EPPO STANDARD ON PHYTOSANITARY PROCEDURES

PM 3/97 (1) Inspection of consignments of plants for planting for invasive alien plants

Specific scope: This Standard describes inspection procedures for consignments of plants for planting imported with soil or other growing medium (and aquatic plants with water) to avoid the import of regulated invasive plants. The Standard does not cover inspection of seeds and plants for planting such as tubers, rhizomes imported as the commodity itself, without soil or growing medium, nor does it cover inspection of pests other than plants.¹ The Standard describes (1) the inspection to check whether the plants for planting are regulated or prohibited as invasive alien plants, and (2) the inspection and sampling of soil or other growing medium associated with plants for planting to ensure it is free from invasive alien plant as contaminants. The Standard provides guidance that may be relevant to inspections for exports. Specific Approval: This Standard was first approved in 2024-09.

1 | INTRODUCTION

Invasive alien plant (IAPs) species are considered a major threat to biodiversity and ecosystem services (Early et al., 2016; Huisman et al., 2021). These species can have negative impacts on agricultural systems, reducing crop yields and degrading pastures (Eschen et al., 2021). One of the main pathways for the entry of IAPs into the EPPO region is via the horticulture trade (Hulme et al., 2018). Although most ornamental species do not cause any adverse impacts, some may become invasive and cause ecological and economic impacts to the areas where introduced (van Kleunen et al., 2015).

Invasive alien plants may be imported as a commodity themselves. The species indicated on the import documents (e.g. a phytosanitary certificate) may be the correct species name, a synonym, or a misapplied scientific or common name. Incorrect labelling and misidentification of plants for planting in trade is widespread and may be deliberate or by neglect (Brunel, 2009; Hulme et al., 2018; Neucker & Scheers, 2022; Thum et al., 2012; Verbrugge et al., 2014). Mislabelling may consist of simple misspelling

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of names or considering a variety as a true species, or just preferring one name over another (Van Valkenburg et al., 2022, 2023). Using synonyms, rather than the preferred scientific name can also lead to confusion. Detecting mislabelled IAPs requires some taxonomic knowledge of the species in question by the inspector.

Invasive alien plants may be imported accidently as contaminants of soil or other growing medium associated with plants for planting, including water for aquatic plants. ISPM 5 Glossary of phytosanitary terms (IPPC, 2024) defines growing medium as 'Any material in which plant roots are growing or intended for that purpose'. Soil and water are included in this definition of growing media and consequently this Standard will refer to growing media.

The EPPO pest risk analysis for *Lygodium japonicum* details a high risk of entry for this species along the pathway contaminant of growing media (EPPO, 2018). Both *Parthenium hysterophorus* (Asteraceae: EPPO A2 List) and *Persicaria perfoliata* (Polygonaceae: EPPO A2 List) have been intercepted by the Dutch NPPO as contaminants of potted plants (EPPO, 2016). Additionally, inspection of consignments of bonsai plants (*Podocarpus macrophyllus* and *Pseudolarix amabilis*) have intercepted *P. perfoliata*, *Alternanthera philoxeroides* (Amaranthaceae: EPPO A2 List), as well as *Humulus scandens* (Cannabaceae: EPPO A2 List).

At import, consignment freedom of pests is usually verified by inspection and, where appropriate, by testing for particular pests before the consignment is released. Similar procedures may be applied in the exporting country before dispatching the consignment, if the importing country requires consignment freedom or verification of the efficacy of other phytosanitary measures (e.g. treatment).

When consignments of plants for planting imported with growing media are inspected at points of entry, inspection for contamination with invasive alien plants is not normally the priority.

The information in this Standard may be used as part of a routine inspection and can be used in conjunction with the EPPO Standard *PM 3 Inspection of growing media associated with consignments of plants for planting* (EPPO, 2024).

¹For inspection of pests other than invasive alien plants, refer to PM 3/98 (1) Inspection of growing media associated with consignments of plants for planting (EPPO, 2024).

1.1 | Types of growing media

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 the survival of invasive alien plants.

Soil: Natural untreated soil presents a high risk irrespective of where the material was collected. Soil can contain seeds or viable fragments (nodes, stems, roots, rhizomes) of IAPs. The import of soil is prohibited in many EPPO countries.

Compost: Commercial compost is processed from plant-based material and can include peat, coconut or rice husk, 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 viable propagules of IAPs.

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, the risk of peat can depend on the origin of the peat and its exposure to IAPs. Peat can contain seeds of plants as pests (IPPC, 2017a). The pest risk is considered lower when the peat is sourced from certified bogs (IPPC, 2017a).

Water: Can be associated with imported plants for planting, especially with aquatic plants which may be found in containers with water. Generally, water is considered a low risk.

Other plant based growing media: Types of media can include paper, coconut fibres, sawdust, wood shavings, cork, moss (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.

Other non-plant based growing media: Sand can potentially harbour propagules of invasive alien plants. However, in general, the risk is generally low. Synthetic media (e.g. polystyrene, vermiculite) generally has a low risk.

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

2 | PHYTOSANITARY INSPECTIONS

In ISPM 5 *Glossary of phytosanitary terms* (IPPC, 2024), inspection is defined 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 pests and compliance with specific phytosanitary measures (for example freedom from contaminants, treatment, or origin in a pest free area).

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 (2002) Standard *PM* 5/2: Pest risk analysis on detection of a pest in an imported consignment, should be followed to allow the NPPO to decide the phytosanitary action to take.

Inspection of consignments of growing media attached to 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.

It is important to pay attention to consignments that are likely to include IAPs as contaminants (e.g. place of origin of the plants for planting imported with growing medium where IAP of concern occur, and from areas/ producers where there are known instances of noncompliance of consignments).

Risk based inspections can be applied for growing media and should take into account the following factors:

- Type 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).

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, 2016).

General background information on inspection of consignments is given in the EPPO (2009) Standard PM/3 72 (2) Elements common to inspection of places of

production, area-wide surveillance, inspection of consignments and lot identification.

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

Information on the presence and status of an IAP in a country of origin may be found in EPPO Global Database (EPPO, 2023).

3 | COMMODITIES CONCERNED

Examples of commodities of plants for planting with associated growing media covered by this Standard are: plants for planting (including bulbs, tubers and rhizomes) in pots or where the roots are contained in another substance, i.e. large shrubs or trees with root balls wrapped in a hessian fabric (e.g. burlap) or aquatic plants which may be bunched together wrapped in paper or other material to preserve moisture and boxed

Plants for planting imported with growing media are usually traded in large lots and transported by sea in palletized boxes, ship holds or containers, or 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

Plants for planting may have been grown outdoors in natural soil (or water) 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 IAPs. Alternatively, plants may have been permanently grown in growing media other than natural soil which presents a lower risk depending on production conditions of the plants.

4 | INVASIVE ALIEN PLANTS OF CONCERN FOR THE EPPO REGION

This Standard covers mainly those IAPs which are listed in the EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests, and which can enter the EPPO region as a plant for planting imported with growing medium or as a contaminant of plants for planting imported with growing medium (see Tables 1 (terrestrial plants) and 2 (aquatic plants)). IAPs that are listed on the EPPO List of Invasive Alien Plants and the EPPO Observation List and are regulated by specific EPPO Member countries are also included. EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests are subject to annual additions and deletions. The species listed in Tables 1 (terrestrial plants) and 2 (aquatic plants) should therefore be revised whenever IAPs are identified and added to the lists.

The phytosanitary procedures described in the Standard are primarily aimed at preventing the introduction of these IAPs into a country via imported consignments of plants for planting imported with growing media.

Inspectors should be aware of regulations and listed regulated species for their countries. For example, for European Union, Regulation (EU) 1143/2014 on the prevention and management of the introduction and spread of invasive alien species contains a list of invasive alien plants of Union concern (European Commission, 2016). The same regulation provides European Union Member countries to develop national lists.

For an indication of the status of these IAPs, the EPPO Global Database (EPPO, 2023) may be consulted. For additional up to date information, the respective scientific literature should be consulted.

Some EPPO countries may have other lists of species which can be included in the inspection process. For example, national or regional alert lists (e.g. EPPO Alert List).

Further information on IAPs of quarantine concern for the EPPO region which can enter as contaminants of plants for planting can be found in Appendix 1.

5 | LOT IDENTIFICATION

General background information on lot identification is given in the EPPO Standard PM 3/72(1) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009).

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.

In the case of the inspection of a consignment of plants for planting with growing media attached, the lot identification depends on the species, variety or size, and place of production. Additionally, in the case of inspection for invasive alien plants contaminants, the type of growing media can be considered.

Lots identified on the phytosanitary certificate should be the starting point for planning the inspection. When a consignment comprises of more than one lot, the inspection to determine compliance should be done on each lot, and each lot should be sampled separately. 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.

Packaging may contain an indication of the country of origin and additional information which may be used to identify individual lots. -

Species	Plants for planting	Contaminant of growing media	Categorization
Acacia dealbata	Х		Portugal, Spain (Regulated Invasive Alien Plant); Switzerland (Observation List)
Acacia saligna	Х		Morocco (Quarantine pest); EU (IAS of Union concern)
Acer rufinerve	Х		Morocco (Quarantine pest)
Acroptilon repens		Х	Estonia (Regulated Invasive Alien Plant); Belarus, Israel, Moldova (Quarantine Pest); Jordan (A1 List); Azerbaijan, Georgia, Kazakhstan, Russia, Ukraine, Uzbekistan (A2 List)
Ailanthus altissima	Х		Russia, Ukraine (Regulated Non-quarantine pest); Switzerland (List Invasive Alien Plants); EU (IAS of Union concern)
Aizoon pubescens	Х		Morocco (Quarantine pest)
Amaranthus tuberculatus		Х	EPPO A2 List
Ambrosia artemisiifolia		Х	Spain, Switzerland (Regulated Invasive Alien Plant); Belarus (Quarantine Pest); Jordan (A1 List); Azerbaijan, Kazakhstan, Russia, Ukraine, Uzbekistan (A2 List)
Ambrosia confertiflora		Х	EPPO A2 List
Ambrosia trifida		Х	EPPO A2 List
Amorpha fruticosa	Х		Jordan (A1 List); Switzerland (List Invasive Alien Plants)
Andropogon virginicus	Х	Х	EPPO A2 List
Araujia sericifera	Х		Portugal, Spain (Regulated Invasive Alien Plant)
Arctotheca calendula		Х	Portugal (Regulated Invasive Alien Plant)
Asclepias syriaca	Х		Switzerland (List Invasive Alien Plants); EU (IAS of Union concern)
Asparagus asparagoides	Х		Spain (Regulated Invasive Alien Plant)
Baccharis halimifolia	Х	Х	EPPO A2 List
Baccharis spicata		Х	Morocco (Quarantine Pest); Portugal (Regulated Invasive Alien Plant)
Bidens frondosa		Х	Estonia, Portugal (Regulated Invasive Alien Plant); Jordan (A1 List)
Bidens subalternans		Х	Morocco (Quarantine pest)
Broussonetia papyrifera	Х		Morocco (Quarantine pest)
Buddleia davidii	Х	Х	Spain (Regulated Invasive Alien Plant); Switzerland (List Invasive Alien Plants)
Cardiospermum grandiflorum	Х		EPPO A2 List
Carpobrotus acinaciformis	Х		Portugal, Spain (Regulated Invasive Alien Plant); Switzerland (List Invasive Alien Plants)
Carpobrotus edulis	Х		Portugal, Spain (Regulated Invasive Alien Plant)
Celastrus orbiculatus	Х	Х	EPPO A2 List
Cenchrus longispinus		Х	Jordan, Kazakhstan (Al List); Russia, Ukraine (A2 List); Morocco (Quarantine Pest)
Cenchrus setaceus	Х		EU (IAS of Union concern)
Cortaderia jubata	Х	Х	EPPO A1 List
Cortaderia selloana	Х	Х	Portugal (Regulated Invasive Alien Plant); Switzerland (Observation List)
Cyperus esculentus		Х	Jordan (A2 List); Switzerland (List Invasive Alien Plants)
Ehrharta calycina		Х	EPPO A2 List
Fallopia baldschuanica	Х	Х	Estonia, Portugal, Spain (Regulated Invasive Alien Plant); Jordan (A1 List)
Fallopia japonica		Х	Estonia, Poland, Portugal, Spain, Switzerland (Regulated Invasive Alien Plant)
Fallopia sachalinensis		Х	Estonia, Poland, Portugal, Switzerland (Regulated Invasive Alien Plant)
Fallopia x bohemica		Х	Estonia, Poland, Portugal, Switzerland (Regulated Invasive Alien Plant)
Gunnera tinctoria	Х		EU (IAS of Union concern)
			(Continues)

TABLE 1 Terrestrial invasive alien plants of quarantine concern for the EPPO region which can enter as plants for planting or as contaminants of growing media. When a species is an EPPO listed A1 or A2 pest, no further country or regional categorization is listed.

TABLE 1 (Continued)

Species	Plants for planting	Contaminant of growing media	Categorization
Hakea sericea	Х		EPPO A2 List
Heracleum mantegazzianum	Х		Switzerland (Regulated Invasive Alien Plant); EU (IAS of Union concern)
Heracleum persicum	Х		EPPO A2 List
Heracleum sosnowskyi	Х		EPPO A2 List
Humulus scandens	Х	Х	EPPO A2 List
Impatiens edgeworthii		Х	Morocco (Quarantine Pest)
Impatiens glandulifera		Х	Switzerland (Regulated Invasive Alien Plant); EU (IAS of Union concern)
Koenigia polystachya	Х	Х	Switzerland (Regulated Invasive Alien Plant)
Lespedeza cuneata	Х		EPPO A1 List
Lupinus polyphyllus	Х		Switzerland (List Invasive Alien Plants)
Lygodium japonicum	Х	Х	EPPO A1 List
Lysichiton americanus	Х		Switzerland (Observation List); EU (IAS of Union concern)
Microstegium vimineum		Х	EPPO A2 List
Miscanthus sinensis	Х	Х	Morocco (Quarantine pest)
Oxalis pes-caprae		Х	Jordan (A1 List); Poland, Portugal, Spain (Regulated Invasive Alien Plants)
Parthenium hysterophorus		Х	EPPO A2 List
Paspalum distichum		Х	Jordan (A1 List)
Persicaria perfoliata		Х	EPPO A2 List
Prunus serotina	Х		Switzerland (List Invasive Alien Plants)
Senecio inaequidens		Х	Portugal, Spain, Switzerland (Regulated Invasive Alien Plant); Jordan (A1 List)
Sicyos angulatus		Х	Uzbekistan (A1 List); Switzerland (List Invasive Alien Plants)
Solanum elaeagnifolium		Х	EPPO A2 List
Solidago canadensis		Х	Estonia, Switzerland (Regulated Invasive Alien Plant); Jordan (A1 List)
Solidago gigantea		Х	Estonia, Switzerland (Regulated Invasive Alien Plant)
Solidago nemoralis		Х	Switzerland (List Invasive Alien Plants)
Triadica sebifera	Х		EPPO A1 List

6 | INSPECTION AND SAMPLING

This section contains guidance on:

- (1) Inspection to check whether the plants for planting are prohibited IAPs,
- (2) Inspection and sampling of growing media associated with plants for planting to ensure it is free from IAP contaminants.

6.1 | General guidance for inspection

Inspectors should be aware to the possible presence of all regulated IAPs.

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

Inspections and sampling can themselves be a pathway for spreading pests. Therefore, inspectors should take appropriate precautions during inspection and sampling, such as wearing protective clothes (coat, overshoes, gloves, etc.).

Good hygiene procedures when collecting samples for the laboratory should be followed by decontaminating tools and hands.

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 lot.

6.2 | Sampling

An adequate proportion of plants for planting from each lot should be subjected to a systematic

TABLE 2	Aquatic invasive alien plants of quarantine concern for the EPPO region which can enter as plants for planting or as
contaminants	s of growing media. When a species is an EPPO listed A1 or A2 pest, no further country or regional categorization is listed.

Species	Plants for planting	Contaminant of growing media	Categorization
Alternanthera philoxeroides		Х	EPPO A2 List
Azolla filiculoides	Х	Х	Jordan (A1 List); Poland, Portugal (Regulated Invasive Alien Plants)
Cabomba caroliniana	Х	Х	Switzerland (List Invasive Alien Plants); EU (IAS of Union concern)
Crassula helmsii	Х	Х	EPPO A2 List
Egeria densa	Х	Х	Portugal (Regulated Invasive Alien Plant)
Elodea nuttallii	Х	Х	Switzerland (Regulated Invasive Alien Plant); Jordan (Al List); EU (IAS of Union concern)
Gymnocoronis spilanthoides	Х	Х	EPPO A2 List
Hydrilla verticillata	Х	Х	Jordan (A1 List); Portugal (Regulated Invasive Alien Plant)
Hydrocotyle ranunculoides	Х	Х	EPPO A2 List
Hygrophila polysperma	Х	Х	Jordan (A1 List)
Lagarosiphon major	Х	Х	Jordan (A1 List); EU (IAS of Union concern)
Limnophila sessiliflora	Х	Х	Jordan (A1 List)
Ludwigia grandiflora	Х	Х	EPPO A2 List
Ludwigia peploides	Х	Х	EPPO A2 List
Myriophyllum aquaticum	Х	Х	Jordan (A1 List); Switzerland (List Invasive Alien Plants); EU (IAS of Union concern)
Myriophyllum heterophyllum	Х	Х	EPPO A2 List
Pistia stratiotes	Х	Х	EPPO A2 List
Pontederia crassipes	Х	Х	EPPO A2 List
Salvinia molesta	Х	Х	EPPO A2 List

examination to check whether the plants for planting are prohibited IAPs and/or IAPs are present as contaminants.

The sample (as minimum number of individuals selected from the lot or consignment to be examined) should be determined based on lots, taking into account the statistical background provided in ISPM 31 Methodologies for sampling of consignments (IPPC, 2008). The number of units to be inspected will depend on the overall size of the consignments, the desired level of confidence and the level of infestation to be detected. Appendix 2 of ISPM 31 provides tables with sample sizes for various levels of confidence and detection. For IAPs, the level of confidence should allow reliable detection of a level of infestation which is as low as possible. The intensity of inspections and selected number of plants to be inspected should be adapted to the risk associated with the introduction of IAPs with plants for planting imported with growing media.

The NPPO should determine the sample size. For example, if 448 potted plants with soil or other 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.

6.3 | Inspection to check whether the plants for planting are prohibited invasive alien plants

The first step in confirming a species identity is to check the phytosanitary certificate and where applicable the import permit. The inspector should check additional documentation to verify the identity and quantity of the plants (the species name, variety, origin, type of commodity, number of plants). Then the inspector should inspect the plants to confirm that the plants are the species as described on the documents.

Some aquatic plants are imported in sealed plastic bags where the plant material may be wrapped in paper to ensure it remains moist (Figures 1 and 2). Plants may be wrapped individually or bunched. The inspector should remove any wrapping and inspect the plant material for any contamination.



FIGURES 1 AND 2 Example of packaging used for aquatic plant imports into the Netherlands (Images courtesy of Johan van Valkenburg, NPPO, NL).

Q-bank Invasive Plant Database² includes look-alike pages that have been developed to distinguish IAPs from similar looking species.

In addition, Q-bank Invasive Plant Database includes Border inspection tools which show plants, at stages most likely to be intercepted at import, and closely related plants or similar species.

Inspectors can also consult the following Q-bank identification guides:

- (1) Invasive aquatic plants
- (2) Invasive terrestrial plants
- (3) *Pennisetum* cultivars

Species misidentification and mislabelling can occur, and inspectors should be aware of commonly misidentified plant groups (see Appendix 2 for further information).

If the identity of the plants cannot be confirmed, specimens should be taken from each lot and sent to the laboratory. If the plants have flowers, fruits or other distinguishable characteristics, it is advisable to include them in the sample which is sent to the laboratory to confirm identification.

When a sample has been taken from the consignment because the presence of a prohibited IAP is suspected, the consignment should remain under official control and should not be released until the final laboratory result confirms that no prohibited invasive alien plants were detected.

Molecular identification, or other rapid identification methods, should be promoted in order to allow for the prompt release of consignments, in cases where no quarantine organism is identified. A process to secure the integrity of the consignment should be considered for cases where rapid identification is not possible.

6.4 | Inspection and sampling of growing media for invasive alien plant contaminants

Visual examination of growing media associated with plants for planting is usually adequate to detect the presence or absence of contaminant IAPs. However, a magnifying lens (at least 10^{\times}) or binocular magnifier should be used to assist identification of the contaminant.

IAPs as contaminants may be present in different lifecycle stages including seeds, rhizomes, seedlings or whole (parts of) plants (Appendix 1). Seeds or rhizomes may be present within the growing media whereas seedlings may be visible growing from the growing medium.

For plants with growing media attached, the inspector should examine the surface of the growing media to check for any contamination. Scraping the surface of the media may also reveal contamination just below the surface.

For potted plants with growing media attached, the pot may be removed, and the growing media can be removed from the roots and spread out on a white tray to inspect for contamination.

Inspectors can also consult the following Q-bank identification guides:

- (1) Seeds of invasive plants
- (2) Weeds in bonsai plants

²Q-bank Invasive Plant Database (2022) is an important resource that can be used for the identification of invasive alien plants.

(3) Seedlings of invasive plants

Additional information on sampling for identification of contaminants that might be present in consignments of plants for planting can be found in Appendix 1.

When a sample has been taken from the consignment because the presence of a prohibited IAP is suspected, the consignment should remain under official control and should not be released until the final laboratory result confirms that no prohibited invasive alien plants were detected.

Reliable rapid identification methods (molecular or morphological), which can provide results within a time frame of 24-48 hours should be promoted in order to allow for the prompt release of plant consignments, in cases where no quarantine organism is identified. A process meant to secure the integrity of the plant consignment should be considered for cases where rapid identification is not possible.

If the presence of an IAP is confirmed, the NPPO should assess the risk of the consignment and propose appropriate measures.

7 | SAMPLING FOR LABORATORY IDENTIFICATION

If an IAP is suspected, either as a commodity itself or as a contaminant, a sample should be taken and sent to the laboratory to confirm its identity.

Samples for laboratory identification should preferably be composed of the whole plant (including flowers and fruits if present). If the plants are small, several plants should be sent. Growing media can be added to small seedlings and young plants. The plant material should ideally be wrapped in paper in order to prevent proliferation of saprotrophic bacteria and fungi. Then, the material should be placed in a sealed airtight box or plastic bag and sent directly to the laboratory. Keep at cool temperatures to avoid exposing samples to stress conditions (see specific procedure in Appendix 1). All packaging should be clearly labelled.

For some IAPs, identification up to species level can be complicated. Also, plants in an early development stage may not exhibit all morphological characters required for an accurate identification up to species level solely based on a visual inspection at the point of entry. For these plants, molecular analysis may provide additional information to support an identification up to species level (see for example EPPO, 2021).

The EPPO-Q-bank Invasive Plants database (2022) includes curated sequence data on vascular plants (excluding algae and mosses), with special focus on aquatic (non-marine) plants. It also provides information on where to find specimens of plants for which DNA sequences are available in herbarium collections.

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APPENDIX 1 - SPECIFIC PROCEDURES

Sampling for identification of contaminants that might be present in consignments of plants for planting. The species detailed in this appendix are species that have been intercepted in consignments of plants for planting imported with soil or other growing medium.

For useful resources to identify IAPs misidentified see Appendix 2.

For each of the IAPs of concern mentioned below, information on biology, detection and identification can be found in Q-bank Invasive Plants Database (2022), EPPO-Q-bank and EPPO (2023).

Further information can be found in relevant EPPO Standards and in named scientific references.

In the following description of seeds and seedlings, the text is taken from Q-bank Invasive Plants.

Alternanthera philoxeroides (EPPO A2 List)

Alternanthera philoxeroides (Amaranthaceae) is a perennial riparian species that has been cultivated as an aquarium plant. It has been intercepted as a contaminant of bonsai plants from China. It does not always set viable seed but reproduces vegetatively from axillary buds at each node. Each node has two axillary buds. Stem nodes, portions of thicker roots, and underground stems are all capable of growth, dispersal is by fragmentation. Leaves are petiolate, without stipules; petioles glabrous or slightly hairy; blades entire, narrowly elliptic, or oblanceolate, herbaceous, glabrous, upper side muricate, base attenuate, margins entire, apex acute to obtuse, with a mucro. Leaves dark-green, waxy (Figure A1).



FIGURE A1 Small plants of *Alternanthera philoxeroides* (Image courtesy of J. van Valkenburg).

Alternanthera philoxeroides can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.

Azolla filiculoides (Jordan (A1 List); Poland, Portugal (Regulated Invasive Alien Plants))

Azolla filiculoides (Salviniaceae) is a floating aquatic fern which due to its small size, it can be a contaminant of aquatic plant imports (Figure A2). It can also be the commodity itself. The leaves are bluish-green, but becoming bright red in autumn, small, not spirally coiled when young, of 1 sort, 2-lobed. The upper lobe floating, lower thin, submerged and bearing the sori in pairs on cylindrical receptacles, covered by the flange of the upper lobe. Upper leaf lobes ± 1 mm, ovate, obtuse, covered above with unicellular hairs ($2.5 \times 0.9-1.4$ mm) which make the surface non-wettable, margins hyaline.

Azolla filiculoides can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.



FIGURE A2 *Azolla filiculoides* a floating aquatic fern (EPPO Global Database Image courtesy of Iñigo Sánchez).

Humulus scandens (EPPO A2 List)

Humulus scandens (Cannabaceae) is a herbaceous annual vine which has been intercepted as a contaminant of bonsai plants from China. The stem is twining clockwise, hexangular ridged or winged, branched, with rigid 2-armed stalked hairs. Leaves with petiole usually longer than blade, with rigid 2-armed stalked hairs (Figure A3). Blade palmately (3-)5-9-lobed, sometimes simple, papery, light green, sometimes variegated (cultivated), base cordate, adaxially pubescent but not densely so, abaxially with yellowish brown resinous glands and dots and with rigid spinulose hairs on veins; lobes ovate-triangular, margin serrate and upper margins of younger leaf blades with stiff cystolithic hairs (i.e. mineral concretions: calcium carbonate or calcium oxalate), apex acuminate.

Humulus scandens can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.



FIGURE A3 *Humulus scandens* seedling (Image courtesy of J. van Valkenburg).

Hydrilla verticillata (Jordan (A1 List))

Hydrilla verticillata (Hydrocharitaceae) is an herbaceous aquatic plant species that is occasionally encountered in the aquarium trade. The species spreads horizontally by means of branches which grow over the bottom of a waterbody. Vertical branches and roots are produced at nodes on these runners. The leaves are grouped in whorls of 5 with internodes of 3–50mm long (the internodes tend to elongate in flowing water). The leaves are linear to lanceolate, with a conspicuous midrib and conspicuously sharply toothed margins and spines on the vein on the lower side of the leaves (Figure A4). The plant can easily reproduce by fragmentation of the stem or specialized storage organs that are formed subterraneous or in the leaf axils under disadvantageous conditions.

Hydrilla verticillata can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.



FIGURE A4 *Hydrilla verticillata* (Image courtesy of J. van Valkenburg).

Hydrocotyle ranunculoides (EPPO A2 List)

Hydrocotyle ranunculoides (Araliaceae) is a herbaceous riparian species that is sometimes sold as an aquarium plant, and also as an outdoor pond plant. It has been intercepted as a contaminant in potted pond plants. Leaves are stipulate, leaves emergent and held above the horizontal stem on long fleshy petioles, non-peltate, sub-orbicular to reniform with cordate base, sometimes lobes overlapping at base and leaf seemingly peltate (Figure A5). Frequently broader than long, shallowly or deeply 3–7 lobed, lobes rounded, crenate or lobulate and subequal; diameter (2) 4–10 (-18) cm in eutrophic conditions.

Hydrocotyle ranunculoides can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.



FIGURE A5 *Hydrocotyle ranunculoides* (Image courtesy of J. van Valkenburg).

Hygrophila polysperma (Jordan (A1 List))

Hygrophila polysperma (Acanthaceae) is a rhizomatous perennial riparian plant with stems four angled



FIGURE A6 *Hygrophila polysperma* (Image courtesy of J. van Valkenburg)

and opposite leaves (Figure A6). The roots are either rooted in the sediment or float freely in the water column from floating shoots. The leaves are oblong to elliptic, sparsely hairy and broader to the tips. Stems are often prostrate, 4-angled, slightly swollen above nodes. Leaves are hairless, opposite, petiole to 5 mm; leaf blade oblong-lanceolate to ovate, $2-3.5 \times 0.6-1.3$ cm.

Lagarosiphon major (Jordan (A1 List); EU (IAS of Union concern))

Lagarosiphon major (Hydrocharitaceae) is an aquatic plant species that is sometimes traded for use in outside water features. It has been intercepted as a mislabelled plant at import in the Netherlands. It forms a branching stem with alternate leaves spirally arranged, closer together towards the top of the stem; dark green, denticulate, leaf tip acute, stiff, strongly curved (Figure A7).

Lagarosiphon major can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.



FIGURE A7 *Lagarosiphon major* (Image courtesy of J. van Valkenburg).

Lygodium japonicum (EPPO A1 List)

Lygodium japonicum (Lygodiaceae) is a climbing fern, with a twining rachis. It has been intercepted as a contaminant in potted plants (Figure A8). Fronds borne 2-7 mm apart on rhizome, vinelike with a twining rachis with continuous growth, climbing up to 12(-30) m long, alternately pinnate, central rachis ca. 2mm in diameter, glabrous, but adaxalially minutely hairy; pinnae reduced to short stalks 3-10mm long and dormant apex covered with pale hairs; each pinna bearing a pair of opposite pinnules. Pinnules 1-pinnate when young, 2-3-pinnate when full grown, 6-17×5.5-17 cm, rachis shortly hairy (more dense adaxially), primary divisions pinnately to palmately lobed or divided, or if undivided with distinct basal lobes; lobes directed towards leaf apex; ultimate segments 4-6mm wide, petiolules not articulate or thickened at apex, not leaving wiry stalks when detached; blade tissue sparsely to moderately pubescent abaxially. Fertile pinnae towards the apex of fertile fronds, pinnules lanceolate-triangular, 2-3-pinnate, $5-18 \times 4-14$ cm; ultimate segments ovate to lanceolate, fringed with 1-5mm long fertile lobes, otherwise similar to sterile segments. Vernation circinate. Evergreen in climates with no dry season, possibly dying down in dry season.

Lygodium japonicum can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.



FIGURE A8 *Lygodium japonicum* (Image courtesy of J. van Valkenburg).

Myriophyllum heterophyllum (EPPO A2 List)

Myriophyllum heterophyllum (Haloragaceae) is an aquatic plant species which is sometimes utilized for aquariums and outdoor ponds. Stem is green, often with a reddish tinge in older parts of the stem, internodes up to $\frac{1}{4}$ of the length of the leaves, upper part of the stem rising above the surface of the water (up to



FIGURE A9 *Myriophyllum heterophyllum* (Image courtesy of J. van Valkenburg).

10-15 (35) cm) (Figure A9). The submersed leaves are oblong in outline, pinnatisect, with 3-20 pairs of segments; leaves seemingly grouped in whorls of 4-5.

Myriophyllum heterophyllum can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.

Parthenium hysterophorus (EPPO A2 List)

Parthenium hysterophorus (Asteraceae) is an annual terrestrial plant species that can produce up to 100000 seeds (EPPO, 2014). Parthenium hysterophorus have been intercepted by the Dutch NPPO as contaminants of potted plants (EPPO, 2016). Fruits are nutlets obovoid, pappuslike persistent corolla appendages and style erect, deltate to ovate, 0.5–1mm (sometimes a third, subulate spur near apex adaxially). Seedling description: Cotyledons 7–10mm long, orbicular to ovate, glabrous or sparsely pubescent, a bit glossy bright green (Figures A10 and A11). First pair of leaves 12–15mm long, ovate to rhomboid, shallowly lobed to 3-lobate, pubescent with white patent hairs, margins ciliate, dull light green. Third and fourth leaf pinnately lobed to pinnatisect, dull dark

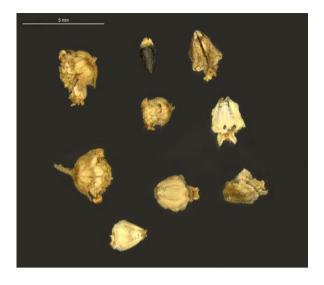


FIGURE A11 Parthenium hysterophorus seeds (Image courtesy of J. van Valkenburg).

green, pubescent with white patent hairs, margins ciliate. Young plants produce rosettes at first.

Parthenium hysterophorus can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.

Persicaria perfoliata (EPPO A2 List)

Persicaria perfoliata (Polygonaceae) is a herbaceous vine which has been intercepted as a contaminant of bonsai plants and other potted plants. Fruits are berry-like, pale, metallic blue as they ripen, shiny, globose, 3–5mm in diameter. Seeds are shiny, black or reddish-black, nearly rounded, 2mm in diameter. The characteristic ochrea at the base of the petiole is cupshape; those of the upper leaves are conspicuous expanded with a green herbaceous wing at apex (1.5–3 cm in diameter) (Figure A12). Petiole long and perfoliate, with curved, retorse barbs. Lamina pale green, thin, glabrous, shaped like an equal-sided triangle, base



FIGURE A10 *Parthenium hysterophorus* (Image courtesy of J. van Valkenburg).



FIGURE A12 Seedling *Persicaria perfoliata* (Image courtesy of J. van Valkenburg).

truncate or subcodate, apex subacute; the veins on the underside of the lamina with curved, retorse barbs.

Persicaria perfoliata can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.

Pistia stratiotes (EPPO A2 List)

Pistia stratiotes (Araceae) is a free-floating aquatic plant with a rosette of obovate to spatulate, short haired leaves (up to 35 cm in European thermal waters). *P. stratiotes* is a plant that forms small colonies with daughter plants attached to the mother plant through stolons. Leaves are sessile to subsessile in a dense rosette, floating to ascending or nearly erect (Figure A13). Leaves obovate to spatulate, light green to greyish green, base obtuse or rounded, apex subtruncate, or notched, pubescent densely on upper-, undersides and on margins, nerves 5-13(-15), radiating fan-wise, prominent on the underside.

Pistia stratiotes can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.



FIGURE A13 Small floating plants of *Pistia stratiotes* (EPPO Global Database Image courtesy of Anne-Sophie Roy).

Pontederia crassipes (EPPO A2 List)

Pontederia crassipes (Pontederiaceae) is a free-floating aquatic plant. Plants in uncrowded situations tend to have short, spreading petioles with pronounced swelling, while in a dense stand they are taller, more erect and with little or no swelling of the petioles. *P. crassipes* is a clonal plant that forms small colonies with daughter plants attached to the mother plant through stolons. Leaves consist of petiole (often swollen, 2–5 cm thick) and blade (roughly round, ovoid or kidney-shaped) (Figure A14). The base of the petiole and any subsequent leaf is enclosed in stipule up to 6 cm long. Roots develop at the base of each leaf and



FIGURE A14 *Pontederia crassipes* (Image courtesy of J. van Valkenburg).

form a dense mass; usually 20-60 cm long, though they can extend to 3 m. Seedlings have leaves that are only a few cm across or high.

Pontederia crassipes can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.

Salvinia molesta (EPPO A2 List)

Salvinia molesta (Salviniaceae) is a free-floating aquatic fern. Plants are mat-forming, mat usually up to 2.5 cm thick. Leaves in whorls of 3. The 2 upper leaves are floating, photosynthetic, entire, elliptic-ovate to rounded, with a distinct midvein, covered with papillae, apices rounded or emarginate and the submerged leaf is



FIGURE A15 Salvinia molesta (Image courtesy of J. van Valkenburg).

finely divided into linear segments (feathery), segments appearing as and functioning as roots (Figure A15). Papillae on the upper side of the floating leaves are either fairly uniform in size throughout, or inner longer than outer; papillae apex split into several, more celled hairs that form a birdcage-like structure; the cage traps an air bubble when submerged, creating a non-wetting upper surface. Lower leaves submerged, subsessile or petiolate, with or without sporocarps.

Salvinia molesta can be identified through molecular techniques and DNA sequences are available in EPPO-Q-bank.

A PPENDIX 2 - MISIDENTIFICATION AND MISLABELLING OF PLANTS FOR PLANTING INTENDED FOR HORTICULTURE

Mislabelling of plants for planting intended for the horticulture industry can result in the entry of regulated invasive alien plants which would otherwise be intercepted at the border and prohibited from entry. Mislabelling may be due to spelling mistakes, using a synonym (which could also be misspelt) and misidentification (Table A1).

Available resources should be consulted to ensure correct identification, in particular for those cases where confusion with invasive alien plants can occur (EPPO, 2009).

Inspectors can check synonyms and common names in EPPO Global Database https://gd.eppo.int/. Additionally, other databases can be consulted including, Plants of the World Online.

TABLE A1Examples of misapplied plant names in horticulture(from Neucker & Scheers, 2022).

Horticultural name	Misapplied name	
Azolla filiculoides	Azolla caroliniana	
Cabomba caroliniana	Cabomba	
Crassula helmsii	Crassula recurva	
Egeria densa	Elodea densa	
Gunnera tinctoria	Gunnera manicata	
Lagarosiphon major	Elodea crispa	
Myriophyllum rubricaule	Myriophyllum brasiliensis (+ Myriophyllum Red Stem)	
Salvinia molesta	Salvinia natans	

APPENDIX 3 - SHORT PROCEDURE FOR INSPECTORS

General

Visual examination of imported consignments of plants for planting imported with growing medium is usually adequate to detect the presence or absence of contaminant IAP. However, a magnifying lens (at least $10\times$) or binocular magnifier should be used to assist identification of the contaminant.

Hygiene measures

Inspections and sampling can themselves be a pathway for spreading pests. Therefore, inspectors should take appropriate precautions during inspection and sampling, such as wearing protective clothes (coat, overshoes, gloves, etc.). Good hygiene procedures when collecting samples for the laboratory should be followed by decontaminating tools and hands.

Inspection

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

In the case of the inspection of a consignment of plants for planting with growing media attached, the lot identification depends on the species, variety or size, and place of production. Additionally, in the case of inspection for invasive alien plants contaminants, the type of growing media can be considered.

Lots identified on the phytosanitary certificate should be the starting point for planning the inspection. When a consignment comprises of more than one lot, the inspection to determine compliance should be done on each lot, and each lot should be sampled separately. Packaging may contain an indication of the country of origin and additional information which may be used to identify individual lots.

An adequate proportion of plants for planting from each lot should be subjected to a systematic examination to check whether the plants for planting are prohibited IAPs and/or IAPs are present as contaminants.

The NPPO should determine the sample size. For example, if 448 potted plants with soil or other 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.

If the presence of an IAP is confirmed, the NPPO should assess the risk of the consignment and propose appropriate measures.

Visual examination

The visual examination should begin with an overall examination of the consignment.

Confirmation of a species identity

The first step in confirming a species identity is to check the phytosanitary certificate and where applicable the import permit. The inspector should check additional documentation to verify the identity and quantity of the plants (the species name, variety, origin, type of commodity, number of plants). Then the inspector should inspect the plants to confirm that the plants are the species as described on the documents.

If there are any doubts on the identity of a species, specimens should be taken from each lot and sent to the laboratory. If the plants have flowers or fruits, it is advisable to include them in the sample which is sent to the laboratory.

Inspection for contamination

IAPs as contaminants may be present in different lifecycle stages including seeds, rhizomes, seedlings or whole plants (Appendix 1). Seeds or rhizomes may be present within the growing media whereas seedlings may be visible growing from the growing medium.

For plants with growing media attached, the inspector should examine the surface of the growing media to check for any contamination. Scraping the surface of the media may also reveal contamination just below the surface. For potted plants with growing media attached, the pot may be removed, and the growing media can be removed from the roots and spread out on a white tray to inspect for contamination.

Sampling for laboratory identification

If an IAP is suspected, either as the commodity itself or as a contaminant, a sample should be taken and sent to the laboratory to confirm its identity.

Samples for laboratory identification should preferably be composed of the whole plant (including flowers and fruits if present). If the plants are small, several plants should be sent. Growing media can be added to small seedlings and young plants. The plant material should ideally be wrapped in paper in order to prevent proliferation of saprotrophic bacteria and fungi. Then, the material should be placed in a sealed airtight box or plastic bag and sent directly to the laboratory. Keep at cool temperatures to avoid exposing samples to stress conditions (see specific procedure in Appendix 1). All packaging should be clearly labelled.