DOI: 10.1111/epp.12771

PM 3/76 (2) 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. 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. This Standard equally applies to production sites.
Specific approval: First approved in 2015–09. Revision approved in 2021–09.
Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

Trees of the genera *Malus*, *Pyrus*, *Prunus* and *Cydonia* are among the most important fruit trees in the EPPO region. Plants for planting of these trees are produced in the region and are also exported to other parts of the world. Trees in these genera are used to produce fruits like apples, pears, cherries, almonds, apricots and plums.

Plants for planting may potentially carry regulated pests specific to these genera as well as polyphagous or contaminating pests. These may 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 (1) should be free from plant debris, (2) should be free from insect pests at any stage of development and (3) should fulfil the provisions set out in ISPM no. 40 *International movement of growing media in association with plants for planting* (IPPC, 2017).

Consignment freedom can be verified by inspection and testing, where appropriate, before issuing the phytosanitary certificate or a document for internal movement. However, import requirements often specify an inspection of the place of production for plants for planting during the growing period and verification of the efficacy of other phytosanitary measures by plant or soil testing for specified relevant pests.

2 | 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 this Standard are specific to inspection of a place of production 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 example the same quarantine pests are concerned, or for international movement.

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

Inspections at the place of production should be undertaken during the growing period and at least once a year. However, two or three inspections per year are recommended depending on crop history and origin of the plants for planting at the place of production. 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 national legislation, inspection of the whole place of production including inspection of the immediate vicinity may be required for a particular pest. For example, many countries require this for '*Candidatus* Phytoplasma mali' (EPPO A2 List), '*Candidatus* Phytoplasma pyri' (EPPO A2 List) and Plum pox virus (EPPO A2 List).

If the export destination of the stock is known from the onset of production, producers and exporters in the exporting country should be aware of the specific phytosanitary regulation of the importing country. This will enable them to report to the NPPO any suspicious symptoms of the pest relevant for the importing country. If the importing country requires inspections at the place of production during the growing period, these should 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, 2016) 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 reliably detectable by visual examination, the inspection procedure may consist only of lot identification and sampling for laboratory testing. This latter point applies particularly to fruit trees, which are susceptible to a large number of pests that 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 to prevent the introduction or spread of pests during inspection and sampling. Such precautions may include wearing of protective clothes (coat, overshoes, gloves, etc.), changing of gloves between different lots and disinfection of used sampling equipment between different lots following national guidelines.

3 | TYPES OF MATERIAL CONCERNED

This Standard covers plants for planting of the genera *Malus*, *Pyrus*, *Prunus* and *Cydonia*, but at the place of production different types of material may be present. The most common ones are listed here.

3.1 | 'Mother' trees

These are trees of known variety[1] and health status that are 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).

3.2 | 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 and then harvested mechanically before being split into individual rootstocks for planting or for sale. For some species, particularly *Prunus* spp., the material can 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.

3.3 | Seed production trees

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

3.4 | Seedling rootstocks

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

3.5 | Tissue culture

Plants in tissue culture are usually intended for further propagation and the method is used primarily for rootstocks, 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 go unnoticed during micro-propagation.

Inspection of plants in tissue culture at the time of sale or export is difficult to perform and unreliable. It is recommended that this material is inspected before it is propagated in tissue culture or after transplanting into growing medium and growth continued to a stage where symptoms could be detected.

3.6 | Grafted trees (varieties)

These are the final budded or grafted trees grown in the field, greenhouse or a temporary structure, usually for 2 or 3 years, before marketing either as 'bare

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

rooted' plants or in individual pots as finished young trees.

4 | PESTS RECOMMENDED FOR REGULATION AS QUARANTINE PESTS OR REGULATED PESTS IN COUNTRIES IN THE EPPO REGION

This Standard mainly relates to important pests affecting all species and hybrids of *Malus*, *Pyrus*, *Prunus* and *Cydonia*, which are listed in the EPPO A1 and A2 Lists of pests recommend for regulation as quarantine pests. Some of the listed EPPO A2 pests are classified by EPPO countries as Regulated Non-Quarantine Pests (RNQPs). It also covers important pests which are listed in specific EPPO countries even if not mentioned in the EPPO A1 or A2 Lists. For a more comprehensive list of pests associated with species and hybrids of *Malus*, *Pyrus*, *Prunus* and *Cydonia* the EPPO Global Database should be consulted (EPPO, 2021a). Specific pests of *Malus*, *Pyrus*, *Prunus* and *Cydonia* trees (EPPO A1 and A2 Lists and pests listed by specific EPPO countries) are described in Table 1. Polyphagous pests affecting *Malus*, *Pyrus*, *Prunus* and *Cydonia* trees (EPPO A1 and A2 Lists and pests listed by specific EPPO countries) are listed in Table 2. 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. 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 and 2) will therefore need to be revised whenever relevant new quarantine pests are identified.

Details on all these pests can be found in *Quarantine Pests for Europe*, 2nd edition (CABI/EPPO, 1997) and in the book *Viruses and Virus-Like Diseases of Pome and Stone Fruits* (APS, 2011). Additional information, including more recent EPPO datasheets, can be found in the EPPO Global Database (EPPO, 2021a) and in EPPO Standards regarding specific pests or crops. The relevant scientific literature should be consulted for additional up-to-date information.

For plants in growing medium, attention should be paid to nematodes (see Appendix 1, section B), which may act as virus vectors.

For trees grown in open ground, NPPOs may apply additional controls to reduce the risk of moving soilborne pests such as *Globodera* spp. (e.g. *G. pallida* and *G. rostochiensis*), *Synchytrium endobioticum*, *Meloidogyne* spp. and *Phytophthora fragariae*.

TABLE 1 Specific pests of *Malus*, *Pyrus* and *Cydonia* and *Prunus* spp.

Specific pests of	EPPO A1 pests	EPPO A2 pests	Other pests regulated by specific EPPO member countries[a]
Malus	**Fungi:** *Alternaria mali*, *Gymnosporangium juniperi-virginianae*, *Gymnosporangium yamadae*, *Phyllosticta solitaria*, *Gymnosporangium clavipes*, *Gymnosporangium globosum*	**Bacteria (including phytoplasmas):** '*Candidatus* Phytoplasma mali'	**Insects:** *Grapholita funebrana* (Israel Quarantine Pest 2009; Jordan A1 List 2013)
Pyrus* and *Cydonia	**Fungi:** *Gymnosporangium clavipes*, *Gymnosporangium globosum*	**Bacteria (including phytoplasmas):** '*Candidatus* Phytoplasma pyri'	**Fungi:** *Venturia nashicola* (Israel Quarantine Pest 2009; Turkey A1 List 2016; EU (A1 QP (Annex II A 2019)))
Prunus	**Insects:** *Aromia bungii* **Fungi:** *Apiosporina morbosa*, *Phyllosticta solitaria*	**Bacteria (including phytoplasmas):** *Pseudomonas syringae* pv. *persicae*, *Xanthomonas arboricola* pv. *pruni* **Viruses:** Plum pox virus	**Insects:** *Grapholita funebrana* **Bacteria (including phytoplasmas):** '*Candidatus* Phytoplasma prunorum' (Israel (2009), Tunisia (2012) Quarantine Pest; Jordan A1 List 2013; Turkey A2 List 2016; EU RNQP 2019) **Viruses:** Cherry necrotic rusty mottle virus (Israel Quarantine Pest 2009; Jordan A1 List 2013; Turkey A1 List 2016; EU RNQP 2019)
	Bacteria (including phytoplasmas): '*Candidatus* Phytoplasma phoenicium', '*Candidatus* phytoplasma pruni', Peach yellows phytoplasma, Peach rosette phytoplasma **Viruses:** American plum line pattern virus, Peach mosaic virus		

[a]Countries are listed on first mention of the species.

TABLE 2 Polyphagous pests of *Malus*, *Pyrus* and *Cydonia*, and *Prunus* spp.

Polyphagous pests of	EPPO A1 pests	EPPO A2 pests	Other pests regulated by specific EPPO member countries[a]
Malus	**Insects:** *Anoplophora glabripennis*, *Conotrachelus nenuphar* *Choristoneura rosaceana*, *Diabrotica speciosa*, *Grapholita packardi*, *Grapholita prunivora*, *Malacosoma americanum*, *Saperda candida*	**Insects:** *Anoplophora chinensis*, *Comstockaspis perniciosa*, *Lepidosaphes ussuriensis*, *Lopholeucaspis japonica*, *Lymantria mathura*, *Malacosoma parallela*, *Platynota stultana*, *Popillia japonica*, *Spodoptera littoralis*, *Trichoferus campestris*	**Insects:** *Anarsia lineatella* (Azerbaijan A1 List 2007), *Anthonomus quadrigibbus* (Turkey A1 List 2016; EU (A1 QP (Annex II A 2019)), *Epiphyas postvittana* (Jordan A1 list 2013; Morocco Quarantine Pest 2018), *Grapholita molesta* (Israel (2009) and Tunisia (2012) Quarantine Pest), *Halyomorpha halys* (Kazakhstan (2017) and Ukraine (2019) A1 List), *Hyphantria cunea* (Azerbaijan (2007), Georgia (2018), Russia (2014), Ukraine (2019) A2 List; Belarus (1994), Israel (2009), Tunisia (2012) Quarantine pest; Jordan (2013), Uzbekistan (2008) A1 List), *Lymantria dispar* (Israel Quarantine Pest 2009; Azerbaijan (2007), Georgia (2018) A1 Pest; Russia A2 List), *Parabemisia myricae* (Jordan A2 List 2018; Georgia A1 List 2018; EU RNQP 2019), *Pseudococcus calceolariae* (Israel (2009), Belarus (1994) Quarantine Pest; Azerbaijan (2007), Uzbekistan (2008) A1 list; Georgia (2018) A2 list).
	Fungi: *Phymatotrichopsis omnivora*	**Fungi:** *Monilinia fructicola*	**Fungi:** *Botryosphaeria kuwatsukai* (Tunisia (2012), Israel (2009) Quarantine Pest; EU (A1 QP (Annex II A 2019)), Turkey A1 List), *Monilinia fructigena* (Jordan A2 List), *Neonectria ditissima* (Israel Quarantine Pest 2009; EU RNQP)
	Viruses: Cherry rasp leaf virus	**Bacteria (including phytoplasmas):** *Erwinia amylovora*	
Pyrus* and *Cydonia	**Insects:** *Aleurocanthus woglumi*, *Conotrachelus nenuphar*, *Choristoneura rosaceana*, *Grapholita packardi*, *Grapholita prunivora*, *Saperda candida*	**Insects:** *Comstockaspis perniciosa*, *Lopholeucaspis japonica*, *Platynota stultana*, *Trichoferus campestris*	**Insects:** *Anarsia lineatella*, *Anthonomus quadrigibbus*, *Epiphyas postvittana*, *Grapholita molesta*, *Halyomorpha halys*, *Hyphantria cunea*, *Lymantria dispar*, *Parabemisia myricae*, *Pseudococcus calceolariae*
		Fungi: *Monilinia fructicola*	**Fungi:** *Botryosphaeria kuwatsukai*, *Monilinia fructigena*, *Neonectria ditissima*
		Bacteria (including phytoplasmas): *Erwinia amylovora*	
Prunus	**Insects:** *Conotrachelus nenuphar*, *Choristoneura rosaceana* *Diabrotica speciosa Grapholita packardi*, *Grapholita prunivora*, *Homalodisca vitripennis*, *Malacosoma americanum*, *Saperda candida*	**Insects:** *Comstockaspis perniciosa*, *Euwallacea fornicates*, *Frankliniella occidentalis*, *Malacosoma parallela*, *Lopholeucaspis japonica*, *Platynota stultana*, *Popillia japonica*, *Trichoferus campestris*	**Insects:** *Anarsia lineatella*, *Anthonomus quadrigibbus*, *Epiphyas postvittana*, *Grapholita molesta*, *Halyomorpha halys*, *Hyphantria cunea*, *Lymantria dispar*, *Parabemisia myricae*, *Pseudococcus calceolariae*
	Fungi: *Phymatotrichopsis omnivora*	**Fungi:** *Monilinia fructicola*	**Fungi:** *Monilinia fructigena*
	Viruses: Cherry rasp leaf virus, Peach rosette mosaic virus	**Bacteria (including phytoplasmas):** *Erwinia amylovora*, '*Candidatus* Phytoplasma solani', *Xylella fastidiosa*	**Viruses:** Cherry leaf roll virus (Tunisia (2012) Israel (2009), Norway (2012) Quarantine Pest; Jordan A1 List 2013; Turkey A2 List 2016; EU RNQP 2019); Tomato black ring virus (Israel (2009), Norway (2012), Tunisia (2012) Quarantine Pest; Jordan A2 List (2013), Turkey A2 List (2016); EU RNQP 2019)
		Viruses: Raspberry ringspot virus, Tomato ringspot virus	

[a]Countries are listed on first mention of the species.

5 | IDENTIFICATION OF LOTS

General background information on lot identification 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). 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. When information on different batches of grafted trees or origins of budwood or rootstocks is available this should also be taken into account.

6 | SELECTION OF PLANTS FOR INSPECTION AND SAMPLING FOR LABORATORY TESTING

This section contains guidance on 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 documentation.

6.1 | Selection of plants for 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). 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 inspection, either alone or in combination with sampling for laboratory testing. Testing may be performed on symptomatic material or on asymptomatic material to detect a latent infection which may be present.

Depending on the specific requirements (including those of importing countries) inspections may target the place of production and its vicinity, the place of production alone or a consignment of plants.

6.1.1 | Inspection of the place of production

The number of plants that should 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, 2009). For example, if 294 plants are inspected from a lot of 10,000 this provides 99% confidence of detecting evident symptoms present in 1% of the plants, provided that symptoms are seen and are uniformly distributed, and the plants are selected at random or higher-risk plants are targeted, e.g. those at the outer edge of the nursery. This may be sufficient as part of a national survey. If 3689 plants are inspected from a lot of 10,000 this provides 99% confidence of detecting evident symptoms present in 0.1% of the plants, provided the symptoms are seen and are uniformly distributed, and the plants are selected at random. This level of inspection may be more appropriate, for example, in supporting the issue of a phytosanitary certificate.

For small lots (fewer than 1000 plants), all plants should be inspected.

For a number of fruit tree pathogens, for example Plum pox virus (EPPO A2 List) and '*Candidatus* Phytoplasma prunorum' [formerly EPPO A2; currently EU (RNQP 'Annex IV'), also listed in other countries], the regulations for some countries, including EU members, require that the place of production and susceptible plants in its immediate vicinity should be subject to inspection. Therefore, all susceptible species at the place of production and relevant plants around the place of production should also be inspected. The definition of '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 resources to carry out inspections.

6.2 | Sampling for laboratory testing (general aspects)

6.2.1 | 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 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 may mean that phytosanitary measures shall be applied to ensure that the plants do not present any risk of spreading the pest or may mean that plants are no longer allowed to be marketed. 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.

6.2.2 | Sampling of asymptomatic material

For the purposes of declaring a pest-free place or site of production or a pest-free area (PFA) or for export purposes, sampling of asymptomatic plants and vectors may be required to detect latent or hidden infections. If varieties are present that are known to be susceptible to latent infection or origins include potential high risk or areas with high vector populations sampling for laboratory testing may be required.

Sometimes a country's legislation may specify that plants are derived from tested material, for example for '*Candidatus* Phytoplasma mali' (EPPO A2 List) and Plum pox virus (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 (see ISPM 31 for the number of plants that should be selected for inspection) and lengths of parent rootstock beds so that propagating material is only taken from those plants with a known history of testing and found to be pest free during the required 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[2] Methodologies for sampling of consignments (IPPC, 2009). Further details are given for individual pests in Appendix 1.

Information to determine the sample size for asymptomatic material is largely unknown and depends also on the pest to be detected. In the event of positive results it should be possible to trace back all samples 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.

6.3 | Selection of plants for 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.

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

Plants in a nursery will generally be grown 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 between pest species depending on the optimum time for expression of symptoms. In general, plants should be in active growth and have sufficient time after breaking dormancy to display symptoms if an 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.

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

For the inspection procedure, a general inspection of the place or lot should be carried out first and any noticeable areas with poor growth or plants with obvious symptoms (e.g. paler or with other types of 'patches') should be examined first. If none are apparent, a representative number of plants should be thoroughly examined [see 'Selection of plants for 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 blotches or pale areas and veinal or interveinal necrosis on leaves, lesions, cankers, necrosis, wilting and die back on stems, and spots and lesions on fruits. Plants may be lifted out of containers or dug up to inspect the roots for necrosis and/or poorly developed roots (e.g. due to *Phytophthora* or nematodes). This could be risk targeted by above-ground symptoms or by taking a representative sample.

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. Plum pox virus will sometimes show symptoms on the fruits).

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

ACKNOWLEDGEMENTS

This Standard was first drafted by Mr P. Reed of the Department for Environment, Farming and Rural Affairs (Defra) (GB). The Standard was updated in 2021 by Mr D. Eyre (Defra). The Panel on Phytosanitary Inspections reviewed the revised Standard.

REFERENCES

Aldrich JH, Gould AB & Martin FG (1992) Distribution of *Xylella fastidiosa* within roots of peach. *Plant Disease* 76, 885–888.

Alston DG, Murray M & Reding ME (2011) San Jose Scale (*Quadraspidiotus perniciosus*). *Utah Pests, Fact Sheet* 153(6), 5.

Anderson H, Eyre D & Giltrap N (2016) Plant pest factsheet: Citrus longhorn beetle, *Anoplophora chinensis*. https://planthealthportal.defra.gov.uk/assets/factsheets/CLB-Plant-Pest-Factsheet-update-May2016v5.pdf [Accessed 6 November 2020].

APS (2011) *Viruses and Virus-Like Diseases of Pome and Stone Fruits*. American Phytopathological Society, St. Paul (USA).

Burke HR & Anderson RS (1989) Systematics of species of *Anthonomus* Germar previously assigned to *Tachypterellus* Fall and Cockerell (Coleoptera: Curculionidae). *Annals of the Entomological Society of America* 82, 426–437.

CABI (2019) *Popillia japonica* (Japanese beetle) datasheet. CABI http://www.cabi.org/isc/datasheet/43599 [Accessed on 1 February 2016].

CABI. (2021) Nectria canker (apple, pear) *Neonectria ditissima*. CABI Plantwise Datasheet, https://www.plantwise.org/knowledgebank/datasheet/35964 [Accessed 6 November 2020].

CABI/EPPO (1996a) Pear decline phytoplasma. In *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (UK).

CABI/EPPO (1996b) Peach rosette phytoplasma. In *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (UK).

CABI/EPPO (1996c) Peach yellows phytoplasma. In *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (UK).

CABI/EPPO (1996d) Plum American line pattern ilarvirus. In *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (UK).

Quarantine Pests for Europe, 2nd edn (ed. Smith IM, McNamara DG, Scott PR & Holderness M), CAB International, Wallingford (UK).

CFIA (2019) *Lymantria dispar* (Gypsy moth) – factsheet. https://www.inspection.gc.ca/plant-health/plant-pests-invasive-species/insects/gypsy-moth/fact-sheet/eng/1330355335187/1335975909100. [Accessed 4 November 2020].

Desvignes JC (1976) The virus diseases detected in greenhouse and in field by the peach seedling GF 305 indicator. *Acta Horticulturae* 67, 315–323.

Dias HF (1975) *Peach rosette mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 150*. Association of Applied Biologists, Wellesbourne, UK.

Dickler E (1982) The distribution of the quarantine pests *Anarsia lineatella* Zell. and *Grapholita molesta* Busck in the Federal Republic of Germany. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 34, 145–152.

EFSA (2017) Pest categorisation of *Venturia nashicola*. Available at: https://doi.org/10.2903/j.efsa.2017.5034 [Accessed 6 November 2020].

EFSA (2019) Pest survey card on *Globodera rostochiensis* and *Globodera pallida*. *EFSA Supporting Publications* 16: 1566E. [Accessed 6 November 2020].

EPPO (1999) PM 4/27 Certification scheme for pathogen-tested material of *Malus, Pyrus* and *Cydonia*. *EPPO Bulletin* 29, 239–252.

EPPO (2001a) PM 4/29 Certification scheme for pathogen-tested material of cherry. *EPPO Bulletin* 31, 447–461.

EPPO (2001b) PM 4/30 Certification scheme for pathogen-tested material of almond, apricot, peach and plum. *EPPO Bulletin* 31, 463–478.

EPPO (2002) PM 7/11 Diagnostic protocol for regulated pests – *Frankliniella occidentalis*. *EPPO Bulletin* 32, 241–292.

EPPO (2004) EPPO Standard PM 7/32 (1) Diagnostic protocol for regulated pests: Plum pox virus. *EPPO Bulletin* 34, 155–157.

EPPO (2005a) PM 7/54 *Lopholeucaspis japonica*. *EPPO Bulletin* 35, 271–273.

EPPO (2005b) *Lymantria mathura*. *EPPO Bulletin* 35, 464–467.

EPPO (2005c) PM 7/43 (1) Diagnostic protocol for regulated pests: *Pseudomonas syringae* pv. *persicae*. *EPPO Bulletin* 35, 271–273.

EPPO (2005d) American plum line pattern ilarvirus. *EPPO Bulletin* 36, 157–160.

EPPO (2005e) PM 7/49(1) Tomato ringspot nepovirus. *EPPO Bulletin* 35, 313–318.

EPPO (2006a) PM 7/69 *Lepidosaphes ussuriensis*. *EPPO Bulletin* 36, 165–166.

EPPO (2006b) PM 7/73 *Gymnosporangium* spp. (non-European). *EPPO Bulletin* 36, 441–446.

EPPO (2006c) PM 7/64 (1) *Xanthomonas arboricola* pv. *Pruni*. *EPPO Bulletin* 43, 471–495.

EPPO (2009a) PM 3/72 (2) Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification. *EPPO Bulletin* 39, 260–262.

EPPO (2009b) 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*. *EPPO Bulletin* 39, 284–288.

EPPO (2013a) EPPO Standard PM 7/20 (2) Diagnostic protocol for regulated pests: *Erwinia amylovora*. *EPPO Bulletin* 43, 21–45.

EPPO (2013b) PM 7/119 (1) Nematode extraction. *EPPO Bulletin* 43, 471–495.

EPPO (2015a) Pest risk analysis for the *Ambrosia* beetle *Euwallaceae* sp. Available at: https://gd.eppo.int/taxon/XYLBFO/documents [Accessed on 1 February 2020].

EPPO (2015b) PM 7/124 (1) *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania*. *EPPO Bulletin* 45, 410–444.

EPPO (2016) PM 9/21 (1) *Popillia japonica*: procedures for official control. *EPPO Bulletin* 46, 543–555.

EPPO (2017a) PM 7/40 (4) *Globodera rostochiensis* and *Globodera pallida*. *EPPO Bulletin* 47, 174–197.

EPPO (2017b) Diagnostic protocol PM 7/95(2) *Xiphinema americanum* sensu lato. *EPPO Bulletin* 47, 198–210.

EPPO (2017c) PM 7/28 (2) *Synchytrium endobioticum*. *EPPO Bulletin* 47, 420–440.

EPPO (2018) PM 3/84 (1) Inspection of places of production for '*Candidatus* Phytoplasma pyri'. *EPPO Bulletin* 48, 323–329.

EPPO (2019) PM 7/24 (4) *Xylella fastidiosa*. *EPPO Bulletin* 49, 175–227.

EPPO (2020a) PM 7/18 (3) *Monilinia fructicola*. *EPPO Bulletin* 50, 5–18.

EPPO (2020b) PM 7/78 (2) *Verticillium nonalfalfae* and *V. dahliae*. *EPPO Bulletin* 50, 462–476.

EPPO (2020c) Standard PM 7/62 (3) '*Candidatus* Phytoplasma mali', 'Ca. P. pyri' and 'Ca P. prunorum'. *EPPO Bulletin* 50, 69–85.

EPPO (2020d) PM 3/81(2) Inspection of consignments for *Xylella fastidiosa*. *EPPO Bulletin*, 50, 401–414.

EPPO (2020e) PM 3/82(2) Inspection of places of production for *Xylella fastidiosa*. *EPPO Bulletin*, 50, 415–428.

EPPO Global Database (2021a). Available at: https://gd.eppo.int/

EPPO (2021b) Cherry rasp leaf virus. EPPO datasheets on pests recommended for regulation. Available at: https://gd.eppo.int.

Fleming WE (1972) Biology of the Japanese beetle. USDA Technical Bulletin 1449, Washington, DC

Forest Research (2020) Gypsy moth (*Lymantria dispar*). https://www.forestresearch.gov.uk/tools-and-resources/pest-and-disease-resources/gypsy-moth-lymantria-dispar/. [Accessed 4 November 2020].

Garonna AP, Nugnes F, Epinosa B, Griffo R & Benchi D (2013) *Aromia bungii*, nuovo tarlo asiatico ritrovato in Camapania [*Aromia bungii*, a new Asian worm found in Campania]. *Informatore Agrario* 69, 60–62.

Gressitt JL (1942) *Destructive long-horned beetle borers at Canton, China. Special Publication 1*. Lingnan Natural History Survey and Museum, Lingnan University, Canton, China: 1–60.

Hopkins DL (1981) Seasonal concentrations of Pierce's disease bacterium in grapevine stems, petioles, and leaf veins. *The American Phytopathological Society* 71, 415–418.

IPPC (1995) *Requirements for the establishment of pest free areas. ISPM no. 4*. IPPC Standards, IPPC Secretariat, Rome (IT).

IPPC (1999) *Requirements for the establishment of pest free places of production and pest free production sites. ISPM no. 10*. IPPC Standards, IPPC Secretariat, Rome (IT).

IPPC (2009) *Methodologies for sampling of consignments. ISPM no. 31* IPPC Standards, IPPC Secretariat, Rome (IT).

IPPC (2016) *Glossary of phytosanitary terms. ISPM no. 5*. IPPC Standards, IPPC Secretariat, Rome (IT).

IPPC (2017) *International movement of growing media in association with plants for planting. ISPM no. 40*. IPPC Standards, IPPC Secretariat, Rome (IT).

ISHS (1980) Detection of viruses and other graft-transmissible virus-like diseases of fruit trees. *Acta Phytopathologica Academiae Scientiarum Hungaricae* 15, 407–413.

ISHS (1983) Detection of virus and virus-like diseases of fruit trees. *Acta Horticulturae.* 130, 319–326.

Jones AT (1987) Cherry rasp leaf virus in Rubus. In: *Virus diseases of small fruits* (ed. Converse RH). Agriculture Handbook No. 631, 241–242. US Department of Agriculture, USA.

Kato K (1973) Studies on Physalospora canker of Japanese pear with special reference to ecology and control. Special research Bulletin of the Aichi-Ken Agricultural Research Centre Nagakute, Aichi, Japan, Series B, 1-70.

Klos EJ. (1964) How to recognize and control black knot of plum and cherry. Extension Bulletin, Michigan State University E-469, 2 pp.

Koganezawa H & Sakuma T (1984) Causal fungi of apple fruit rot. *Bulletin of the Fruit Tree Research Station. C (Morioka)* 11, 49–62.

Larsen HJ & Oldfield GN (1995) Peach mosaic. In *Compendium of Stone Fruit Diseases*. APS, St Paul (US).

Malumphy C, Anderson H & Korycinska A (2016) Plant pest factsheet: Japanese beetle, *Popillia japonica*. https://planthealthportal.defra.gov.uk/assets/factsheets/popillia-japonica-factsheet.pdf. [Accessed 26 January 2021].

Malumphy C, Korycinska A & Ostoja-Starzewski J (2012) Differentiating *Anoplophora* longhorn beetle damage from that of native wood-boring insects. https://planthealthportal.defra.gov.uk/assets/factsheets/anplophoraLonghornBeetle.pdf. [Accessed 6 November 2020].

Mircetich SM, Lowe SK, Moller WJ & Nyland G (1976) Etiology of almond leaf scorch disease and transmission of the causal agent. *Phytopathology* 66, 17–24.

Mizell RF, Andersen PC, Tipping C & Brodbeck BV. (2015) *Xylella fastidiosa* Diseases and their Leafhopper Vectors available online: http://edis.ifas.ufl.edu/pdffiles/IN/IN17400.pdf [Accessed on 26 February 2016]

Oloumi-Sadeghi H & Esmaili M (1983) The moth population study of peach twig borer (*Anarsia lineatella* Zeller) in Ghazvin and Karadj from 1975–80. *Entomologie et Phytopathologie Appliquees* 50(1/2), pp. 1–16; en? pp.

Osman F, Rwahnih M & Rowhani A (2017) Real-time RT-qPCR detection of cherry rasp leaf virus, cherry green ring mottle virus, cherry necrotic rusty mottle virus, cherry virus A and apple chlorotic leaf spot virus in stone fruits. *Journal of Plant Pathology* 99, 279–285.

Ostojá-Starzewski JC, Malumphy Eyre D & Anderson H. (2017) Plant pest factsheet: red-necked longhorn beetle, *Aromia bungii*. 2017. https://planthealthportal.defra.gov.uk/assets/factsheets/Aromia-bungii-Defra-PP-Factsheet-May-2017-2.pdf. [Accessed 23 October 2019].

Pine TS. (1976) Peach mosaic. In Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. USDA Agriculture Handbook no. 437, 61-70. USDA Washington (US).

Ramsdell DC (1995) Peach rosette mosaic virus. In *Compendium of Stone Fruit Diseases*(eds. Ogawa JM, Zehr EI, Bird GW, Ritchie DF, Uriu K & Uyemoto JK), p. 70, American Pytopathological Society Press, St. Paul, USA.

Redak RA, Purcell AH, Lopes JRS, Blua MJ, Mizell RF III & Anderson PC (2004) The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annual Review of Entomology* 49, 243–270.

Russo E, Nugnes F, Vicinanza F, Garonna AP & Bernardo U (2020) Biological and molecular characterization of *Aromia bungii* (Faldermann, 1835) (Coleoptera: Cerambycidae), an emerging pest of stone fruits in Europe. *Scientific Reports* 7112, 10.

Salem NM, Tahzima T, Odeh S, Abdeen AO, Massart S, Goedefroit T & De Jonghe K (2020) First report of '*Candidatus* Phytoplasma solani' infecting plum (*Prunus domestica*) in Jordan. *Plant Disease* 104, 563.

Sourakov A & Paris T (2011) University of Florida featured creatures: fall webworm, *Hyphantria cunea*. http://entnemdept.ufl.edu/creatures/trees/moths/fall_webworm.htm. [Accessed 4 November 2020].

St-Pierre RG & Lehmkuhl DM (1990) Phenology of *Hoplocampa montanicola* Rohwer (Tenthredinidae) and *Anthonomus quadrigibbus* Say (Curculionidae) on their host plant *Amelanchier alnifolia* Nutt. (Rosaceae) in Saskatchewan. *Canadian Entomologist* 122(9–10), 901–906.

Vail KM, Hale F, Williams HE & Mannion CM (1999) The Japanese beetle and its control. University of Tennessee Extension PB 946.

USU Extension (2021) Western flower thrips. https://extension.usu.edu/pests/ipm/notes_ag/fruit-west-flower-thrips. Accessed 26 January 2021.

Van der Zwet T & Beer S (1995) Fire blight – its nature, prevention and control. A Practical Guide to Integrated Disease Management.

Van der Zwet T & Keil HL (1979) Fireblight: a bacterial disease of rosaceous plants. USDA Agriculture Handbook No. 510.

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 (CABI/EPPO, 1997) or in more recent EPPO datasheets via the EPPO Global Database (EPPO, 2021a). Illustrations are available in the EPPO Global Database (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) *Anarsia lineatella* (Azerbaijan A1 List), peach twig borer

Symptom description

Prunus is a major host. Young larvae bore into buds and developing shoots causing them to wilt and die (Figure 1). When populations are high, spring larval feeding can cause substantial damage to trees. Larvae of the summer generations attack the fruit, usually making several entry holes near the stem end. Damaged fruit and twigs exude gum.

FIGURE 1 Larva in tender shoot of almond tree. Photo: EPPO Global Database. Courtesy: Carlos Lozano Tomás, Centro de Sanidad y Certificación Vegetal, Gobierno de Aragón (ES)

Sampling and identification

Anarsia lineatella have two to four generations a year depending on climate (Dickler, 1982; Oloumi-Sadeghi & Esmaili, 1983). Adult moths have a wingspan of 14–16 mm, with light and dark grey mottled wings. There are scales on the front of the head giving it a pointed appearance. Young larvae are pale with light brown rings and a black head. Older larvae are chocolate brown with a dark-brown head and prothorax. Mature larvae are roughly 10–12 mm long. Adults can be caught in pheromone traps. Laboratory examination of genitalia is needed to confirm the identification of specimens.

Larvae can be sent alive with host plant material in an airtight and secure labelled container, except if the phytosanitary risk is high. In that case, specimens can be sent with the host plant material in 70% ethanol. If adults are collected, they can be placed in hermetic containers before being sent to the laboratory. Care should be taken to preserve wings that are very fragile.

(2) *Anoplophora chinensis* (EPPO A2 List) and *Anoplophora glabripennis* (EPPO A1 List), citrus longhorn beetle and Asian longhorn beetle

Symptom description

The most obvious symptoms of *A. chinensis* damage are adult exit holes, which are typically 6–11 mm in diameter and are generally found towards the base of trunks and exposed roots. These holes are circular and on smooth-barked trees resemble drilled holes. *A. glabripennis* create similar emergence holes, but they are generally found higher up on the trunk or branches of host trees. Other less obvious symptoms include scars/slits on the bark at the site that eggs have been laid and piles of frass (sawdust like droppings). These will generally be towards the base of the trunk or on exposed roots for *A. chinensis* and higher up the tree for *A. glabripennis* (Anderson et al., 2016).

The tunnels created by *A. chinensis* and *A. glabripennis* larvae are linear, circular in cross-section and located within the pith and vascular layers of the host trunk, branches or roots. As the larvae grow, the tunnels become progressively wider, before a pupal chamber is formed in which the larvae pupate. The presence of the pupal chamber may be indicated by a bulge in the bark. As the longhorn beetle larvae feed they back-fill their larval tunnel with their own sawdust-like waste amongst which can sometimes be found cast larval skins and their dark-brown sclerotized mandibles. Several tunnels may occur in the same tree. Piles of the sawdust-like waste may collect at the base of infested trees (Malumphy et al., 2012).

Sampling and identification

Adult beetles of both species are large and black with variable white markings (Figures 2 and 3). Their antennae, which are longer than their bodies (between 1.2 and 2 times the body length) are particularly distinctive and are black with white/light blue bands. The adults can be trapped on pheromone traps although traps are not as efficient for *Anoplophora* as they are for some other pests such as Lepidoptera. To take samples of juvenile stages it may be necessary to cut down and split open potentially infested trees. For *A. chinensis* it may be necessary to pull up the stump of the tree and uproot major roots to detect the pest. The eggs are about 5–7 mm in length, off-white and oblong. Just before hatching, they turn yellowish-brown. The larva are legless grubs up to 50 mm long when fully grown. They are creamy white, with a chitinized brown mark on the prothorax. Collected adults can be transferred to 70% ethanol before being sent to the laboratory for identification. Larvae can be sent alive with host plant material in airtight and secure containers, or alternatively specimens can be sent in 70% ethanol.

FIGURE 2 *Anoplophora chinensis* adult. Photo: EPPO Global Database. Courtesy: M. Maspero, Fondazione Minoprio, Como (IT)

FIGURE 3 *Anoplophora glabripennis* adult. Photo: EPPO Global Database. Courtesy: Franck Hérard, European Biological Control Laboratory, Montferrier-sur-Lez (FR)

(3) *Anthonomus quadrigibbus* (Turkey A1 List; EU (A1 QP (Annex II A))), apple curculio

Symptom description

Malus is a major host. Feeding and oviposition by *A. quadrigibbus* cause misshapen and undersized fruit. The first signs of injury are usually tiny punctures of 0.5 mm diameter and often 2.5 mm deep (St-Pierre & Lehmkuhl, 1990). Punctures made for oviposition are covered in a pellet of frass. These punctures lead to deep funnel-shaped pits as the fruit grows. Mature fruit can contain larvae, pupae and adults. Infested fruit generally drop prematurely. Adult feeding on mature fruit can cause collapsed brown spots, which coalesce to areas of around 2.5 cm in diameter.

Sampling and identification

The adult beetles are 5–11 mm long including a rostrum of 2.5–5.5 mm (Burke & Anderson, 1989). They are brown and have an antennal club which is as long or longer than the six preceding segments of the funicle (the part of the antenna between the scape and the club) combined. In North American, adults overwinter in the ground litter and emerge in May when the ground temperature reaches around 16°C. They initially feed on leaf petioles and flowers, and can then been found on developing fruit. Yellow sticky traps can be used to capture flying adults. Collected adults can be killed and transferred to 70% ethanol before being sent to the laboratory for identification.

(4) *Aromia bungii* (EPPO A1 List), peach borer

Symptom description

Aromia bungii cause economic damage to apricot, cherry, peach, plum and ornamental *Prunus* species. Mature larvae are 42–52 mm long, pale yellowish-white, broadest across the thorax, with body segments which taper towards the abdominal apex. Pupae are light yellow, 22–38 mm long, becoming darker as the adult develops legs and long coiled antennae form. Adults (Figure 4) are 24–33 mm long, elongated and shiny blue-black except for the pronotum, which is usually a distinctive red colour, although some *A. bungii* have a black pronotum (Ostojá-Starzewski et al., 2017; Russo, 2020).

Sampling and identification

Young larvae create small galleries under the bark of host trees which may be detectable. Larger larval tunnels may be seen in sap wood of the trunk and in larger branches (Gressitt, 1942; Garonna et al., 2013). The larvae push large amounts of frass (pellets of sawdust-like waste) through exit holes which may be visible on the outside or base of host trees (Figure 5). If adults cannot be found it may be necessary to destructively sample

FIGURE 4 *Aromia bungii* adult. Photo: EPPO Global Database. Courtesy: Raffaele Griffo, Plant Health Service of Campania Region, Napoli (IT)

FIGURE 5 Damage caused by *Aromia bungii* on a plum tree (*Prunus domestica*). Photo: EPPO Global Database. Courtesy: Daniela Benchi, Plant Health Service of Campania Region, Napoli (IT)

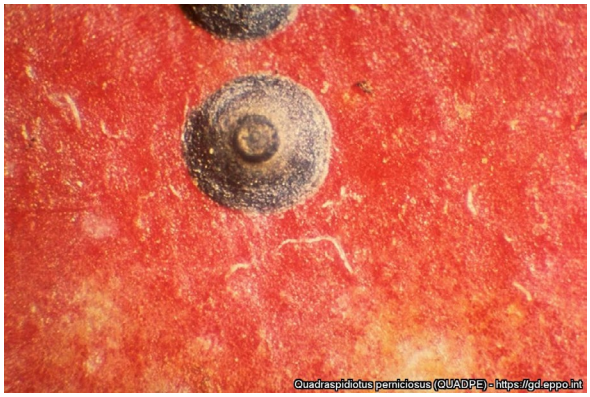

FIGURE 6 Close up of adult female *C. perniciosa*, showing circular grey scale. Photo: EPPO Global Database. Courtesy Biologische Bundesanstalt (DE)

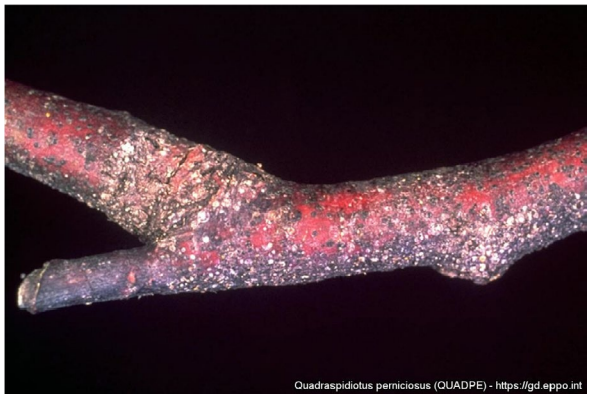

FIGURE 7 Severe infestation of apple branch. Photo: EPPO Global Database. Courtesy: Biologische Bundesanstalt (DE) [Colour figure can be viewed at wileyonlinelibrary.com]

host trees by removing bark or cutting through the trunk to reveal galleries and larvae. Samples of insects should be submitted to a laboratory in dry sealed plastic containers. Any larvae, pupae or adults should be sent in individual pots to prevent individuals attacking others.

(5) *Comstockaspis perniciosa* (EPPO A2 List), San Jose scale

Symptom description

Comstockaspis perniciosa is a polyphagous pest with *Malus*, *Prunus* and *Pyrus* being major hosts. 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. 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 (Figures 6 and 7) should be removed or sections cut away with the scales still attached and sent to the laboratory in a dry condition in plastic containers. The scale covering of females is grey, circular, slightly raised, often with a ringed appearance from moulting, and can reach 2 mm in diameter. Adult males are winged and can be caught in pheromone traps (Alston et al., 2011).

(6) *Epiphyas postvittana* (Jordan Quarantine Pest), leaf roller moth

Symptom description

Malus is a major host. 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

The adults are variable in colour and may be confused with other leaf roller moths and similar species. 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. 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. Sampling should follow the methods described for *Anarsia lineatella*.

FIGURE 8 *Frankliniella occidentalis* adult. Photo: EPPO Global Database. Courtesy: Blandine Delbourse, Point of Entry Roissy CDG airport (FR)

(7) *Euwallacea fornicatus* (EPPO A2 List), tea shot-hole borer

Symptom description

Prunus is a host. *Euwallacea fornicatus sensu lato* is a complex of *Ambrosia* beetles which associate with symbiotic fungi (EPPO, 2015a). Each host tree shows different symptoms, mostly depending on the response to the fungus infection. Attack symptoms including staining, sugary exudate, gumming and/or frass may be noticeable before the tiny beetles are observed. Beneath or near these symptoms, the beetle's entry/exit holes may also be seen. The abdomen of the female beetle can sometimes be seen sticking out of the hole (EPPO, 2015a).

Sampling and identification

Adult females are 1.9–2.5 mm long and 2.3 times as long as wide. Males are smaller. Eggs are approximately 0.3 mm long. If adults cannot be found it may be necessary to destructively sample host trees by removing bark or cutting through the trunk to reveal galleries and larvae. Samples should be submitted to a laboratory in dry, sealed plastic containers.

Collected adults can be killed and transferred to 70% ethanol before being sent to the laboratory for identification. Pupae can be sent alive with host plant material in airtight and secure container except if the phytosanitary risk is high. In that case, specimens can be sent with the host plant material in 70% ethanol.

(8) *Frankliniella occidentalis* (EPPO A2 List), alfalfa thrips

Symptom description

Frankliniella occidentalis (Figure 8) 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 (they are routinely attracted to bright floral colours, particularly white, blue and yellow) 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 therefore be transported with any traded material.

Symptoms of infestation, caused by adult oviposition and feeding damage by adults and nymphs, can be areas of silvery discoloration of the upper leaf surface, speckling and halo spotting on leaves, discoloration and scarring of open blooms and petals, and distortion of newly developed fruit. *F. occidentalis* can cause 'pansy spot' symptoms (a group of rounded blotches) on apple fruit and white netting or russetting on nectarines (USU Extension, 2021).

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

(9) *Grapholita molesta* (Quarantine Pest Belarus, Israel, Tunisia), oriental fruit moth

Symptom description

Malus, *Prunus* and *Pyrus* are hosts. First-generation larvae are mostly found in buds and shoots of peaches, but occasionally also on shoots of apricots, plums, almonds, cherries, apples, pears and quinces. In young trees when terminal twigs are attacked, several lateral shoots will appear below them and grow rapidly. Infested trees can become somewhat bushy after persistent attacks. Severe attacks on shoots of recently budded peaches result in crooked stems.

In harvested peaches there are two distinct types of injury. One is caused by larvae that have abandoned the twigs, feeding on, or entering into, the side of the fruit early in the season when the fruit is small. The second type of damage is caused by entrance at the stem and occurs when the fruit is almost fully grown. This injury is caused by newly hatched larvae that go directly to the fruit. It can be difficult to spot the presence of maggots in the fruit during external examination.

Sampling and identification

Final-instar larvae are approximately 10–12 mm in length with a pinkish abdomen and large pale pinacula (flattened sclerotized plates on a caterpillar that bear the setae). The head and prothoracic shield are yellowish brown. The anal shield is light brown without mottling. *G. molesta* can appear similar to other *Grapholita* and *Cydia* spp. Adults have dull greyish brown forewings with a row of black dots near the apex and are similar to other species of *Grapholita*. They can be caught in pheromone traps. Sampling should follow the methods described for *Anarsia lineatella*.

(10) *Halyomorpha halys* (Kazakhstan, Ukraine A1 List), brown marmorated stink bug

Symptom description

Malus, *Prunus* and *Pyrus* are hosts. Like other true bugs, *H. halys* feeds by sucking plant juices. Adults generally feed on fruit, whereas nymphs feed on leaves, stems and fruit. Leaf feeding is characterized by small lesions (3 mm diameter) which may become necrotic and coalesce. Attacked fruits may present small necrotic spots or blotches, grooves and brownish discolorations.

Sampling and identification

Adults are 12–17 mm long, brownish or greyish, mottled and variable in size and colour (Figure 9). In summer, females lay eggs (usually 50–150 eggs and occasionally up to 400 eggs, clustered in groups of 20–30) on the underside of the leaves. There are five larval stages (nymphs) (Figure 10). The pronotum of the younger nymphs is armoured with spines, and the tibiae of instars three to five show a white band. Nymphs can be sent alive with host plant material in airtight and secure containers, except if the phytosanitary risk is high. In that case, specimens, including adults, can be sent with the host plant material in 70% ethanol.

FIGURE 9 Upper side of adult. Photo: EPPO Global Database. Courtesy: Dalia del Nista, Regione Toscana (IT)

FIGURE 10 Newly hatched nymphs. Photo: EPPO Global Database. Courtesy: Iris Bernardinelli, ERSA, Servizio fitosanitario, Friuli Venezia Giulia (IT)

(11) *Hyphantria cunea* (Quarantine Pest Belarus, Israel, Tunisia), mulberry moth

Symptom description

Major pest of *Malus*, *Prunus* and *Pyrus*. The larvae are gregarious and form large tents enclosing a leaf or number of leaves. By late summer or early autumn the tents can become larger and may join.

FIGURE 11 *Hyphantria cunea larvae*. Photo: EPPO Global Database. Courtesy: Iris Bernardinelli, ERSA, Servizio fitosanitario, Friuli Venezia Giulia (IT)

Sampling and identification

In the north part of their range in North America, *H. cunea* adults are bright white with a hairy body whereas in the southern part of their range the moths are white with dark wing spots. Mature larvae are hairy and either have a lime green body with black spots or a darker colour, especially in the later instars (Figure 11). The head capsules in some populations can be either red or black. In other populations, they are entirely black. The black-headed larvae are thought to be more prevalent in the northern areas, while the red-headed larvae are thought to be dominant in the southern areas (Sourakov & Paris, 2011). Sampling should follow the methods described for *Anarsia lineatella*.

(12) *Lepidosaphes ussuriensis* (EPPO A2 List), ussuri oystershell scale

Symptom description

Malus is a host. Females (Figure 12) and larvae of *L. ussuriensis* are easily detected on leaves and branches. The scales located on the bark and small populations are difficult to detect. Bark can be inspected for female scales, eggs, feeding marks and sap-sucking (female scale brownish black, protuberant, about 2.5 mm long, eggs violet). Severe attacks by *L. ussuriensis* result in dieback and premature leaf fall.

Sampling and identification

The taxonomy of the Coccoidea is based almost entirely on the adult female and a good slide preparation of a female is required for identification to the species level by light microscopy. PM 7/69 *Lepidosaphes ussuriensis* (EPPO, 2006a) provides a key for identification.

Collected specimens (females) can be transferred to 70% ethanol before being sent to the laboratory for identification. Alternatively, sections of infested bark can be sent to the laboratory in sealed containers.

(13) *Lopholeucaspis japonica* (EPPO A2 List), Japanese long scale

Symptom description

On *Prunus* and *Pyrus*, *Lopholeucaspis japonica* can produce dieback symptoms in hosts along with premature leaf fall due to senescence of all infested branches. It infests the bark and adults may be found in the cracks of the bark (Figure 13), though low-level infestations can be difficult to detect as obvious symptoms are not always detectable and close examination is required. Heavy infestations give bark a greyish white appearance.

Sampling and identification

The taxonomy of the Coccoidea is almost entirely based on characters of the adult female and a good slide preparation of a teneral female is required for identification to species level. The EPPO diagnostic protocol PM 7/54 *Lopholeucaspis japonica* (EPPO, 2005a) provides a morphological description of the species.

Collected specimens can be transferred to 70% ethanol before being sent to the laboratory for identification. Alternatively, sections of infested bark can be sent to the laboratory in sealed containers.

FIGURE 12 *Lepidosaphes ussuriensis* slide-mounted adult female. Photo: EPPO Global Database. Courtesy: Jean-François Germain, Plant Health Laboratory Montpellier (FR)

FIGURE 13 *Lopholeucaspis japonica* on Zelkova stem. Photo: EPPO Global Database. Courtesy: Central Science Laboratory, York (GB), British Crown copyright

FIGURE 14 *Lymantria dispar* adult male. Photo: EPPO Global Database. Courtesy: Ilya Mityushev, Department of Plant Protection of the Russian State Agrarian University, Moscow Timiryazev Agricultural Academy

FIGURE 15 *Lymantria mathura* adult male. Courtesy: David Mohn, Bugwood.org

(14) *Lymantria dispar* (Israel Quarantine Pest), gypsy moth

Symptom description

Malus, *Prunus* and *Pyrus* are hosts. Gypsy moth eggs are laid in a large mass (3–4 cm long) and covered in yellowish/brown hairs. The egg mass is usually found on crevices of bark, but can also be found on walls, fences or any other sheltered rough surface. Early-instar larvae excavate small holes in leaves and feed gregariously. As the larvae grow, they make larger holes and also consume the leaf margin. Final-instar larvae will consume the entire leaf. At high populations, larvae can strip all leaves from a tree.

Sampling and identification

When freshly hatched, gypsy moth larvae or caterpillars are small (about 2 mm long), dark coloured and very hairy. As the caterpillar grows, its body colour lightens and becomes a brownish-yellow grey with black markings. The yellow and black head is easily seen from the front.

The mature caterpillar develops a series of distinctly coloured 'warty spots' along its back: five pairs of blue spots behind the head and six pairs of red spots to the rear. These spots are an easy way to distinguish the caterpillars from other similar species (Forest Research, 2020). Male moths are much smaller than females and have a wingspan of 35–40 mm. Females have a wingspan of 55–70 mm. Males are brown (Figure 14) whereas females are mainly white. Both sexes have a dark, crescent-shaped mark on the forewing (CFIA, 2019). Pheromone-baited traps can be used to detect very low-density populations that could not be detected using any other method. Sampling should follow the methods described for *Anarsia lineatella*.

(15) *Lymantria mathura* (EPPO A2 List), pink gypsy moth

Symptom description

Malus is a host. The moth *Lymantria mathura* (Lymantriidae) (Figure 15) is on the EPPO A2 List. Eggs of this moth are laid in small masses underneath bark scales. After hatching, the larvae feed gregariously on the foliage of host trees. Defoliation of host trees is usually spectacular. The presence of caterpillars is easily detected. Adult males can be captured using light traps (EPPO, 2005b).

Sampling and identification

Male wingspan 40–50 mm, female wingspan 70–90 mm. Males: antennae strongly pectinate, yellow-grey with black segments; head and thorax yellow-grey with grey strokes; abdomen yellow with bundles of grey hairs on tergites; ventral side of abdomen and thorax yellow; fore wings yellow-grey with many grey and white transversal stripes, yellow veins and yellow-grey fringe; hind wings dull, grey-yellow, with light yellow fringe; upper side yellow, sometimes slightly pinkish. Females: white-pink; hind wings, abdomen, base of antennae, legs and tops of veins on front wings bright pink; other parts of the body pinkish; fore wings pink with white longitudinal strokes along veins. Sampling should follow the methods described for *Anarsia lineatella*.

(16) *Malacosoma parallela* (EPPO A2 List), mountain ring silk moth

Symptom description

Host plants include *Cydonia oblonga*, *Malus domestica*, *Prunus dulcis* and *Pyrus communis*. Defoliation of host plants is usually spectacular. The presence of egg masses, nests and individual caterpillars is easily detected. Moths are attracted by sources of light. Eggs are laid in an egg mass and encircle thin branches of host plants similar to the egg masses of the closely related European

species *Malacosoma neustria*, but they are covered by a thick layer of special female secretion (spumaline), which is a shiny whitish grey when fresh and then turns dark. Neonate caterpillars appear from the end of March at the same time as young leaves of host plants. They usually all hatch over 1–2 days and begin to make a web nest on branches. They feed on young leaves around the nest.

Sampling and identification

The neonate caterpillar is brown-black, 2–2.5 mm long, with a black head 0.3 mm wide. The caterpillar of the sixth instar is 40–50 mm long before pupation with a head that is 4.0–4.5 mm wide. The adult wingspan is 30–45 mm. The front wings vary from yellowish ochre to brown-red with two transverse stripes. Moths are attracted by sources of light. Sampling should follow the methods described for *Anarsia lineatella*.

(17) *Parabemisia myricae* (Georgia A1 List; Jordan A2 List; EU (RNQP)), bayberry whitefly

Symptom description

Prunus and *Pyrus* are hosts. Whiteflies suck phloem sap, which in some cases can cause leaves to wilt and drop when there are high numbers of whiteflies. However, the primary concern is the honeydew they produce. Honeydew excreted by nymphs and adults collects dust and supports the growth of sooty mould as well as attracting ants, which interfere with the biological control of whiteflies and other pests. The sooty mould can affect tree yields by reducing photosynthesis and requiring extra handling time for fruit.

Sampling and identification

Eggs are laid on leaf margins or upper surfaces of very young leaves, each attached by a short pedicel; they are white initially and turn black in a few days. The first-instar larva has six legs, but the three subsequent larval stages have legs reduced to stubs and are held on the leaf by their mouthparts. Larvae are translucent, white to yellowish, each with a clear wax marginal fringe and are difficult to see. The fourth immature instar or 'pupa' is 0.89–0.97 mm long and is most easy to see. This is the pre-adult stage required for accurate identification; it has 30–32 marginal setae, including the caudal setae. Adults are whitish yellow to dusty grey or lavender in colour, with opaque wings. Branches, shoots, leaves and fruit with suspect juvenile whiteflies should be removed or sections cut away with the scales still attached and sent to the laboratory in a dry condition in plastic containers. Adults can be submitted in sample tubes.

(18) *Popillia japonica* (EPPO A2 List), Japanese beetle

Symptom description

Malus and *Prunus* are hosts. *Popillia japonica* (Figure 16) is a chafer or scarab beetle. Symptoms indicative of adult beetles include feeding holes in host plants extending to skeletonization of leaves when population numbers are high. Often the mid-vein of leaves is left intact. Severely damaged leaves soon turn brown. They may drop or remain attached. On some plants with thin leaves and fine venation, and on petals of flowers, the beetles consume irregularly shaped sections in the same manner as many Lepidoptera (CABI, 2019). The adults are gregarious (Figure 17) and have been reported from the USA as usually beginning to feed on foliage at the top of a plant and working downward (Fleming, 1972). Evidence of the presence of *P. japonica* larvae in the soil can be seen in the discolouration of grass in patches which gradually enlarge over time. Severe infestations can result in the death of the turf (Vail et al., 1999).

FIGURE 16 Adult *P. japonica*. Photo: EPPO Global Database. Courtesy: Martino Buonopane, Plant Protection Service, Lombardia (IT)

FIGURE 17 Adults showing gregarious behaviour on apple shoot. Photo: EPPO Global Database. Courtesy: M.G. Klein, USDA/ARS, Wooster (US)

Sampling and identification

Eggs are laid in soil cavities. Newly deposited eggs are variable in size and shape: they may be round, elliptical or nearly cylindrical, with a length of about 1.5 mm. The colour can be translucent to creamy white and the external surface is marked with hexagonal areas. The larvae can be

distinguished from other scarab larvae by the characteristic V-shaped arrangement of two medial rows of 67 spines on the 10th abdominal segment (EPPO, 2016). The pupae are 14 mm long and 7 mm wide, and resemble adults, but the wings, legs and antennae are held close to the body and are functionless. The colour changes from cream to tan and eventually the metallic green observed in the adult. The larvae are highly cryptic (living in the soil) and could easily be accidently moved with rooted plants. The adult beetles can be easily detected in the field by visual examination of plant foliage. Adults are brightly coloured with a metallic green thorax and head and coppery bronze wing cases (elytra), oval in shape and vary from 8 to 11 mm in length, and 5 to 7 mm in width. Along each lateral side of the wing cases are five tufts of white setae present and two dorsal spots of white setae on the last abdominal segment (pygidium) (Malumphy et al., 2016). Traps containing a combination of floral attractant and synthetic pheromone lures can be used to monitor populations of *P. japonica*. EPPO (2016) provides detailed guidance on inspection and sampling (including traps).

(19) *Pseudococcus calceolariae* (Quarantine Pest Israel, Belarus), citrophilus mealybug

Symptom description: *Malus*, *Prunus* and *Pyrus* are hosts

Besides the insects themselves, other signs of a mealybug infestation are the presence of ants and sooty moulds which form on the honeydew secreted by mealybugs and premature leaf fall, which can result from heavy infestations.

Sampling and identification

The body outline of female *P. calceolariae* is elongate-oval to oval around 3–4 mm long and all stages are covered with a rather coarse, powdery, white wax. There are 17 pairs of short white lateral filaments, and two relatively short and stout terminal filaments. The body is a dark purplish-red, and there are usually two distinct lines (of reduced wax cover) running along the back. A characteristic feature is that the honeydew *P. calceolariae* produce is dark red. Most other mealybug species produce cream to yellow honeydew. They live in colonies composed of adult females, eggs and nymphs (juveniles). Morphological identification usually requires adult female specimens to be cleared and slide mounted for examination. 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.

(20) *Spodoptera littoralis* (EPPO A2 List), cotton leaf worm

Symptom description

Spodoptera littoralis (Figure 18) is a highly polyphagous lepidopteran species which is widespread from Africa and Southern Europe to the Arabian Peninsula and into Iran. *Malus* is a major host. Symptoms of the presence of larvae are holes in leaves with the presence of excrement. Symptoms caused by the larvae are generic for most primarily foliage feeding Lepidoptera. Under natural conditions, pupation takes place in the soil where the pupae are difficult to detect. Pupae can incidentally be found in commodities without soil, since larvae will always start pupating when fully grown, regardless of the presence of soil.

FIGURE 18 *Spodoptera littoralis* adult. Photo: EPPO Global Database. Courtesy: O. Heikinheimo (FI)

Sampling and identification

All stages of the pest can be detected visually, with a hand lens for early stages, and specimens can be collected by hand or a sweep net (adults). In the field and in production, storage, handling and other facilities adults can also be detected with the aid of light traps and pheromone baited traps. Further details are available in the EPPO Global Database (EPPO, 2021a) and in EPPO Standard PM 7/124 (1) *Spodoptera littoralis*, *Spodoptera litura*, *Spodoptera frugiperda*, *Spodoptera eridania* (EPPO, 2015b).

(21) *Trichoferus campestris* (EPPO A2 List), mulberry borer

Symptom description

Attacks *Malus* and *Pyrus*. The characteristic symptoms of infestation by *T. campestris* include large entrance and emergence holes in trunks, peeling bark, waste from borings at the base of infested trees and tunnels made by large larvae. The leaves of attacked trees often show yellowing and wilting.

Sampling and identification

The adult is elongated, with parallel-sided elytra, 11–20 mm long (Figure 19). The whole body, elytra and legs vary from dark brown to brownish-orange, the legs and antennae usually being lighter than the body. There are

FIGURE 19 *Trichoferus campestris* adult. Photo: EPPO Global Database. Courtesy: Pascal Reynaud, Marseille (FR)

specific protuberances at the base of the antennae. The adult is easily recognized by the irregularly distributed hairs on elytra, which form spots. Eggs are slightly elongated, 1.9 mm long and 0.6 mm wide. Larvae are white-yellow and up to 30 mm long.

Collected adults can be killed and transferred to 70% ethanol before being sent to the laboratory for identification. Pupae can be sent alive with host plant material in airtight and secure containers, except if the phytosanitary risk is high. In that case, specimens can be sent with the host plant material in 70% ethanol.

(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 soil sampling and testing of the field or the field is not under official control due to previous findings, then the soil in the relevant area should be sampled and tested for *G. pallida* and *G. rostochiensis*. Recommendations on sampling can be found in the EFSA pest survey card (EFSA, 2019). Additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/40(4) *Globodera rostochiensis and G. pallida* (EPPO, 2017a).

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

Symptom description

The crop should be planted in a plot that has been found to be free of *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* (EPPO, 2009b). Additional information for personnel carrying out diagnostics is available in EPPO Standard PM 7/95 *Xiphinema americanum sensu lato* (EPPO, 2017b).

(C) Fungi

(1) *Alternaria mali* (EPPO A1 List)

Symptom description

The fungus causes leaf spots mainly on *Malus*, which enlarge in zonate circular or crescent-shaped rings. Hyphae are normally scant or lacking on the host surface, but abundant light-grey mycelium can be produced on the surface under moist storage conditions.

Sampling and identification

Leaf and/or fruits samples showing typical symptoms can be placed flat between two sheets of absorbent paper and placed in an airtight package, 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.

(2) *Apiosporina morbosa* (EPPO A1 List)

FIGURE 20 *Prunus domestica* severely infected by *Apiosporina morbosa*. Photo: EPPO Global Database. Courtesy: D. Ritchie (US)

Symptom description

On *Prunus*, in early spring, branches infected the previous year develop small, light-brown swellings which gradually enlarge (Figure 20) (Klos, 1964). The intercellular fungal cells are elongate with thick walls. Early symptoms are similar to those caused by *Agrobacterium tumefaciens* on *Prunus* spp. Knots first become visible just below the point of attachment of the leaf petioles to the stem. They are corky and covered by a velvety olive-green growth due to the conidial stage. Later in the summer, they turn black and become hard and brittle. Knots naturally range from 1 to 15–20 cm in length and from 0.5 to 4 cm in diameter; they very often coalesce to form larger knots and may even girdle the stem. The green coloration on young knots is far less pronounced on peaches than plums.

Sampling and identification

Plants for planting of *Prunus* should be inspected for the presence of knots or cankers. Infected material should be excised at least 10 cm below the visible swelling. Samples of infected material 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.

(3) *Botryosphaeria kuwatsukai* (Quarantine Pest Israel, Tunisia; A1 List Turkey; EU A1 QP (Annex II A))

Symptom description

On *Malus* and *Pyrus*. On Japanese pears (Kato, 1973), the fungus forms wart-like protuberances (wart bark) on the surface of trunks and branches, rather than typical Botryosphaeria cankers. These are subsequently surrounded by dark-brown spots. Infected twigs eventually wither and die back. Large contoured dark-brown spots are formed on the leaves and also on the fruits. The warts on trunks and branches damage the tree, reducing its growth and productivity.

Sampling and identification

Pieces of infected stems and branches, fruits and leaves showing typical symptoms should be collected. According to Koganezawa and Sakuma (1984), the morphology of the fungus is identical to that of *B. dothidea*. The fungus is rather variable in the size of the stroma, asci and ascospores. Sampling should follow the methods described for *Alternaria mali*.

(4) *Gymnosporangium* spp. (*Gymnosporangium asiaticum*, *G. clavipes*, *G. globosum*, *G. juniperi-virginianae*, *G. yamadae*, EPPO A1 List)

Symptom description

Gymnosporangium species are heteroecious species requiring a second host for completion of the life cycle. A detailed description of symptoms caused by *Gymnosporangium* spp. on different hosts is reported in EPPO PM 7/73 *Gymnosporangium spp. (non-European)* (EPPO, 2006b).

Sampling and identification

Identification of *Gymnosporangium* species is based on the host pathogen relationship. EPPO (2006b) provides a detailed table on hosts and spore morphology of the five *Gymnosporangium* species for reference. Samples of infected material 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.

(5) *Monilinia fructicola* (EPPO A2 List) and *M. fructigena* (Jordan A2 List)

Symptom description

Occurring on stone fruits, but also *Malus*, *Prunus and Pyrus* spp., *M. fructicola* has occurred in a few countries in Europe and is similar to two other widespread species: *Monilinia fructigena* and *Monilinia laxa*. Blossoms become infected, 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 and having sharp edges. Infected leaves show more or less circular brown dead areas that may later drop 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 (3) *Monilinia fructicola* (EPPO, 2020a).

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 (3) *Monilinia fructicola* (EPPO, 2020a).

(6) *Neonectria ditissima* (Quarantine Pest Israel; EU (RNQP))

Symptom description

On *Cydonia*, *Malus* and *Pyrus*. Trees can be infected in the nursery and or during propagation. First symptoms of infection on stem and branches of apple consist of a discoloration of the bark. The point of infection can become reddened over a 1 cm^2 area. Tissue may shrink around the lesion and young stems can rapidly become girdled. Large canker lesions on main stems or branches usually originate at the junction with side shoots. Infections at the base of these side shoots extend into the larger branches (CABI, 2021).

Sampling and identification

Samples of infected material 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.

(7) *Phyllosticta solitaria* (EPPO A1 List)

Symptom description

On *Malus* and *Pyrus*. On leaves: tiny white spots, 1.5–3 mm in diameter, first appear between or on the veins and petioles. The spots enlarge, up to 6 mm, and become elliptical, sunken, tan or buff lesions with a black spot (pycnidium) forming in the centre. This infection is of little consequence in itself, but infection at the petiole base may cause defoliation by midsummer. Leaves often remain uninfected. On twigs roughly circular, dark, raised spots studded with tiny projecting pycnidia develop. These infections may either be the result of a direct spore infection or may arise from the fungus passing from the petiole of the leaf to the wood. Slightly sunken, brown to black cankers develop. In the second year, the central part of the canker is surrounded by a dark border which indicates the extent of the fungus. Pycnidia form in the border area.

Sampling and identification

Samples of infected material 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.

FIGURE 21 Symptoms of *Venturia nashicola* on *Pyrus pyrifolia* cv. Hosui. Photo: EPPO Global Database. Courtesy: H. Ishii, Fruit Tree Research Station, MAFF (JP)

(8) *Venturia nashicola* (Quarantine Pest Israel; A1 List Turkey; EU (A1 QP (Annex II A)))

Symptom description

EFSA (2017) state that on *Pyrus*, *V. nashicola* infects fruit, leaves and young shoots, causing typical scab symptoms. The first symptoms appear on either side of the leaves as olive green to brown, velvety spots with abundant conidia. Lesions are well-defined circular areas (5–10 mm in diameter). Similar but more elongate lesions appear on the main veins of the leaves and on petioles. Lesions on young actively growing shoots appear early in the growing period as black to brown velvety spots. Later in the season, the twig lesions become corky and canker-like. Scab lesions on fruit are superficial and occur first on the calyx end adjacent to the sepals and later on the side of fruit (Figure 21). As the lesions expand and coalesce, large, dark-brown to black patches are produced. Infections of petioles and peduncles result in premature abscission of leaves and fruit, respectively. Infected fruits often become misshapen.

Sampling and identification

Samples of infected material 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.

(9) 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 or fungus-like organisms 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 (2) *Synchytrium endobioticum* (EPPO, 2017c)), *Verticillium*

nonalfalfae and *Verticillium dahliae* (additional information for personnel carrying out diagnostics can be found in EPPO Standard PM 7/78 (2) *Verticillium nonalfalfae and V. dahliae* (EPPO, 2020b)). 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 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 (Figure 22). 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, paler and be flattened with longer peduncles compared to those from 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 depending on the diagnostic methods available. During early summer to autumn (June to the end of October) leaves can be sampled and molecular identification methods are detailed in EPPO (2020c). 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 plant because of uneven distribution.

The samples should be placed in a plastic bag (without any paper), which is then inflated slightly and sealed, and 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.

FIGURE 22 Enlarged stipules of apple leaf (cv. Golden Delicious) affected by '*Candidatus* Phytoplasma mali', healthy leaf on right. Photo: EPPO Global Database. Courtesy: Institut für Pflanzenschutz im Obstbau, Dossenheim (DE)

FIGURE 23 Almond shoots affected by '*Candidatus* Phytoplasma phoenicium' (left) and healthy shoots (right). Photo: EPPO Global Database. Courtesy: Piero A. Bianco (University of Milan) and Marina Molino Lova (AVSI-Lebanon)

Further details are available in EPPO Standard PM 7/62 (3) *'Candidatus Phytoplasma mali', 'Ca. P. pyri', 'Ca. P. prunorum'* (EPPO, 2020c).

(2) '*Candidatus* Phytoplasma phoenicium' (EPPO A1 List)

Symptom description

Symptoms vary between hosts. On *Prunus dulcis*, shoot proliferation occurs at several points on the main trunks of affected trees, or from the roots, with an occasional appearance of witches' broom (Figure 23). Proliferation symptoms are always observed, but witches' broom symptoms may appear only on some trees. Proliferation and witches' broom are also observed on branches. Perpendicular development of many axillary buds on the branches, with small and yellowing leaves (pale green), and shoots becoming stunted with short internodes (rosetting). Early flowering (20–30 days earlier than normal) can occur. Other symptoms include general decline of affected trees, severe dieback, off-season growth and in dry weather the leaves may appear brownish-red.

On peaches and nectarine, phyllody can occur, which is the abnormal development of floral parts into leafy structures. Other symptoms are the proliferation of shoots from the collar of the trunk and occasionally witches' broom on branches, early flowering (15–20 days earlier than normal) and development of buds some months after the normal flowering period and smaller light green leaves. Early senescence of trees (reddening of leaves and leaf fall) can occur. Symptoms initially affect only some branches and in subsequent years all branches.

In apricot, symptoms include leaf roll and proliferation.

Sampling and identification

Sampling should follow the methods described for '*Candidatus* Phytoplasma mali'.

(3) '*Candidatus* Phytoplasma pruni' (EPPO A1 List)

FIGURE 24 Dieback and partial yellowing of a peach tree affected by '*Candidatus* Phytoplasma pruni'. Photo: EPPO Global Database. Courtesy: R. Bernhard, INRA, Bordeaux (FR)

Symptom description

The first symptoms of infection of peach are yellow spotting and rolling of the leaves (Figure 24). Shortly after, the whole tree becomes chlorotic and its leaves fall, leaving a few rosettes at the tips of the shoots. Young trees die 1–3 years after first symptom appearance. Chronically infected older trees may survive for several years but yield little or no fruit. Infected cherry trees on *Prunus mahaleb* rootstocks die rapidly because the rootstock is resistant and a hypersensitive reaction occurs at the graft union. On other rootstocks, decline is slower. Leaves are smaller and red tinged, sometimes with enlarged stipules; fruits mature late, have short pedicels and bland flavoured watery flesh.

Sampling and identification

Sampling should follow the methods described for '*Candidatus* Phytoplasma mali'.

(4) '*Candidatus* Phytoplasma pyri' (EPPO A2 List)

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

FIGURE 25 Premature reddening of leaves with '*Candidatus* Phytoplasma pyri'. Photo: EPPO Global Database

FIGURE 26 Japanese pear (nashi) cv. Hosui showing enlarged vein with leafcurl induced by '*Candidatus* Phytoplasma pyri'. Photo: EPPO Global Database. Courtesy: L. Giunchedi, University degli Studi, Bologna (IT)

Symptom description

As detailed in EPPO (2020b, 2018), in general the first visual symptoms occur in late summer when the leaves of affected trees develop a premature red colour followed by early leaf fall (Figure 25). Occasionally, some cultivars may develop premature yellowing of the leaves. There may be some leaf cupping or curling (Figure 26). It should be noted that the autumn symptoms associated with pear decline may be caused by other biotic or abiotic causes (EPPO, 2020c). Second-stage symptoms are seen in the following spring when developing leaves remain small and pale with little or no shoot growth and no fruit production (EPPO, 2020c). Spring symptoms can vary in severity from death or severe stunting to a complete absence of symptoms (EPPO, 2020c).

A typical diagnostic symptom of pear decline is a dark phloem ring immediately below the graft union in bark sections from infected trees, visible by microscopic examination of stained transverse sections.

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. Molecular identification methods are detailed in EPPO (2020b).

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 (CABI/EPPO, 1996a).

Further details on inspection are available in EPPO Standard PM 3/84 *Inspection of places of production for 'Candidatus Phytoplasma pyri'*.

The samples should be sent to the laboratory as described for '*Candidatus* phytoplasma mali'.

Further details are available in EPPO Standard PM 7/62 (3) *'Candidatus Phytoplasma mali', 'Ca. P. pyri', 'Ca. P. prunorum'* (EPPO, 2020c).

(5) '*Candidatus* Phytoplasma prunorum' (Quarantine Pest Israel, A1 List Jordan, EU RNQP)

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

FIGURE 27 Characteristic leafroll symptoms induced by '*Candidatus* Phytoplasma prunorum' on apricot Photo: EPPO Global Database. Courtesy: G. Morvan, INRA, Montfavet (FR)

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 (Figure 27) 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 absent in trees less than 5 years old.

Sampling and identification

Inspection of susceptible hosts in the vicinity of the nursery should also be carried out where possible. Molecular identification methods are detailed in EPPO (2020b). 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'.

Further details are available in EPPO Standard PM 7/62 (3) *'Candidatus Phytoplasma mali', 'Ca. P. pyri', 'Ca. P. prunorum'* (EPPO, 2020c).

(6) '*Candidatus* Phytoplasma solani' (EPPO A2 List)

Symptom description

'*Candidatus* Phytoplasma solani' has been reported as infecting *Prunus domestica* in Jordan (Salem et al., 2020). Symptoms on other hosts include yellowing, reddening, decline, dwarfism, leaf malformation and degeneration diseases. Its main vector is the cixiid planthopper *Hyalesthes obsoletus*. Samples should be handled similar to *Alternaria mali*.

FIGURE 28 Dieback of shoots of *Malus* sp. infected with *Erwinia amylovora*

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.

(7) Erwinia amylovora (EPPO A2 List)

Symptom description

This common 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 (Figure 28). 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

FIGURE 29 Canker on a pear tree after removing bark showing necrotic inner tissues Photo: EPPO Global Database

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 (Figure 29) (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 fire blight, 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, 2013a).

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 should be taken to avoid cross-contamination between samples.

The samples should be placed in plastic bags (with dry absorbent paper) which are 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 (2) *Erwinia amylovora* (2013a).

(8) Peach rosette phytoplasma and Peach yellows phytoplasma (EPPO A1 List)

Symptom description

For peach rosette phytoplasma, symptoms are very similar to those caused by peach rosette mosaic nepovirus (Dias, 1975; CABI/EPPO, 1996b). On affected peach, new shoots have very short internodes. The leaves of the older shoots fall in early summer, leaving only bunches of young leaves on the tips of naked shoots. Flowers rarely set fruit. The most severely affected trees die during their first year of disease. Other fruit trees (almond, plum) show similar symptoms. Infected plum trees are seriously stunted.

For peach yellow phytoplasma, at the beginning of the growing period, the foliage is greener and proliferation of leaves on short side branches gives the tree a bushy appearance. The leaves become chlorotic during the season. The symptoms are first seen on one branch, or part of the tree, then spread to the whole tree (CABI/EPPO, 1996c).

Sampling and identification

Pathogenicity tests for both phytoplasmas can be done on peach seedlings (cv. Elberta or GF305) in the field, but 4 years are needed for results to be certain. It can also be tested on the same indicators in the glasshouse, symptoms appearing up to 3 months after inoculation. Infected plant material should be collected and sent to the laboratory in sealed containers.

FIGURE 30 Typical necrosis caused by *Pseudomonas syringae* pv. *persicae*, developing in early winter around dormant buds on young peach shoots. Photo: EPPO Global Database. Courtesy: INRA, Angers (FR)

FIGURE 31 Leaf spots on peach in spring. Photo: EPPO Global Database. Courtesy: J.L. Gaignard and J. Luisetti, INRA, Angers (FR)

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

Symptom description

In nectarine and peach, symptoms include necrosis and shoot dieback (Figure 30), infections of branches and main trunk, leaf spots (Figure 31) 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. *morsprunorum*), 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 area 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, 2005c).

(10) *Xanthomonas arboricola* pv. *pruni* (EPPO A2 List)

Symptom description

Symptoms of bacterial spot can be observed on leaves, fruits, twigs and branches (CABI/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 affected leaves turn yellow and drop off, resulting in acute 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 the shot-hole effect can be pronounced. On almonds, apricots and cherries, leaf symptoms are similar to those on peach, but are rarely as prominent. Inspection should also be carried out on all other host plants on the nursery, in particular cherry laurel (*Prunus laurocerasus*) and Portuguese laurel (*Prunus lusitanica*), and may also be required on all host plants in the boundary of the nursery and the surrounding area.

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 fruit 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 to the laboratory 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* (EPPO, 2006c).

(11) *Xylella fastidiosa* (EPPO A2)

Xylella fastidiosa is a bacterial pathogen of a wide range of hosts including *Prunus* spp. and is a quarantine pest in the EU, Georgia, Israel, Jordan, Turkey, Russia, the United Kingdom and the Ukraine. It is the cause of a number of important plant diseases, including phony peach disease, plum leaf scald and leaf scorch on almond. Inspection for *X. fastidiosa* is covered in detail in two other EPPO Standards, one for imported consignments (PM 3/81(2); EPPO, 2020d) and a second for inspection of places of production (PM 3/82(2); EPPO, 2020e). There is also a diagnostic standard for *X. fastidiosa* (PM 7/24; EPPO, 2019). These Standards include a number of photos of *X. fastidiosa* symptoms. As of October 2019, in the EPPO region, *X. fastidiosa* is currently found in limited areas of France, Italy, Spain and Portugal and it is under eradication in most mainland locations and under containment in island locations (Corsica, Ibiza, Menorca and Majorca) and in Puglia in the far south-east of Italy. There have also been findings in Germany, where it has been eradicated, and Belgium, where it was detected on imported olive trees. The EU has established special requirements for the import and movement of certain high-risk plants of which *Prunus dulcis* is one. Sites growing high-risk plants need to be inspected annually and tested to confirm with 99% reliability that the level of presence of *X. fastidiosa* is <5%.

FIGURE 32 Leaf scorch symptoms caused by *Xylella fastidiosa* on almond. Courtesy: D. Boscia, CNR-Institute for Sustainable Plant Protection (IT)

FIGURE 33 Phony peach: typical 'phony peach' symptom on peach leaves caused by *Xylella fastidiosa*. Courtesy: M. Scortichini, Instituto Sperimentale per la Frutticoltura, Rome (IT)

Symptom description

On *Prunus dulcis* the most characteristic symptoms of almond leaf scorch are leaf scorching followed by decreased productivity and general decline of the tree. A narrow band of yellow (chlorotic) tissue usually develops between the brown necrotic tissue and the green tissues of the leaves, but when the sudden appearance of leaf scorch symptoms is prompted by hot weather the narrow chlorotic band may not develop. As the disease progresses, affected twigs on branches die back from the tip (Mircetich et al., 1976). Even highly susceptible varieties take many years to die, but nut production is severely reduced within a few years in most varieties. Leaf scorching symptoms have been also reported on almond in late summer/autumn in Italy (Figure 32).

On infected peach trees, young shoots are stunted and bear greener, denser foliage than healthy trees (Figure 33). Lateral branches grow horizontally or droop, so that the tree seems uniform, compact and rounded. Leaves and flowers appear early and remain on the tree for longer than on healthy trees. Early in summer, because of shortened internodes, infected peach trees appear more compact, leafier and darker green than normal trees. Affected trees yield increasingly fewer and smaller fruits until, after 3–5 years, they become economically worthless. Fruits may also be more strongly coloured and will often ripen a few days earlier than normal. Infected peach and plum trees bloom several days earlier than healthy trees and tend to hold their leaves later into the autumn. The leaves of infected peach never display the typical symptom of leaf scorching seen on infected plum trees. Symptoms of plum leaf scald on leaves are a typical scorched and scalded appearance. Plum leaf scald also increases the susceptibility of the tree to other problems. Phony peach disease and plum leaf scald can limit the life of peach and plum orchards (Mizell et al., 2015).

Insects belonging to the order Hemiptera, sub-order Auchenorrhyncha (Redak et al., 2004), that feed on xylem sap are considered as potential vectors of *X. fastidiosa*.

Sampling and identification

Visual observations alone are not sufficient for the detection of *X. fastidiosa* due to the fact that latent infections could be present and secondary infections caused by other organisms may hide the symptoms of the pest. As *X. fastidiosa* is confined to the xylem tissue of its hosts, the petiole and midrib recovered from leaf samples are the best source for diagnosis as they contain larger amounts of xylem vessels (Hopkins, 1981). However, other sources of tissue include small twigs and roots of peach (Aldrich et al., 1992).

When sampling individual asymptomatic plants, a minimum of between four and 10 branches should be taken, depending on the size of the plant. These branches should be taken to be representative of the entire aerial part of the plant because *X. fastidiosa* can be localized in part of host plants. For sampling of asymptomatic *Prunus dulcis* or *P. avium*, samples of up to 100 plants can be pooled in a single sample with a minimum of two shoots per sample. Symptomatic material should preferably be collected from a single plant, but a pooled sample may also be collected from several plants showing similar symptoms.

Additional information for personnel carrying out diagnostics can be obtained from EPPO Standard PM 7/24 (4) *Xylella fastidiosa* (EPPO, 2019).

(12) 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 sepedonicus* (EPPO A2 List) and *Ralstonia solanacearum*. This should be confirmed by examination of the NPPO records of occurrences and previous inspections of relevant host plants carried out at the site.

(E) Viruses

(1) American plum line pattern virus (EPPO A1)

American plum line pattern virus (APLPV) is the least extensively documented Ilarvirus reported to infect stone fruits. The virus infects stone fruits, in particular Japanese plum, peach and flowering cherry, generally causing clear-cut symptoms.

Symptom description

From CABI/EPPO (1996d): on Japanese plum, there is a regular sequence of pattern types, starting with chlorotic ring and oak-leaf type pattern and finally yellow vein banding. In the early summer, the yellow pattern fades to a creamy-white one. In other cases, leaf borders are first chlorotic and then turn golden. The symptoms do not

FIGURE 34 Symptoms of American plum line pattern virus on Japanese plum. Photo: EPPO Global Database. Courtesy: A. Myrta, IAM Bari (IT)

disappear during the hot season, but new leaves emerging during this period are symptomless.

On peach, in spring and early summer, there are fine irregular, pale-green, wavy bands on each side of the main veins of the leaves. These either form a symmetrical pattern or are broken and turn back on themselves to form figures of various shapes. Some leaves develop a network of fine lines, or a golden net pattern, fine confluent rings, vein banding or an oak-leaf pattern. Symptoms usually disappear in summer.

On *P. serrulata*, whitish, yellowish or pinkish discoloured areas of various forms occur (Figure 34), sometimes large rings but more often oak-leaf pattern. Leaf borders are faintly chlorotic to pronounced golden or white.

Sampling and identification

In spring, leaves are a better virus source than flowers and cortical tissues, whereas in summer, mature fruits are better than leaves. Dormant buds represent a reliable tissue source for testing in winter. If typical symptoms are present in leaves, symptomatic leaves should be collected. If the tree is symptomless, leaves should be collected from different parts of the canopy. When sampling is done in spring, the location of leaves on 1-year branches seems not to have any effect in virus detection. During the hot season, basal mature leaves are a slightly better source than those of central and apical positions. Leaf samples, as for the other stone fruit ilarviruses, can be stored at 4°C for not more than 7 days before processing.

Additional information for personnel carrying out diagnostics and details on identification and symptoms are included in EPPO Standard PM 7/67 *American plum line pattern ilarvirus* (EPPO, 2005d).

(2) Cherry necrotic rusty mottle virus (formally EPPO A2: Quarantine Pest Israel; A1 List Jordan, Turkey; EU RNQP)

Symptom description

Cherry necrotic rusty mottle disease is characterized by the appearance of brown necrotic spots on the leaves from 3 to 5 weeks after petal fall. These are followed by yellowish or rust-coloured areas, the necrotic areas falling out, giving a conspicuous shot-hole effect. In some cultivars, on young branches, cankers or blister-like lesions develop in the cortex and cause a pronounced roughening of the bark. Only certain cultivars show symptoms.

Sampling and identification

For cherry necrotic rusty mottle, detection is possible by indexing on woody indicators, following the recommendations of ISHS (ISHS, 1983). The method consists of graft-inoculating indicator plants with budwood from candidate nuclear-stock plants or plants suspected to be infected and observing the new growth and/or fruits on the indicator plants for symptoms. Such symptoms are normally specific and highly diagnostic for many diseases. Real-time RT-PCR has been developed for the detection of cherry necrotic rusty mottle (Osman et al., 2017). Infected plant material should be collected and sent to the laboratory in sealed containers.

(3) Cherry leaf roll virus (Quarantine Pest Israel, Norway; A2 List Turkey; A1 List Jordan; EU RNQP)

Symptom description

Trees infected with cherry leaf roll virus will produce yields of late-ripening fruit and inevitably the tree will die in 4 or 5 years. Foliar symptoms on cherries include yellowing and chlorosis of leaves, leaf rolling and bunching of shoots. Cherry leaf roll virus causes blackline disease in walnuts. Necrosis at the graft union can lead to dieback of branches and a general decline of the infected tree.

Sampling and identification

Infected plant material should be collected and sent to the laboratory in sealed containers Enzyme-linked immunosorbent assay (ELISA) has been used to detect infection in tissues of natural and experimental hosts (Jones, 1987).

FIGURE 35 Symptoms of cherry rasp leaf nepovirus in cherry. Photo: EPPO Global Database. Courtesy: R. Stace-Smith, Vancouver (CA)

(4) Cherry rasp leaf virus (EPPO A1 List)

Symptom description

Cherry rasp leaf virus is a nepovirus, transmitted by nematode vectors in the *Xiphinema americanum* group (EPPO, 2021b). Cherry rasp leaf virus is readily transmitted by sap inoculation. Seed transmission has been shown to occur in some herbaceous hosts. The virus has been detected in pollen from infected cherry trees, but transmission by pollen has not been confirmed.

On peaches and cherries [from EPPO (2021b)] leaves become deformed, narrow, folded, puckered or distorted, shortening of the internodes (Figure 35), and a general decline of the tree. On the underside of the leaves between the lateral veins and along the midrib develop prominent leaflike growths called enations can be observed. Affected leaves are distorted but remain green.

The virus spreads slowly within infected trees so symptoms are often sporadic and may not appear on all leaves or shoots. Symptoms begin on the lower part of the tree and move upward as the virus spreads. Because fewer leaf buds develop on infected wood, limbs become bare near the base of the tree, while leaves higher up develop rasp leaf symptoms.

On apples [from EPPO (2021b)] leaf symptom consists of a rolling of the leaf margins toward the midrib; they become small, long and narrow and appear to be dry. The leaves also tend to point toward the terminus of the spur or shoot. The resulting appearance is one of water stress or drought. The fruit is flattened along the longitudinal axis, but has a normal seed count. The calyx basin is more prominent and the stem cavity is shallow. Reaction severity varies considerably among cultivars. Symptoms of flat apple occur mainly on cultivars Delicious, Golden Delicious, Jonagold and Gala. Cultivars Fuji, Empire and Granny Smith exhibit relatively mild symptoms.

Sampling and identification

EPPO (2021b) states infection can be confirmed by sap inoculation to herbaceous indicators (ISHS, 1980). *Chenopodium quinoa* and *C. murale* are the most reliable indicator species. ELISA can be used to detect the virus. Real-time RT-PCR has been developed for the detection of cherry rasp leaf virus (Osman et al., 2017). Infected plant material should be collected and sent to the laboratory in sealed containers.

(5) Peach mosaic virus (EPPO A1)

Symptom description

The first signs of disorder become apparent on the trees during the second year after planting and include delays of 4–6 days in leaf emergence, flowering and maturity; pink broken lines on the white petals in warm temperatures; irregularly shaped, flattened, colourless fruit, with cracked sutures and enlarged pits; open habit; bud necrosis. Some isolates cause mosaic, blotch, calico and necrosis of the leaves, whereas others induce stem pitting and leaf twisting. For further descriptions of the disease see Larsen & Oldfield (1995) and Pine (1976).

Sampling and identification

FIGURE 36 Symptoms of peach rosette mosaic virus on peach. Photo: EPPO Global Database. Courtesy: W.R. Allen Agriculture Canada (CA)

Peach mosaic virus can be detected in the glasshouse on indicator plants (peach seedlings, e.g. cvs. Elberta, GF305, Rio Oso Gem) by grafting (Desvignes, 1976). Indirect ELISA using the monoclonal antibody prepared for cherry mottle leaf closterovirus can also be used as a useful interim detection tool for peach mosaic virus.

(6) Peach rosette mosaic virus (EPPO A1)

Symptom description

In infected peach trees bud break is delayed, leaves produced in spring are mottled, narrower than normal and distorted (Figure 36). Shoot internodes are shortened, thus giving rise to rosettes which represent the characterizing trait of the disease. Infected trees are stunted and produce little or no fruit (Ramsdell, 1995).

Sampling and identification

Infected plant material should be collected and sent to the laboratory in sealed containers. Peach rosette mosaic virus (PRMV) is serologically unrelated to any other nepovirus. ELISA works very well in directly detecting PRMV in infected peaches. Alternatively, PRMV-infected young leaves of peach can be ground and rub-inoculated to *C. quinoa* test plants.

(7) 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 (Figure 37).

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, 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 five 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 one 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'.

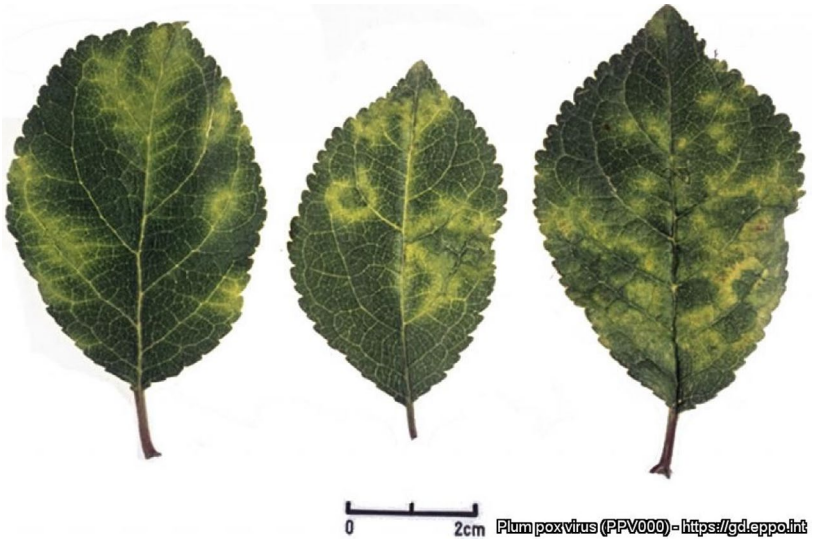

FIGURE 37 Leaf symptoms of plum pox virus infection on plum cv. St Julien. Photo: EPPO Global Database. Courtesy: Biologische Bundesanstalt (DE)

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

(8) 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 contaminated 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

Visual examination is not sufficient as a method for detecting ToRSV in mother plants. These should be sampled and tested in line with the diagnostic Standard PM 7/49 (EPPO, 2005e).

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) which are 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 Standard PM 7/49 *Tomato ringspot nepovirus* (EPPO, 2005e).

(9) 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 inspection of relevant host plants or by soil testing of fields where such host plants have been previously grown but not inspected.

(10) 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 leaf-roll virus, prune dwarf virus or *Prunus* necrotic ringspot virus. In mixed infections 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 (prominent leaflike growths) 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 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* (EPPO, 2013b) 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* (EPPO, 2009b), 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).

(11) Other viruses

Tomato black ring virus (TBRV) (Quarantine Pest Israel, Norway; A1 List Turkey; EU RNQP) is a nepovirus, which may be involved in the aetiology of the diseases.

APPENDIX 2- SHORT PROCEDURE FOR INSPECTORS

To ensure that inspections during active growth are carried out at the optimum time of year inspectors 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 given 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 plum pox virus 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. Depending on 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 denser 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 on 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 inspections to save resources.