Phytosanitary procedures
Procédures phytosanitaires

Draft commodity-specific phytosanitary procedure

Consignment inspection of *Fragaria* plants for planting

Specific scope

This standard describes the procedure by which consignments of *Fragaria* spp. plants for planting are subjected to import control including sampling and identification.

Specific approval and amendment

First approved in 2008-09.

Introduction

Fragaria spp. is one of the most important small-fruit crops in the EPPO region. Plants for planting of *Fragaria* are produced in the EPPO region but are also imported from other parts of the world (e.g. USA, South America). Imported consignments may carry regulated pests specific to plants of *Fragaria* spp. as well as polyphagous or contaminating pests either included in the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests or otherwise regulated by member countries (e.g. those listed in the EU Directive 2000/29/EC). Many EPPO countries also require that consignments of *Fragaria* plants for planting, should be 1) free from plant debris, 2) free from insect pests at any stage of development and 3) should fulfil the provisions set out in the Phytosanitary Procedure PM 3/54 (1) *Growing plants in growing medium prior to export.*

At import, consignment freedom is usually verified by visual inspection and testing, where appropriate, before release of the consignment. Similar procedures may be applied in the exporting country just before forwarding the consignment, if the importing country requires consignment freedom only (place of production inspection is usually required for plants for planting) or as a verification of the efficacy of other phytosanitary measures (e.g. treatment). Post-entry inspections are recommended for certain pests.

Phytosanitary inspections

General background information on phytosanitary inspection of consignments 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.*

The procedures described in this standard are mainly specific to consignment inspection in an EPPO importing country, but may also be applicable for export inspection (when the importing country requirements are similar e.g. same quarantine pests concerned). General elements of this inspection procedure apply to both inspection in the exporting or the importing country.

In the exporting country inspections should be done at the premises of the producer or exporter as long as the whole consignment is still accessible i.e. before packing or loading. Inspection of plants in cool storage should be avoided as this greatly influences detection of insect pests because of their reduced mobility. For quarantine organisms likely to be present on the consignment in a latent stage, field inspections and sampling should be done at the most appropriate time according to EPPO Standard PM 3/xx. *Field inspection* (in preparation). Producers, exporters and transporters in the exporting country should be informed of the particular requirements of the importing country for strawberry plants.

Inspections of consignments of *Fragaria* plants in the importing country may be done at the point of entry or at the point of final destination, depending on the possibilities of carrying out efficient inspections and provided that the plants remain under official control. In case of inspection at the point of final destination there should be no risk of escape of quarantine pests during transport. When a sample has been taken from the consignment the imported *Fragaria* plants should remain under official control and should not be planted until the final laboratory result confirms absence of the relevant quarantine pests.

Plants for planting produced according to the EPPO Standard PM 4/11 (2) *Certification Scheme on 'pathogen-tested strawberry'* or any equivalent phytosanitary certification system, are generally considered to provide higher phytosanitary guarantees and this

should be taken into account. Consequently intensity of inspections and testing may be reduced and inspection may mainly focus on pests not covered by the respective certification scheme.

Commodities concerned

Plants of *Fragaria* spp. are usually traded either in the form of plants in tissue culture (microplants), 'frigo' plants (young plants after cold storage), green plants (runners) or seeds. They are intended for further propagation or breeding purposes, for commercial fruit production or for dispatch to the final (private) consumer.

Specifically for this Standard, the different types of imported commodities are defined as follows:

Plants in tissue culture

Plants in tissue culture are usually intended for further propagation. As this material is the starting point for large-scale multiplication in principle it may contribute to efficient spread of plant pests. However, due to the special growing conditions the majority of potential contaminating pests and diseases will be excluded. This may not be the case with viral or bacterial pathogens which could persist undetected during micropropagation. Visual inspection of plants in tissue culture at the time of import is difficult to perform and unreliable. Inspections at the place of final destination, preferably after transplanting into growing medium are therefore recommended.

'Frigo' plants

'Frigo' plants are obtained from runners or plants in tissue culture which have been stored for several months under cool conditions (1–2°C) before planting into the field for fruit production. The plants are imported without leaves in a dormant stage. Bundles (usually 25 plants per bundle) of plants may be delivered without soil wrapped in plastic bags or stacked in boxes filled with soil. Production of 'frigo' plants is done under field conditions where they are potted in growing medium (soil, peat). The plants can be contaminated by a broad spectrum of different pests. As the plants at the time of import are in a dormant stage, usually without leaves, visual inspection relying only on visible symptoms or signs of plant pests may be difficult and unreliable.

Fragaria green plants

Green plants, with and without roots, are obtained from runners or plants in tissue culture and are usually intended for fruit production. The plants are produced under field conditions and are in active growth. Rooted plants may be traded in bags, with or without growing medium. Due to these growing conditions green plants also can be contaminated by a broad range of potential plant pests.

Seeds of *Fragaria* spp.

There may also be imports of *Fragaria* seeds which are intended to be used for breeding purposes. Plants or seeds of various *Fragaria* species, other than *Fragaria* × *ananassa*, may be imported for use as ornamental plants or for breeding purposes. Seed of *Fragaria* spp. can serve as a pathway for the seed-transmissible nepoviruses, such as *Tomato ringspot nepovirus* (TomRSV), and are also included in this standard. These viruses can only be detected by laboratory testing.

Regulated pests of *Fragaria* spp.

This standard mainly relates to pests of the EPPO Lists of A1 and A2 pests recommended for regulation recognized as of primary importance for strawberry plants and it also covers those pests listed in the EU Directive 2000/29/EC but not included in the EPPO lists. The phytosanitary procedures described in the standard are primarily aimed at preventing the introduction and spread of these specific pests in the EPPO region via imported consignments of *Fragaria* plants. This standard also covers polyphagous quarantine pests which have *Fragaria* as an economically-relevant host and it includes hitchhikers which have the possibility of being introduced as contaminants.

For plants in growing medium particular attention should be paid to nematodes which may act as virus vectors.

Details on all these pests can be found in *Quarantine Pests for Europe, 2nd edition* (*EPPO/CABI, 1997*). For additional up to date information the relevant scientific literature should be consulted.

The EPPO Lists of A1 and A2 pests recommended for regulation as well as the lists of Directive 2000/29/EC are subject to additions and deletions. The present list will therefore be revised whenever new quarantine pests are identified.

a) Specific pests of *Fragaria* spp.*

A1 pests	A2 pests	Other pests regulated by some EPPO member countries†
Insects	Nematodes	Nematodes
Anthonomus bisignifer	*Aphelenchoides besseyi*	*Aphelenchoides fragariae*
Anthonomus signatus		
	Fungi	Fungi
	Phytophthora fragariae var. *fragariae*	*Glomerella acutata*
	Procaryotes	Procaryotes
	Xanthomonas fragariae	Strawberry witches' broom phytoplasma
	Viruses	Viruses
	Strawberry vein banding virus (caulimovirus)	*Strawberry crinkle virus (cytorhabdovirus)*
		Strawberry mild yellow edge virus (potexvirus)

*Although listed in the EPPO A1 List, Strawberry latent C virus is omitted from this document as it is not referred in the Eighth Report of the International Committee on Taxonomy of Viruses (2005). The phytosanitary categorization of this virus will be revised.

†Several other viruses and phytoplasmas are regulated as 'non-European viruses' because a disease is known, although the taxonomy of the causal organisms for some of them is unclear.

b) Polyphagous pests also attacking *Fragaria* spp.

A1 pests	A2 pests	Other pests regulated by some EPPO member countries
Insects	Insects	Viruses
Helicoverpa zea	*Frankliniella occidentalis*	*Arabis mosaic virus* (nepovirus)
Heteronychus arator	*Scirtothrips dorsalis*	*Strawberry latent ringspot virus* (sadwavirus)
Naupactus leucoloma	Nematodes	*Tomato black ring virus* (nepovirus)
	Ditylenchus dipsaci	
	Viruses	
	Raspberry ringspot virus (nepovirus)	
	Tomato ringspot virus (nepovirus)	

c) Possible contaminating pests on *Fragaria* spp. (examples)

Nematodes
Globodera pallida, *G. rostochiensis*, *Xiphinema americanum sensu stricto*, *X. bricolense*, *X. californicum* and *X. rivesi*.

d) Other harmful and/or exotic pests on *Fragaria* spp.

As mentioned in the introduction many countries require that *Fragaria* plants for planting should be free from plant debris and free from insects at any stage of development. The inspection should also be carried out for the detection of organisms 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* should be followed to allow the NPPO to make a decision as to what phytosanitary action to take.

For an indication of the status of these pests consult the latest version of PQR (Plant Quarantine Retrieval System) http://www.eppo.org/DATABASES/pqr/pqr.htm.

Identification of lots

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*. For *Fragaria* cultivars are the primary criteria for lot identification. If information on different producers is available this should also be taken into account.

Sampling for visual inspection and laboratory testing

This section contains guidance on visual inspection of consignments of *Fragaria* plants for planting, on the proportion of items to be inspected (sample size) and on sampling for laboratory testing. Phytosanitary inspections are done after checking the documents associated with the consignment (in particular the phytosanitary certificate) and the integrity of the consignment. The general background for carrying out import inspections is included in ISPM no. 20 *Guidelines for a phytosanitary import regulatory system* and ISPM no. 23 *Guidelines for inspection*.

Sampling for visual inspection (general aspects)

Phytosanitary inspections should start with an overall examination of the consignment, container, packaging and means of conveyance in order to obtain indications of adverse conditions during transport (e.g. temperature, wetness), to check the physical condition of the *Fragaria* plants and to look for plant debris and live or dead insects.

An adequate proportion of plants from each lot should be subjected to a systematic examination in order to detect the presence or signs of quarantine pests for *Fragaria* plants or contaminating pests as listed above. If appropriate, samples should be taken to the laboratory for identification.

The size of the unit of inspection (= minimum number of individuals to be examined) should be determined on the basis of lots taking into account the statistical background provided in EPPO Standard PM 3/65 *Sampling of consignments for visual phytosanitary inspection*. One should keep in mind that sampling can never prove that a pest is truly absent.

Plants for planting usually present a higher risk of introduction and spread of regulated pests than fruits or other parts of plants not intended for planting. Therefore the size of the unit of inspection should be determined depending on the status of the regulated pest in the area of import. For regulated pests absent from the area of import the objective should be of aiming at detecting an infection level of 1% or more with a confidence level of at least 99%. For pests present in the area of import the aim should be to detect an infection level of 1% with a confidence level of at least 95%. Sample sizes are indicated in Table 2(b) of EPPO Standard PM 3/65 *Sampling of consignments for visual phytosanitary inspection*.

Sampling for laboratory testing (general aspects)

Visual examination of imported consignments of *Fragaria* plants alone is not considered to be sufficient as this will only result in the detection of visually detectable pests like insects. Since many of the nematodes and the fungal, bacterial or viral diseases (e.g. all viruses, *Glomerella acutata* and *Xanthomonas fragariae*) may be present in a latent stage and can hardly be detected on young plants usually traded with few or without leaves or even on plants in tissue culture by means of visual inspection, laboratory testing should be done as a complementary check whenever possible.

Firstly samples should be taken from those plants on which harmful organisms or signs of them are present and cannot be immediately identified by the inspector and from those plants

showing conspicuous symptoms, deformations or general weakness. In these cases the sample consists of the suspect plant(s).

Regardless of the presence of symptoms and especially in those cases where no obvious symptoms are recorded, random sampling of asymptomatic plants should be done in order to detect latent or hidden infections. The size of the sample to be taken depends on the potential distribution of the pests within the consignment or lot and on the capacity of the laboratory (e.g. the method to be applied for diagnosis). Sampling should be done preferably on a lot basis with plants evenly collected throughout the lot. Since the plant parts most suitable for detection greatly differ for the different quarantine organisms, samples for laboratory testing should always contain the complete plants in order to give a possibility of testing for the whole range of potential pests. Sampling should target susceptible varieties or countries of origin at risk. Sampling plans should be formulated to determine the frequency of submission of samples for laboratory testing.

If a pest is found which the inspector suspects to be a quarantine pest, the lot or consignment should be detained under official control. As previously indicated, 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* should be followed to allow the NPPO to make a decision as to what phytosanitary action to take.

Sampling for visual inspection and for laboratory testing (specific aspects)

For further details on symptoms, sampling and identification of the relevant quarantine pests of *Fragaria* spp. see Appendix 1.

Fragaria *plants in tissue culture*

In the closed environment in which plants in tissue culture are kept or produced there is less risk of infestation with insect or nematode pests but there may be infections with fungal, bacterial or viral pests which usually are difficult to detect by visual inspection. Therefore laboratory testing of a representative sample of plantlets taken from several vessels should be done. For practical reasons sampling may be performed under sterile conditions (clean bench) at the place of final destination. Relevant quarantine pests for which testing should be done are: *Glomerella acutata*, *Phytophthora fragariae* var. *fragariae*, Strawberry witches' broom phytoplasma, *Xanthomonas fragariae*, all quarantine viruses of *Fragaria*. It should be ensured that the plants in tissue culture will not be used for further propagation while awaiting the results from the laboratory.

'Frigo' plants

'Frigo' plants are usually shipped in a dormant stage without leaves after cold storage. Visual inspection of those plants at the point of entry is difficult to perform and should therefore preferably be done at the place of final destination. For practical reasons complete bundles (usually 25 plants/bundle) should be sampled from different bags or cases and subjected to visual inspection and sampling for laboratory testing.

The aerial parts of the plants should be thoroughly examined for the presence of different live stages (eggs, larvae, pupae, adults) of insects using magnifying equipment e.g. a magnifying lens or a binocular. Plants should be shaken over sheets of white paper. Small insects present on the surface of plants fall onto the paper and can be collected for further identification. If there are some leaves on the plants left, attention should also be paid to signs of infection with micro-organisms. The root systems of the plants should be examined for external symptoms of infection by *Phytophthora fragariae* var. *fragariae* i.e. the characteristic 'rat-tail'. At least 100 plants (except for small consignments) taken from different bundles (5 plants per bundle), including any plants with external symptoms, should be selected for examination of internal symptoms ('red-stele'). The upper, unrotted parts of the roots should be cut open and examined for wine-red to brick-red coloration of the stele. Roots should also be examined for possible signs or symptoms of nematodes using magnifying equipment (living nematodes or swellings, see Appendix 1).

Samples from pests detected on the plants or from plants with suspicious alterations or symptoms are taken for further identification in the laboratory. Visual inspections should be complemented by laboratory testing of representative randomly-collected samples from different lots in order to detect hidden or latent infections of the relevant nematodes, fungi, bacteria and viruses.

Green plants

Green young plants are usually shipped in a rooted stage without growing medium attached. The plants are in active growth with mostly younger leaves. Visual inspection is only suitable for detecting harmful organisms which are present on the plants (e.g. insects, nematodes) or which induced conspicuous symptoms to the plants, e.g. Red Core Disease induced by *Phytophthora fragariae* var. *fragariae* or under certain circumstances leaf symptoms of *Xanthomonas fragariae*. Many of the other quarantine pests still may be present in a latent stage without any visible symptoms. Determination of the size of the unit for inspection is in principle the same as for 'frigo' plants.

The leaves (top and bottom side) and the crown of the plants should be thoroughly examined for the presence of different live stages (eggs, larvae, pupae, adults) of insects using magnifying equipment. Equally, possible signs or symptoms of nematodes (e.g. swelling, distortion, crinkling or stunting, see Appendix 1) could be seen. Plants should be shaken over sheets of white paper. Small insects present on the surface of plants fall onto the paper and can be collected for further identification. When inspecting the aerial parts of the plants, special attention should be paid to signs of infection by micro-organisms (fungi, bacteria, viruses) such as various kinds of leaf spots, leaf crinkling, leaf mottling, distortions, stunting, general decline or wilting. The root systems of the plants should be examined for symptoms of *Phytophthora fragariae* var. *fragariae* as described for 'frigo' plants.

Sampling for laboratory testing of plants with suspicious alterations or symptoms and of plants without visible symptoms is done as described above ('frigo' plants).

Where green plants are potted into growing medium, it is also recommended to take a sample of the growing medium adhering to the plants in order to test for the presence of nematodes of the genus *Longidorus* and *Xiphinema* (which are known to be vectors of nepoviruses) and also *Globodera pallida* and *G. rostochiensis*. Sampling of the growing media may not be necessary when it is composed of pure peat. The sample should combine growing media from a representative number of boxes. If any of these vectors are found there should be additional testing for nepoviruses.

Seeds of Fragaria *spp*.
Imports of *Fragaria* seeds are rare. However, if such consignments are imported e.g. for breeding purposes, attention should be paid to possible infections with seed-transmissible nepoviruses. These viruses either can be detected by direct assay of the dormant seed samples by means of ELISA or preferentially during a post-entry quarantine period at the place of final destination. Since seedlings grown from infected seed rarely produce recognizable symptoms, laboratory testing of a representative number of the seedlings is recommended (ELISA).

Reference

EPPO/CABI (1997) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

Enquiries

Any question about this standard should be sent to hq@eppo.fr

Appendix 1

Symptoms and sampling for identification of quarantine pests of *Fragaria* spp.

For each of the quarantine pests mentioned below basic information on host range, biology, detection and identification can be found in *Quarantine Pests for Europe*, *2nd edition* (*EPPO/CABI, 1997*). Illustrations are available on the EPPO website (http://www.eppo.org). When an EPPO diagnostic protocol exists it is mentioned in the text. The fact that there is no EPPO diagnostic protocol does not mean that no method for diagnosis is available in the scientific literature.

A) Insects

1) *Anthonomus bisignifer* and *A. signatus*

Symptom description
This pest is only likely to be detected at import on green plants. Partially severed buds can be seen hanging from infected plants.

Sampling and identification
The severed buds may contain larvae or pupae of the pest which should be sent to the laboratory for identification. Both species have to be distinguished from the commonplace and widespread European *Anthonomus rubi* which is very similar in appearance and habits. There is no EPPO Diagnostic protocol available.

2) *Frankliniella occidentalis*

Symptom description
Damage is not likely to be seen on imported plants as much of it is caused by feeding on flowers or at early fruit sets.

Sampling and identification
Look for adults and larvae. Details on identification of *F. occidentalis* are included in the EPPO Standard PM 7/11 (1) *Diagnostic protocol for regulated pests:* Frankliniella occidentalis.

3) *Helicoverpa zea*

Symptom description
Helicoverpa zea is a polyphagous pest, the host range of which includes *Fragaria* spp. The caterpillars mainly attack flowers and fruit from a broad range of host plants but also feed on buds. Specific symptoms on *Fragaria* plants are not described in the literature.

Sampling and identification
Plant material should be inspected carefully to detect different life stages of the pest (eggs, larvae) or signs of it such as bore holes on buds or flowers. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. A specific EPPO Diagnostic protocol is not yet available but the EPPO Standard PM 7/19 (1) *Diagnostic protocol for regulated pests:* Helicoverpa armigera provides some useful information on the identification of similar *Helicoverpa* species.

4) *Heteronychus arator*

Symptom description
Since all life-stages of the pest are subterranean the feeding damage mainly occurs on the lower parts of the plants (roots and stems). The major pathway of this pest is soil attached to imported plants.

Sampling and identification
Fragaria plants for planting potted into growing medium or with growing medium attached to the roots should be thoroughly inspected for the presence of soil-inhabiting larvae or adult beetles. If appropriate, samples for identification of the pest in the laboratory should be taken. An EPPO Diagnostic protocol is not yet available.

5) *Naupactus leucoloma*

Symptom description
Naupactus leucoloma is a highly polyphagous pest. Whereas the feeding of the adult weevils is restricted to the bases of the leaf margins leading to characteristic 'notching', the main damage is caused by the larvae. They gnaw at tap roots, small lateral roots and the basal parts of stems. When feeding is severe, plants turn yellow, wilt and die. Since eggs are laid on many parts of host plants and remain viable for more than seven months, they can be transported in trade. Eggs, larvae and pupae may also be transported with soil attached to *Fragaria* plants.

Sampling and identification
Fragaria plants for planting potted into growing medium or with growing medium attached to the roots should be thoroughly inspected for the presence of soil-inhabiting larvae, pupae or adult weevils. There also may be eggs present on the lower parts of the plants or in the adhering soil. If appropriate, samples for identification of the pest in the laboratory should be taken. An EPPO Diagnostic protocol is not yet available.

6) *Scirtothrips dorsalis*

Symptom description
Scirtothrips dorsalis is not a pest specific to *Fragaria* plants but because of its highly polyphagous nature imported consignments may be contaminated by this organism. Feeding of the pest often results in considerable distortion of young leaves.

Sampling and identification
Since *Scirtothrips* spp. primarily infest young growing buds, these should be examined particularly careful using appropriate magnifying equipment. If appropriate, samples for identification of the pest in the laboratory should be taken.

Details on identification of *Scirtothrips dorsalis* are included in the EPPO Standard PM 7/56 (1) *Diagnostic protocol for regulated pests:* Scirtothrips aurantii, Scirtothrips citri, Scirtothrips dorsalis.

B) Nematodes

1) *Aphelenchoides besseyi*

Symptom description
On *Fragaria* plants *Aphelenchoides besseyi* is ectoparasitic, feeding on young tissue thus inducing leaf crinkling, leaf discoloration, distortion and dwarfing of the plants.

Sampling and identification
Isolation and identification of *A. besseyi* can be done from infested plant tissues (apex, leaves or stems). Details on identification of *A. besseyi* are included in the EPPO Standard PM 7/39 (1) *Diagnostic protocol for regulated pests:* Aphelenchoides besseyi.

2) *Aphelenchoides fragariae*

Symptom description
The symptoms caused by *Aphelenchoides fragariae* are stunting and deformation of the buds, leaves and flowers.

Sampling and identification
Isolation and identification of *A. fragariae* can be done from infested plant tissues (apex, or leaves). There is no diagnostic protocol for *A. fragariae* but some diagnostic information on this species is provided in the EPPO Standard PM 7/39 (1) *Diagnostic protocol for regulated pests:* Aphelenchoides besseyi in the section 'Confusion with similar species'.

3) *Ditylenchus dipsaci*

Symptom description
Ditylenchus dipsaci is a pest with a very broad host range (over 450 plant species), including *Fragaria* spp. It mostly can be found as an endoparasite in aerial parts of the plants (stems, leaves, flowers) but may also attack rhizomes. Common symptoms of infestation are swelling, distortion, discolouration and stunting of above-ground plant parts.

Sampling and identification
Samples for laboratory testing should be taken from plants showing conspicuous symptoms. Isolation and identification of *D. dipsaci* can be performed from infested plant tissues, mainly leaves or stems. An EPPO Diagnostic protocol is in preparation for *D. dipsaci*.

4) *Xiphinema americanum sensu stricto, X. bricolense, X. californicum* and *X. rivesi*

Symptom description
These nematodes have a very broad host range, including *Fragaria* spp. The direct damage induced to the roots of the plants is usually negligible, except for very high nematode populations when roots may show swellings close to the root tips. However, the main concern with these nematodes is their ability to transmit nepoviruses such as *Tomato ringspot nepovirus*.

Sampling and identification
In the case of imports of *Fragaria* plants for planting potted into growing medium or with growing medium attached to the roots, a sample of 500 to 1000 g of growing medium taken from a representative number of boxes or plants should be tested for the presence of soil-inhabiting nematodes. If nematodes of the genus *Longidorus* or *Xiphinema* are detected, it is recommended to test the plants for infections with nepoviruses. An EPPO Diagnostic protocol is in preparation for *Xiphinema* spp.

5) *Globodera pallida* and *G. rostochiensis*

Globodera pallida and *G. rostochiensis* are not pests of *Fragaria* sp. but some EPPO countries require that plants for

planting of *Fragaria* sp. should have been produced in a place of production free from these nematodes.

Sampling and identification
In the case of imports of *Fragaria* plants for planting potted into growing medium or with growing medium attached to the roots, a sample of 500 to 1000 g of growing medium taken from a representative number of boxes or plants should be tested for the presence of soil-inhabiting nematodes.

Details on the identification of *Globodera rostochiensis* and *G. pallida* are included in the EPPO Standard PM 7/40 (1) *Diagnostic protocol for regulated pests:* Globodera rostochiensis *and* G. pallida.

C) Fungi

1) *Glomerella acutata* (*Colletotrichum acutatum*)

Symptom description
Symptom expression of *G. acutata* is unlikely to be seen on imported consignments of *Fragaria* plants as the fungus is usually inactive in living vegetative tissues. Active infections, in the form of small, black, elongated and sunken lesions on petiols, leaf and flower stalks, or small irregular spots on leaves, are occasionally present but not always observed as they are typically inconspicuous or easily confused with damage caused by other factors. Thus, infections of consignments of *Fragaria* with *G. acutata* cannot be detected reliably by visual examination and it is therefore essential that those consignments be sampled for laboratory testing to detect any latent infection.

Sampling and identification
Details on the identification of *G. acutata* are included in the EPPO Standard PM 7/25 (1) *Diagnostic protocol for regulated pests:* Glomerella acutata. At least one plant should be sampled per 1000 plants with a minimum of 50 plants for small lots and a maximum of 300 plants taken from different parts of the lot.

2) *Phytophthora fragariae* var. *fragariae*

Symptom description
Fragaria plants are only attacked by *Phytophthora fragariae* var. *fragariae* which induces characteristic symptoms to the root system of affected plants. Lateral feeder roots are usually badly rotted and are commonly lost by the time plants are harvested. The adventitious roots rot from the tips upwards and often have a grey to brown appearance at their distal ends, giving the characteristic 'rat-tail' symptom. Cutting open the upper, white, unrotted parts of such roots reveal steles wine-red to brick-red in colour – hence the name red core. The colour can extend for quite long distances above the rotted parts of the roots, right into the crown in highly susceptible cultivars. As a consequence of the root damage, the upper parts of the plant often grow in a stunted manner, younger leaves can have a blue-green coloration and older leaves turn yellow or red.

Sampling and identification
The root systems of the plants should be examined for external symptoms of infection by *Phytophthora fragariae* var. *fragariae* i.e. the characteristic 'rat-tail'. At least 100 plants (except for small consignments) taken from different bundles (5 plants per bundle), including any plants with external symptoms, should be selected for examination of internal symptoms ('red-stele'). The upper, unrotted parts of the roots should be cut open and wine-red to brick-red coloration of the stele should be looked for. Whenever there is a doubt suspicious plants should be sent for confirmation to a laboratory. An EPPO Diagnostic protocol is in preparation for *Phytophthora fragariae* var. *fragariae*.

D) Prokaryotes

1) Strawberry witches' broom phytoplasma

Symptom description
Strawberry witches' broom phytoplasma is only known to infect *Fragaria* spp. Diseased plants are dwarfed, very bushy in appearance, and have numerous branched crowns with small leaves on erect spindly petioles.

Sampling and identification
Plants for planting of *Fragaria* spp. should be thoroughly inspected for the presence of conspicuous symptoms of possible infestation by phytoplasmas and suspicious plants should be sent to the laboratory. Since *Fragaria* plants in tissue culture may also be infested with strawberry witches' broom phytoplasma testing of a representative sample of plantlets taken from different vessels is also recommended. No EPPO Diagnostic protocol is available.

2) *Xanthomonas fragariae*

Symptom description
Visual symptoms on *Fragaria* plants for planting are mainly confined to leaves where 1–4 mm, angular, shiny, water-soaked spots appear surrounded by the smallest veins. In early stages of infection the spots are only visible on the lower surface and appear translucent against the light. After enlarging and coalescing of the spots they may be also visible on the upper surface as water-soaked, angular spots, which become reddish-brown in colour. These spots may be covered by bacterial exudate which when dry turns brown and appears as gum-like scales. In very severe cases crown infection pockets may be seen inside after dissection. They appear as localized, water-soaked zones, frequently confined to one side of the crown.

Sampling and identification
Thorough visual inspections on older leaves of green plants or on their remains still attached to the runners should be done looking for typical angular spots. However young plants and, in particular, 'frigo' plants or plants in tissue culture frequently do not show any symptoms of infections at this stage. Therefore,

samples, consisting of one plant for 1000 plants from different parts of the lot with a minimum of 50 plants for small lots and a maximum of 300 plants, should be taken for examination in the laboratory. Details on identification of *X. fragariae* are included in EPPO Standard PM 7/65 (1) *Diagnostic protocol for regulated pests:* Xanthomonas fragariae.

E) Viruses

1) Nepoviruses and sadwaviruses (*Arabis mosaic virus, Raspberry ringspot virus, Strawberry latent ringspot virus, Tomato black ring virus, Tomato ringspot virus*)

All nepoviruses relevant for *Fragaria* spp. and *Strawberry latent ringspot virus* (SLRSV) are readily transmitted by various nematode vectors of the genus *Xiphinema* or *Longidorus* as well as via pollen and seed. They can induce heavy damage to the crop in the field but may also occur in a latent stage, especially in young plants. Reliable visual inspection of consignments of *Fragaria* plants for planting is therefore not possible and laboratory testing is necessary in order to evaluate the presence of nepoviruses. Infection of the same plant with more than one nepovirus is not a rare event. These mixed infections may increase the damage induced to the respective plants.

Symptom description
Arabis mosaic virus (nepovirus) (ArMV) can induce heavy damage to strawberry crops rendering them worthless within one or two years of infection. The most common symptoms are leaf mottling, mosaic or flecking, various degrees of stunting and several forms of deformation including enations. The symptoms vary depending on cultivar, virus isolate, season and year. Many infections with ArMV are latent and the plants are asymptomatic.

Symptoms induced by *Raspberry ringspot virus* (nepovirus) (RpRSV) vary according to season and virus strain. In general, progressive dwarfing and ultimate death may be expected. There may also be irregularly-shaped, chlorotic blotches or local necrotic spots on the leaves. The virus causes few or no symptoms in the early stages of infection e.g. in young plants.

Strawberry latent ringspot virus (sadwavirus) can sometimes be found together with ArMV as both viruses share the same nematode vector, *Xiphinema diversicaudatum*. SLRSV usually causes latent infections in *Fragaria* plants but there are some cultivars showing varying degrees of mottling and decline.

Plants infected with *Tomato black ring virus* (nepovirus) (TBRV) usually show few or no symptoms especially in the year of infection. Nevertheless, plant growth and vigour in such plants may be impaired. TBRV may induce chlorotic mottling and/or ringspots in leaves of *Fragaria* plants.

There is also a broad variety of symptoms with *Tomato ringspot virus* (nepovirus) (ToRSV). Depending on the strawberry cultivar and the season, symptoms range from none to leaf mottling, dwarfing, reduction in runner production and to death of whorls of outer leaves and subsequent death of plants.

Sampling and identification
As outlined above visual inspection of young *Fragaria* plants, especially of those without leaves, is not feasible. Representative samples preferably of leaves, or of whole plants if necessary, of the different lots should be taken and subjected to laboratory testing. The same applies for imports of seeds from *Fragaria* spp. as there may be infections with these seed-transmissible nepoviruses. An EPPO Diagnostic protocol for regulated pests: *Tomato ringspot nepovirus* PM 7/49 (1) is available. Diagnostic protocols for the other nepoviruses are not available.

2) *Strawberry crinkle virus* (cytorhabdovirus)

Symptom description
The host range of *Strawberry crinkle virus* (cytorhabdovirus) (SCV) is confined to *Fragaria* spp. where it is one of the most damaging viruses. The symptoms vary considerably in relation to the virus strain and the strawberry cultivar. Mild strains are asymptomatic in all cultivars, whereas severe strains, in susceptible cultivars, cause distortion of the leaves, with leaflets unequal in size and small irregularly shaped chlorotic spots, often associated with the veins. Vigour and productivity of the plants is considerably reduced.

Sampling and identification
Visual inspection of young *Fragaria* plants, especially of those without leaves, is not feasible. Representative samples of the different lots should be taken and subjected to laboratory testing. This is especially recommended if the aphid vector *Chaetosiphon fragaefolii* was present on the plants. There is no EPPO Diagnostic protocol available.

3) *Strawberry mild yellow edge virus* (potexvirus)

Symptom description
Strawberry mild yellow edge disease is one of the most widespread virus diseases of cultivated *Fragaria* spp. Most of the cultivars grown today are tolerant to infection with this virus. Sensitive cultivars may develop dwarfing, marginal chlorosis, leaf distortion and small fruit. It is quite common for this virus to occur together with other viruses, like *Strawberry mottle virus*, *Strawberry crinkle virus (*cytorhabdovirus) or *Strawberry vein banding virus* (caulimovirus). These virus complexes can cause severe loss of plant vigour, yield and fruit quality and lead to a decline of the plants.

Sampling and identification
Visual inspection of young *Fragaria* plants, especially of those without leaves, is not feasible. Representative samples of the different lots should be taken and subjected to laboratory testing. This is especially recommended if the aphid vectors of the genus *Chaetosiphon* were present on the plants. There is no EPPO Diagnostic protocol available.

4) *Strawberry vein banding virus* (caulimovirus)

Symptom description
The host range of *Strawberry vein banding virus* (caulimovirus) (SVBV) is restricted to *Fragaria* spp. with symptoms mostly induced on *F. vesca*. Symptoms on *F. vesca* initially appear on the youngest developing leaf. There may be an epinasty of midribs and petiols, a tendency for opposite half leaves to be appressed, irregularly wavy leaflet margins, and slight crinkling of the laminae. Usually these symptoms are mild and not all present simultaneously. On expanded leaves clearing and yellowish banding of some or all of the veins may be visible. The coloration often occurs in scattered discontinuous streaks of variable lengths along the main and secondary veins. Only some leaves of the plant may show symptoms. On commercial strawberries there are usually no characteristic symptoms which allow diagnosis of the virus, but this may be influenced by the cultivar and by potential co-infections with other viruses.

Sampling and identification
As with the other viruses, visual inspection of young *Fragaria* plants, especially of those without leaves, is not feasible. Representative samples of the different lots should be taken and subjected to laboratory testing. This is recommended especially where aphid vectors of the genus *Chaetosiphon* were present on the plants. An EPPO Diagnostic protocol for *Strawberry vein banding virus* (caulimovirus) is not yet available.

6. Other viruses

Other viruses have been described on strawberry and some EPPO member countries require that *Fragaria* plants for planting should be free from non-European viruses. Consequently inspectors should take additional care when inspecting *Fragaria* plants.

Appendix 2

Short procedure for inspection of imported consignments of *Fragaria* plants for planting to be used by the inspector

This short procedure includes the main elements for practical work of an inspector when carrying out phytosanitary inspections of consignments of *Fragaria* plants for planting at the point of entry or at the place of final destination. It is supposed that documentary checks and identity checks are already accomplished. A general outline of the inspection procedure is included in Fig. 1.
1) A decision should be made whether the consignment may be inspected at the point of entry or at the place of final destination
2) Lots to be inspected should be identified, preferably based on cultivar, origin, producer or category
3) The appropriate number of individual plants to be inspected per lot should be selected taking into account statistical requirements as much as possible.

Plants in tissue culture (microplants)

Phytosanitary inspections should be done at the place of final destination.

Visual inspection
– an overall examination of the physical condition of the plants in tissue culture should be carried out but visual inspection to detect specific pests is not reliable.

Sampling for laboratory testing
– a representative sample of plantlets should be taken under sterile conditions from several vessels and subjected to laboratory testing for possible infestations with *Glomerella acutata*, *Phytophthora fragariae* var. *fragariae*, *Xanthomonas fragariae*, phytoplasmas or viruses.

'Frigo' plants (usually rooted and without leaves)

Phytosanitary inspections may be carried out at the point of entry or the place of final destination.

Visual inspection
– examination of the aerial parts of the plants for insects and mites in any stage
– plants should be shaken over sheets of white paper and small insects that fall onto the paper should be collected for further identification
– if there are any leaves, attention should be paid to signs of infestation with *Aphelenchoides* spp., *Ditylenchus* spp., *Xanthomonas fragariae*, *Glomerella acutata* or viruses
– examination of roots for symptoms of *Phytophthora* spp. and for signs or symptoms of nematodes.

Sampling for laboratory testing
– if suspicious symptoms or signs are detected, samples should be taken and subjected to laboratory testing in order to properly identify the pest and distinguish it from species already present in the country
– samples of 1 per 1000 plants with a minimum of 50 plants (small lots) and a maximum of 300 randomly-collected asymptomatic plants per lot should be taken for laboratory testing in order to detect hidden or latent infestations with harmful organisms like *Glomerella acutata*, *Phytophthora fragariae* var. *fragariae*, *Xanthomonas fragariae*, phytoplasmas or viruses.

Green plants (usually rooted and with leaves)

Phytosanitary inspections usually carried out at the point of entry.

Visual inspection
– examination of leaves (top and bottom side) and the crown of the plants for insects and mites in any stage and for signs or symptoms of nematodes

– plants should be shaken over sheets of white paper and small insects that fall onto the paper should be collected for further identification
– examination of core leaves for symptoms of *Aphelenchoides* spp.
– examination of older leaves for symptoms of *Xanthomonas fragariae*
– examination of older leaves and petioles for symptoms of *Glomerella acutata* and *Ditylenchus* spp.
– examination of roots for symptoms of *Phytophthora* spp.
– examination of the plants for suspicious symptoms of viral or phytoplasma infections
– if appropriate, examination of soil for larvae, pupae or adults of insect pests.

Sampling for laboratory testing
– if suspicious symptoms or signs are detected, samples should be taken and subjected to laboratory testing in order to properly identify the pest and distinguish it from species already present in the country
– samples of 1 per 1000 plants with a minimum of 50 plants (small lots) and a maximum of 300 randomly-collected asymptomatic plants per lot should be taken for laboratory testing in order to detect hidden or latent infestations with harmful organisms like *Glomerella acutata*, *Phytophthora fragariae* var. *fragariae*, *Xanthomonas fragariae*, phytoplasmas or viruses
– if plants are traded in growing medium or growing medium is adhering to the roots, a sample of 500 to 1000 g of growing medium should be taken and tested for the presence of nematode vectors of nepoviruses (*Longidorus* spp. or *Xiphinema* spp.) and for *Globodera pallida* and *G. rostochiensis*.

Seeds of *Fragaria* spp.

Phytosanitary inspections may be carried out at the point of entry but should preferably be done at the place of final destination.

Visual inspection
– visual inspection to detect specific pests is not suitable.

Sampling for laboratory testing
– a representative sample of seeds should be taken and tested for the presence of seed-transmissible nepoviruses
– alternatively, seedlings grown from the imported seed lots could be tested for the respective viruses

4) All consignments should remain under official control until the visual inspections are finalized and the results of the laboratory tests are available

5) According to the final outcome of the phytosanitary inspections the consignments may be released or appropriate phytosanitary measures applied.

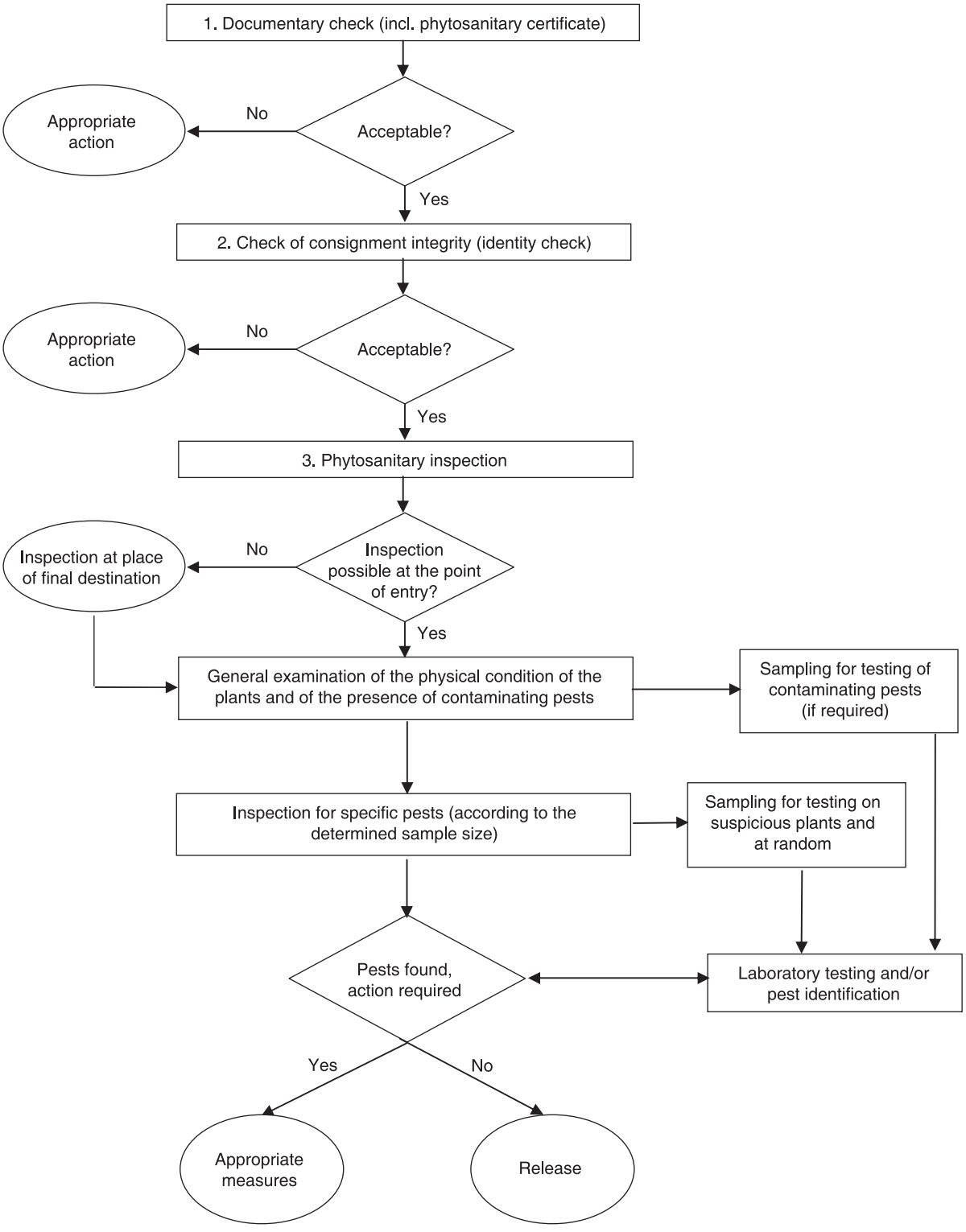

Fig. 1 General import inspection procedure for consignments of *Fragaria* plants for planting.

Corrigendum

EPPO would like to update the reference in the following standards:

EPPO Standard PM 3/70 (1) Export certification and import compliance checking for potato tubers

In the section ''Inspection procedure in importing countries'', 4th paragraph, the sentence ''*For further guidance on sampling*, *see EPPO Standard PM 3/66* 'Sampling for visual inspection of consignments' is replaced by ''*For further guidance on sampling*, *see ISPM no. 31* Methodologies for sampling of consignments (FAO, 2008).''

The following reference should be added to the reference list:

FAO (2008) ISPM No. 31, *Methodologies for sampling of consignments*, IPPC Secretariat, Rome (IT).

EPPO Standard PM 3/71 (1) General crop inspection procedure for potatoes

In the section ''1.4 Inspection of harvested tubers (including tubers shortly before harvest and tubers presented for marketing)'', 1st paragraph, the sentence ''*For further guidance on sampling*, *see EPPO Standard PM 3/66* Sampling of consignment for visual phytosanitary inspection *and Battilani* et al. (2005)'' is replaced by ''*For further guidance on sampling*, *see ISPM no. 31* Methodologies for sampling of consignments *and Battilani* et al. (2005)''

The following reference should be added to the reference list:

FAO (2008) ISPM No. 31, *Methodologies for sampling of consignments*, IPPC Secretariat, Rome (IT).

EPPO Standard PM 3/73 (1) Consignment inspection of *Fragaria* plants for planting

In the section ''Sampling for visual inspection and laboratory testing'', subsection ''Sampling for visual inspection (general aspects)'', 3rd paragraph the sentence ''*The size of the unit of inspection* (= *minimum number of individuals to be examined*) *should be determined on the basis of lots taking into account the statistical background provided in EPPO Standard PM 3/65* Sampling of consignments for visual phytosanitary inspection'' is replaced by ''*The size of the unit of inspection* (= *minimum number of individuals to be examined*) *should be determined on the basis of lots taking into account the statistical background provided in ISPM no. 31* Methodologies for sampling of consignments.''

In the 4th paragraph of the same section and subsection the last sentence ''*Sample sizes are indicated in Table 2*(*b*) *of EPPO Standard PM 3/65 Sampling of consignments for visual phytosanitary inspection*.'' Is replaced by ''*Sample sizes are indicated in Table 1 of ISPM no. 31* Methodologies for sampling of consignments.''

The following reference should be added to the reference list:

FAO (2008) ISPM No. 31, *Methodologies for sampling of consignments*, IPPC Secretariat, Rome (IT).

References

OEPP/EPPO (2006) Export certification and import compliance checking for potato tubers. *Bulletin OEPP/EPPO Bulletin* **36**, 423–424.
OEPP/EPPO (2007) General crop inspection procedure for potatoes. *Bulletin OEPP/EPPO Bulletin* **37**, 592–597.
OEPP/EPPO (2008) Consignment inspection of *Fragaria* plants for planting. *Bulletin OEPP/EPPO Bulletin* **38**, 396–406.

