lots, the numbers required will often mean that all plants should be inspected.

The place where the inspection is conducted should be well lit and equipped with an inspection table.

The visual examination should begin with an overall examination of the consignment. Visual examination of the container, packaging and means of conveyance can provide indications of adverse conditions during transport (e.g. adverse temperatures or signs of damp or wetness) which may affect the physical condition of the consignment.

If there is an abnormal die-off of plants in a consignment or lot, or there are other anomalies within the plants present (e.g., abnormal growth, differences in colour), these lots should be inspected with specific attention.

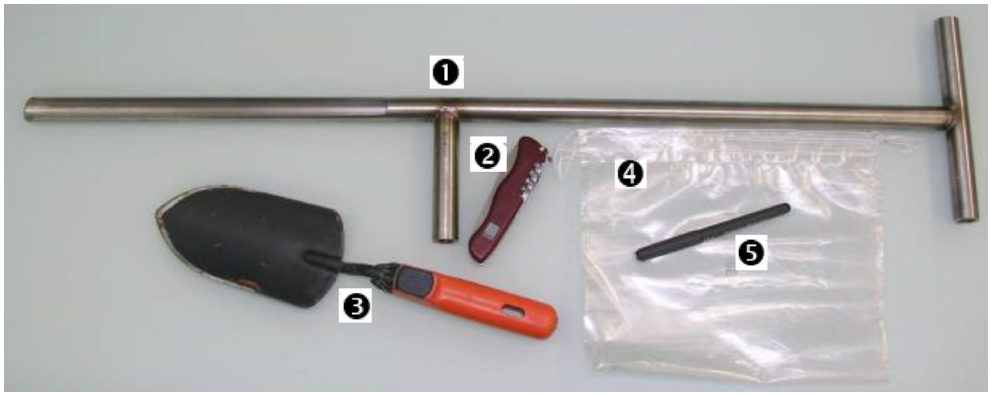

FIGURE A15 Equipment for growing medium sampling: Sampling probe (for large plants) (❶), knife (❷), planting shovel (❸), plastic bag (❹), permanent marker (❺).

Any wrapping surrounding the plant should be removed. The inspector should examine the surface of the media to check for any sign of pests. Scraping the surface of the media may also reveal any contamination just below the surface.

For potted plants, the pot should be removed, and the growing media can be removed from the roots and spread out on a white tray to inspect for pests. A brush can assist in dislodging the growing media from plant roots.

SAMPLING FOR LABORATORY TESTING

Sampling for laboratory testing is required to detect and identify certain pests (e.g. nematodes). This section contains guidance on sampling for laboratory testing of growing medium.

It is necessary to use adequate equipment to sample growing medium (Figure A15).

VISIBLE PESTS AND/OR SYMPTOMS

Samples should be taken from the growing media where pests are visible, or where signs of pests are present and cannot be immediately identified by the inspector. In these cases, the sample consists of the plant(s) and associated growing media.

Samples of adult insects, larvae, pupae and to a lesser extent eggs should be put in a pot with screw cap. Living organisms should be sent to the laboratory together with plant material (roots with growing media) of the host plant in a suitable container. Insects may also be collected in small tubes with ethanol.

NON-VISIBLE PESTS

When no pests are visible, the presence of plant pests in growing media can only be detected and identified by laboratory testing. For parasitic nematodes different amounts of soil may be required depending on the extraction method (e.g. 250–1000 mL) (EPPO, 2013). When it is not possible to sample the required quantity of growing media, the sample should be as big as possible. In this case, the roots and growing media can be sampled with a knife.

For large plants, trees or shrubs with big root balls, sampling should be conducted using a soil sampling probe. After removal of the top 5 cm of growing media, a sample should be taken from the next 25 cm where nematodes are more active. If this layer can be reached easier from the side of the root ball, the probe can be entered from the side. It is important to avoid taking samples from only the outer part and surface of the root ball.

For the presence of other non-visible pests (e.g. fungi), growing media and/or root samples should be sent to the laboratory.

Growing media samples should be placed in a labelled and sealed plastic bag. Samples should be tested in a laboratory as quickly as possible.