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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 3/85 (1)

Procedures phytosanitaires
Phytosanitary procedures

PM 3/85 (1) Inspection of places of production – *Vitis* plants for planting

Specific scope

This Standard describes the procedure for inspection of places of production of *Vitis* plants for planting and includes relevant sampling criteria and the main regulated pests. It mainly focuses on the pests which are present in the EPPO region and affect *Vitis* plants. Additional information on some soil-borne pests is also reported, as they could be of phytosanitary relevance, including those that are vectors of pests. Evidence gathered from inspections carried out according to this Standard may be used for export, for internal country movements of materials, for general surveillance or to help demonstrate freedom from relevant pests. The Standard does not cover any provision about the adoption of phytosanitary measures.

Specific approval

This Standard was first approved in 2018-09.

Introduction

The Eurasian grapevine (*Vitis vinifera*) is one of the most widely cultivated and economically important fruit crops in the world. Other species of *Vitis* are also cultivated, mainly for rootstock production for grafting. Plants for planting are commonly grafted onto interspecific hybrids which are tolerant to phylloxera (*Viteus vitifoliae*), although grapevine can be grown on its own root systems as own-rooted (or self-rooted) vines. Plants for planting are usually vegetatively propagated, starting from cuttings, in order to preserve the characters of the genotype. As a result, grapevines are rarely propagated from seedlings, except within breeding programmes.

Plants for planting are generally considered to pose a higher pest risk than other regulated articles (FAO, 2012). Such plants are thus an important pathway for the spread of pests over a very wide area.

There is a risk of spread of pests through the grafting of cuttings deriving from infested rootstock and scion mother plants. Pests could be further spread through activities such as green or winter pruning, mechanical removal of the suckers, physical contact between different lots and contact with infected plant debris. Pests may also naturally spread by vectors, wind or rain splash.

Plants for planting produced according to EPPO Standard PM 4/8 (2) *Certification scheme on pathogen-tested material of grapevine varieties and rootstocks* (EPPO, 2008), or any equivalent phytosanitary certification system, are generally considered to provide high phytosanitary guarantees, which is especially important for certain viruses. This Standard does not cover pests which are relevant only for marketing purposes within EPPO countries. Nevertheless, some pests are categorized as a quarantine pest in some EPPO countries and pests of quality concern in other EPPO countries, on a risk-based approach. This is the case for some viruses (e.g. Grapevine fanleaf virus, Arabis mosaic virus and strains of Grapevine leafroll-associated virus), which are of quality concern for the European Union (EU), and of quarantine relevance for countries which are not in the EU.

This Standard may be applicable to maintain freedom of places of production or freedom of crops from specified pests, as requirements are frequently provided for *Vitis* plants for planting in several countries. It may also provide guidance for export inspection when the requirements of the importing country are similar to those in the country of origin.

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).

It is important to carry out the inspection at the most appropriate time depending on the biological characteristics of pests, and the most suitable period for detecting the symptoms and collecting suitable samples for testing. Guidance can be found in the relevant EPPO Diagnostic Standards which are mentioned in Appendix 1 and in the references, when available for specific pests.

Inspections should be carried out at the places of production and in different lots of plants at least once a year in order to ensure official control of plantations against the relevant pests of quarantine concern. Nevertheless, more frequent inspections may need to be carried out, depending on the crop history, type of materials, origin and type and distribution of the pest.

ISPM no. 5 *Glossary of Phytosanitary Terms* (FAO, 2017) 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 easily detectable, the inspection procedure may consist of sampling for laboratory testing.

Mother plants of *Vitis* remain in the field for several years and are exposed and susceptible to a large number of pests, such as viruses, bacteria and phytoplasmas. In the case of the latter (as for Grapevine flavescence dorée), a pest may remain undetected with no symptoms being exhibited, particularly in the first year of contamination or when present at low levels of infection or on rootstocks.

Inspection may also be carried out for the detection of organisms which are not yet regulated as pests but which could include potential pests (EPPO 2009a).

Types of material concerned

This Standard covers all type of *Vitis* propagating material, which is used both for the production of rootstock and scion cuttings, and young rooted plants. Visual inspection of the different types of material is recommended during the most appropriate period to detect symptoms, depending on the biological cycle of the surveyed pest.

Rootstock mother plants

These are permanent plants of selections of *Vitis* species, or interspecific hybrids of *Vitis*. A wide range of varieties and clones are available. Roots of rootstock plants are tolerant to phylloxera (*Viteus vitifoliae*). The foliage of these varieties is generally tolerant to downy mildew and more or less tolerant to gall-forming phylloxera, and they are suitable for growing in different pedo-climatic conditions (e.g. they are drought tolerant, tolerant to poor soil drainage, salt tolerant and active limestone tolerant). Rootstocks also have influence on scion vigour.

Common rootstocks are hybrids of *Vitis berlandieri* × *Vitis riparia* [e.g. Kober 5BB (5BB), Selection Oppenheim (SO4), Millard et de Grasset 420A (420A Mgt), Couderc161-49 (161-49C), Teleki 5C (5C)], *V. berlandieri* × *Vitis rupestris* [e.g. Paulsen 1103 (1103P), Richter 110 (110R), Ruggeri 140 (140Ru)], *V. riparia* × *V. rupestris* [e.g. Millard et de Grasset 101-14 (101-14 Mgt), Couderc 3309 (3309C), Schwarzmann]. A selection of *V. rupestris* is Rupestris du Lot (St George) and a selection of *V. riparia* is Riparia Gloire de Montpellier.

Vitis vinifera × *V. berlandieri* rootstocks are also used (e.g. Millard et de Grasset 41B (41B Mgt) and Fercal).

Rootstock mother plants are maintained individually *in situ* for the production of graftable rootstock cuttings, but also for the production of own-rooted young plants to be grafted in the field.

Scion mother plants

These are permanent plants of known variety and, where relevant, the clone for grape production, more often from *V. vinifera* (e.g. Chardonnay, Cabernet Sauvignon, Cabernet Franc, Merlot, Pinot Noir).

Scion mother plants are maintained individually *in situ* for the production of scion wood. Scion wood is commonly used for the production of grafted young plants and sometimes for the production of self-rooted young plants.

Young plants

These are the final grafted and self-rooted vines grown in the open field, for one vegetative season. Most often they are marketed as 'bare rooted' plants, but sometimes young plants are individually produced in pots, in glasshouses or temporary structures.

Micropropagated rootstocks

These are plants resulting from *in vitro* propagation through axillary bud multiplication of different varieties of rootstocks.

This method of plant propagation is not widespread and not in use for scions because it induces the phenomenon of juvenility which could distort the behaviour of the clone at the agronomic level. As this material is the starting point for large-scale multiplication it could in principle contribute to major spreading of plant pests. However, due to the special growing conditions, the majority of potential contaminating invertebrate pests will be excluded. This may not be the case with viral or bacterial pathogens, which could persist undetected during the micropropagation process.

It is recommended that visual inspection of micropropagated plants is carried out after transplanting into the growing medium and after growing on until a phase where symptoms of diseases could be detected.

Outline of the scheme for production of young plants in the open field

Growing young *Vitis* plants in the open field is the most common practice; less commonly production is in pots or via micropropagation (rootstocks). In winter, during the dormant stage, cuttings are collected from rootstocks and scion mother plants. Cuttings of rootstocks are used for grafting with scions and as self-rooted rootstock plants, while cuttings of scions are usually grafted on rootstocks. Any scion is grafted, often with a grafting machine, onto a rootstock in the form of a small piece of woody shoot carrying a single bud.

The grafted cuttings are then paraffinized with special waxes to protect the grafting point from infections and to prevent dehydration. Following this, the grafted cuttings are stratified in bins containing wet sawdust (e.g. very pure fir sawdust), peat and a sterile substratum (e.g. Agroperlite) or water. Bins are kept in glasshouses for two to four weeks at a temperature of around 30°C, at which grafting callus occurs with budding and rooting.

After a phase of acclimation of the plants to environmental conditions at the beginning of spring (starting from the first days of May in Southern Europe), the grafted cuttings are planted in the field, where they are grown for a whole vegetative season.

At the end of autumn, during leaf fall, the young grafted plants are harvested, sampled to evaluate their quality and sanitation conditions and packaged and stored in cold storage rooms before marketing. An outline of grafted plant production is described in Fig. 1.

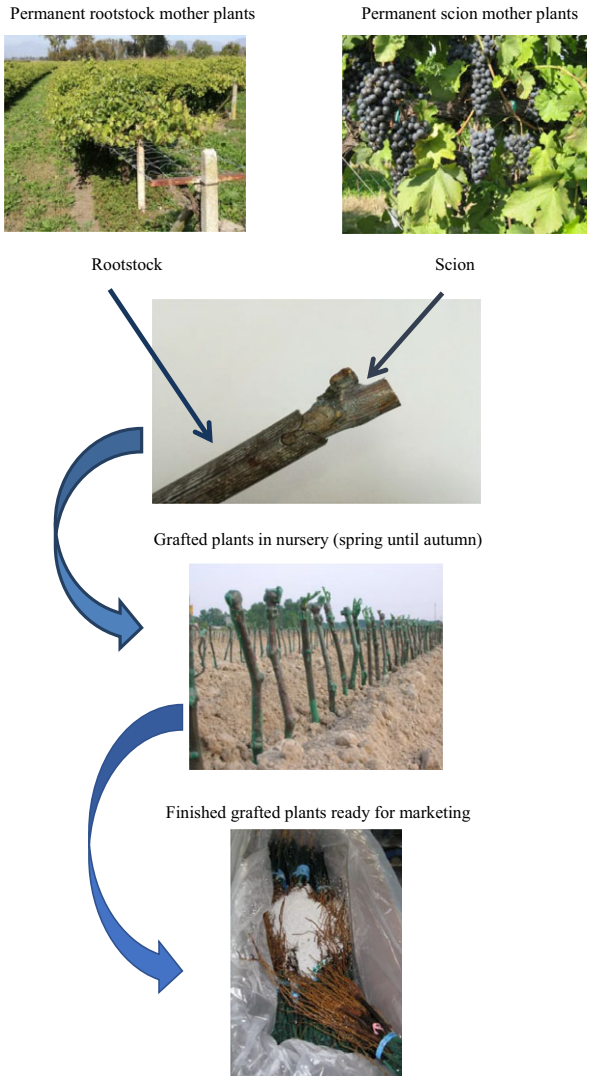

Fig. 1 Outline scheme for production of grafted young plants.

Pests of concern for the EPPO region

This Standard mainly relates to those organisms affecting *Vitis* plants, which are listed in the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests. It also considers those pests which are listed in specific EPPO countries, even if not mentioned in the EPPO A1 or A2 Lists. It does not include those pests affecting fruits (e.g. *Drosophila suzukii* or *Bactrocera* spp.). Specific pests of *Vitis* spp. (EPPO A1 and A2 Lists, and pests listed by specific EPPO countries) are described in Table 1. Polyphagous pests affecting *Vitis* (EPPO A1 and A2 Lists) and pests listed by specific EPPO countries are described in Table 2.

Other EPPO A1 and A2 listed pests, and pests listed by specific EPPO countries for which their status on *Vitis* spp. is unclassified or incidental on plants for planting, as cited in the EPPO Global Database (EPPO, 2017a), are not highlighted in this Standard (Table 3).

For woody plants, national plant protection organizations (NPPOs) may apply additional controls to reduce the risk of moving soil-borne pests, such as *Meloidogyne chitwoodi* and *Meloidogyne fallax*, *Xiphinema rivesi* and other non-listed *Xiphinema* species which are vectors of Nepoviruses, even if not always specifically injurious for plants of the genus *Vitis*. Therefore, they have also been taken into consideration (Table 4).

A brief comment on the syndrome associated with the Grapevine Pinot Gris virus (GPGV) is also reported.

Details of all the pests concerned can be found in the EPPO Global Database (EPPO, 2017a), in EPPO Standards regarding the specific pests or crops and in relevant scientific references.

Identification of lots

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

For non-grafted plants (e.g. rootstock mother plants), the cultivar, and where relevant the clone, are the primary lot-distinguishing characters.

Table 1. Specific pests of *Vitis* spp.

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects: *Margarodes prieskaensis*, *Margarodes vitis*, *Margarodes vredendalensis*	**Insects:** *Viteus vitifoliae* **Bacteria and phytoplasmas:** Grapevine flavescence dorée phytoplasma, *Xylophilus ampelinus*	**Insects:** *Eupoecilia ambiguella* (Israel, quarantine pest, 2009; Jordan, quarantine pest, 2007), *Scaphoideus titanus* (Israel, quarantine pest, 2009; Turkey, A1 List, 2007) **Fungi:** *Eutypa lata* (Israel, quarantine pest, 2009; Jordan, quarantine pest, 2007), *Phakopsora euvitis* (Israel, quarantine pest, 2009; Jordan, quarantine pest, 2007), *Phyllosticta ampelicida* (Israel, quarantine pest, 2009; Jordan, quarantine pest, 2007) **Viruses:** Arabis mosaic virus (ArMV) (Israel, quarantine pest, 2009; Norway, quarantine pest, 2012; Turkey, A2 List, 2007), Grapevine chrome mosaic virus (GCMV) (Jordan, quarantine pest, 2007), Grapevine fanleaf virus (GFLV) (Turkey, A2 List, 2007), Grapevine leafroll-associated virus 1 (GLRaV 1) (Turkey, A2 List, 2007), Grapevine leafroll-associated virus 2 (GLRaV 2) (Turkey, A2 List, 2007), Grapevine leafroll-associated virus 3 (GLRaV 3) (Turkey, A2 List, 2007), Grapevine leafroll-associated virus 4 (GLRaV 4) (Turkey, A2 List, 2007, including GLRaV 5)

Table 2. Polyphagous pests affecting *Vitis* spp.

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects: *Aleurocanthus woglumi*, *Homalodisca vitripennis*, *Lycorma delicatula* **Viruses:** Peach rosette mosaic virus (PRMV)	**Insects:** *Aleurocanthus spiniferus*, *Frankliniella occidentalis*, *Maconellicoccus hirsutus*, *Platynota stultana*, *Popillia japonica*, *Scirtothrips dorsalis*, *Spodoptera littoralis* **Bacteria and phytoplasmas:** '*Candidatus* Phytoplasma solani', *Xylella fastidiosa* **Viruses and viroids:** Blueberry leaf mottle virus (BLMV), Raspberry ringspot virus (RRV), Tomato ringspot virus (ToRSV)	**Insects and mites:** *Draeculacephala minerva* (EU, Annex I/A1; Turkey, A1 List, 2007), *Epiphyas postvittana* (Jordan, quarantine pest, 2007), *Graphocephala atropunctata* (EU, Annex I/A1; Turkey, A1 List, 2007), *Hyphantria cunea* (Azerbaijan, A2 List, 2007; Belarus, quarantine pest, 1994; Israel, quarantine pest, 2009; Jordan, quarantine pest, 2007; Kazakhstan, A2 List, 2009; Russia, A2 List, 2014; Ukraine, A2 List, 2010; Uzbekistan, A1 List, 2008), *Jacobiasca lybica* (Turkey, A1 List, 2007), *Naupactus xanthographus* (Jordan, quarantine pest, 2007), *Parthenolecanium corni* (Israel, quarantine pest, 2009), *Xyphon fulgidum* (EU, Annex I/A1; Turkey, A1 List, 2007) **Viruses:** Artichoke Italian latent virus (AILV) (Jordan, quarantine pest, 2007), Strawberry latent ringspot virus (SLRV) (Israel, quarantine pest, 2009; Norway, quarantine pest, 2012; Turkey, A1 List, 2007), Tomato black ring virus (TBRV) (Israel, quarantine pest, 2009; Norway, quarantine pest, 2012; Turkey, A2 List, 2007)

For grafted plants (e.g. scion mother plants), the grafting combination, cultivar and rootstock, and their clones when relevant, are the primary criteria for lot identification.

A lot should also include all plants originating from the same propagating material (both scion and rootstock for grafted plants), of the same age and cultivated in a single field (or set of plants in the case of potted plants).

Selection of plants for visual inspection and sampling for laboratory testing

This section contains guidance on visual inspection of places of production of *Vitis* 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 location of fields and assessing the regulations or NPPO requirements for the purpose of the inspection. This may be for monitoring or survey purposes, for the issue of a phytosanitary certificate or for internal movement certification, such as for the issue of an EU plant passport.

Selection of plants for visual inspection (general aspects)

Inspection of plants at a place of production is covered in general terms by EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009a). For the purposes of this procedure, these principles also apply for different types of plant propagating material, as for rootstock and scion mother plants or rooted cuttings, irrespective of whether they are grafted or own-rooted or if grown in the open field

Table 3. Pests unclassified or incidental on *Vitis* spp.

UNCLASSIFIED
Oemona hirta (EPPO A1 List)
Otiorhynchus sulcatus (Israel, quarantine pest, 2009; Jordan, quarantine pest, 2007)
Pseudococcus comstocki (Azerbaijan, A2 List, 2007; Belarus, quarantine pest, 1994; Israel, quarantine pest, 2009; Kazakhstan, A2 List, 2009; Moldova, A2 List, 2006; Uzbekistan, A2 List, 2008)
Tetranychus pacificus (Israel, quarantine pest, 2009)
INCIDENTAL
Scirtothrips aurantii (EPPO A1 List)
Scirtothrips citri (EPPO A1 List)
Tobacco ringspot virus (EPPO A2 List)

Table 4. Soil-borne pests of concern

Insects
Heteronychus arator (EPPO A1 List)
Nematodes
Meloidogyne chitwoodi (EPPO A2 List)
Meloidogyne fallax (EPPO A2 List)
Meloidogyne mali (EPPO A2 List)
Xiphinema rivesi (EPPO A2 List) and other *Xiphinema* species
Fungi
Phymatotrichopsis omnivora (EPPO A1 List)

(e.g. nurseries) or inside glasshouses (e.g. potted plants). The aim of these inspections is to detect the presence of plant pests by visual inspection, either alone or in combination with sample collection for laboratory testing to obtain confirmation of the diagnosis.

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

Recommendations for visual inspection

The number of plants that must be visually 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* (FAO, 2009). For instance, from a lot of 10 000 plants, 3689 plants need to be inspected to provide a 99% confidence of detecting symptoms present in 0.1% of the plants, provided the symptoms are uniformly distributed and the plants are randomly selected. For small lots, the numbers required will often mean that all plants should be visually inspected.

In practice, in scion mother plants, symptoms of grapevine pests are commonly more frequent and more easily detectable than in rootstock plants. In this case, inspection of whole plants may be performed, because lots are generally not very large.

For the inspection of rootstock mother plants and rooted cuttings, where pest symptoms are often latent, hidden or less specific, inspection may be randomly carried out on a set of plants, accordingly to ISPM 31 (FAO, 2009), and sampling for laboratory testing for detection of latent infection may be recommended.

Monitoring and sampling for known vectors of pathogens can be a complementary activity to visual inspection of plants.

Inspection of the place of production in the case of exports

Procedures for the inspection of consignments of plants, plant products and other regulated articles at import and export are described in the ISPM no. 23 *Guidelines for inspection*, which states that inspection can be used to verify compliance with some phytosanitary regulations (FAO, 2005). Therefore, place of production and pre-export inspections may be carried out to verify that the exporting lot meets phytosanitary requirements of the importing country.

For export, a common practice to prevent the spread of soil-borne pests is to uproot and remove soil residues from plants using pressure washers. As a result, plants are delivered as bare rooted plants with a lower phytosanitary risk.

Sampling for laboratory testing (general aspects)

The following of good hygiene procedures is important when collecting samples for the laboratory; in particular, tools should be disinfected between sample collections. Samples should be sent to the laboratory as soon as possible after collection.

Sampling of symptomatic material

Inspectors should be familiar with the symptoms of the listed pests they may encounter, and if any are observed suspected samples should be taken for laboratory testing. Details of the procedures for sampling for the individual pests are given in Appendix 1.

In general, samples should be taken from individual plants and these should be kept separate in order to aid diagnosis and obtain a measure of the number of plants that are infested. Nevertheless, pooling of items is acceptable for sampling of certain pests.

If the inspector is confident in 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 plants.

Sampling of asymptomatic material

In situations where it is difficult to find symptoms, or to declare a pest-free place of production or determine area freedom, sampling of asymptomatic plants and vectors may be required in order to detect latent or hidden infections for

regulated pests. Sample size should be increased if varieties are likely to have asymptomatic infections of specific pests or the origin of material includes potential high-risk or areas with high vector populations.

The analytical sensitivity of each test to be used should be known in order to organize and perform the sampling protocol according to laboratory needs. In general, pooling of samples from no more than five plants is acceptable for the detection of viruses, bacteria and phytoplasmas in the most commonly used diagnostic protocols.

Sampling of asymptomatic plants may reinforce the outcomes of visual inspections. In the absence of any symptoms, a number of plants within each lot may be selected for the detection of latent infections. Samples from 60 plants, from any lot size, may be taken to detect a latent infection level of 5% with a confidence of at least 95%.

All samples must be traceable back to the original plant or lot.

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

For further details on symptoms, sampling and identification of the relevant pests of grown *Vitis* varieties see Appendix 1.

Young plants, rootstock and scion mother plants

The young plants for inspection in a nursery (grafted or own-rooted) will usually be growing in the field, or sometimes in pots under protection. Each lot should be individually inspected because it may have a different origin, grafting combination and specific features in terms of disease resistance, history, previous treatments and potentially different infestations levels.

The time of year for inspection will vary with the pest species, depending on the optimum time for expression of symptoms for a specific pest survey programme. In general, plants should be in active growth and have sufficient time after breaking dormancy to be showing symptoms of diseases.

Inspections should be completed before general senescence, which starts in the autumn, or before defoliating operations, such as green pruning or mechanical harvesting in scion mother plants. Timing of inspection should be linked to symptoms of specific pests. For instance, while symptoms of Grapevine flavescence dorée phytoplasma are detectable starting from flowering until the end of the season, strains of Grapevine leafroll-associated virus, are visually detectable late in the growing season. However, dormant plants may be sampled, even during the winter months when there are no apparent symptoms on plants, as in the case of cane sampling for the detection of viruses.

Visual inspection will only detect pests which are apparent on the plants, such as insect stages, galls or mildews, or are systemic but showing symptoms in the foliage or stems, as for phytoplasmas and viruses. Symptoms are best detected when the timing, climatic conditions or variety susceptibility are appropriate.

For the inspection procedure, a look over the relevant lot should be carried out and any abnormal symptom should be examined thoroughly.

Inspectors should particularly look for symptoms on shoots, leaves and fruits, if present. Additionally, inspectors should look for vectors that transmit the disease. Symptoms caused by insects are often generic for most primarily foliage feeding pests. Sometimes leaf or root galls appear. Larval damage is commonly not specific, especially for those insects which have powerful mouthparts to cut plant tissues.

Diseases may exhibit different symptoms, which can be apparent via reduced growth, stem grooving, stunting and other deformations. Leaves may be thicker than normal, or may show many other symptoms, such as ring spots, discoloration, pale or reddish areas, clearing of veinlets, veinal or interveinal necrosis, rolled margins or mild deformation. Shoots may also exhibit several symptoms, with deformed, flexible and drooping aspects, or incomplete lignification.

It should be highlighted that the same infected plant may not show symptoms systematically, as it may exhibit signs of disease one year and no symptoms the following year. If asymptomatic infection is suspected (as for Grapevine flavescence doreé), or plants are being indexed for possible latent infection, then random samples representative of the whole lot should be collected.

On grape-producing mother plants, inflorescences, peduncles and berries may desiccate and drop off. Bunches may appear shrivelled or brown, or have mildew, depending on the pest.

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Appendix 1 – Symptoms and sampling for identification of quarantine pests of *Vitis* plants

For each pest mentioned below, basic information on host range, distribution, symptom description and elements for sampling are detailed. 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/ (EPPO, 2017a).

Further information can be found in relevant EPPO Standards and in named scientific references. When an EPPO Diagnostic Protocol exists it is mentioned in the text. However, the fact that there is no EPPO Diagnostic Protocol does not mean that there is no method for diagnosis available in the scientific literature.

Insects

Aleurocanthus spiniferus (orange spiny whitefly) (EPPO A2 List)

Aleurocanthus spiniferus (Fig. 2) is a polyphagous pest and *Citrus* spp. are the main hosts of economic importance. It is distributed mainly in Asian countries and a few other

Fig. 2 Adult female *Aleurocanthus spiniferus.* Photo: M. A. van den Berg, ITSC (ZA).

countries in Africa and Oceania and in Hawaii. In the EPPO region it has been recorded in Italy and Montenegro.

Symptom description

Dense colonies of immature stages develop on leaf undersides; the adults fly actively when disturbed. Leaves and fruit have spots of sticky, transparent honeydew, which become covered in black sooty mould fungus. A heavy infestation gives trees an almost completely black appearance.

Sampling and identification

Infested samples showing the presence of various stages or debris of the insects (e.g. adults, pre-imaginal whitefly stages, puparia or pupal cases) 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 a diagnostic laboratory as soon as possible.

Further information on *A. spiniferus* can be found in the EPPO Global Database (EPPO, 2017a) and in EPPO Standard PM 7/7 (1) Aleurocanthus spiniferus (EPPO, 2002b).

Eupoecilia ambiguella **(European grape berry moth) (quarantine pests for Israel and Jordan)**

Eupoecilia ambiguella (Fig. 3) is a polyphagous species and a quarantine pest for Israel and Jordan. It is considered a key pest of grapevine. It is widespread in Europe and in Asia (EPPO, 2017a).

Symptom description

The insect overwinters as pupae concealed in the bark of grapevines. The first-generation larvae develop on inflorescences (flower buds are held together by a silken web). Second-generation larvae cause severe damage to fruit clusters. Sometimes another partial generation occurs.

Sampling and identification

The flight period can be monitored effectively by using pheromone traps. The flight of the overwintering generation starts after bud-break. Once collected, each trap should be marked with the nursery reference number and date in order to collate survey results and determine the rate of infestation.

Fig. 3 *Adult European grape berry moth. Photo: Todd M. Gilligan and Marc E. Epstein, TortAI: Tortricids of Agricultural Importance, USDA APHIS ITP, Bugwood.org.*

Spread of *E. ambiguella* by plants for planting is of low probability since the insect overwinters in the pupal stage in crevices of the grapevine bark, which is missing in plants from the nursery. Therefore, monitoring for the presence of adult berry moths in nurseries could be considered only when strictly requested by the importing country.

Additional information on monitoring and control programmes for *E. ambiguella* are included in EPPO Standard PP 2/23 (1) *Grapevine* (EPPO, 2002a).

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

Frankliniella occidentalis (Fig. 4) is a polyphagous pest which is widespread in many countries in Asia, Africa, Central and South America, Europe and Oceania (EPPO, 2017a). In Northern European countries it is found mainly in glasshouses, but in southern regions it is mainly recorded as field pest.

Symptom description

Frankliniella occidentalis infests vineyards of table grapes. *Frankliniella occidentalis* multiplies on the flowers and young bunches, causing scarring of grapes.

Sampling and identification

During inspection of plant material for the presence of *F. occidentalis*, aerial parts of plants should be shaken over sheets of white paper. Thrips and other small insects present on the surface of plants and in flowers fall onto the paper, where they can be collected with small brush-pencils or by an insect aspirator ('pooter') and preserved in AGA (10:1:1 60% alcohol:glycerine:acetic acid). It is also possible to place them immediately in 10% ethanol and Teepol, and after a week transfer them into 70% ethanol. Stronger alcohol should be avoided as the thrips are likely to contract and become very rigid.

Further details are available in the EPPO Global Database (EPPO, 2017a), EPPO Standard PP 2/23 (1)

Fig. 4 *Frankliniella occidentalis* adults. Photo: EPPO Global database. Courtesy: P. M. J. Ramakers, PTG, Waaldwiik (NL).

Grapevine (EPPO, 2002a) and in EPPO Standard PM 7/11 (1) Frankliniella occidentalis (EPPO, 2002c).

Maconellicoccus hirsutus (pink hibiscus mealybug) (EPPO A2 List)

Maconellicoccus hirsutus is a highly polyphagous pest which is widespread in Australia, Africa, the Middle East, Central America, northern South America and the USA. In the EPPO region it has been recorded in Tunisia and Cyprus (EPPO, 2017a).

Symptoms and identification

Plant material, in particular twigs, should be examined for distorted, stunted, bunchy growths containing white woolly wax, curled leaves, tiny salmon-pink eggs and sooty mould or sticky honeydew (Fig. 5). The honeydew produced may attract attendant ants. The entire mealybug colony tends to become covered by white, sticky, elastic, woolly, ovisac wax material. When the sticky ovisac wax is parted with a needle, clusters of pink eggs and pink to grey females become visible.

Sampling and identification

Samples of leaves and soft tissues of plants 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 a diagnostic laboratory as soon as possible.

Further details are available in the EPPO Global Database (EPPO, 2017a), and in EPPO Standard PM 7/70 (1) Maconellicoccus hirsutus (EPPO, 2006a).

Fig. 5 *Maconellicoccus hirsutus. Photo: Jeffrey W. Lotz, Florida Department of Agriculture and Consumer Services, Bugwood.org.*

Margarodes prieskaensis, *Margarodes vitis* and *Margarodes vredendalensis* (EPPO A1 List)

Margarodes prieskaensis (Fig. 6) and *M. vredendalensis* infest roots of *V. vinifera* and both are only known to be present in South Africa. *Margarodes vitis* is lives on the

Fig. 6 Female *Margarodes prieskaensis*. Photo: EPPO Global Database. Courtesy C. A. de Klerk, Nietvoorbij Institute for Viticulture and Oenology Stellenbosch (ZA).

Fig. 7 Damage on grapevine caused by *Margarodes* sp. Photo: EPPO Global Database. Courtesy C. A. de Klerk, Nietvoorbij Institute for Viticulture and Oenology Stellenbosch (ZA).

roots of many wild plants, and grapevine is the main economically important host plant. *Margarodes vitis* is only recorded in South America.

Symptom description
All *Margarodes* species have subterranean stages. These usually live at depths of 20–60 cm, but can occur at depths of up to 120 cm. Infested plants show a progressive decline where shoots become thinner and shorter and leaves smaller. One or more of the branches of the vine may die, followed in severe infestations by the eventual death of the whole plant (Fig. 7). Infestations of vineyards are usually patchy. The symptoms resemble those caused by grapevine phylloxera (*Viteus vitifoliae*), but in the case of *Margarodes* no galls are formed.

Sampling and identification
Nymphs of the species attach themselves to roots and feed on them. Once feeding is complete, the nymphs are capable of secreting a protective waxy covering to form pearl-like cysts. For the sampling of cysts, soil and roots may be washed with water through a sequence of sieves. Live cysts sink into the water, dead cysts float. Live cysts of various sizes can be collected with small brush-pencils and placed on moist filter paper in plastic boxes for gathering females on emergence.

Further details are available in De Clerk (1980), in the EPPO Global Database (EPPO, 2017a) and in EPPO Standard PM 7/82 (1) Margarodes prieskaensis, Margarodes vitis, Margarodes vredendalensis (EPPO, 2007a).

Platynota stultana (EPPO A2 List)

Platynota stultana is a highly polyphagous leafroller tortricid from Mexico and the Southwestern USA, found in Spain in 2009.

Symptom description
Platynota stultana has been recorded on more than 25 plant families with many economically important plants, including *Vitis vinifera*. Larvae of *P. stultana* tie leaves together and feed inside.

Sampling and identification
Adults can be detected by using pheromone traps and larvae by visual examination of leaves, while nests can be found in flower clusters and bunches, as well as on leaves and in shoot tips. In Spain, damage has exclusively been reported on *Capsicum annuum*.

Further details are available in the EPPO *Mini data sheet on* Platynota stultana (EPPO, 2017b) and other documents available in the EPPO Global Database https://gd.eppo.int/.

Popillia japonica (Japanese beetle) (EPPO A2 List)

Popillia japonica is a highly polyphagous species. It originates from Northeastern Asia and was introduced into North America. In the EPPO region it is recorded in Italy and the Azores (Portugal).

Symptom description
Defoliation symptoms caused by adults are easily noticed (see Fig. 8). The beetle skeletonizes the leaves by chewing out the tissue between the veins and leaving a vein skeleton. Leaves may turn brown and fall. The larvae cause feeding damage to the roots of host plants, and the symptoms caused are not specific.

Fig. 8 Adults of *Popillia japonica* feeding on grapevine leaves. Photo: EPPO Global Database. Courtesy: Japanese Beetle Research Laboratory, USDA (US).

Sampling and identification
Adults can be detected by visual examination of green parts of plants and larvae by visual examination of roots in soil. Traps containing food-type lures and/or sex attractants may be used. Where appropriate, samples for laboratory testing should be taken for final identification of the pest.

Further details are available in the EPPO Global Database (EPPO, 2017a), in EPPO Standard PM 7/74 (1) Popillia japonica (EPPO, 2006b) and in EPPO Standard PM 9/21 (1) Popillia japonica*: procedures for official control* (EPPO, 2016a).

Scaphoideus titanus (quarantine pests for Israel and Turkey)

This insect is considered the main vector of the Grapevine flavescence dorée phytoplasma. It originates from North America, but is now present in several countries in Europe, mainly in the southern regions.

Symptom description
Scaphoideus titanus feeds on leaves but causes non-characteristic symptoms (Fig. 9).

Sampling and identification
Sampling of nymphs may be carried out looking at the underside of sucker leaves at the beginning of the growing

Fig. 9 Fifth instar nymph of *Scaphoideus titanus* on grapevine leaf. Photo: EPPO Global Database. Courtesy Ilya Mityushev, Department of Plant Protection of the Russian Timiryazev State Agrarian University.

season (in Southern Europe starting from early June). Three yellow sticky traps per vineyard may also be placed within the canopy of stock plants for trapping adults, starting from early summer until the autumn (from the beginning of July until the middle of October in Southern Europe).

Further details are available in *Grapevine flavescence dorée phytoplasma* (CABI/EPPO, 1997), EPPO Standard PP 2/23 (1) *Grapevine* (EPPO, 2002a) and in Lessio *et al.* (2011).

Scirtothrips dorsalis (Assam thrips) (EPPO A2 List)

Scirtothrips dorsalis (Fig. 10) is a highly polyphagous species with its range in tropical Asia, but it is widespread in Northern Australia and the Solomon Islands, Hawaii (US) and South Africa. In the EPPO region it has been recorded on different host plants in Israel, Spain and England (GB).

Symptom description

Symptoms are silvering of the leaf surface, linear thickening of the leaf lamina and brown excreta on the leaves and fruits of host plants. The species can cause distortion to young leaves and premature leaf fall.

Sampling and identification

All stages of *S. dorsalis* feed on epidermal and sometimes palisade cells of young leaves. They do not feed on mature leaves. They could be carried on plants for planting, in particular seedlings or cuttings with young growing leaf buds.

Further details are available in the EPPO Global Database (EPPO, 2017a) and in EPPO Standard PM 7/56 (1) *Diagnostics.* Scirtothrips aurantii, Scirtothrips citri, Scirtothrips dorsalis (EPPO, 2005a).

Fig. 10 Scirtothrips dorsalis. *Photo: Andrew Derksen, USDA APHIS, Bugwood.org.*

Spodoptera littoralis (Mediterranean brocade moth) (EPPO A2 List)

Spodoptera littoralis (Fig. 11) is a highly polyphagous lepidopteran species which is widespread from Africa and Southern Europe to the Arabian Peninsula and into Iran.

Symptom description

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.

Sampling and identification

The species can be found on plants or above-ground plant parts. All stages of the pest can be detected visually (with

Fig. 11 Adult *Spodoptera littoralis*. Photo: EPPO Global Database. Courtesy O. Heikinheimo (FI).

a hand lens for early stages), and specimens can be collected by hand or with 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. Pheromone-baited traps allow adult males to be caught and light traps catch both adult females and males. Adults can sometimes be found and collected by hand, especially when plants are transported or stored in cool conditions. Eggs can be found on all above-ground plant parts, mostly on the underside of leaves.

Further details are available in the EPPO Global Database (EPPO, 2017a) and in EPPO Standard PM 7/124 (1) Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania (EPPO, 2015).

Viteus vitifoliae (phylloxera, grapevine leaf louse) (EPPO A2 List)

The main economically important hosts of *Viteus vitifoliae* (Fig. 12) are *Vitis* spp. The pest is native to North America but is now widespread in all continents of the world, and in many countries of the EPPO region.

Symptom description

Viteus vitifoliae infests the root system and leaves of *Vitis* species, and is a cecidogenic insect (gall-forming). Phylloxera is responsible for the formation of numerous knots and tuberosities on roots of *Vitis* plants. On *V. vinifera* it causes serious damage to the radical system which degenerates into a slow decline, until the death of the plant. Therefore, grapevine is grafted onto selections and hybrids of American rootstocks which are tolerant of root system infestations.

The full life-cycle of *V. vitifoliae* on American *Vitis* spp. is a complex alternation between an aerial, leaf-feeding form, gallicolae, and the root-feeding form, radicicolae. On *V. vinifera,* the radicicolae form predominates, while the gallicolae form is less common, even if observed in several varieties without serious damage.

Gallicolae form

Small galls develop on the leaf surface, sometimes so numerous as to cover practically the entire leaf. Although leaf galling by phylloxera does not normally cause significant losses in grape production, severe infestations do cause considerable distortion and falling of affected leaves, especially in rootstock mother plants if not properly managed, which can affect cutting yield.

Radicicolae form

Numerous knots or galls form on grapevine roots, with rotting of the roots, yellowing of the foliage and a general reduction in vigour of the vines. Death of the vines may result within 3–10 years.

Sampling and identification

Small leaf galls can be detected by visual inspection of rootstock plants, and also on sucker leaves of grafted vines. Leaf galls may also be observed on leaves in some European varieties, with no significant or minimal damage. Inspection of root-galling forms of phylloxera is most likely in regions where grafting of *V. vinifera* onto tolerant rootstocks is not a common practice. Emergence traps have been shown to be effective both in grafted and ungrafted *V. vinifera* vineyards for detection of the insects.

Further details are available in the EPPO Global Database (EPPO, 2017a), in Powell *et al.* (2013), Benheim *et al.* (2012) and in EPPO Standard PP 2/23 (1) *Grapevine* (EPPO, 2002a).

Nematodes

Meloidogyne chitwoodi (Columbia root-knot nematode) and *Meloidogyne fallax* (false Columbia root-knot nematode) and *Meloidogyne mali* (EPPO A2 List)

Meloidogyne chitwoodi has been recorded in some countries of Central Southern Europe, and in North America and a few countries of Africa, while *M. fallax* has been reported for a few countries in Central and Northern Europe and Australia.

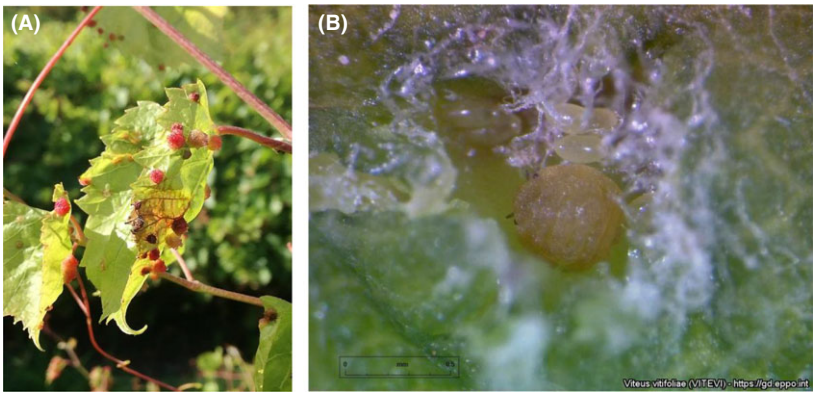

Fig. 12 (A) Leaf galls of *Viteus vitifoliae*. Photo Gianluca Governatori, Ersa-Friuli Venezia Giulia (IT). (B) *Viteus vitifoliae* within its gall. Photo: EPPO Global Database. Courtesy Jean-Francois Germain, Plant Health Laboratory, Montpellier (FR).

Meloidogyne mali originates from Japan and was probably introduced into the EPPO region with elm trees.

Symptom description
Meloidogyne chitwoodi and *M. fallax* have a very wide host range that includes many crop species. Even if *M. chitwoodi* and *M. fallax* are not specific pests of *Vitis* plants, several countries within and outside the EPPO require that plants for planting should have been produced in a place of production or field known to be free from these root-knot nematodes. *Meloidogyne mali* induces large root galls on its host plants (e.g. apple, elm), resulting in malformed root systems and retarded plant growth.

Sampling and identification
If the history of the field is not known, and if there are consequently no records of sampling and testing of the field or the field is not under official control due to previous findings, then the relevant area should be sampled and the samples found to be free from the relevant nematodes.

Additional information can be found in data sheets on quarantine pests *Meloidogyne chitwoodi* and *Meloidogyne fallax* (EPPO, 2017a), in EPPO Standard PM 9/17 (1) Meloidogyne chitwoodi *and* Meloidogyne fallax (EPPO, 2013a), in EPPO Standard PM 7/41 Meloidogyne chitwoodi *and* Meloidogyne fallax (EPPO, 2016b), in the mini data sheet on *Meloidogyne mali* (EPPO, 2017c) and other documents available in the EPPO Global Database https://gd.eppo.int/.

Virus transmitting *Xiphinema rivesi* (EPPO A2 List) and other *Xiphinema* species

Xiphinema rivesi has been recorded in many European countries and other *Xiphinema* species such as *Xiphinema index*, which are vectors of Nepoviruses and are widely present in the EPPO region.

Symptom description
Root tips which are attacked may become hook-shaped or swell to form terminal galls. Root tissues darken with cortical hyperplasia and lateral root proliferation; secondary and feeder roots are often lost. Often the obvious symptoms of infestation are from the virus rather than the nematode. Virus symptoms are commonly seen on grape, raspberry and strawberry, and include: chlorotic mottling and defoliation of leaves; bright yellow discoloration of foliage; chrome yellow flecks along main veins; spots, blotches and crinkling of leaves; and stunting (Martelli and Taylor, 1990).

In the cases of exports of plants to those countries in which Nepoviruses (such as Arabis mosaic virus, Artichoke Italian latent virus, Grapevine chrome mosaic virus and Grapevine fanleaf virus) are listed, plants should be planted in plots known to be free of *X. rivesi* or other *Xiphinema* species. If the status of this nematode is unknown, then the field should be sampled and examined for the presence of *Xiphinema* species.

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 can be found in the EPPO Global Database (EPPO, 2017a), and in EPPO Standard PM 7/95 Xiphinema americanum sensu lato (EPPO, 2009c).

Fungi

Eutypa lata (dieback of grapevine) (quarantine pest for Israel and Jordan)

Eutypa dieback is caused by *Eutypa lata*, a fungus which has many host plants, including many woody plants.

Symptom description
The disease infects grapevines older than 8 years. Shoots arising from the cankered area then generally show the typical symptoms of deformation and discoloration during the first 2 months of growth. Young leaves are small and

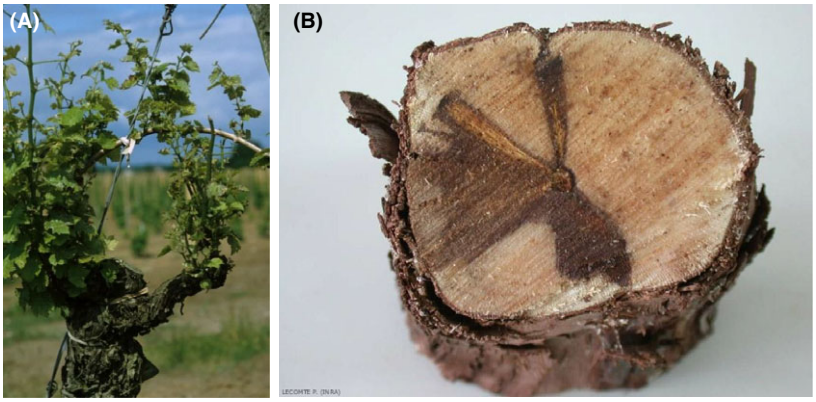

Fig. 13 (A) Shoots from an infected *Eutypa lata* branch have slowed growth, short internodes and tight, chlorotic leaves and leaf galls. (B) The wood necrosis generated by *Eutypa lata* always appears in a sectoral form. Photo INRA e-phytia, http://ephytia.inra.fr

chlorotic and dwarfing of the internodes can be observed (Fig. 13A). Bunches often have a mixture of small and large berries.

Sampling and identification
Ascospores of the fungus infect fresh wounds during pruning, giving rise to a canker. Wood samples should be collected from cordons of symptomatic plants showing dead spurs, discolored vascular tissues and cankers (Fig. 13B).

Further details are available in EPPO Standard PP 2/23 (1) *Grapevine* (EPPO, 2002a).

Phakopsora euvitis **(quarantine pests for Israel and Jordan)**

Phakopsora euvitis occurs mainly in tropical and subtropical areas, and it is reported that it is more damaging in these areas than in temperate areas. *Vitis* plants have been recorded as hosts of this fungus, mainly *V. labrusca* and *V. vinifera*, but also other species. *Phakopsora euvitis* is recorded from the north and far east of Russia (EPPO, 2007b).

Symptom description
Yellowish to brownish lesions of various shapes and sizes appear on the leaves. Yellowish orange masses of urediniospores are produced on the lower leaf side (Fig. 14), with dark necrotic spots on the upper surface. Heavy infection causes early senescence of the leaves and premature leaf fall. The disease can cause poor shoot growth, reduction of fruit quality and yield loss.

Sampling and identification
If there are symptoms or risky materials have been introduced, sampling of mycelium may be carried out in dormant grapevine shoots. Symptomatic leaves may also be sampled and delivered to laboratories for testing.

Further details are available in the EPPO Global Database (EPPO, 2017a).

Fig. 14 *Phakopsora euvitis*. Photo: EPPO Global Database. Courtesy: Regina Sugayama (Agropec).

Phyllosticta ampelicida **(black rot of grapevine) (quarantine pest for Israel and Jordan)**

The black rot disease caused by the fungus *Phyllosticta ampelicida* (teleomorph *Guignardia bidwellii*) can have a high economic impact in some European countries (especially in an Atlantic climate).

Symptom description
Symptoms on leaves (spots 2–10 mm in size) become cream-coloured, deepening to reddish brown. Leaf spots are bordered by a narrow band of dark brown tissue. Lesions on peduncles and pedicels are small darkened depressions, which turn black later. Black cankers develop on young shoots. Whitish dots indicate the infection of berries, later surrounded by reddish brown rings. The berries then develop into blue-black mummies.

Sampling and identification
Samples of symptomatic leaves, stems and fruits should be collected, placed in a plastic bag and kept in cool boxes until they have been sent to the laboratory.

Further details are available in EPPO Standard PP 2/23 (1) *Grapevine* (EPPO, 2002a).

Bacteria and phytoplasmas

Grapevine flavescence dorée phytoplasma (EPPO A2 List)

Grapevine flavescence dorée phytoplasma is one of an important complex of diseases affecting *Vitis*. It is part of the complex of diseases associated with the presence of phytoplasmas known as grapevine yellows. It has been recorded in Central Southern Europe. Its spread occurs through infected grapevine planting material and through its main vector, the cicadellid *Scaphoideus titanus*.

Symptom description
Symptoms are not specific for Grapevine flavescence dorée but are more or less the same for all phytoplasma diseases of grapevine (grapevine yellows). Symptoms are not present at the beginning of the disease and become apparent from spring onwards, involving reduced growth and, sometimes, the absence of shoot formation. However, they usually develop after flowering. The whole plant may show symptoms or only a group of shoots.

On shoots. When infected early, shoots fail to lignify and are thin, rubbery and hang pendulously. They later become brittle and there may be necrosis of the apical and lateral buds. During winter, the non-lignified branches blacken and die. If infected later in the growing season, lignification is interrupted. Numerous small black pustules form along the diseased branches of susceptible cultivars.

On leaves. The leaves show colour aberrations and downward-rolled margins. In white-fruited cultivars, there is a yellowing of the portion of the lamina exposed to the sun that confers a metallic lustre to the leaf surface (Fig. 15A). Later in the season, well-defined creamy-yellow spots a few millimetres in diameter appear along the main veins. These spots enlarge and form continuous yellow bands along the veins, which gradually extend over large parts of the leaf surface. Red-fruited cultivars develop a similar pattern of colour changes of the leaves, but the discolorations are reddish (Fig. 15B). The central portion of the discoloured areas becomes necrotic and dries out. These brittle rigid leaves are frequently detached in wind, but they appear to withstand autumn frosts well and fall later than healthy leaves.

In rootstock varieties, symptoms are less apparent or absent and are more difficult to detect by visual inspection.

On fruits. Early in the season, fruits can fail to develop and only the shrivelled inflorescence is visible (Fig 16A). The infection can be observed later in the season on mature fruits, which can appear brown and shrivelled (Fig. 16B).

Sampling and identification

Samples should be collected in July–October, selecting leaves showing symptoms but in good condition (no necrotic areas) and not affected by other pests. Approximately 20 leaves per plant should be collected. It is possible to test asymptomatic plants (rootstocks, canes, asymptomatic leaves), but no validation data are available. Material for testing should be used fresh, or stored at −20°C (or lower) depending on the storage time. Pooling leaves from up to five plants is possible. Surveys should be conducted in order to find any presence of the main vector *S. titanus*.

Further details are available in the EPPO Global Database (EPPO, 2017a), in EPPO Standard PM 7/079 (2) *Grapevine flavescence dorée phytoplasma* (EPPO, 2016c), in EPPO Standard PP 2/23 (1) *Grapevine* (EPPO, 2002a) and in EPPO Standard PM 4/8 (2) *Pathogen-tested material of grapevine varieties and rootstocks* (EPPO, 2008).

'*Candidatus* Phytoplasma solani' (= grapevine bois noir phytoplasma, Stolbur phytoplasma) (EPPO A2 List)

'*Candidatus* Phytoplasma solani' is the causal agent of bois noir disease, one of the diseases of the grapevine yellows complex. It is widespread in wild plants in the EPPO region (from Central and Southern Europe to Asia) other than *Vitis*. It has also been recorded in regions of Chile, Niger, India and China. Its main vector is the cixiid planthopper *Hyalesthes obsoletus.*

Symptom description

The symptoms caused by bois noir, or black wood and other grapevine yellows pathogens are very similar to those of flavescence dorée. The name bois noir refers to the

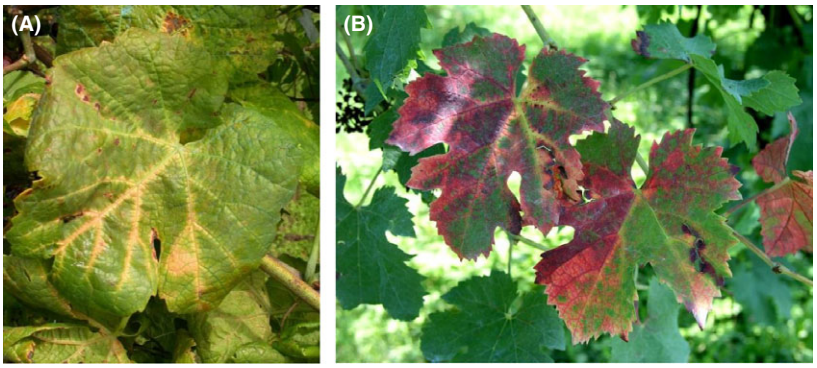

Fig. 15 (A) Clear foliar symptoms of Grapevine flavescence dorée phytoplasma showing coloured bands along the veins in (A) the white variety and (B) the red variety, Photo Gianluca Governatori, ERSA-Friuli Venezia Giulia (IT).

Fig. 16 Symptoms of infection on grapes at (A) an early stage and (B) a late stage with brown shrivelled fruits. Photo Gianluca Governatori, ERSA-Friuli Venezia Giulia (IT).

blackening of non-lignified shoots in winter, but it is not a specific symptom. It seems that bois noir symptoms appear later in the growing season than those of Grapevine flavescence dorée phytoplasma.

Sampling and identification

See the sampling procedures described for Grapevine flavescence dorée phytoplasma.

Further details are available in the EPPO Global Database (EPPO, 2017a) and in EPPO Standard PM 4/8 (2) *Pathogen-tested material of grapevine varieties and rootstocks* (EPPO, 2008).

Xylella fastidiosa (EPPO A2 List)

Xylella fastidiosa is highly polyphagous and widely distributed in the world. It has been reported in North, Central and South America, in Asia (Iran, Taiwan) and in Europe (France, Italy, Spain).

On grapevine, *X. fastidiosa* subsp. *fastidiosa* is the plant pathogenic bacterium causing Pierce's disease. The subspecies *fastidiosa* caused serious damage in Californian vineyards in the 1990s. It was detected in Mallorca (Spain, Balearic Islands) in 2016 on one plant of table grapes for home consumption and again in 2017 on 30 plants of table grapes for home consumption (EPPO, 2017d).

Symptom description on grapevine

Disease symptoms detailed below are from the USA and therefore may be more or less specific to those detailed for the EPPO region. On grapevine, the most characteristic symptom of primary infection is leaf scorch (Fig 17A). An early sign of infection is a sudden drying of part of a green leaf, which then turns brown while adjacent tissues turn yellow or red. The leaf symptoms can be confused with fungal diseases. The desiccation spreads over the whole leaf causing it to shrivel and drop, leaving only the petiole attached. Diseased stems often mature irregularly, with patches of brown and green tissue (Fig 17B). Chronically infected plants may have small, distorted leaves with interveinal chlorosis and shoots with shortened internodes. Fruit clusters shrivel. In later years, infected plants develop late and produce stunted chlorotic shoots. Symptoms involve a general loss of plant vigour followed by death of part of or the entire vine. Highly susceptible cultivars rarely survive for more than 2–3 years, although signs of recovery may be seen early in the second growing season. Young vines succumb more quickly than mature vines. More tolerant cultivars may survive chronic infection for more than 5 years.

Sampling and identification

The most suitable time to look for symptoms in grapevine is late summer to early autumn when the weather conditions are predominantly hot and dry or when grape plants are exposed to drought stress. Samples for the laboratory should be composed of cuttings with 10–25 leaves, depending on leaf size. The sample should include mature leaves. Young growing shoots should be avoided. For small plants, the entire plant can be sent to the laboratory. The sample should consist of branches representative of the symptoms seen on the plant(s). For testing individual asymptomatic plants at least 4–10 branches need to be collected, depending on the host and plant size.

The only known vector of *X. fastidiosa* in the EPPO region is *Philaenus spumarius* (Hemiptera Aphrophoridae), which transmits the CoDiRO strain of *X. fastidiosa* subsp. *pauca* in olive groves of Southern Apulia, in Italy (EPPO, 2016d). It may be collected with sweeping nets and/or aspirators. Insects may be trapped accidentally with sticky traps, and collected specimens can be used for testing. Vectors can be removed from the traps using small forceps/pincers and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol/acetone. Traps should be inspected on a weekly basis. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detection of the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol or at −20°C or −80°C. Sticky traps can also be stored at −20°C.

In California, the main vectors of Pierce's disease of grapevine are the sharpshooters *Homalodisca vitripennis* (EPPO A1 List) and *Graphocephala atropunctata*, EU quarantine pest, not recorded in the EPPO area.

Further details are available in the EPPO Global Database (2017a), in EPPO Standard PM 7/24 (3) Xylella fastidiosa (EPPO, 2018), in EPPO Standard PM 3/81 (1) *Inspection of consignments for* Xylella fastidiosa (EPPO, 2016d) and in EPPO Standard PM 3/82 (1) *Inspection of places of production for* Xylella fastidiosa (EPPO, 2016e).

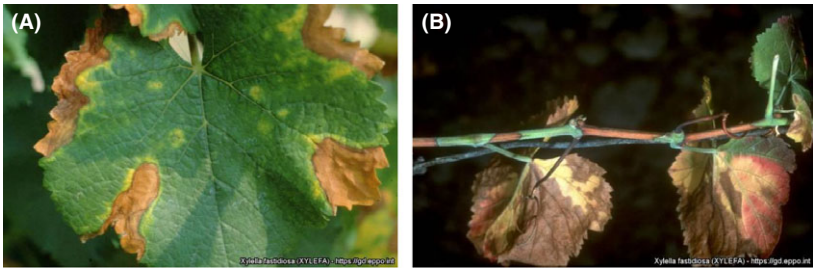

Fig. 17 Symptoms of *Xylella fastidiosa* on grapevine showing (A) marginal necrosis surrounded by a chlorotic halo on the leaf and (B) irregular ripening of bark Photo: EPPO Global Database. Courtesey M. Scortichini, Istituto Sperimentale per la Frutticoltura, Rome (IT) (A) and J. Clark, University of California, Berkeley (US) (B).

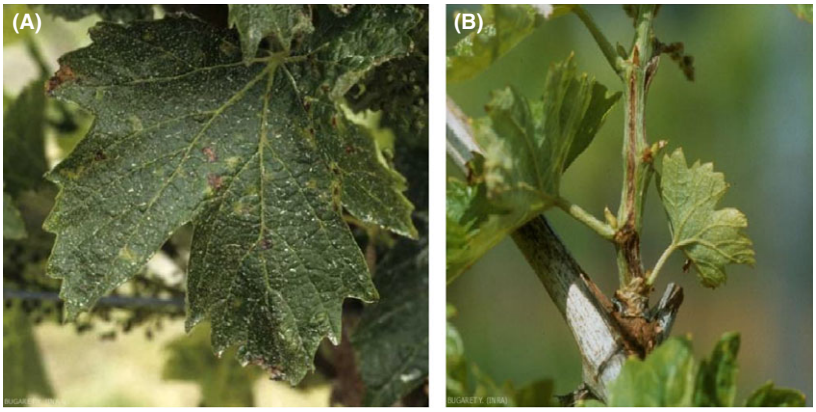

Fig. 18 Symptoms of *Xylophilus ampelinus* on grapevine showing (A) symptoms on leaf and (B) symptoms on shoots. Photo: INRA e-phyitia, http://ephytia.inra.fr.

Xylophilus ampelinus (EPPO A2 List)

Xylophilus ampelinus is the plant pathogenic bacterium that causes 'bacterial blight' of grapevine. The bacterium is specific to *Vitis*. *Xylophilus ampelinus* has been recorded mainly in Southern Europe (Greece, France, Italy, Moldova, Slovenia, Spain) and South Africa.

Symptom description

In the field, symptoms can appear on all aerial parts of plants. On leaves, necrotic spots surrounded by a discolored halo can be observed. Eventually, the central dried part of the spot drops out and the 'shot hole' symptom appears. However, when contamination reaches the leaf via the petiole, necrotic sectors surrounded by a halo occur (Fig. 18A).

Buds in infected shoots either fail to sprout or show stunted growth in the spring (Fig. 18B). Cracks appear along infected shoots, mainly in the lowest parts of the shoots. Infection spreads along the branches, which show a brown discoloration of tissues and may eventually die. Young shoots on infected spurs develop pale yellowish-green areas on the lower internodes. These expand upwards to become darker, crack and develop into cankers. When these cankers split, the xylem tissues are revealed. Later in summer, cankers are often seen on one side of petioles, causing a characteristic one-sided necrosis of the leaf. Infected canes can have no visible symptoms when they are latently infected. Almost all plant parts with disease symptoms exhibit a brown discoloration of the xylem tissues in longitudinal sections.

The symptoms described, especially on new vegetation and leaves, are typical of the disease but not specific, and confusion may occur with other diseases or disorders. Bacterial blight can affect both *V. vinifera* varieties and rootstocks.

Sampling and identification

The bacterium may be found in stems and leaves up to 10 cm, or even 40 cm, above visibly infected areas. Nursery and mother plant stocks should be inspected and handled using suitable equipment. Pruning tools should be disinfected.

Further details are available in the EPPO Global Database (EPPO, 2017a), in EPPO Standard PM 7/96 (1) Xylophilus ampelinus (EPPO, 2009d) and in EPPO Standard PP 2/23 (1) *Grapevine* (EPPO, 2002a).

Viruses

Tomato ringspot virus (ToRSV) (EPPO A2 List)

ToRSV occurs mostly in woody and ornamental plants, including grapes. The virus is present in North America and in several countries in the other continents. It has a very restricted distribution in the EPPO region, with no occurrences in fruit trees.

Symptom description

In general the symptoms cannot be taken as proof of the presence of ToRSV. Symptoms are difficult to diagnose early in the season unless vines are severely affected, in which case they have many winter-killed buds and weak, stunted shoot growth. By about 9 weeks after the start of vine growth, shoot and foliage symptoms are conspicuous on one or more shoots. Leaves develop ringspots and mottling, are reduced in size and rosetted due to the shortening of internodes. Fruit clusters are reduced in size with many berries aborting. Removal of bark from trunks and stems of diseased vines may reveal thickened, spongy phloem tissue with numerous necrotic pits.

Sampling and identification

Plants should be inspected for the symptoms described above. Where appropriate, samples for laboratory testing (plant parts with symptoms) should be taken for final identification. As the infection can be latent, representative samples of different lots should be taken and subjected to laboratory testing. Double antibody sandwich-ELISA and molecular tests can be conducted to detect ToRSV (EPPO, 2005b).

Further details are available in the EPPO Global Database (EPPO, 2017a), in EPPO Standard PM 7/49 (1) *Tomato ringspot nepovirus* (EPPO, 2005b) and in EPPO

Standard PM 3/32 (2) *Tomato ringspot virus in fruit trees and grapevine: inspection* (EPPO, 2013b).

Grapevine fanleaf virus (GFLV) (quarantine pest for Turkey)

This is a worldwide virus with a natural host range restricted to *Vitis* species. GFLV is transmitted by the nematode *Xiphinema index*.

Symptom description

Infected plants may show several symptoms, such as reduced vigour, short internodes, malformation of leaves and canes, chlorotic mottling, fewer and smaller bunches with shot berries, and bright yellow discoloration of the foliage, varying from scattered spots to total yellowing.

Sampling and identification

Sources of antigens for ELISA tests can be grapevine buds, roots, leaves and wood shavings. Wood shavings, however, are advantageous because: (i) they can be used throughout the year without apparent loss of efficiency due to the seasonable variation of antigen titre in vegetative organs, (ii) give low and consistent background readings, and (iii) are much more reliable for identification in American rootstocks, especially *Vitis rupestris* and its hybrids (Fig. 19).

Further details are available in Frison & Ikin (1991), EPPO Standard PM 4/8 (2) *Pathogen-tested material of grapevine varieties and rootstocks* (EPPO, 2008) and INRA e-phytia (2018).

Grapevine leafroll-associated viruses 1, 2, 3 and 4 (GLRaV 1, GLRaV 2, GLRaV 3 and GLRaV 4) (quarantine pest for Turkey)

These are worldwide viruses with a host range restricted to *Vitis* species. Vectors are insects feeding on the aerial parts of the plants, such as some scale insects.

Symptom description

Infected plants show downward rolling and discoloration of the leaves, which turn reddish-purple or yellowish in red- and white-fruited cultivars (Fig. 20). Bunches may be small and with discolored and tasteless berries. Symptoms are most noticeable in late summer to autumn. American *Vitis* spp. and their hybrids used as rootstocks can be symptomless carriers.

Sampling and identification

The same as for Grapevine fanleaf virus.

Further details are available in Frison & Ikin (1991) and in EPPO Standard PM 4/8 (2) *Pathogen-tested material of grapevine varieties and rootstocks* (EPPO, 2008).

Fig. 19 (A) leaf discolorations, (B) which can extend to a large surface area of the leaf. Photo INRA e-phyitia, http://ephytia.inra.fr.

Fig. 20 Foliar symptoms of Grapevine leafroll-associated virus, with downward rolling and reddish-purple interveinal colour in red grape variety. Photos Gianluca Governatori, ERSA – Friuli Venezia Giulia, IT.

Other viruses involved in the aetiology of European Nepovirus diseases

1) Arabis mosaic virus (ArMV) (quarantine pest, Israel and Norway; A2 List. Turkey).
2) Artichoke Italian latent virus (AILV) (quarantine pest, Jordan).
3) Blueberry leaf mottle virus (BLMV) (EPPO A2 List).
4) Grapevine Bulgarian latent virus (GBLV).
5) Grapevine chrome mosaic virus (GCMV) (quarantine pest, Jordan).
6) Raspberry ringspot virus (RRV) (EPPO A2 List).
7) Strawberry latent ringspot virus (SLRV) (quarantine pest: Israel, Norway; A1 List, Turkey).
8) Tomato black ring virus (TBRV) (quarantine pest, Israel, Norway; A1 List, Turkey).

These are Nepoviruses, which separately, or in combination, may be involved in the aetiology of the diseases. These viruses are spread throughout the Central Europe, parts of Eastern Europe and the Balkans.

Symptom description
Similar to those induced by Grapevine fanleaf virus, with leaf and cane deformation, chlorotic mottling, reduced vigour, heavy (chromogenic) strains, crop losses and bright yellow discolorations.

Sampling and identification
The same as for Grapevine fanleaf virus.

Further details are available in Frison & Ikin (1991) and in the EPPO Global Database (EPPO, 2017a).

Grapevine Pinot Gris virus (GPGV)

Since 2003, a new syndrome characterized by symptoms of stunting, chlorotic mottling, leaf deformation, reduced yields and quality has been reported in grapevine (*V. vinifera*) in Italy, Trentino-Alto Adige. A virus (GPGV) has been identified and related to the presence of the syndrome. Since the first description, the presence of GPGV has been discovered in several countries in Europe, North America, Asia and Australia.

At present, the occurrence of the virus in many symptomatic and asymptomatic plants in different grapevine cultivars highlights uncertainties on this issue. More scientific data are needed to better understand the causes of the syndrome and the role of GPGV in its expression. Further details are available in the EPPO Global Database (EPPO, 2017a) and in the relevant scientific literature.

Appendix 2 – Short procedure for inspectors

Time of inspection

Inspections should be carried out at the proper time during active growth to ensure the best chance of detecting pest symptoms. Some pests of *Vitis* show symptoms during the whole growing season, which is the case for phytoplasmas causing grapevine yellows and for the leaf-galling phylloxera. In other cases, symptoms are detectable at the beginning of the season, as in the case of infestations of *Frankliniella occidentalis* on table grape varieties, or late in the season, as is the case for symptoms caused by *Xylella fastidiosa* or strains of Grapevine leafroll-associated virus.

Where possible, inspections should be undertaken during overcast days because symptoms of viruses and phytoplasmas may be obscured by bright sunlight. Plants showing visual symptoms should be sampled for laboratory testing. If no symptoms are seen, it is recommended to sample asymptomatic host plants for latent or hidden infections.

Hygiene measures

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

Good hygiene procedures when collecting samples for the laboratory should be followed by decontaminating tools and hands. This is particularly relevant for handling of diseased plants, and in particular for handling samples with symptoms of *Xylophilus ampelinus* which may be transmitted with cutting tools.

Lot identification

A lot should also include all plants originating from the same propagating material (both scion and rootstock for grafted plants), of the same age, cultivated in a single field (or set of plants in the case of potted plants).

Visual inspection

Inspectors should be well equipped and trained to recognize symptoms of the listed pests of *Vitis*, for different varieties of scions and rootstocks, at different moments during the same growing season because the expression of symptoms may change over time, in plants and parts of plants.

Inspectors should also be trained to identify the morphological characteristics of the main varieties of scions and rootstocks, in order to recognize different lots at the same place of production.

Information on distribution, biology, host range, sampling criteria, diagnostics, symptoms and pathways for pests of *Vitis* are available in the EPPO Datasheets and in the relevant EPPO Standards referred to for each pest in Appendix 1. All documentation can be found in the EPPO Global Database (https://gd.eppo.int/) (EPPO, 2017a).

Details about the phytosanitary history of the crop and the use of plant protection products should also be gathered.

Indeed, the application of insecticides, fungicides and other products may affect pest presence, even at level of a single lot.

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 distinguishing each stock.

Inspection of mother plant stocks is achieved by walking between two rows and inspecting either side to ensure that all plants may be inspected. Plants in two or three rows close together, as is often the case for nurseries of rooted cuttings, may be inspected together. If necessary the inspector may move across rows to check plants in a neighbouring row.

If freedom of the place of production and its vicinity is required, then all plants in the nursery and its boundary and in the immediate area should be inspected. The regulations of some EPPO countries require regular testing of asymptomatic material used for propagation, and these sampling activities can be combined with visual inspections to save resources.

Monitoring and sampling for known vectors can be a complementary activity to visual inspection of plants.

Sampling for laboratory testing

Plants from which samples have been taken should be marked, to enable follow-up in the case of positive test results. All samples for laboratory testing should be clearly labelled for traceability of information, with identification by location (possibly with GPS coordinates), plant information (e.g. variety, clone, grafting combination), sampling date, parts or part of plants sampled, symptoms (possibly with images), the owner's details and the name of the sampler. A lot of the necessary information may be expressed by recording the unique code of the lot.

Symptomatic plant material should preferably be collected from individual plants. However, a pooled sample may also be collected from several plants showing similar symptoms.

Test results are highly dependent on the quality of the sample which arrives at the laboratory. All sampled material should be stored in a cooler, in a manner that allows it to arrive at the laboratory in a fresh condition, without overheating or desiccation. Samples should be transported to the diagnostic laboratory as soon as possible after collection, before the plant tissues deteriorate. It is important to make sure that the samples will not be received by the laboratory on a non-working day, and to inform the laboratory of when they are likely to arrive.

Insects (including vectors) should preferably be collected with sweep nets, beating trays or aspirators. Insects may be collected using traps – yellow sticky traps or traps baited with attractant, depending on the pest.

Live insects for analysis (including vectors) can be killed by freezing or by exposure to ethyl acetate. The quality of the dead insects from sticky traps mainly depends on the period of time for which the traps have been hanging in the field (the shorter the period, the better the sample).

If insects cannot be processed immediately, they should be stored in 95–99% ethanol or at −20°C or below. Entire traps may be stored at −20°C or below.