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Phytosanitary procedures
Procedures phytosanitaires

PM 3/83 (1) *Fragaria* plants for planting – inspection of places of production

Specific scope

This Standard describes the procedure for inspection of places of production of *Fragaria* plants for planting.

The Standard includes relevant sampling procedures. The Standard focuses on pests of concern for the EPPO region which are present in the EPPO region. The purposes of a place of production inspection may be for export or for internal 'within country' use. Alternatively, inspection may be carried out as part of a national survey for monitoring or to determine country or area freedom for specified pests. The Standard also provides guidance which may be relevant to exports to non-EPPO countries, in which case the pest lists and requirements for the importing country should be consulted.

A procedure for inspecting imported plants from outside the EPPO region is covered in EPPO Standard PM 3/73 *Consignment inspection of* Fragaria *plants for planting* (EPPO, 2008).

Specific approval

This Standard was first approved in 2017-09.

Introduction

Fragaria spp. comprise an important fruit crop in the EPPO region, for which plants for planting are mainly largely produced in the EPPO region. *Fragaria* plants for planting represent an important pathway for the entry and spread of pests, since the plants are propagated vegetatively and infested *Fragaria* plants for planting from a single lot can be planted in many different locations. There is a risk of introduction of pests into the propagating system through infested mother plants. In the field, an outbreak can be further spread through activities such as cutting off runners, removing flowers, physical contact between different lots and spread by vectors. Delivering infested plants for planting to fruit producers may result in the spread and establishment of pests over a wide area. It is thus very important to ensure that only healthy plants for planting are placed on the market.

Place of production freedom or crop freedom for specified pests is a frequent requirement in phytosanitary legislation for *Fragaria* plants for planting.

Phytosanitary inspections

General background information on phytosanitary inspection of places of production is given in EPPO Standard PM 3/72 *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009) and in ISPM 23 *Guidelines for inspection* (FAO, 2005).

The general elements of the procedure may also be useful to member countries when they perform inspection of places of production for commodities exported to other countries.

Visual inspection also serves to detect organisms for which the phytosanitary risk has not yet been determined. When an unfamiliar pest or a pest from the EPPO Alert List is detected, the procedures specified in EPPO Standard PM 5/2 *Pest risk analysis on detection of a pest in an imported consignment* (EPPO, 2002) should be followed to allow the NPPO to make a decision as to what phytosanitary action to take.

For an indication of the status of these pests, consult the latest version of the PQR (Plant Quarantine Retrieval System, http://www.eppo.int/DATABASES/pqr/pqr.htm) or the EPPO Global Database (https://gd.eppo.int/) (EPPO, 2017a).

Visual inspection should be done at the most appropriate time of year. This is spring to early summer (mother plants) and autumn (plants for planting on a waiting bed[1], in trays).

[1]Waiting bed = a temporary planting site for growing plants before cold store or dispatch.

Visual examination of *Fragaria* plants for planting alone is not considered to be sufficient for pests which may be present in a latent stage and/or are difficult to detect. Where the risk of latent infection being present is high or a high degree of assurance is required, laboratory testing should be carried out to provide additional evidence of freedom from pests. This is particularly applicable for viruses, bacterial infections and nematodes, which can remain undetected due to a lack of symptoms, especially when infection levels are low.

Phytosanitary inspection should start with an overall examination of the place of production in order to check the physical condition of the plants. If there is an abnormal die-off in a place or lot, or there are other anomalies within the crop (e.g. abnormal growth, differences in colour, spots on leaves), these lots/places should be checked with specific attention. If no symptoms are seen, a systematic inspection of the field should be made, each lot being examined as a separate unit. Large lots should be walked in a 'W' transect.

Within this Standard a lot should be defined as a number of plants of the same type (e.g. garden strawberry, pineberry, strasberry; see Figs 1–3) and variety (e.g. Elsanta, Dar Select) from the same origin and planted at the same time.

It is also recommended to inspect plants in the vicinity of the place of production, for example weeds of host plants. Inspection of the discarded plant waste heap may give an indication of the diseases present.

An adequate proportion of plants should be subjected to a systematic examination in order to detect the presence or signs of pests. If appropriate, samples should be taken to the laboratory for identification.

The size of the unit of inspection, or sample (the minimum number of individual plants to be examined), should be determined on the basis of lots undergoing inspection, taking into account the statistical background provided in ISPM 31 *Methodologies for sampling of consignments* (FAO, 2008). Inspection of a sample of 4600 plants selected at random provides at least 99% confidence of detecting a level of infection present in 0.1% of plants, where symptoms are evident (assuming 100% efficacy of detection).

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

Trapping for the monitoring of pests such as thrips, moths and beetles is also an important supplement to visual inspection. Specific details are listed in Appendix 1.

Inspections and sampling can themselves be a pathway for spreading infestations. Therefore, inspectors should take all necessary precautions during inspection and sampling, such as wearing appropriate protective clothing – coat, overshoes, gloves etc. Gloves must be changed between different lots. All sampling equipment used must be disinfected between different lots.

Fig. 1 Garden strawberry. Photo: Hoogstraten (BE).

Fig. 2 Pineberry. Photo: Hoogstraten (BE).

Fig. 3 Strasberry. Photo: Hoogstraten (BE).

Production sector concerned

The production of *Fragaria* plants generally takes place in specialized plant-propagating nurseries for sale to strawberry fruit producers (professionals and private persons), and plants are also produced by strawberry fruit producers for their own use. The plants are grown from mother plants usually brought in by the nurseries or producers. The mother plants derive from nuclear stock plants that have been tested and found to be free from different pathogens, especially viruses, and are produced under conditions that minimize infestation by other pests (aphid-free glasshouses). More information on the reproduction of *Fragaria* plants is given in EPPO Standard PM 4/11 *Certification scheme for strawberry* (EPPO, 2008b).

Propagation of *Fragaria* plants for planting is done by planting the mother plants in the propagation fields, mainly in spring. During late spring fruiting is prevented by removing the flowers; this results in the faster development of runners. In late summer (or earlier in southern EPPO countries) runners are transplanted into rows, beds or trays. During winter, when the plants are dormant, the runners are generally kept in cold storage (−1°C), before planting into the field for fruit production.

Pests of concern for the EPPO region

This Standard mainly relates to pests in the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests, which are present in the EPPO region. The pests covered in this Standard are recognized as of primary importance for *Fragaria* plants for planting (Table 1). The phytosanitary procedures described in this Standard are primarily aimed at preventing the spread of these specific pests in the EPPO region through trade in *Fragaria* plants for planting. This Standard also covers polyphagous quarantine pests which have *Fragaria* plants as economically relevant hosts and which may be introduced as contaminants (Table 2). For plants in growing medium, attention should be paid to nematodes which may act as virus vectors.

Details on all of these pests can be found in *Quarantine Pests for Europe* (2nd edition; EPPO/CABI, 1997) and in EPPO Datasheets. For additional up-to-date information the relevant scientific literature should be consulted, as well as the PQR and the EPPO Global Database.

The EPPO Lists of A1 and A2 pests are subject to additions and deletions. The present list needs to be revised whenever relevant new quarantine pests are identified.

Sampling for laboratory testing

Samples should be taken from plants on which pests, or signs of them, are present that cannot be immediately identified by the inspector, and from plants showing suspicious symptoms or deformations. In these cases the sample consists of the suspect plant(s).

Table 1. Specific pests of *Fragaria*

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects *Anthonomus bisignifer*		Insects *Chaetosiphon fragaefolii*
	Bacteria and phytoplasmas *Xanthomonas fragariae*	
	Fungi and fungi like organisms* *Phytophthora fragariae* var. *fragariae*	
	Viruses and viroids *Strawberry vein banding virus*	Viruses and viroids *Strawberry crinkle virus*, *Strawberry mild yellow edge virus*, *Strawberry mottle virus*

**Phytophthora fragariae* var. *fragariae* belongs to the Kingdom Chromista.

Table 2. Polyphagous pests

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects *Naupactus leucoloma*	Insects *Frankliniella occidentalis*, *Popillia japonica*, *Scirtothrips dorsalis*	Insects *Epiphyas postvittana*
	Bacteria and phytoplasmas *Phytoplasma solani*	
		Fungi *Colletotrichum acutatum*
	Nematodes *Aphelenchoides besseyi* *Ditylenchus dipsaci* *Meloidogyne fallax*	*Aphelenchoides fragariae* *Aphelenchoides ritzemabosi*
	Viruses and viroids *Raspberry ringspot virus* *Tomato ringspot virus*	Viruses and viroids *Apple mosaic virus* *Arabis mosaic virus* *Strawberry latent ringspot virus* *Tomato black ring virus*

The size of the sample to be taken depends on the potential distribution of the pests within the lot and on the method selected for diagnosis in the laboratory. The analytical sensitivity of each test to be used should be known before sampling commences so that a single known infested item should be detected even when mixed with several uninfested items. In this way, the maximum number of items (e.g. leaves) can be included in each sample (which will increase the efficiency and economy of testing) without reducing sensitivity.

Sampling should normally be done on a lot basis with plants evenly collected throughout the lot. In general, since

the plant parts most suitable for detection differ greatly for different pests, samples for laboratory testing should contain the complete plants in order to give the possibility of testing for the whole range of potential pests.

Nevertheless, in some cases only parts of plants (e.g. leaves) can be taken instead of the whole plant. Appendix 1 specifies the plant part to be sampled for the relevant pests.

Sampling plans should be formulated to determine the frequency of sample submission for laboratory testing.

Each sample should be individually labelled to ensure a link is made to the nursery name (or reference number), place of sampling (parcel, place within the lot, plan ...), sample number, date, plant species, plant variety, if relevant, and lot number, symptoms seen in the field and suspected harmful organism, so follow-up action can be taken if necessary.

In order to identify the pest, sampled material such as plants and plant parts should be kept in good condition and placed in plastic bags together with a piece of absorbent paper. If the plant parts are dry, a piece of slightly damp absorbent paper should be added; for wet plant parts a piece of dry absorbent paper should be added (to avoid rotting of the plant parts). Plants with roots in potting compost, substrate, etc. do not easily dry out and as a consequence absorbent paper is not needed.

Samples of adult insects, larvae, pupae and eggs should be put in a secure pot with a screw cap and appropriate additional containment. Living organisms should be sent to the laboratory together with plant material of the host plant in a suitable container. Dead organisms should be kept in alcohol in order to prevent decomposition during transport.

If a pest found during inspection is suspected by the inspector to be a quarantine pest, the suspect lot should be detained under official control pending a test result. All other lots potentially at risk of infestation and lots which are related to the suspect lot (e.g. the same mother plant, contact by means of manipulation of the plants, contact by irrigation etc.) should also be detained under official control.

For further details on symptoms, sampling and identification of the relevant pests of *Fragaria* plants for planting, see Appendix 1.

Acknowledgements

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Appendix – Symptoms and sampling for the identification of quarantine pests of *Fragaria* plants for planting

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), as well as in EPPO Datasheets and EPPO Diagnostic Standards. Illustrations are available on the EPPO website (http://www.eppo.int or https://gd.eppo.int/). When an EPPO Diagnostic Standard exists it is mentioned in the text. The fact that there is no EPPO Diagnostic Standard does not mean that no method for diagnosis is available in the scientific literature. Information on the current distribution of relevant pests and photographs of symptoms, where available, can be found on the EPPO Global Database (https://gd.eppo.int/).

Fig. 4 *Anthonomus bisignifer*. Photo: Pest and Diseases Image Library, Bugwood.org.

a) Insects

1) *Anthonomus bisignifer* (Japanese strawberry weevil) (EPPO A1 List)

Symptom description

The most obvious symptoms of damage are partially severed buds hanging from the plants and severed buds on the ground. The adult weevil is dark brown and 2.5–4 mm long (Fig. 4).

Sampling and identification

Look for adults and inspect the severed buds; they may contain eggs, larvae or pupae of the pest. Where appropriate, samples for laboratory testing should be taken for final identification of the pest.

2) *Chaetosiphon fragaefolii* (strawberry aphid) (Norway quarantine pest)

Symptom description

Adult of this species are translucent yellow white to pale greenish in colour with an antenna 0.9–1.1 times the length of the body (Fig. 5). The body is covered with conspicuous capitate hairs. The life cycle of the strawberry aphid includes overwintering eggs, nymphs and adult apterae (wingless), and alatae. Eggs are white-yellowish in colour when deposited, but hours later they become shiny and black. Nymphs are small (0.8–1.1 mm long) and morphologically similar to the adults. Nymphs vary in colour from light green to pale yellow. The pre-reproductive period averages less than 13 days at 25°C.

Fig. 5 *Chaetosiphon fragaefolii*. Photo: Jeffrey W. Lotz, Bugwood.org.

Sampling and identification
Look for adults and damage by larvae. Typical symptoms of aphid damage include curled leaves, yellowish spots and the presence of sticky honeydew excreted by the aphid. A black sooty mould may develop on the leaves, affecting photosynthesis and possibly reducing plant yields.

3) *Epiphyas postvittana* (leaf roller moth) (Jordan quarantine pest)

Symptom description
The adults are variable in colour and may be confused with other leaf roller moths and similar species (Fig. 6). Males are either uniformly light brown or have a forewing with a light brown area at the base, which is distinguishable from a much darker, red-brown area at the tip. Females have only slightly darker oblique markings distinguishing the area at the tip of the wing.

Eggs are laid in clusters on the leaves. Larvae damage leaves. Early instar larvae often spin a finely webbed protective cover for feeding, or a leaf roll, on the underside of nearby leaves. The late-stage larvae feed on all leaf tissue except the main veins. The casing of the pupae is often found within a leaf roll, or a silken cocoon spun and woven between two leaves.

Sampling and identification
Look for adults and damage by larvae. For the detection and monitoring of *E. postvittana*, moth traps with specific pheromones can be used. Where appropriate, samples for laboratory testing should be taken for final identification of the pest.

4) *Frankliniella occidentalis* (alfalfa thrips) (EPPO A2 List)

Symptom description
The major symptoms of *F. occidentalis* infestation include a discoloration of the upper leaf surface, speckling and halo spotting on leaves and discoloration and scarring of open blooms and petals.

Sampling and identification
Look for adults and check the upper leaf surface for discoloration. During inspection of plant material for the presence of *F. occidentalis*, aerial parts of plants should be shaken over sheets of white paper. Thrips and other small insects present on the surface of plants, and especially in flower blossoms, fall onto the paper, where they can be collected with small brush-pencils and put in a tube with 10% alcohol plus wetting liquid or by an insect aspirator ('pooter') (EPPO, 2016). Blue sticky traps can be used for the detection and monitoring of *F. occidentalis*. These traps should be placed 20–30 cm above the crop. Morphological identification may be limited for insects collected from sticky traps. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. Details on the identification of *F. occidentalis* are included in the EPPO Standard PM 7/11 Frankliniella occidentalis. Figure 7 shows an image of a male and 2 females of *F. occidentalis.*

Fig. 7 *Frankliniella occidentalis* adults. Photo: EPPO Global database.

Fig. 6 *Epiphyas postvittana*. Photo: Department of Primary Industries and Water, Tasmania, Bugwood.org.

5) *Naupactus leucoloma* (white-fringed beetle) (EPPO A1 List)

Symptom description

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. Root feeding extends from the soil surface to a depth of about 12 cm. When feeding is severe, plants turn yellow, wilt and die. Eggs, larvae and pupae may also be transported with the soil attached to *Fragaria* plants. Eggs are laid in clusters and may be present on the lower parts of the plants or in the adhering soil. Adults cannot fly but they actively crawl and climb.

Sampling and identification

Inspect the roots for damage by larvae. *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. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. Figure 8 shows an image of an adult *N. leucoloma.*

Fig. 8 *Naupactus leucoloma* adult. Photo: Lesley Ingram, Bugwood.org.

6) *Popillia japonica* (Japanese beetle) (EPPO A2 List)

Symptom description

Symptoms caused by adults of *P. japonica* are easily recognizable (defoliation). The beetle skeletonizes the leaves by chewing out the tissue between the veins and leaving a vein skeleton (Figs 9 and 10). Leaves may turn brown and fall. On flower petals, the beetle consumes large and irregularly shaped parts. The larvae simply cause feeding damage to the roots of host plants.

Sampling and identification

Adults can be detected by visual examination of green parts of plants and larvae by visual examination of roots in soil. Larvae may be transported in soil around the roots of plants for planting (see Fig. 11). For the detection and monitoring

Fig. 9 Adult *Popillia japonica* feeding. Photo: Matteo Maspero, Centro MiRT – Fondazione Minoprio (IT).

Fig. 10 Adult of *Popillia japonica*. Photo: Matteo Maspero, Centro MiRT – Fondazione Minoprio (IT).

Fig. 11 Larvae of *Popillia japonica*. Photo: Japanese Beetle Research Laboratory, USDA (US).

of *P. japonica*, traps containing food-type lures and/or sex attractants can be used. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. Details on the identification of *P. japonica* are included in EPPO Standard PM 7/74 (1) Popillia japonica (EPPO, 2006a).

7) *Scirtothrips dorsalis* (Assam thrips) (EPPO A2 List)

Symptom description
Feeding of *S. dorsalis* often results in considerable distortion of young leaves. They do not feed on mature leaves.

Sampling and identification
Since *Scirtothrips* spp. primarily infest young leaves and growing buds, these should be examined particularly carefully using appropriate magnifying equipment. During inspection of plant material for the presence of *Scirtothrips* spp., aerial parts of plants should be shaken over sheets of white paper. Thrips and other small insects present on the surface of plants fall onto the paper, where they can be collected with small brush-pencils and put in a tube with 10% alcohol plus wetting liquid. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. Details on the identification of *S. dorsalis* are included in EPPO Standard PM 7/56 (1) Scirtothrips aurantii, Scirtothrips citri, Scirtothrips dorsalis (EPPO, 2005a). Figure 12 shows an image of *S. dorsalis* larvae.

Fig. 12 *Scirtothrips dorsalis* larvae. Photo: Kevin Ong, Texas AgriLife Extension Service, Bugwood.org.

b) Bacteria and phytoplasmas

1) *Phytoplasma solani* (black wood of grapevine) (EPPO A2 List)

Symptom description
'*Candidatus* Phytoplasma solani' is naturally dispersed by its leafhopper vectors, such as *Hyalesthes obsoletus*. Affected strawberry plants show conspicuous stunting and poor root systems. Older leaves show upward rolling and a marked purple discoloration, while young leaves are cupped, chlorotic and generally reduced in size with shortened petioles (EPPO 2008c).

Sampling and identification
Plants, especially the leaves, should be inspected for symptoms. Attention should be paid to the presence of the leafhopper vectors. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for the final identification of the pest.

2) *Xanthomonas fragariae* (angular leaf spot) (EPPO A2 List)

Symptom description
Small (1–4 mm) angular water-soaked spots appear initially only on the lower leaf surface in between the smallest veins. In the early stage, the spots are only visible on the lower surface and appear translucent when viewed with transmitted light (see Fig. 13). They enlarge, coalesce and after about 2 weeks are also visible on the upper surface as water-soaked, angular spots, which become reddish-brown in colour (see Fig. 14). The bacteria are disseminated from the spots by irrigation, rain or dew to initiate new infections, frequently along the main veins of the leaf. With high relative humidity, white, milky, cream-coloured or yellow exudates can appear, which when dry turn brown and appear as parchment-like scales. Dead tissues appear as reddish-brown irregular spots; these tear and break off, causing holes in the leaves (Fig. 15).

Symptoms of angular leaf spot caused by *X. fragariae* may be confused with those caused by fungi such as

Fig. 13 Symptoms of *Xanthomonas fragariae* showing angular water-soaked spots on lower leaf surface. Photo: Elphinstone.

Fig. 14 Symptoms of Xanthomonas fragariae showing advanced lesions on upper leaf surface. Photo: P. Llop, IVIA (ES).

Fig. 15 Leaf severely affected by *Xanthomonas fragariae*, ragged appearance and coalescence of the spots along the main veins. Photo: U. Mazzucchi, Università di Bologna (IT).

Mycosphaerella fragariae and with the symptoms caused by *Xanthomonas arboricola* pv. *fragariae*.

Sampling and identification

Particularly inspect the lower surface of 2-week-old to 2-month-old leaves. For a good observation the leaves should be viewed with transmitted light; the pattern of the water-soaked spots then looks like a stained-glass window. Where appropriate, samples for laboratory testing (consisting of leaves, including leafstalks) should be taken for final identification of the pest. Details on the identification of *X. fragariae* are included in EPPO Standard PM 7/65 (1) Xanthomonas fragariae (EPPO, 2006b).

c) Fungi and fungi like organisms

1) *Colletotrichum acutatum* (anthracnose of strawberry) (Israel quarantine pest)

Symptom description

Affected strawberry plants shows small, black, elongated and sunken lesions on petioles, leaf and flower stalks, or small irregular spots on leaves. Conidia are normally dispersed by water splash. They may lie dormant in the soil for some time, often overwintering in this fashion. Survival is longest under relatively cool, dry conditions. The fungus can also remain viable for long periods in dead plant material on the surface or buried in the soil.

Sampling and identification

Inspect the leaves for irregular spots and the leaf stalks for sunken lesions (Figs 16 and 17). Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification of the pest.

Fig. 16 *Colletotrichum acutatum* (other scientific name *Glomerella acutata*): lesions on strawberry stolons. Photo: Central Science Laboratory, Harpenden (GB), British Crown.

Fig. 17 *Colletotrichum acutatum* (other scientific name *Glomerella acutata*): lesions in strawberry crown. Photo: Central Science Laboratory, Harpenden (GB), British Crown.

1) *Phytophthora fragariae* var. *fragariae* (red core of strawberry) (EPPO A2 List)

Symptom description

Phytophthora fragariae var. *fragariae* induces characteristic symptoms in the root system of affected plants. Lateral feeder roots are usually badly rotted and are commonly lost by the time plants are harvested (Figs 18–20). 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.

Disease outbreaks often start from small foci of infected strawberry plants. They increase in size, especially down slopes where spread in water can lead quickly to large areas being affected. Symptoms can be apparent on the roots from late autumn onwards but generally do not become noticeable on the above-ground parts of the plants until late spring or early summer.

Fig. 18 Healthy root system of young strawberry runner, showing normal lateral root development. Photo: SCRI, Dundee (GB).

Fig. 19 Strawberry runner root system infected by *P. fragariae* var. *fragariae*, showing typical rat's tail appearance. Photo: SCRI, Dundee (GB).

Fig. 20 Longitudinal section of roots of strawberry plants showing typical red-core symptoms. Photo: SCRI, Dundee (GB).

Phytophthora fragariae var. fragariae can spread in surface or drainage water (caution must be exercised when irrigating crops) and can also be moved in soil on implements and machinery. However, the most important means of spread is in planting material.

More information on the inspection of *P. fragariae* is given in EPPO Standard PM 3/22 Phytophthora fragariae*: inspection* (EPPO, 2013a).

Sampling and identification

A general inspection of the field should be made looking for patches of uneven growth, especially in lower-lying or wet areas of the field. Plants that appear unhealthy should be lifted and their roots examined. The lifted plants should either be mother plants or well-established runners. The root systems of the plants should be examined for external symptoms of infection, i.e. the characteristic 'rat-tail'. At least 100 plants per lot should be selected for examination of internal symptoms ('red-stele') [see EPPO (2008a) PM 3/73 Consignment inspection of Fragaria plants for planting]. For larger lots refer to ISPM 31. 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. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification of the pest.

d) Nematodes

1) *Aphelenchoides besseyi* (strawberry crimp disease nematode) (EPPO A2 List)

Symptom description

On *Fragaria* plants, *A. besseyi* is ectoparasitic, feeding on young tissue. The nematode is the causal agent of summer dwarf or crimp disease. Symptoms include leaf crinkling and distortion, and dwarfing of the plant with an associated reduction in flowering. More information on a treatment for *A. besseyi* in strawberry plants is included in EPPO (1993)

Fig. 21 *Aphelenchoides besseyi*: leaf crinkling and distortion. Photo: Ministry of Agriculture (HU).

Standard PM 3/52 Aphelenchoides besseyi – *Treatment method for strawberry plants* and EPPO Standard PM 10/19 (1) *Hot water treatment of strawberry plants to control* Aphelenchoides besseyi *and* Aphelenchoides fragariae (EPPO, 2012).

Sampling and identification

Inspect the leaves for crinkling and distortion and the plants for dwarfing (Figs 21 and 22). Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification of the pest (see 'Phytosanitary Inspections' section). Details on the identification of *A. besseyi* are included in EPPO Standard PM 7/39 Aphelenchoides besseyi (EPPO, 2004). Details on nematode extractions are included in EPPO Standard PM 7/119 *Nematode extraction* (EPPO, 2013b).

Fig. 22 *Apelenchoides besseyi* causing dwarfing in Fragaria plants. Photo: J. McCulloch, Queensland Department of Primary Industries, Indooroopilly (AU).

2) *Aphelenchoides fragariae* (Turkey A2 List, Jordan quarantine pest)

Symptom description

Aphelenchoides fragariae lives and feeds mainly in runner buds and growing points but also in leaves, causing stunting and deformation of leaves, shoots and flowers (Fig. 23). Typical leaf symptoms are discoloration between the veins. More information on a treatment for *A. fragariae* in strawberry plants is included in EPPO Standard PM 10/19 (1) *Hot water treatment of strawberry plants to control* Aphelenchoides besseyi *and* Aphelenchoides fragariae.

Fig. 23 Leaf damage caused by *Apelenchoides fragariae* Photo: Penn State Department of Plant Pathology and Environmental Microbiology Archives, Penn State University, Bugwood.org.

Sampling and identification
Inspect the leaves and buds for stunting, deformation and discoloration between the veins. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification of the pest (see 'Phytosanitary Inspections' section). Some diagnostic information on this species is provided in EPPO Standard PM 7/39 (1) Aphelenchoides besseyi. In addition, there is an IPPC protocol including the species (IPPC, 2016). Details on nematode extractions are included in EPPO Standard PM 7/119 (1) *Nematode extraction* (EPPO, 2013b).

3) *Aphelenchoides ritzemabosi* (chrysanthemum eelworm) (Israel quarantine pest)

Symptom description
Aphelenchoides ritzemabosi lives and feed mainly in runner buds and growing points but also in leaves, causing stunting and deformation of leaves, shoots and flowers. A typical leaf symptom is discoloration between the veins.

Sampling and identification
Inspect the leaves and buds for stunting, deformation and discoloration between the veins. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification of the pest (see 'Phytosanitary Inspections' section). Some diagnostic information on this species is provided in EPPO Standard PM 7/39 Aphelenchoides besseyi. Details on nematode extractions are included in EPPO Standard PM 7/119 *Nematode extraction*. There is an IPPC protocol including the species (IPPC, 2016).

4) *Ditylenchus dipsaci* (stem nematode) (EPPO A2 List)

Symptom description
Ditylenchus dipsaci 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, discoloration and stunting of above-ground plant parts and necrosis or rotting of stem bases and rhizomes. In the field, the nematode can survive for years without a host plant. Cool, moist conditions favour invasion of young plant tissue by this nematode. The nematode can also survive on a number of weeds. Irrigation water and cultivation by contaminated farm tools and machinery are other sources of inoculum dissemination.

Sampling and identification
Look for stunted plants and swollen, distorted or discoloured stems. Look for necrosis or rotting of the stem bases and rhizomes. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification of the pest (see 'Phytosanitary Inspections' section). Details on the identification of *Ditylenchus dipsaci* are included in EPPO Standard PM 7/87 Ditylenchus destructor *and* Ditylenchus dipsaci (EPPO, 2008d).

5) *Meloidogyne fallax* (false Columbia root-knot nematode) (EPPO A2 List)

Symptom description
The juveniles of the second stage are attracted to the roots and penetrate the roots closely behind the root tip. The parasites finally start feeding on cells, which are rapidly turned into multinucleated giant cells. At the same time as the giant cells are formed, the cells of the neighbouring pericycle start to divide, giving rise to a typical gall or root knot with no secondary roots emerging from it. Unspecific above-ground symptoms such as stunting or a general lack of vigour may be observed due to an impaired uptake of water and nutrients. Root-knot nematodes are able to move only a few metres annually on their own, but they can be spread readily through the transport of infested plants, in soil, adhering to farm implements and in irrigation water. Details on a national regulatory control system for *M. fallax* are included in EPPO Standard PM 9/17 (1) Meloidogyne chitwoodi *and* Meloidogyne fallax (EPPO, 2013c).

Sampling and identification
Inspect the roots for galls. As the symptoms are not always present or clear, a soil or root sample should be taken to provide additional assurance of pest freedom. It should be noted that detection of the nematodes through field inspection and soil sampling is more sensitive if done as closely as possible to the time of harvest of a host crop, and preferably before the 15 November [based on Dutch and Belgian circumstances (see Belgian Federal Agency for the safety of the Food Chain, 2017)].

During the growing season, an area of 1 ha should be selected from the field. From that area 60 plants with roots should be lifted at random and sent to the laboratory. After harvesting, a root sample should consist of roots from a minimum of 200 plants.

A soil sample should be taken for each hectare according to a grid pattern of 10 m × 10 m.

Each core should contain 40 mL of soil from the top 25 cm of soil. The diameter of the auger should be chosen accordingly (between 1 and 2 cm). The total soil sample should be 4000 mL of soil per hectare. The soil sampling should be done according to a systematic partition of the field, but with a higher density in high-risk areas (e.g. field entrances, headlands and waste heaps).

Where appropriate, samples for laboratory testing (plant parts with symptoms, roots, soil) should be taken for final identification of the pest. Details on the identification of *M. fallax* are included in EPPO Standard PM 7/41 Meloidogyne chitwoodi *and* Meloidogyne fallax. Details on nematode extractions are included in EPPO Standard PM 7/119 *Nematode extraction*. Details on soil sampling

are included in EPPO Standard PM 9/17 Meloidogyne chitwoodi *and* Meloidogyne fallax.

e) Viruses and viroids

1) *Apple mosaic virus* (Turkey A2 List, Israel quarantine pest)

Symptom description
The symptoms on strawberry plants are not well known. Possible symptoms could be leaf roll or a peacock pattern on the leaves.

Sampling and identification
Inspect the leaves for leaf roll symptoms and peacock patterns. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification of the pest.

2) *Arabis mosaic virus* (EU Annex II/A2, Turkey A2 List, Israel and Norway quarantine pest)

Symptom description
Arabis mosaic nepovirus is transmitted by the soil nematode *Xiphinema diversicaudatum*.

The most common symptoms are leaf mottling, a mosaic pattern or flecking, stunting and several forms of deformation including enations. The symptoms vary depending on cultivar, virus isolate, season and year. Many infections are latent and plants do not show symptoms.

Sampling and identification
Inspect the leaves for mottling, a mosaic pattern, flecking, stunting and deformation (see Fig. 24). Attention should be paid to the presence of the nematode vector. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for the final identification of the pest. As the infection can be latent, representative samples of different lots should be taken and subjected to laboratory testing.

3) *Raspberry ringspot virus* (EPPO A2 List)

Symptom description
Raspberry ringspot virus is transmitted by the free-living, soil-inhabiting nematodes *Longidorus elongatus* or *Longidorus macrosoma*. The symptoms induced vary according to season and virus strain. In general, progressive dwarfing and mortality 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. Symptoms are less clear on leaves developed in summer or at high temperatures.

Sampling and identification
Look for dwarfed and dead plants. Inspect the leaves for irregularly shaped, chlorotic blotches or local necrotic spots (Fig. 25). Attention should be paid to the presence of the

Fig. 25 Raspberry ringspot virus infection in strawberry cv. Huxley, showing irregularly shaped chlorotic blotches, some with a necrotic centre. Photo: SCRI, Dundee (GB).

Fig. 24 Leaf mottling caused by Arabis mosaic virus. Photo: CSL, York (GB), British Crown.

nematode vector. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for the final identification of the pest. As the infection can be latent, representative samples of different lots should be taken and subjected to laboratory testing.

4) *Strawberry crinkle virus* (EU Annex II/A2, Turkey A1 List, Israel, Jordan and Norway quarantine pest)

Symptom description

Strawberry crinkle virus (see Fig 26) is transmitted by the principal natural aphid vector *C. fragaefolii* (see Fig. 5). The symptoms vary in relation to the strawberry cultivar and the virus strain. Mild strains are symptomless in all cultivars, whereas in susceptible cultivars severe strains cause distortion and crinkling of the leaves, with leaflets that are unequal in size and small irregularly shaped chlorotic spots, often associated with the veins. The vigour and productivity of the plants are considerably reduced.

Fig. 26 Symptoms of Strawberry crinkle virus showing leaf distortion. Photo: Dr Jelkmann, Biologische Bundesanstalt, Dossenheim (DE).

Sampling and identification

Look for leaflets that are unequal in size. Inspect the leaves for distortion, crinkling and irregularly shaped chlorotic spots (Fig. 26). Attention should be paid to the presence of the aphid vector. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for the final identification of the pest. As the infection can be latent, representative samples of different lots should be taken and subjected to laboratory testing.

5) *Strawberry latent ringspot virus* (EU Annex II/A2, Turkey A2 List, Israel and Norway quarantine pest)

Symptom description

Strawberry latent ringspot virus is transmitted by various nematode vectors of the genus *Xiphinema* or *Longidorus*. Infection with this virus is usually latent in strawberries. Some strawberry cultivars show varying degrees of mottling and decline.

Sampling and identification

Attention should be paid to the presence of the nematode vector. As the infection is usually latent, representative samples of different lots should be taken and subjected to laboratory testing.

6) *Strawberry mottle virus* (Norway quarantine pest)

Symptom description

The major vector for strawberry mottle virus is the strawberry aphid *C. fragaefolii* (see Fig. 5). Strawberry mottle virus can cause a reduction in yield and runner production of up to 30%. Symptoms include stunting, chlorosis and/or necrosis on newer leaves, reddening of older leaves and leaf distortion.

Sampling and identification

Attention should be paid to the presence of the aphid vector. As the infection is usually latent, representative samples of different lots should be taken and subjected to laboratory testing.

7) *Strawberry mild yellow edge virus* (EU Annex II/A2, Turkey A1 List, Norway quarantine pest)

Symptom description

Strawberry mild yellow edge virus is transmitted by the strawberry aphid *C. fragaefolii*. Cultivated strawberries usually remain symptomless. Sensitive cultivars may develop dwarfing, marginal chlorosis, leaf distortion and small fruit.

Sampling and identification

Look for dwarfing, marginal chlorosis and leaf distortion (Fig. 27). Attention should be paid to the presence of the aphid vector. As the infection is usually latent, representative samples of different lots should be taken and subjected to laboratory testing.

Fig. 27 Chlorosis caused by Strawberry mild yellow edge virus. Photo: Dr Jelkmann, Biologische Bundesanstalt, Dossenheim (DE).

8) *Strawberry vein banding virus* (EPPO A2 List)

Symptom description

Strawberry vein banding virus is transmitted by aphids. On commercial strawberries, there are usually no characteristic symptoms that allow diagnosis of the virus, but this may be influenced by the cultivar and by potential co-infections with other viruses. Symptoms on *Fragaria vesca* (wild, woodland strawberry) 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.

Sampling and identification

Inspect the youngest developing leaves for epinasty, mild crinkling and wavy margins. Inspect the mature leaves for vein-banding (Fig. 28). Attention should be paid to the presence of the aphid vector. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for the final identification of the pest. As the infection is usually latent for commercial strawberries, representative samples of different lots should be taken and subjected to laboratory testing (Fig. 28).

9) *Tomato black ring virus* (EU Annex II/A2, Turkey A2 List, Israel and Norway quarantine pest)

Symptom description

Tomato black ring virus is transmitted by the free-living, soil-inhabiting nematodes *Longidorus elongates* and *Longidorus attenuatus*. Infected plants usually show few or no symptoms especially in the year of infection. Nevertheless, plant growth and vigour in such plants may be impaired. *Tomato black ring virus* may induce chlorotic mottling and/or ringspots in the leaves.

Sampling and identification

Inspect the leaves for chlorotic mottling and/or ringspots (Fig. 29). Attention should be paid to the presence of the nematode vector. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for the final identification of the pest. As the infection can be latent, representative samples of different lots should be taken and subjected to laboratory testing.

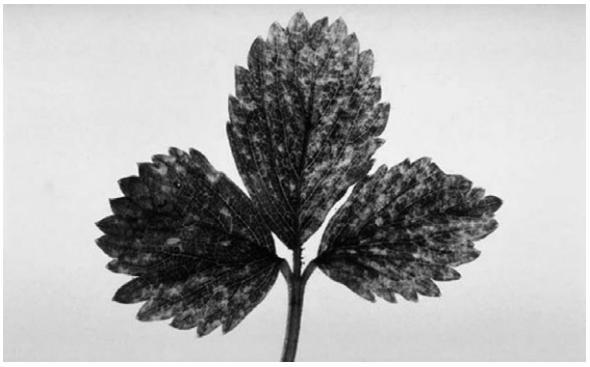

Fig. 29 Irregularly shaped chlorotic spots and mottling caused by Tomato black ring virus. Photo: A. T. Jones, SCRI, Dundee (GB). [Colour figure can be viewed at wileyonlinelibrary.com]

10) *Tomato ringspot virus* (EPPO A2 List)

Symptom description

Tomato ringspot virus is transmitted by the nematode vector *Xiphinema americanum sensu lato* [see EPPO Standard PM 7/95 (1) *Xiphinema americanum sensu lato*] (EPPO, 2017b). There is a broad variety of symptoms. Depending on the strawberry cultivar and the season, symptoms range

Fig. 28 *Strawberry vein banding virus*. Photo: Horticulture Research International, East Malling, Horticulture Research International, Bugwood.org. [Colour figure can be viewed at wileyonlinelibrary.com]

from none to leaf mottling, dwarfing, reduction in runner production and to death of whorls of outer leaves and subsequent plant death.

Sampling and identification
Inspect the plants for dwarfing, reduction in runner production and death of whorls of outer leaves. Inspect the leaves for mottling. Attention should be paid to the presence of the nematode vector. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for the final identification of the pest. As the infection can be latent, representative samples of different lots should be taken and subjected to laboratory testing. Details on the identification of *Tomato ringspot virus* are included in EPPO Standard PM 7/49 (1) Tomato ringspot nepovirus (EPPO, 2005b).

Appendix 2 – Short procedure for inspectors

Time of inspection

The most appropriate time for inspections of *Fragaria* plants for planting at places of production is in spring to early summer (for mother plants) and autumn for plants on waiting beds in trays.

Hygiene measures

In order not to spread and increase infestations, adequate precautions should be taken during inspections and sampling, such as protective clothes – coat, overshoes, gloves, etc. Gloves must be changed between different lots. All equipment for sampling must be disinfected between different lots.

Lot identification

For *Fragaria* spp., a lot should be defined as a number of plants of the same type (i.e. garden strawberry, pineberry, strasberry) and variety (e.g. Elsanta, Dar Select) from the same origin and planted at the same time.

Visual inspection

- Phytosanitary inspection should start with an overall examination of the place of production to check the physical condition of the plants. The plants in the vicinity of the place of production (e.g. weeds) should also be inspected.
- If no symptoms are seen, a systematic inspection of the field should be made, each lot being examined as a separate unit. Large lots should be walked in a 'W' transect. Examination of traps: moth traps (*Epiphyas postvittana*), blue sticky traps (*Frankliniella occidentalis*), traps containing food-type lures and/or sex attractants (*Popillia japonica*), etc.
- Thorough examination of lots with an abnormal level of die off, with differences in colour, plants with an abnormal growth, plants with a flaccid appearance, wilting plants, stunted plants, reduced leaf size, etc.
- Examination of leaves (upper and lower leaf surface), buds, leaf stalks, roots, soil and/or growing medium for insects in any stage.
- Plants should be shaken for the presence of adults of *F. occidentalis*.
- Inspection of the leaves (upper and lower leaf surface) with transmitted light (*X. fragariae*) for
  - water soaked spots (*X. fragariae*)
  - chlorotic spots, discoloration, etc. (fungi, nematodes, viruses)
  - crinkling, distortion, deformation, leaf roll, etc. (nematodes, viruses)
  - vein yellowing (viruses)
- Inspection of the stems for
  - sunken lesions (*C. acutatum*)
  - swelling, distortion, discoloration (*D. dipsaci*).
- Inspection of the roots for
  - rat-tail symptom (*P. fragariae*)
  - Ggalls (*M. fallax*)
- Cut open the upper, unrotted parts of the roots and look for wine-red to brick-red coloration of the stele (*P. fragariae*).
- Pay attention to vectors such as leafhoppers (vector of 'Candidatus *Phytoplasma solani*'), aphids (vector of *Strawberry crinkle virus*, *Strawberry mild yellow edge virus*, *Strawberry vein banding virus*) and nematodes (vector of *Arabis mosaic virus*, *Raspberry ringspot virus*, *Strawberry latent ringspot virus, Tomato black ring virus, Tomato ringspot virus*).

Sampling for laboratory testing

- Samples should be taken from plants on which pests or signs of them are present and cannot be immediately identified by the inspector, and from plants showing suspicious symptoms or deformations. In these cases, the sample consists of the suspect plant(s).
- If a pest is found that is suspected to be a pest recommended for regulation as a quarantine pest, the suspect lot and related lots should be detained under official control pending a test result.
- Sampling should normally be done on a lot basis with plants evenly collected throughout the lot. In general, since the plant parts most suitable for detection greatly differ for different pests, samples for laboratory testing should contain the complete plants in order to give a possibility of testing for the whole range of potential pests.
- Each sample should be individually labelled to ensure a link is made to the nursery name (or reference number), place of

sampling (parcel, place within the lot, plan …), sample number, date, plant species, plant variety if relevant and lot number, symptoms seen in the field and suspected harmful organism, so follow-up action can be taken if necessary.

- Sampled material such as plants and plant parts should be kept in good condition and placed in plastic bags together with a piece of absorbent paper. If the plant parts are dry, a piece of slightly damp absorbent paper should be added; for wet plant parts a piece of dry absorbent paper should be added (to avoid rotting of the plant parts). Plants with roots in potting compost, substrate, etc. do not easily dry out; as a consequence, absorbent paper is not needed. Samples of insects, larvae, pupae and eggs should be put in a pot with screw cap. Living organisms must be sent to the laboratory together with plant material of the host plant in suitable containers. Dead organisms should be kept in alcohol in order to prevent decomposition during transport.