Bulletin OEPP/EPPO Bulletin (2016) **46** (1), 28–39 ISSN 0250-8052. DOI: 10.1111/epp.12268

Procédures phytosanitaires
Phytosanitary procedures

PM 3/76 (1) Trees of *Malus*, *Pyrus*, *Cydonia* and *Prunus* spp. – inspection of places of production

Specific scope

This Standard describes the procedures by which places producing plants for planting of certain genera of trees which are important for fruit production are subjected to inspection, including sampling for testing and pest identification.[1] The crops covered are varieties and rootstocks of all species and hybrids of *Malus*, *Pyrus*, *Prunus* and *Cydonia*, including related ornamental varieties within these genera. Inspection of places of production may be for purposes of export or for internal 'within country' use. Alternatively, inspection may be carried out as part of a national survey for monitoring or to determine freedom for specified pests for countries or areas.

As the longhorn beetles *Anoplophora chinensis* (EPPO A2 List) and *Anoplophora glabripennis* (EPPO A1 List) are, at the time of publication, still unlikely to be present on nursery trees grown in the EPPO region, please refer to EPPO Standard PM 3/79, *Consignment inspection for* Anoplophora chinensis *and* A. glabripennis.

Specific approval

First approved in 2015-09.

Introduction

Fruit trees of the genera *Malus*, *Pyrus*, *Prunus* and *Cydonia* are some of the most important crops in the EPPO region and plants for planting are produced in the region and are also exported to other parts of the world (e.g. the USA, South America, Asia). Cherries, almonds and apricots (*Prunus avium*, *Prunus dulcis*, *Prunus armeniaca*) are example species of these trees along with apples, pears and plums.

Plants for planting may potentially carry regulated pests specific to these genera as well as polyphagous or contaminating pests. These may either be included in the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests or otherwise regulated by specific EPPO countries or third countries.

Many EPPO countries also require that consignments of fruit or ornamental trees 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* (EPPO, 1994).

For export or internal movement, consignment freedom is usually verified by visual inspection and testing, where appropriate, before issue of the phytosanitary certificate or internal national documentation. However, import requirements also often specify a place of production inspection for plants for planting before harvest and verification of the efficacy of other phytosanitary measures such as sampling and plant or soil testing for specified relevant pests.

Phytosanitary inspections

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

The procedures described in the present Standard are specific to place of production inspection in an EPPO country. The Standard may also be applicable for export inspection when the requirements of the importing country are similar to the requirements in the country of origin, for

[1]This Standard forms part of a new series of EPPO Inspection Standards and will be reviewed by the end of 2017. Comments to be taken into account during that review should be sent to the EPPO Secretariat at hq@eppo.int.

example the same quarantine pests are concerned, or for international movement within a 'single market'.

It is important to carry out the inspection at the most appropriate time depending on the symptoms of the pests and possible presence as latent infections. Timing will depend on the pest, and guidance can be found in the relevant EPPO Diagnostic Standards relevant to the crop species.

Inspections should be undertaken during the growing season and at least once a year. However, two or three inspections per year are recommended depending on crop history and origin. Plants or plant material with an unknown history or a history of significant pest occurrence should be inspected more than once.

Depending on the phytosanitary requirements of the importing country or the internal national legislation, inspection of the whole place of production including inspection of the immediate vicinity may be required for a particular pest; for example, for many countries this is required for '*Candidatus* Phytoplasma mali' (Acholeplasmataceae, EPPO A2 List), '*Candidatus* Phytoplasma pyri' (Acholeplasmataceae, EPPO A2 List) and *Plum pox virus* (PPV) (Potyviridae, EPPO A2 List).

If the export destination of the stock is known when commencing production, producers, exporters and transporters in the exporting country should be informed of the particular phytosanitary regulations of the importing country in order for them to be able to report to the NPPO any suspicious symptoms of the relevant pests during the growing season. If the importing country requires inspections at the place of production during the growing season, these must be carried out.

Plants for planting produced according to EPPO Standards PM 4/27 (1) *Certification scheme on pathogen-tested material of* Malus, Pyrus *and* Cydonia (EPPO, 1999), PM 4/29 (1) *Certification scheme for cherry* (EPPO, 2001a) or PM 4/30 (1) *Certification scheme for almond, apricot, peach and plum* (EPPO, 2001b) or any equivalent phytosanitary certification system, are generally considered to provide high phytosanitary guarantees, especially for certain pathogens including viruses, and this should be taken into account.

ISPM no. 5 *Glossary of Phytosanitary Terms* (IPPC 2009a) defines inspection as 'Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations'. For pests that are not visually detectable, the inspection procedure may consist only of lot identification and sampling for laboratory testing or destructive sampling to detect pests, for example cutting portions of trunk into small pieces to detect larval stages of insect pests. This latter point is particularly applicable to fruit trees which are susceptible to a large number of pests, particularly viruses, viroids, virus-like diseases, phytoplasmas and mites which can remain undetected due to their lack of symptoms, particularly when present at low levels of infestation.

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

Types of material concerned

This Standard covers all the genera listed, *Malus*, *Pyrus*, *Prunus* and *Cydonia*, but within those genera the material at the place of production may consist of a range of types.

'Mother' trees

These are trees of known variety[2] and health status maintained individually '*in situ*' for the production of scion wood for grafting or budding onto rootstocks for the production of grafted fruit trees (often known as 'finished' or 'complete' trees).

Parent rootstock beds

These are maintained as beds of plants '*in situ*' for the production of vegetatively propagated rootstocks which are used or sold for the production of grafted fruit trees. These rootstocks are earthed up in ridges then harvested mechanically before being split into individual rootstocks for planting or for sale. Less frequently, for some species, particularly *Prunus* spp., material can alternatively be maintained as 'rootstock hedges' which are taller 'bush-like' plants normally grown in rows. Cuttings of thin stems are removed from these plants and sold for rooting to produce rootstocks for later budding or grafting.

Seed production trees

These are individual trees maintained to produce high-quality seed for growing on to produce rootstocks for later grafting or budding.

Seedling rootstocks

Grown directly from seed, seedling rootstocks are germinated and grown in the field or glasshouse before later budding or grafting to produce grafted fruit trees.

Tissue culture

Plants in tissue culture are usually intended for further propagation and the method is used primarily for rootstocks,

[2]In this scheme, the terms 'variety' and 'rootstock' are used in the traditional fruit-growing sense: the variety is the scion cultivar, while the rootstock may be a cultivar or a species.

especially for apples and pears. As this material is the starting point for large-scale multiplication it could in principle contribute to major spread of plant pests. However, due to the special growing conditions, the majority of potential contaminating pests 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 sale or export is difficult to perform and unreliable. It is recommended that material is inspected before it is propagated in tissue culture or after transplanting into growing medium and growing on until a phase where symptoms could be detected.

Grafted trees (varieties)

These are the final budded or grafted trees grown in the field, glasshouse or a temporary structure, usually for 2 or 3 years, before marketing either as 'bare rooted' plants or in individual pots as finished young trees.

Pests recommended for regulation as quarantine pests or regulated pests in countries in the EPPO region

This Standard mainly relates to pests of the EPPO A2 List recognized as of primary importance for all species and hybrids of *Malus*, *Pyrus*, *Prunus* and *Cydonia* and it also covers those pests listed in European Union (EU) Directive 2000/29/EC (EU, 2000) but not included in the EPPO Lists. The phytosanitary procedures described in this Standard are primarily aimed at preventing the spread of these specific pests in the EPPO region or to third countries via exported consignments of these species. This Standard also covers polyphagous pests which have these species as economically relevant hosts.

For plants in growing medium, 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 (CABI/EPPO, 1997) as well as in more recent EPPO Datasheets and in the book *Viruses and Virus-Like Diseases of Pome and Stone Fruits* (APS, 2011). The relevant scientific literature should be consulted for additional up-to-date information.

Table 1. Specific pests of *Malus* spp.

EPPO A2 pests	Other pests regulated by specific EPPO member countries (including EU-regulated pests)
Insects	
Quadraspidiotus perniciosus	
Fungi	
Monilinia fructicola	
Bacteria (including phytoplasmas)	
'*Candidatus* Phytoplasma mali'	
Erwinia amylovora	

Table 2. Specific pests of *Pyrus* spp. and *Cydonia* spp.

EPPO A2 pests	Other pests regulated by specific EPPO member countries (including EU-regulated pests)
Insects	
Quadraspidiotus perniciosus	
Fungi	
Monilinia fructicola	
Bacteria (including phytoplasmas)	
'*Candidatus* Phytoplasma pyri'	
Erwinia amylovora	

Table 3. Specific pests of *Prunus* spp.

EPPO A2 pests	Other pests regulated by specific EPPO member countries (including EU-regulated pests)
Insects	
Quadraspidiotus perniciosus	
Fungi	
Monilinia fructicola	
Bacteria (including phytoplasmas)	**Bacteria (including phytoplasmas)**
Pseudomonas syringae pv *persicae*	'*Candidatus* Phytoplasma prunorum' (Acholeplasmataceae) [EU ('Annexe I/A2'), Turkey ('A1 List'), Jordan ('quarantine pest'), Israel ('quarantine pest')]
Xanthomonas arboricola pv. *pruni*	
Xylella fastidiosa	
Viruses	
Plum pox virus	
Tomato ringspot virus	
Raspberry ringspot virus	

The EPPO A1 and A2 Lists as well as the lists of regulated pests within countries are subject to additions and deletions. The present list (Tables 1–3) will therefore need to be revised whenever relevant new quarantine pests are identified.

For trees grown in open ground, NPPOs may apply additional controls to reduce the risk of moving soil-borne pests such as *Globodera* spp., *Synchytrium endobioticum*, *Meloidogyne* spp. and *Phytophthora fragariae*.

Identification of lots

General background information on lot identification is given in EPPO Standard PM 3/72 (1) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification.* For mother trees and rootstock beds and hedges, the cultivar is the primary lot-distinguishing character, whereas for grafted trees the cultivar/rootstock combination is the primary criterion for lot identification. If information on different

batches of grafted trees or origins of budwood or rootstocks is available this should also be taken into account.

Selection of plants for visual inspection and sampling for laboratory testing

This section contains guidance on visual inspection of places of production of fruit plants for planting, on the proportion of growing plants to be inspected (sample size) and on sampling for laboratory testing. Inspections are carried out after checking the list of host plants and their location with the nursery supervisor and assessing the regulations or NPPO requirements for the purpose of the inspection. This may be for monitoring or survey purposes, for issue of a phytosanitary certificate or for internal movement certification, such as for the issue of an EU plant passport.

Selection of plants for visual inspection (general aspects)

Inspection of plants at a place of production is covered in general terms by EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production*, *area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009a). For the purposes of this procedure these principles also apply to fruit trees of different types such as mother trees, rootstock beds and grafted ('finished') fruit trees. The aim of these inspections is to detect the presence of plant pests by visual inspection, either alone or in combination with the taking of samples for laboratory testing, which may be of symptomatic material or random sampling of asymptomatic material to detect any cryptic infestations or latent infection which may be present.

Depending on the reason for the inspection and the regulations being applied (including the requirements of importing countries), inspection of the whole place of production and the vicinity, the place of production only or only of a consignment of relevant plants may be required.

Inspection of the place of production

The number of plants that must be selected for inspection to detect a specified level of infection in a specified lot size is indicated in Tables 1, 3 and 4 of ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2009b). (For example: from a lot of 10 000 plants, 2588 plants need to be inspected to provide a 95% confidence of detecting an infection present in 0.1% of the plants, provided the infection is uniformly distributed and the plants are selected at random.) For small lots the numbers required will often mean that all plants should be inspected. Otherwise they should be selected on a random basis. In practice inspection of whole rows randomly or evenly chosen across the field is usually carried out.

Inspection of the place of production and a consignment in the case of exports

Where inspection is needed for regulated pests prohibited by the importing country, the objective should be to detect by visual inspection an infection level of 0.1% or more with a confidence level of at least 99%. For a lot of 10 000 plants this would require inspection of 3689 plants. Again, for small lots the numbers required will mean that all plants should be inspected. Otherwise they should be selected on a random basis. In practice, inspection of whole rows randomly or evenly chosen across the field is usually carried out.

For a number of fruit tree pathogens, for example PPV (EPPO A2 List) and '*Candidatus* Phytoplasma prunorum' [formerly EPPO A2; currently EU ('Annexe I/A2'), also listed in other countries], the regulations for some countries, including EU members, require that the whole place of production and susceptible plants in its immediate vicinity should be inspected. Therefore all susceptible species in the whole place of production and any plants in its surrounding boundary, plus an unspecified area (vicinity) around the place of production should also be inspected. The definition of 'the vicinity' is usually not specified in legislation, and in practice depends on the situation and the organism (including its vectors) involved and its spread capacity, but is usually between 50 and 200 m. Effective inspection of the vicinity depends on the inspector having the right of access and the practical possibility to carry out inspections.

Sampling for laboratory testing (general aspects)

Sampling of symptomatic material

Inspectors should be familiar with the symptoms of the listed pests they may encounter, and if any are observed or suspected samples should be taken for laboratory testing. Details of the procedures for sampling for the individual pests are given in Appendix 1. In general, samples should be taken from individual plants and these should be kept separate in order to aid diagnosis and obtain a measure of the number of plants infested. If the inspector is confident of the diagnosis and there are large numbers of plants in a lot with similar symptoms, sampling may be limited to a representative number of symptomatic trees. A positive finding of a pest will mean that phytosanitary measures shall be applied to ensure that the plants do not present any risk of spreading the pest. Amongst other options these measures may include eradication or containment measures for the lot concerned and possibly for other material in the place of production.

Sampling of asymptomatic material

For the purposes of declaring a pest-free place of production or a pest-free area (PFA) or for export purposes,

sampling of asymptomatic plants and vectors may be required in order to detect latent or hidden infections. Sample size should be increased if varieties known to be susceptible to latent infection are present or origins include potential high risk or areas with high vector populations.

Sometimes a country's legislation may specify that plants are derived from tested material: for example, for '*Candidatus* Phytoplasma mali' (EPPO A2 List) and PPV (EPPO A2 List) some countries require that plants for planting have been derived from host material tested within a certain number of years and found to be free of the pest. Random samples should therefore be taken from individual mother trees and lengths of parent rootstock beds so that propagating material is only taken later from plants known to have been tested and found to be pest free during this period.

Declaration of a PFA as described in ISPM no. 4 (IPPC, 1995) or a pest-free place of production as described in ISPM no. 10 (IPPC, 1999) may also be done by sampling; the number of plants involved should be determined using ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2009b). Further details are given for individual pests in Appendix 1.

The analytical sensitivity of each test to be used should be known before testing commences so that a single known infested item will be detected when mixed with the maximum number of 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. However, all samples must be traceable in the event of positive results in order that they can be traced back to the original plant or plants, for eradication purposes. It is also important to keep in mind that sampling can never prove that a pest is truly absent.

Selection of plants for visual inspection and sampling for laboratory testing (specific aspects)

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

Grafted young trees (in the field or in pots), mother trees and rootstocks

Plants in a nursery to be inspected will normally be growing in individual pots or in the field, and either under protection or outside. Each lot should be individually inspected because it will have different visual characteristics, such as foliar morphology, disease resistance, history and potentially different infestation levels depending on variety and rootstock combinations, origin and previous treatments.

The time of year for inspection will vary with the pest species, depending on the optimum time for expression of symptoms if a specific pest is being targeted for detection. In general, plants should be in active growth and have sufficient time after breaking dormancy to be showing symptoms if infestation is present. Inspections should be completed before general senescence commences in the autumn to prevent disguising of symptoms or the presence of insect pests at life stages that are difficult to detect, for example eggs or larvae.

Visual inspection will only detect harmful organisms which are present on the plants (e.g. insects, mildews or rusts) or are systemic and show symptoms in the foliage or stems (e.g. '*Candidatus* Phytoplasma mali' or PPV) when the climate or variety susceptibility is appropriate. Therefore if asymptomatic infection is suspected, or plants are being indexed for possible infection, then random samples representative of the whole lot will be required (see details in Appendix 1).

For the inspection procedure, a general look over the relevant lot should be carried out first and any noticeable poorer growing areas or those with more obvious symptoms (e.g. paler or with other types of 'patches') should be examined first. If none are apparent then a representative number of plants should be thoroughly examined [see 'Selection of plants for visual inspection (general aspects)' for relevant numbers for the level of detection required].

Individual plants should have their stems and both sides of their leaves examined and also any flowers and fruits, if present. Inspectors should particularly look for leaf blotches or pale areas and veinal or interveinal necrosis.

In addition to the above, mother trees that are fruiting should have the fruit examined for verification of the variety and for any listed pest or disease (e.g. PPV will sometimes show symptoms on the fruits).

Acknowledgement

This Standard was first drafted by Mr P. Reed of the Animal and Plant Health Agency (GB).

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Appendix 1 – Symptoms and sampling for identification of quarantine pests of fruit trees

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) or in more recent EPPO datasheets. Illustrations are available on the EPPO website (http://www.eppo.int or https://gd.eppo.int/). 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. Information on the current distribution of relevant pests and photographs of symptoms can be found on the EPPO Global Database at https://gd.eppo.int/.

(A) Insects

(1) *Frankliniella occidentalis* (EPPO A2 List)

Symptom description. *Frankliniella occidentalis* is an occasional outdoor pest of fruit plants in the EPPO region, especially *Prunus* spp., as well as *Malus* spp., but it is unlikely to be present in traded fruit trees unless they are growing in pots and are in leaf or blossom at the time of movement. This is because the larvae and adults can be present feeding on the blossom tissues and pollen or nectar or living on buds (under the scales) or on foliage (usually the under surface). Nursery stock of fruit trees can also be damaged, the terminal buds being killed or weakened. Eggs can also be present in leaf buds, and so be transported with any traded material.

Symptoms of infestation can be discoloration of the upper leaf surface, speckling and halo spotting on leaves and discoloration and scarring of open blooms and petals.

Sampling and identification. 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 or by an insect aspirator ('pooter'). Alternatively, blue sticky traps can be used to detect infestations and monitor adult population levels.

Additional information can be found at the following links:

Utah State University, *Frankliniella occidentalis*, pest fact sheet http://utahpests.usu.edu/ipm/htm/fruits/fruit-insect-disease/western-flower-thrips

INRA, *Frankliniella occidentalis* http://www7.inra.fr/hyppz/RAVAGEUR/6fraocc.htm.

Additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/11 Frankliniella occidentalis (EPPO, 2002).

(2) *Quadraspidiotus perniciosus* (EPPO A2 List)

Symptom description. All surface parts of young host plant tissue can be infested but attacks are generally on wood. In severe infestations, leaves may also be fed upon. The species injects toxic saliva and, in the absence of control, young apple and pear trees, for example, can be killed within 2–3 years. Within 24 h of a larva arriving, a characteristic violet-red halo appears around the point of attachment. Haloes increase in size as larvae mature and may coalesce and the red cortical tissue swells with accumulating sap. Later in heavy infestations the bark often cracks and exudes gum, resulting in a surrounding dark-brown gelatinous area and cessation of growth. Young nursery stock is less likely to be infested than mother trees, but

budwood from the latter could potentially be infested and hence moved during distribution.

Sampling and identification. Branches, shoots, leaves and fruit with suspect scales should be removed or sections cut away with the scales still attached and sent to the laboratory in a dry condition in plastic containers.

(B) Nematodes

(1) *Globodera pallida* and *Globodera rostochiensis* (EPPO A2 List)

Symptom description. Globodera pallida and *G. rostochiensis* are not pests of fruit tree species and no infestation occurs, and consequently there are no symptoms. However, some EPPO countries require that plants for planting should have been produced in a place of production or field known to be free from these nematodes.

Sampling and identification. If the history of the field is not known and if there are consequently no records of sampling and testing of the field or the field is not under official control due to previous findings, then the relevant area should be sampled and the samples found free from *G. pallida* and *G. rostochiensis.* Recommendations on sampling can be found in the Council Directive 2007/33/EC of 11 June 2007 (EU, 2007) on the control of these two species. Additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/40 Globodera rostochiensis *and* G. pallida (EPPO, 2013a).

(2) Virus transmitting *Longidorus elongates* and *Longidorus macrosoma* and *Xiphinema rivesi* (EPPO A2 List)

Symptom description. The crop should be planted in a plot known not to be infested with *L elongates*, *L. macrosoma* (unlisted vectors of *Raspberry ringspot virus*) or *X rivesi*, or if this is not known then the field should be sampled and examined for the presence of *L. elongates*, *L. macrosoma* and *X rivesi.*

Sampling and identification. Sampling should be carried out according to EPPO Standard PM 4/35 (1) *Soil test for virus–vector nematodes in the framework of EPPO Standard PM 4 Schemes for the production of healthy plants for planting of fruit crops, grapevine*, Populus *and* Salix. Additional information for personnel carrying out diagnostics is available in EPPO Standard PM 7/95 Xiphinema americanum sensu lato (EPPO, 2009b).

(C) Fungi

(1) *Monilinia fructicola* (EPPO A2 List)

Symptom description. Occurring on stone fruits, but also *Malus* and *Pyrus* spp., this species has occurred in a few countries in Europe and is similar to two other widespread species: *Monilinia fructigena* and *Monilinia laxa*. Blossoms become infected and turn brown and die. Under suitable humid or wet conditions, tufts of fungal spores are produced on the dead tissue. This is usually followed by shoot infection with dying bark, usually sunken, having sharp edges. Infected leaves show more or less circular brown dead areas that may later fall away to give a 'shot-hole' appearance, or the entire leaf may be killed. Other than this, the disease may not be clearly visible until the fruits approach maturity, when they either fall to the ground or remain attached to the tree in a 'mummified' form. Pictures of symptoms on fruit are available in EPPO Standard PM 7/18*:* Monilinia fructicola.

Sampling and identification. Samples of flowers, leaves or fruits showing typical symptoms should be collected and placed in a labelled plastic bag together with a piece of slightly damp absorbent paper, kept in cool conditions and sent to the diagnostic laboratory as soon as possible. Each sample should be individually labelled with the nursery name, nursery reference number, date, variety and, if necessary, a way of identifying the individual tree or length of rootstock hedge, so follow-up action can be taken if necessary.

Additional information for personnel carrying out diagnostics and details of isolation and identification of *M. fructicola* are included in EPPO Standard PM 7/18 (2) Monilinia fructicola.

(2) Other quarantine fungi and fungus-like organisms

According to national legislation, fruit plants for planting may be required to be grown in a place of production or field known to be free from soil-borne fungi such as *Phytophthora fragariae* (EPPO A2 List), *Synchytrium endobioticum* (EPPO A2 List, additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/28 Synchytrium endobioticum), *Verticillium albo-atrum* and *Verticillium dahliae* (additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/78 Verticillium albo-atrum *and* V. dahliae). Based on the history of earlier crops at the place of production, freedom from pests should be confirmed by examination of NPPO records of occurrences, previous visual inspection of relevant host plants or soil testing of fields where potential host plants have been grown.

(D) Bacteria (including phytoplasmas)

(1) '*Candidatus* Phytoplasma mali' (EPPO A2 List)

Inspection of susceptible hosts in the vicinity of the nursery should also be carried out where possible.

Symptom description. On established mother trees the most obvious symptoms that may develop are 'witches brooms' which develop in late summer as a result of the suppression of apical dominance. Alternatively, on less vigorous shoots, leaf rosettes may occur, but in either case the leaves are usually more dentate and have enlarged stipules. In autumn early leaf reddening may also occur compared with normal yellowing of healthy trees. Infected young trees in nurseries may be less vigorous and in winter may show a fine hairy root system, while large roots are less frequent in number and size. Symptoms may develop within a year on nursery trees and so these should be inspected in spring and again in late summer.

On established mother trees which are allowed to flower, the fruit may be smaller and of a paler colour and be flattened with longer peduncles compared with healthy trees. They may produce leaves earlier than normal but flowering may be delayed.

Symptoms may vary from year to year depending on weather conditions or management and can also be variable around the tree.

Sampling and identification. Sampling and testing techniques vary depending on the time of year and the diagnostic methods available. During early summer to autumn (June to the end of October) leaves can be sampled and tested by 4′,6-diamidino-2-phenylindole (DAPI) staining or enzyme-linked immunosorbent assay (ELISA) or the DNA can be extracted from leaf midribs, petioles or phloem tissue of shoots for polymerase chain reaction (PCR) testing. Twenty leaves and petioles (with symptoms if available) should be sampled from each tree and sent to the laboratory. Shoot samples for phloem tissue should be taken from around the tree to obtain a representative sample because phytoplasmas may be unevenly distributed.

During the winter and early spring roots can be tested, because at the end of the growing period phytoplasmas move from the apical part of the plant to the roots where they overwinter. Samples should be taken from around the tree or young pot plants because of uneven distribution.

The samples should be placed in a plastic bag (without any paper), inflated slightly and sealed, then packed in strong containers such as cardboard or plastic boxes and padded with paper or similar to prevent movement. Each sample should be individually labelled with nursery name, nursery reference number, date, variety and, if necessary, a way of identifying the individual tree or length of rootstock hedge, so follow-up action can be taken if necessary.

Samples should be kept cool in an icebox if stored in a vehicle and can be stored at 4°C for no more than 7 days before processing. Fruits can be stored for 1 month at 4°C.

Further details are available in EPPO Standard PM 7/62 *Diagnostic protocol for regulated pests:* 'Candidatus *Phytoplasma mali'* (EPPO, 2006a).

(2) '*Candidatus* Phytoplasma pyri*'* (EPPO A2 List)
Inspection of susceptible hosts in the vicinity of the nursery should also be carried out where possible.

Symptom description. The most easily recognized symptoms that may develop on established mother trees occur in late summer with the development of premature autumn leaf colour on affected trees. Most cultivars develop a premature red colour but some may develop a premature yellow colour. There may be some leaf cupping or curling and there is usually premature leaf drop. The following spring, affected trees suffer from weak growth and sparse pale foliage. The spring symptoms can vary from absence of symptoms to death. There may be a line of necrotic tissue in the bark at the graft union between scion and rootstock.

The premature autumn leaf colour symptoms associated with pear decline may also have several other causes: water-logging, root damage, ring barking caused by feeding animals, some bacterial cankers, rootstock and variety incompatibility can all give rise to symptoms resembling those caused by phytoplasma infection.

Sampling and identification. '*Candidatus* Phytoplasma pyri' is found in mature sieve tubes in the phloem of affected trees but moves from the apical part of the plant to roots where it overwinters.

Sampling and testing techniques vary depending on the time of year and the diagnostic methods available. During late spring to early summer (June to the end of September) leaves or shoots can be sampled and the DNA is extracted from leaf midribs, petioles or phloem tissue for testing. Twenty leaves and petioles (with symptoms if available) should be sampled from each tree and sent to the laboratory. Shoot samples for phloem tissue should be taken from around the tree to obtain a representative sample because phytoplasma may be unevenly distributed.

At all times of the year '*Candidatus* Phytoplasma pyri' may also be detected in the roots of affected trees if the trees are grafted onto *Pyrus* rootstocks or are growing on their own roots (if the trees are grafted on the more widespread quince rootstocks, detection in the roots is unreliable). Root samples should be taken from around the tree to obtain a representative sample because phytoplasma may be unevenly distributed.

Bark from 2- to 3-year-old wood from three different parts of the tree can also be examined together with one trunk sample (EPPO, 1996).

The samples should be sent as described for '*Candidatus* Phytoplasma mali'.

Further details are available in EPPO Standard PM 7/63: 'Candidatus *Phytoplasma pyri*' (EPPO, 2006b).

(3) '*Candidatus* Phytoplasma prunorum'

Inspection of susceptible hosts in the vicinity of the nursery should also be carried out where possible.

Symptom description. The earliest symptoms are premature leaf growth during winter, or leaves appearing before or during flowering in spring; growth may also develop from buds on old wood. Symptoms continue to develop and are most visible in late summer and infected shoots are typically shorter with smaller leaves. These curl upwards and longitudinally, so they appear cone shaped (in apricot) or cylindrical (e.g. in Japanese plum). The leaves become either prematurely yellow or red and are usually hard, brittle and drop early. On some hosts leaves develop interveinal chlorosis, swollen yellow midribs with yellow or reddish lateral veins or necrotic lesions. Infested shoots die back in most susceptible hosts, becoming dry and brittle. It should be noted that symptoms are often not present in trees less than 5 years old.

Sampling and identification. Sampling can be carried out from spring onwards and should be taken from symptomatic material as described above. If suspect but symptomless trees are to be sampled, fully expanded leaves (with their petioles) or shoots for phloem tissue should be taken randomly from all around the tree.

The samples should be sent as described for '*Candidatus* Phytoplasma mali'.

(4) *Erwinia amylovora* (EPPO A2 List)

Symptom description. This frequent pathogen (the causal agent of fireblight) infects *Malus*, *Pyrus* and *Cydonia* but not *Prunus* spp. The first symptoms usually appear in spring during warm and humid weather, and progress under favourable conditions. The symptoms may be seen on young grafted fruit trees in the nursery but are more likely to be found in older 'mother trees' kept for budwood. Flowers appear water-soaked, then wilt, shrivel and turn pale brown to black. Peduncles may also appear water-soaked, become dark green and finally brown or black, sometimes oozing droplets of sticky bacterial exudates. Leaves wilt and shrivel, and entire spurs turn brown or 'crooked' in most hosts, or dark brown to black in pear, but remain attached to the tree for some time. Immature fruits (or less frequently mature fruits) may have infected parts that appear oily or water-soaked, becoming brown to black and often exuding droplets of bacterial ooze. Under the bark of infected twigs, branches or trunks, reddish-brown streaks are often found in the subcortical tissues (van der Zwet & Keil, 1979). Brown to black, slightly depressed cankers can develop in the bark of twigs or branches, or even the trunk, in autumn and winter. In young nursery trees symptoms may only be visible on leaves or as crooked shoots if flowering and therefore fruiting does not occur.

Symptoms of other diseases or disorders such as mechanical damage may sometimes appear similar to those of fireblight, especially in blossoms and shoots, and so samples should be taken for laboratory testing.

Inspection should also be carried out on all other hosts in the nursery, and may also be required on all host plants in the boundary of the nursery and the surrounding area. Additional pictures of symptoms (including apple and pear) can be found in EPPO Standard PM 7/20 Erwinia amylovora (EPPO, 2013b).

Sampling and identification. Representative samples of flowers, shoots, leaves or fruits showing typical symptoms should be taken together with some adjacent healthy material.

If analysis of asymptomatic plants is required under a survey or part of a regulatory requirement then these can be taken and tested either individually or in bulk. After favourable conditions for multiplication of the causal agent of fireblight have been confirmed, or at least when the average temperature is higher than 18°C (van der Zwet & Beer, 1995), flowers, shoots, fruitlets or stem segments should be collected. For nursery plants, young shoots, approximately 20 cm long, should be cut from the most susceptible hosts available, scissors or pruning shears need to be disinfected between plants. For plants growing in the field, flowers should be cut when available and/or young shoots about 20 cm long, disinfecting between plants. Flowers or peduncles should be taken as well as the base of the limb of mature leaves or stem segments of selected plants. If analyses need to be performed in winter, collect 5–10 buds per plant. Care must be taken to avoid cross-contamination between samples.

The samples should be placed in plastic bags (with dry absorbent paper), inflated slightly and sealed, then packed in strong containers such as cardboard or plastic boxes and padded with paper or similar to prevent movement. Each sample should be individually labelled with nursery name, nursery reference number, date, variety and, if necessary, a way of identifying the individual tree or length of rootstock hedge so follow-up action can be taken if necessary.

Samples should be kept cool in an icebox if stored in a vehicle and can be stored at 4°C for no more than 7 days before processing. Fruits can be stored for 1 month at 4°C.

Additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/20 Erwinia amylovora.

(5) *Pseudomonas syringae* pv. *persicae* (EPPO A2 List)

Symptom description. In nectarine and peach, symptoms include necrosis and shoot dieback, infections of branches and main trunk, leaf spots and fruit lesions, and in the case of severe infections in young orchards the death of the tree. On Japanese plum, symptoms are mainly confined to dieback and leaf spots. Dieback of terminal shoots can already occur in autumn and in spring following the development

of girdling lesions from nodal infections. Small elliptical lesions may develop at internodes.

The rootstock can also be infected, showing symptoms similar to those on woody shoots.

Leaf infection results in small, mainly angular, brown to necrotic spots, surrounded by a yellow halo. The necrotic tissue subsequently falls out, causing a 'shot-hole' effect. On fruits, small, round, dark, oily spots occur. These can be found within the fruit tissue, causing sunken, deforming lesions that ooze gum.

Some symptoms of bacterial dieback due to *Pseudomonas syringae* pv. *persicae* can be confused with those of bacterial canker of stone fruits (*P. syringae* pv. *syringae*, *P. syringae* pv. *mors-prunorum*), symptoms of leucostoma canker (*Leucostoma* spp.), brown rot (*Monilinia* spp.) and shot-hole disease of stone fruits (*Clasterosporium carpophilum*) or frost injury. Therefore laboratory testing is necessary. Distinctive characteristics of dieback are discoloration of wood in branches above the necrosis and the absence of an obvious boundary between the diseased and healthy tissue in the bark of the tree.

Sampling and identification. Where dieback has occurred wood should be sampled directly from diseased tissue by cutting out tissue from the border between apparently healthy tissue and the necrotic area. Leaves or fruits showing a full range of symptoms should be removed and sampled.

The samples should be packaged and delivered as described for *E. amylovora*.

Additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/43 Pseudomonas syringae *pv.* persicae (EPPO, 2005).

*(6) Xanthomonas arboricola pv. pruni (*Xanthomonadaceae*, EPPO A2 List)*

Symptom description. Symptoms of bacterial spot can be observed on leaves, fruits, twigs and branches (EPPO, 1997).

Symptoms on leaves. On peach leaves, infection is first apparent on the lower surface as small, pale-green to yellow, circular or irregular areas with a light-tan centre. These spots soon become evident on the upper surface as they enlarge, becoming angular and darkening to deep purple, brown or black. The immediate surrounding tissue may become yellow. The diseased areas drop out, usually after darkening in colour, but they may drop out prior to the colour change, giving a shot-hole appearance to the leaf. A dark ring of diseased tissue is often left with the formation of the shot hole. Spots are usually concentrated towards the leaf tip, because the bacteria accumulate in this region in droplets of rain or dew. Bacterial ooze may be associated with the spots. Severely infected leaves turn yellow and drop off, resulting in severe defoliation of trees of susceptible cultivars.

On plum leaves, initial symptoms are angular water-soaked spots, rapidly turning reddish-brown, then dark brown and necrotic, whereas chlorosis is minimal and less apparent than on peach leaves. The necrotic spots frequently perforate, so that the shot-hole effect can be pronounced. On almonds, apricots and cherries, leaf symptoms are similar to those on peach, but are rarely as pronounced.

Symptoms on fruits. On peach fruits, small circular brown spots appear on the surface. They become sunken, the margins are frequently water-soaked and there are often light-green haloes which impart a mottled appearance to the fruit. As fruit enlarge, pitting and cracking occur in the vicinity of the spots. These cracks are often very small and difficult to see, but where heavy infection has occurred on young fruits they can be extensive, severely damaging the surface of the fruit. Gum flow, particularly after rain, may occur from bacterial wounds; this may easily be confused with insect damage. Similar symptoms may appear on apricots and almonds.

On plum fruits, symptoms may be quite varied; large, sunken black lesions are common on some cultivars, while on others only small pit-like lesions occur. On cherries, early fruit infection results in distorted fruit, and bacteria may be found from the epidermis to the stone.

As a general rule, symptoms on fruits appear 3–5 weeks after petal fall and develop until the skin colour changes. Symptoms often occur after hail damage.

Symptoms on twigs. On peach twigs, *X. arboricola* pv. *pruni* occurs on the top portion of overwintering twigs and on water sprouts before green shoots are produced. Initially small, water-soaked, slightly darkened superficial blisters with green shoots extend 1–10 cm parallel to the long axis of the twig and may even girdle it. In this case the tip of the twig may die, while the tissue immediately below the dead area, in which the bacteria are present, is characteristically dark; this is the so-called 'black tip' injury. Twig infections later in the season result in summer cankers, which appear as water-soaked, dark-purplish spots surrounding lenticels. These later dry out and become limited, dark, sunken, circular-to-elliptical lesions with a water-soaked margin.

On plum and apricot twigs and branches, cankers are perennial, in contrast to peach, and continue developing in 2- and 3-year-old twigs. The inner bark is penetrated, resulting in deep cankers which deform and kill twigs.

Sampling and identification. In all *Prunus* species, bacteria can be isolated from symptomatic leaves showing water-soaked angular spots, from immature fruits or from shoots and branches with cankers. The samples should be sent according to the procedure described for *E. amylovora.*

If it is considered necessary to test symptomless mother trees, then 30 twigs from each tree and 100 dormant scion chips should be taken for testing.

Additional information for personnel carrying out diagnostics can be obtained from EPPO Standard PM 7/64 Xanthomonas arboricola *pv.* pruni.

(7) Soil-borne bacteria

Depending on national legislation, fruit plants for planting with roots that have been grown in the open air may be required to be grown at a place of production known not to be infested by quarantine soil-borne bacteria such as *Clavibacter michiganensis* subsp. *sepedonicus* (EPPO A2 List) (Spieckermann & Kotthoff; Davis *et al.*) and *Ralstonia solanacearum*. This should be confirmed by examination of the NPPO records of occurrences and previous visual inspections of relevant host plants carried out at the site.

(E) Viruses

(1) *Plum pox virus* (EPPO A2 List)

Symptom description. Symptoms may appear on leaves, petals, fruits and stones. They are particularly clear on leaves in spring with mild light green discoloration, chlorotic spots, bands or rings, vein clearing or yellowing or even leaf deformation.

On flowers discoloration can occur on petals of some peach varieties.

Mother trees may be allowed to produce fruit and these may show chlorotic spots or lightly pigmented yellow rings or line patterns.

Fruits may become deformed or irregular in shape and develop brown or necrotic small areas and may show internal browning of the flesh and reduced quality. In some cases the diseased fruits drop prematurely from the tree. In general, early varieties have much clearer symptom expression on fruits than late varieties. Stones from diseased apricot fruits show pale rings or spots.

Sampling and identification. Appropriate sample selection is critical for serological or molecular detection. If typical symptoms are present, symptomatic flowers, leaves or fruits should be collected.

In symptomless plants, a standard sample should be taken of 5 shoots or 10 fully expanded leaves collected around the canopy of each individual tree from the middle of each scaffold branch before high temperatures occur at the beginning of summer. Plant material should preferably be selected from the internal structure of the tree.

If the detection method can be demonstrated to be sufficiently sensitive, then larger numbers of leaves can be bulked (e.g. 24 can be collected in order to detect 1 infected leaf in 23 uninfected ones).

Sampling from July to the beginning of September should be avoided in Mediterranean climates.

Samples taken in spring can be of flowers, young shoots or small fruits. Mature leaves can be collected for analysis in the autumn. For certain varieties where symptoms are mostly present on fruit, experience has shown that late inspections focusing on fruit can be relevant.

The samples should be packaged and delivered as described for '*Candidatus* Phytoplasma mali'.

Additional information for personnel carrying out diagnostics and photographs of symptoms are included in EPPO Standard PM 7/32 Plum pox virus (EPPO, 2004).

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

Symptom description. Tomato ringspot virus (ToRSV) does not occur in fruit trees within the EPPO region but does occur occasionally in other hosts and could potentially be introduced in fruit propagating material or infested soil, therefore is included here for information.

Symptoms of ToRSV infection depend on the fruit tree host: all *Prunus* spp. show stem pitting, associated with graft union staining and abnormalities. With peach and cherry it also causes symptoms of a yellow bud mosaic disease (pale green to pale yellow, oblong, feather-edged blotches along the main vein or large lateral veins of the leaves; buds produce rosettes of small and often distorted leaves, with or without mottling, or are pale yellow and later die; fruits may be dwarfed or malformed).

Malus spp. show union necrosis (necrosis of the graft union and symptoms on the tree similar to those following trunk girdling). Symptoms are usually visible in an orchard 4–6 years after planting so could be seen in a mother tree plantation if maintained for this period or longer. Severely affected apple trees may crack and break off at the graft union.

Sampling and identification. Time of testing can be important and should be considered before sampling is initiated. Experience has shown that for woody plants early spring appears to be the most suitable time, since flowers or young leaves can be sampled and tested if no symptoms are present. Samples should be placed in plastic bags (without any paper), inflated slightly and sealed, then packed in strong containers such as cardboard or plastic boxes and padded with paper or similar to prevent movement.

Additional information for personnel carrying out diagnostics and details on identification and symptoms are included in EPPO Standards PM 7/49 Tomato ringspot nepovirus and PM 3/32 Tomato ringspot virus *in fruit trees and grapevine*.

(3) *Beet necrotic yellow vein virus* (EPPO A2 List)

In some countries national legislation may require field-grown fruit plants to have been grown in a field known not to be infested by the soil-borne *Beet necrotic yellow vein virus*. This should be confirmed by previous visual inspection of relevant host plants or by soil testing of fields where such host plants have been previously grown but not inspected.

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

This virus usually occurs in combination or in association with other (non-listed) quality viruses in *P. avium* or *Prunus cerasus* such as *Arabis mosaic virus*, *Cherry leafroll virus*, *Prune dwarf virus* or *Prunus necrotic ringspot virus.* With these it is believed to cause rasp-leaf symptoms in cherry trees, the leaves of which are usually reduced in size, narrow and tough, with abnormally coarse serrations. Depending on varietal sensitivity, enations on the lower surface may be few or numerous, large or small and confined to areas of the leaf close to the midrib. Both types of enations may occur on the same tree, either simultaneously or at different stages of infection.

Any samples taken should be packaged and delivered as described for *Tomato ringspot virus.*

The virus is transmitted by the nematodes *L. elongates* and *L. macrosoma*, so soil from fields to be used should be sampled and tested for these species before planting in order to prevent introduction into clean stock. At the same time this soil sample can also be used for testing for other nematodes, such as *X. diversicaudatum*, that transmit some unlisted quality viruses as included in certification schemes such as *Arabis mosaic virus.* See EPPO Standard PM 7/119 *Nematode extraction* and PM 4/35 *Soil test for virus-vector nematodes in the framework of EPPO Standard PM 4 schemes for the production of healthy plants for planting of fruit crops, grapevine*, Populus *and* Salix, for further details of interest to personnel performing diagnostics.

Details of interest to personnel performing diagnostics on identification and symptoms of *Raspberry ringspot virus* in *Prunus* are included in APS (2011) and in *Quarantine Pests for Europe* (CABI/EPPO, 1997).

Appendix 2 – Short procedure for inspectors

To ensure that inspections during active growth are carried out at the optimum time of year one should take into account the geographical, cultivation and varietal factors of the crop. This ensures the best chance of detecting pathogens prior to autumn senescence which may obscure symptoms or prevent efficient inspection due to the presence of common mildews or other diseases. Where possible, inspections should be undertaken during overcast days because symptoms of viruses and phytoplasmas may be obscured by bright sunlight.

If the inspection is for a specific pathogen, then the visit should take place when symptoms are most likely to be apparent, for example for *E. amylovora* this should be during warm and humid weather so the most appropriate time is between June and October in northern Europe but will generally be earlier in warmer climates. Particular attention should also be made to susceptible mother stock and young plants subject to overhead irrigation.

Inspections for specific pathogens can also be concentrated on more susceptible host species, for example for '*Candidatus* Phytoplasma prunorum' several *Prunus* spp. are tolerant to this pathogen so inspections should concentrate on the more sensitive apricot and Japanese plum varieties to indicate presence in a given area. A similar approach can be made for PPV where some species are more susceptible depending on the host variety or strain of the virus.

On starting the inspections, the correct stock to be examined should be determined using a plan or other documentation and a check of the labels applying to the stock. In general, every row of young trees should be inspected, but this can be varied according to conditions to ensure all material is inspected. Inspection of rootstock beds and hedges is achieved by walking between two rows and inspecting either side to ensure all the stock is inspected. Dependent upon the pathogen being inspected for (e.g. *E. amylovora*), plants in two or three short rows close together may be inspected at the same time. If necessary, the inspector may move across rows to check plants in a neighbouring row. A marker of some sort should be left to ensure to return to the correct location to continue. Symptoms of *E. amylovora* can usually be seen from a few metres away, so where nursery stock is grown in closely spaced rows it will not be necessary to walk each row.

Large mother trees should be inspected individually all around the tree and also 'inside' where the foliage may be more dense or growing under more humid conditions, which may favour development of pathogens.

If freedom of the place of production is required then all host plants in the nursery and its boundary and in the immediate area should be inspected. For example, for *E. amylovora* this would include hawthorn hedges *Crataegus* spp. and trees such as wild *Malus* or *Pyrus*.

In all cases symptoms should be confirmed by laboratory testing and samples can be bulked depending of the sensitivity of the test method and whether results are required for individual stocks or trees, etc. Details of packaging and dispatch can be seen in the section on *E. amylovora* in Appendix 1.

The regulations of some EPPO countries require regular testing of all asymptomatic material used for propagation, and these sampling visits can be combined with visual inspections to save resources.