PEST SURVEY CARD

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Pest survey card on flavescence dorée phytoplasma and its vector *Scaphoideus titanus*

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Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137) at the request of the European Commission. Its purpose is to guide the Member States in preparing data and information for Flavescence dorée phytoplasma (FDp) and its vector *Scaphoideus titanus* that are required to design statistically sound and risk-based surveys, in line with current international standards. Flavescence dorée phytoplasma is a Union quarantine pest known to occur only in Europe, whereas its main vector *S. titanus* is a monophagous leafhopper that has been introduced into the EU and is not regulated in the EU. Both FDp and *S. titanus* are currently present in most of the main grape-growing EU Member States. The alternative hosts and other putative vectors of FDp play a secondary role in the epidemics. Spread of FDp in the EU can occur mainly through the internal movement of plants for planting of grapevine and the spread of infected vectors. Detection surveys for both FDp and *S. titanus* should focus on commercial and productive vineyards and nurseries, wherever present in the EU, while in the event of an outbreak, delimiting surveys should extend to the wild and American *Vitis* spp. plants that can be found in abandoned vineyards, in the wild or grown in backyards and gardens, and that can have a crucial role in primary infections. If *S. titanus* is present in an FDp outbreak area, the delimiting survey could be extended to occasional vectors of FDp and alternative hosts in order to also consider the spread of FDp from those reservoirs. Visual inspections are effective for identifying the vector but not for FDp, which requires confirmation by molecular methods.

Keywords: American grapevines, baco 22A disease, plant pest, risk-based surveillance, Union quarantine pest, vector of flavescence dorée, wild *Vitis* spp.

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Amendment: An editorial correction was carried out, without materially affecting the contents or outcome of this scientific output: the two pictures on Fig. 4 have been inverted. To avoid confusion, the original version of the output has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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Introduction

The information presented in this pest survey card was summarised from the pest categorisation (EFSA PLH Panel, 2014), pest risk assessment (EFSA PLH Panel, 2016) and the priority pest report published by EFSA (2019) on grapevine flavescence dorée phytoplasma (FDp), the European and Mediterranean Plant Protection Organisation (EPPO) Global Database (EPPO, online), the Centre for Agriculture and Bioscience International (CABI) datasheets on FDp (CABI, 2019) and other scientific documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for FDp and its vector *S. titanus* in EU Member States (MSs) following the methodology described in EFSA (2018). It is part of a toolkit that has been developed to assist the MSs with planning a statistically sound and risk-based pest survey approach in line with International standards for phytosanitary measures (ISPM 6: FAO 2018; ISPM 31: FAO, 2016a) and International Plant Protection Convention guidelines for surveillance (FAO, 2016b). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card on flavescence dorée phytoplasma[1] and its vector *Scaphoideus titanus*[1]
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual[2]
   c. The statistical tools RiBESS+ and SAMPELATOR[3].

1. The pest and its biology

1.1. Taxonomy

Flavescence dorée phytoplasma

Scientific name: Flavescence dorée phytoplasma

Class: Mollicutes, **Order**: Acholeplasmatales, **Family**: Acholeplasmataceae, **Genus**: Phytoplasma, **Species:** Not defined. Flavescence dorée phytoplasmas belong to the taxonomic subgroups 16SrV-C and 16SrV-D transmitted by *Scaphoideus titanus*.

In the EU Regulation[4], the pest is referred to as 'Grapevine flavescence dorée phytoplasma'.

Synonyms: Not applicable

EPPO Code: PHYP64

Common names in English: baco 22A disease, flavescence dorée of grapevine

Flavescence dorée phytoplasma is considered to be part of the genus *Candidatus* Phytoplasma. These are pleomorphic, non-culturable bacteria with no cell walls, known as phloem-obligate parasites and transmitted by insect vectors.

Flavescence dorée is a 'sufficiently clearly defined organism', according to the EFSA PLH Panel (2014).

[1] The Pest Survey Card will be updated in the form of Story Map that will be included in the Plant Pests Story Maps Gallery available online: https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://shiny-efsa.openanalytics.eu/
[4] Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. OJ L 319, 10.12.2019, p. 1–279.

For the purposes of this document, the distinction between the two FDp subgroups 16SrV-C and 16SrV-D will not be taken into account.

Flavescence dorée phytoplasma is the causal agent of flavescence dorée and is one of the most important grapevine yellows diseases in the main viticultural areas of Europe. It has a highly species-specific vector, *Scaphoideus titanus* (Chuche and Thiéry, 2014; Quaglino et al., 2019).

Scaphoideus titanus

Scientific name: *Scaphoideus titanus* Ball, 1932

Class: Insecta, **Order**: Hemiptera, **Family**: Cicadellidae, **Subfamily:** Deltocephalinae, **Genus**: *Scaphoideus*, **Species:** *Scaphoideus titanus*

Synonyms: *Scaphoideus littoralis* Ball, 1932

EPPO Code: SCAPLI

Common names in English: American grapevine leafhopper

This leafhopper species is native to the Nearctic regions and reached the EU in the 1950s, where it is a specialist of *Vitis* and is the only known vector of FDp able to spread the pathogen from one grapevine to another.

Conclusions on taxonomy

Both flavescence dorée phytoplasma and its vector *Scaphoideus titanus* are considered distinguishable taxonomic entities.

1.2. EU pest regulatory status

Flavescence dorée phytoplasma is listed under Commission Implementing Regulation 2019/2072 in Annex II, Part B, as a Union quarantine pest known to occur in the EU. Moreover, Annex VI prohibits the introduction of *Vitis* L. plants from third countries other than Switzerland, and Annex VIII details the internal movement requirements for *Vitis* L. plants.

The general requirements for surveys of quarantine organisms in the EU territory are laid down in Regulation (EU) 2016/2031[5].

Overview of the EU regulatory status

Flavescence dorée phytoplasma is a Union quarantine pest, whereas *Scaphoideus titanus* is not a regulated pest. The import into the EU of *Vitis* spp. plants, which are the host of both FDp and *S. titanus*, is prohibited. Both FDp and *S. titanus* are known to be present in the EU.

1.3. Pest distribution

Flavescence dorée phytoplasma

Flavescence dorée phytoplasma only occurs in Europe, with the first symptom observations dating back to the beginning of the 20th century. Its distribution is reported in eight of the main grape-growing EU Member States (Austria, Croatia, France, Hungary, Italy, Portugal, Slovenia and Spain) as well as in Switzerland and in Serbia, according to the most recent pest status reports available from EPPO (online).

[5] Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. OJ L 317 23.11.2016, p. 4-104.

In the past, different phytoplasmas causing similar symptoms and diseases have been identified as 'flavescence dorée' or 'flavescence dorée-like disease'. Therefore, any reports from the 1970s and 1980s of regions colonised by *Scaphoideus titanus* would require review and characterisation with more reliable and recent identification methods (CABI, 2019; see Section 2.2).

Scaphoideus titanus

Scaphoideus titanus, native to North America, has currently reached 12 EU Member States (Austria, Bulgaria, Croatia, Czech Republic, France, Hungary, Italy, Portugal, Spain, Romania, Slovenia and Slovakia) and six more European countries (Bosnia Herzegovina, Moldova, Montenegro, Serbia, Switzerland and Ukraine), making its distribution wider than that of FDp (EFSA PLH Panel, 2016).

Its life cycle requires *Vitis* spp. for its completion; therefore its presence is strictly connected to that of grapevines (EFSA, 2019).

Figure 1 shows the distribution in EU Member States at NUTS2 level, of vineyards, FDp and *S. titanus*, based on the following data:

- Vineyards: Eurostat database. In general, statistical data referred to the year 2015, with a few exceptions due to data availability for specific countries (e.g. Poland, with data from 2010).
- Flavescence dorée phytoplasma: mainly EPPO Global Database (online), with an update for Italy on the Autonomous Provinces of Bolzano (Decreto del direttore[6]) and Trento (Gelmetti et al., 2018). In Campania region (Italy), the presence of FDp at NUTS2 level refers to a distribution restricted to Ischia island.
- *Scaphoideus titanus*: mainly EPPO Global Database (online), with updates on:
    - Italy: added the regions Emilia-Romagna (Tiso and Solmi, 2014) and Toscana (Decreto Dirigenziale n. 9825[7]) and the Autonomous Provinces of Bolzano (Decreto del direttore[6]) and Trento (Gelmetti et al., 2018).
    - Romania: detailed information from Chireceanu (2014), Chireceanu et al. (2017) and Szalárdi et al. (2019).

[6] Decreto del direttore d'ufficio del 3 dicembre 2019, n. 25293 recante l'applicazione del decreto ministeriale del 31 maggio 2000 nel territorio della provincia autonoma di Bolzano – Dichiarazione delle zone di focolaio della Flavescenza dorata. Bollettino Ufficiale n. 50/Sez. gen. del 12/12/2019. Available online: http://www.provincia.bz.it/agricoltura-foreste/agricoltura/downloads/Bollettino_12-12-19.pdf

[7] Decreto Dirigenziale n. 9825, relativo all'aggiornamento per l'anno 2019 delle misure per la lotta obbligatoria contro la Flavescenza dorata della vite nel territorio della Regione Toscana di cui al D.M. n. 32442 del 31.5.2000. Available online: https://www.regione.toscana.it/-/aggiornamento-anno-2019-delle-misure-per-la-lotta-obbligatoria-contro-la-fl

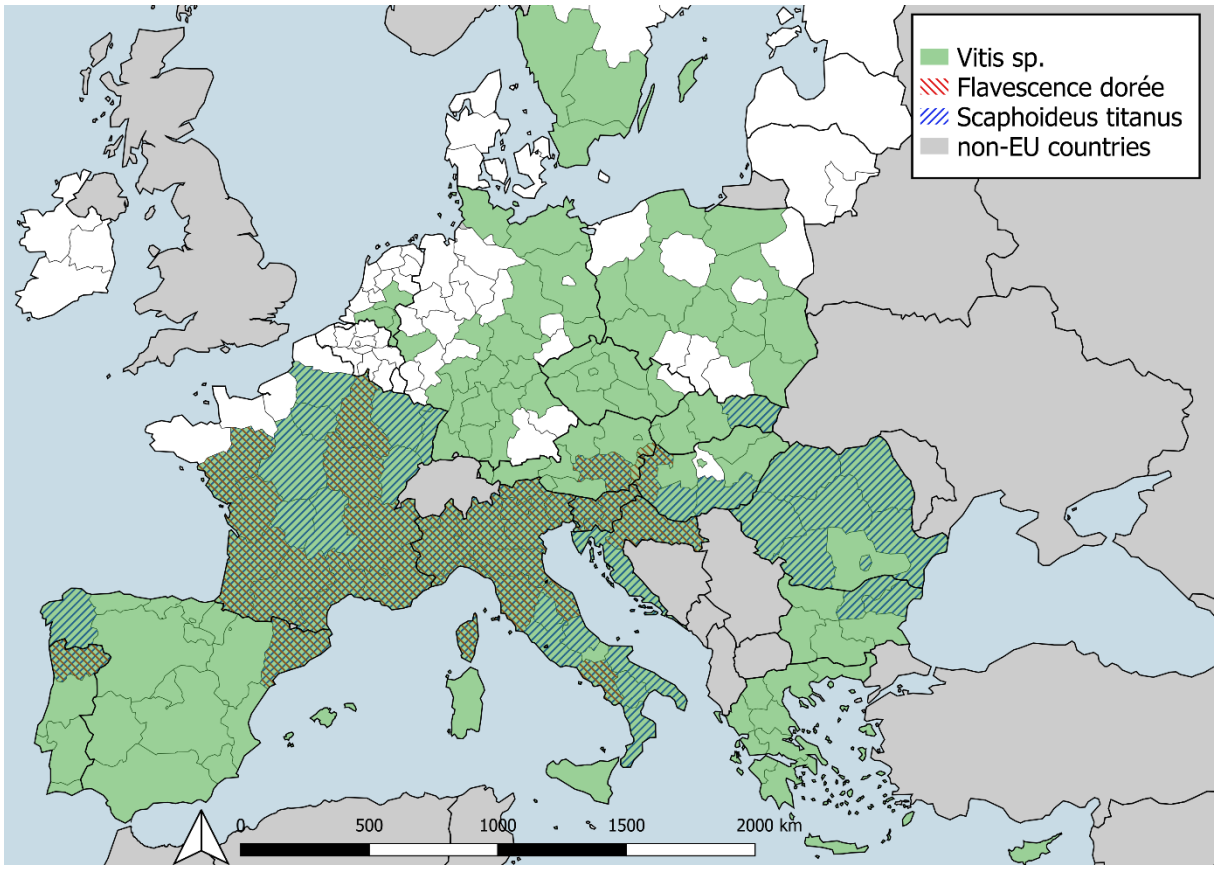

Figure 1: Distribution in the EU of vineyards at NUTS2 level (Eurostat, 2017), flavescence dorée phytoplasma (EPPO Global Database, online) and *Scaphoideus titanus* (EPPO Global Database, online and updates from Italy and Romania based on further publications). The presence of the pathogen and its vector has been indicated at NUTS2 level based on the most recent information, independent of the extension of the infested area and from the transient distribution (i.e. under eradication)

Conclusion on pest distribution

In the EU, flavescence dorée phytoplasma is currently present in the eight main grape-growing EU Member States: Austria, Croatia, France, Hungary, Italy, Portugal, Slovenia and Spain. In the same countries *Scaphoideus titanus* is also present, although its area of distribution also includes Bulgaria, Czech Republic, Romania and Slovakia.

Where *S. titanus* it is not known to be present, FDp surveys should be aimed at substantiating pest freedom for both FDp and *S. titanus.* Whereas where *S. titanus* is already known to be present, surveys should focus only on FDp detection. In the situation of a new outbreak of FDp, both the vector and the pathogen should be monitored to delimit the boundaries of the demarcated areas for FDp (i.e. an infested zone and a buffer zone).

1.4. Life cycle and FDp transmission

The main mechanisms of FDp transmission from one grapevine plant to another are: i) naturally, through the saliva of phloem sap-sucking leafhoppers of species *Scaphoideus titanus*; and ii) by human assistance, although less efficiently, through grafting.

Scaphoideus titanus acquires FDp while feeding on the leaves of infected plants at all its growth stages (from the first nymph to imago) and becomes infectious after a latent period of 4–5 weeks (Chuche and Thiéry, 2014). After having acquired FDp, *S. titanus* remains infectious for the rest of its

life, even after moulting, while its efficiency in FDp acquisition increases throughout the season, with the adult stage being the most efficient (Angelini et al., 2018). Transovarial transmission has not been reported (EFSA PLH Panel, 2014). Once transmitted to a new host plant, FDp multiplies within the phloem until inhibiting sap transport, with an accumulation of carbohydrates and secondary metabolites followed by a source-sink transition and defence response status by the host (Prezelj et al., 2016).

The inoculum abundance in the plant and the insect population size influence the disease propagation (Eveillard et al., 2016).

The incubation period in the plant usually lasts one year, with symptom development during the following year, although the duration is influenced by the variety and age of the grapevine plant (Morone et al., 2007). On the most susceptible varieties, the first symptoms appear in May–June, with stunting and lack of bud break. Later, they evolve into leaf yellowing or reddening, depending on the variety, downward leaf curling, drying of inflorescence and bunches, lack of cane lignification, presence of black spots on new canes and premature leaf fall, on the entire plant or just on individual branches (Caudwell, 1983, 1990; Galetto et al., 2016).

After the first year of disease expression, the further development of symptoms is influenced by the grapevine variety and includes the possibility of recovery, with symptom remission until the next infection event, leaving the plant less productive even when recovered (Morone et al., 2007; EFSA PLH Panel, 2014).

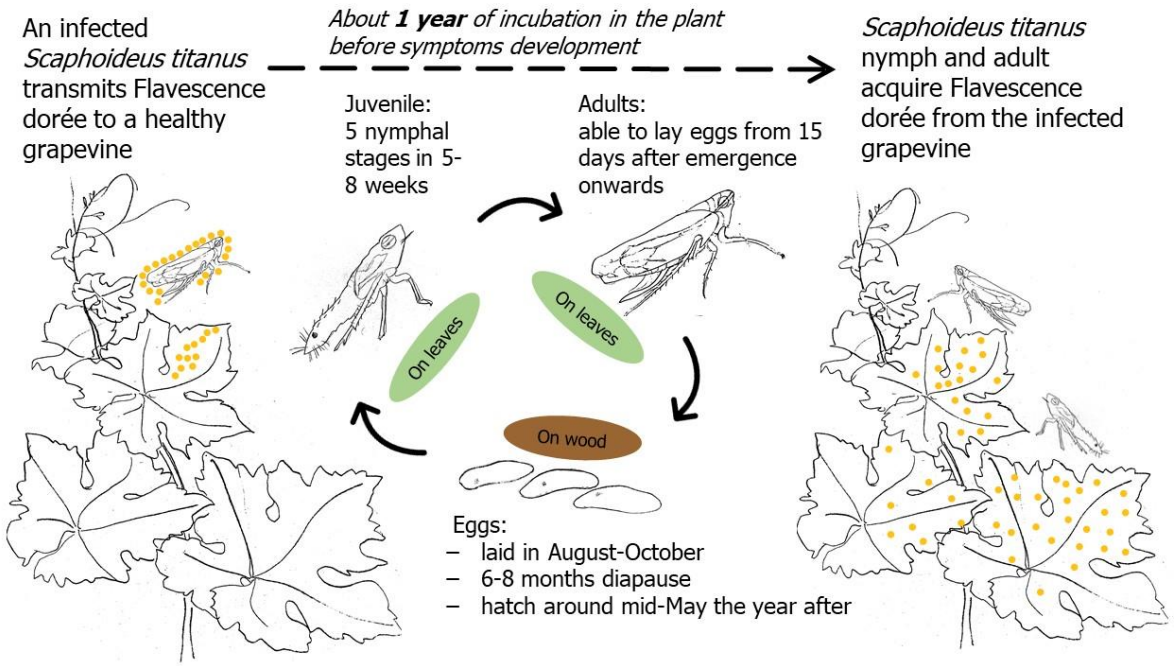

Figure 2: Schematic representation of the life cycle of *Scaphoideus titanus* and its role in transmitting grapevine Flavescence dorée

The whole life cycle of *S. titanus* happens on *Vitis* spp., where it feeds and reproduces, completing one generation per year (Figures 2 and 3):

- **Eggs:** The eggs are laid on branches, preferably of 2-year-old wood, between August and October. Around mid-May of the following year, the eggs start hatching. Chilling favours the precocity and synchrony of hatchings.
- **Nymphs:** The nymphs have five development stages, growing from 1.8 (L1) to 5.2 mm (L5). At L1 they are translucent, go through a milky white stage, then at the end of L2 they are ivory

white, which converts into an increasingly accentuated ivory yellow at L3. At L4 and L5 light to dark brown irregular spots, wings and elytral drafts appear. They have two black spots arranged symmetrically in the dorsolateral position at the posterior end of the abdomen which provide key traits for their identification (Schvester et al., 1962; Vidano 1964; Alma et al., 2016). They are visible from mid-May to August on the underside of grapevine leaves, feeding from the smallest venations at the beginning and, from the fourth instar, on the midribs, green shoots and stems (Vidano, 1964). They almost don't move, unless jumping away when disturbed. In Switzerland, they have been noticed on the herbaceous plants of the inter-rows of vineyards (Trivellone et al., 2013).

- **Adults:** Adult females are larger (5.5–5.8 mm) than males (4.8–5 mm) and have three brown transverse bands at the vertex level while males only have one (Schvester et al., 1962; Vidano 1964). Adults appear from the beginning of July to mid-October, although the highest population level can be observed in July and August. They tend to remain close to their host plant, flying preferably between the evening and the early morning (Lessio and Alma, 2006) and feeding on the midribs, green shoots and stems. Females lay eggs from August to September within the excoriated bark of woody vines where they overwinter.

In the northernmost areas, during short summers, insects have difficulty completing their development and may therefore only form transient populations.

In the FDp epidemics, the most competent vector known, *S. titanus*, monophagous on grapevine, plays a major role and is therefore a main survey component around which FDp-specific surveys should be designed.

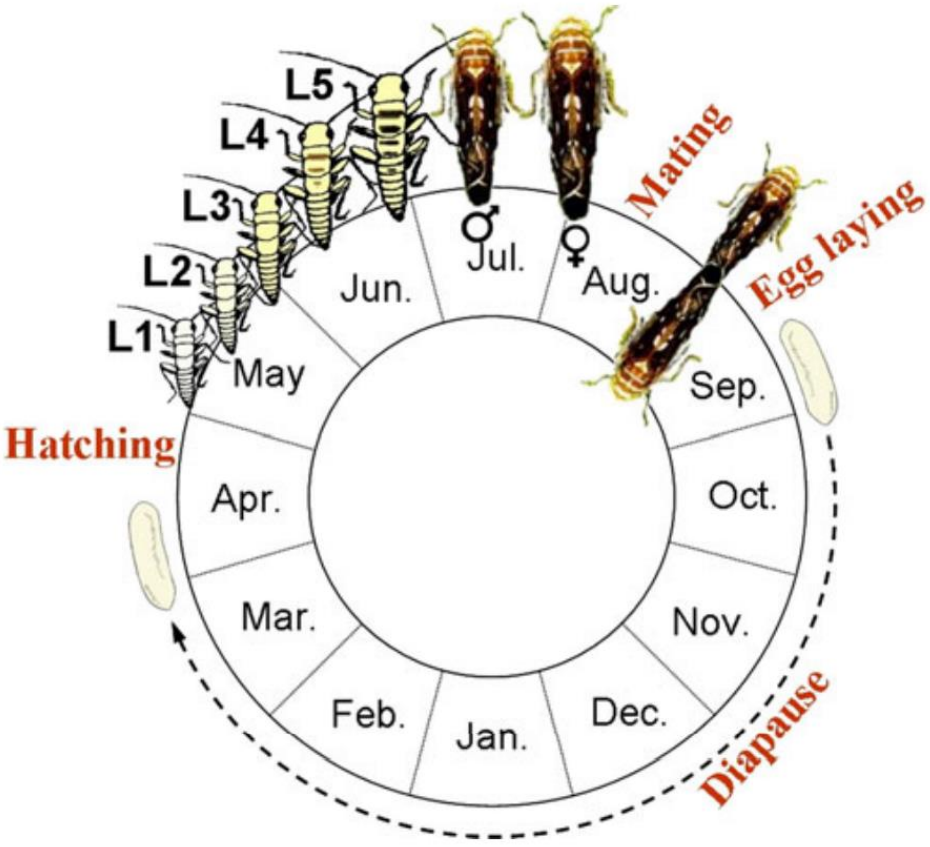

Figure 3: Life cycle of *Scaphoideus titanus*. Dates are indicative and vary depending on the year and location (Source: Chuche and Thiéry, 2014; courtesy of INRA UMR Save)

Apart from *S. titanus*, which plays a major role in spreading FDp from infected wild *Vitis* to cultivated ones, other Auchenorryncha species play a part in transferring FDp from the wild to vineyards (Ripamonti et al., 2020). Still, their importance in FDp epidemics in vineyards and nurseries is

unknown and could be very limited, particularly in the presence of *S. titanus* (EFSA PLH Panel, 2016). The main ones are:

- *Dictyophara europaea* (Hemiptera: Dictyopharidae): the European lantern fly (Figure 4) is a polyphagous species widely distributed in the western Palaearctic. It is able to transmit FDp from *Clematis vitalba* to grapes in natural conditions (Filippin et al., 2009), although the latter is not among its preferred hosts (Lessio and Alma, 2008). Even if its vector ability was proven, it remains only an occasional vector of FDp (Lessio and Alma, 2008; Linder and Cavadini, 2014). Indirect evidence, based on the phytoplasma haplotypes identified in *C. vitalba*, suggest that *D. europaea* might have a negligible role, if any, in spreading FDp in Piemonte region (Rossi et al., 2019).
- *Orientus ishidae* (Hemiptera: Cicadellidae): the mosaic leafhopper (Figure 4) is a polyphagous species that was first reported in the EU in 1998. Its vector ability was confirmed in laboratory experiments (Lessio et al., 2016; Malembic-Maher et al., 2020), although with limited efficiency compared with *S. titanus*. *O. ishidae* is widespread in vineyard agroecosystems, and its eggs can be found on *Vitis* spp. wood, mainly from abandoned vineyards and/or wild rootstocks. However, the insect doesn't move frequently to grapevine and is therefore considered only a very occasional vector of FDp (Jermini et al., 2019; Lessio et al., 2019).
- *Phlogotettix cyclops* (Hemiptera: Cicadellidae): this Asian leafhopper species (Figure 5) is currently present in some EU grapevine-growing countries (Austria, Croatia, Czech Republic, France, Hungary, Italy and Romania). Recent findings from Austria highlighted its putative vector role (Strauss and Reisenzein, 2018) and infected specimens have been found on grapevines as well as on *C. vitalba* and *Ulmus laevis* that were growing near the vineyards (Reisenzein and Strauss, 2019). The first transmission trials proved its capacity to acquire FDp from *C. vitalba* (Reisenzein and Strauss, 2019) and other preliminary results support the hypothesis that *P. cyclops* has the capacity to infect grapevines with FDp. However, further studies are needed to verify whether this species serves as a vector of FDp (Reisenzein, personal communication, April 2020).

Figure 4: Adults of *Dictyophara europaea* (left) and *Orientus ishidae* (right) (Source: DISAFA Entomology, University of Turin)

Figure 5: Adult of *Phlogotettix cyclops* (Source: Gudrun Strauss, AGES)

Conclusion on life cycle

The main mechanism of flavescence dorée phytoplasma transmission from one grapevine plant to another is through the sap-sucking leafhopper *Scaphoideus titanus*. After having acquired FDp, *S. titanus* remains infectious for the rest of its life. FDp presence in grapevine can be observed about one year after the infection. The infection of a few plants could be caused by occasional vectors, such as *Dictyophara europaea*, *Orientus ishidae* and *Phlogotettix cyclops*, which can transfer FDp to grapevines from other plant species, but, if the disease is spreading from grapevine to grapevine only *S. titanus* is involved.

The detection of *S. titanus* nymphs should be performed in June by observing the underside of the leaves. They can be distinguished from other species, besides their morphology, for their jumping behaviour in reaction to disturbance. The adult trapping should be performed from July until the end of the productive season.

1.5. Host range and main hosts

Species belonging to the genus *Vitis* are the most economically important FDp hosts: *V. vinifera*, *V. armurensis*, *V. champinii*, *V. doaniana*, *V. labrusca*, *V. longii*, *V. pentagona*, *V. riparia*, *V. rubra*, *V. rupestris*, *V. simpsonii*, *V. sylvestris* and interspecific hybrids used as rootstocks (EFSA PLH Panel, 2014; EPPO, 1996).

Scaphoideus titanus, the main and most efficient FDp vector, is monophagous on grapevines.

All *V. vinifera* develop symptoms in response to FDp infection, but with different levels of severity: the level of susceptibility expressed by the infected grapevines is cultivar-dependent, such as the observed FDp titres. Even if a *V. vinifera* cultivar expresses severe symptoms, it doesn't necessarily correspond to a strong multiplication of the phytoplasma in its tissues (Eveillard et al., 2016).

Plant species other than cultivated and uncultivated *Vitis* spp. could also be a source of FDp inoculum:

- *Ailanthus altissima*: largely symptomless; it is abundant across the Mediterranean region (Enescu et al., 2016).
- *Alnus* spp.: it hosts FDp belonging to the taxonomic group 16SrV-C, largely symptomless; *A. cordata* is restricted to Italy, northern France and Corsica (Caudullo and Mauri, 2016); *A. glutinosa*, another asymptomatic host, is widespread in most of Europe, except in northern

Norway, Sweden and Finland, and in south-eastern Spain (Houston Durrant et al., 2016a); *A. incana* is present in most of central Europe, Scandinavia, eastern France and northern Italy (Houston Durrant et al., 2016b). A very recent paper (Malembic-Maher et al., 2020) shows how most FDp genotypes are related to alder more than to grapevines and that epidemics in the latter involve a limited number of FDp genetic variants found in black alders (*A. glutinosa*).

- *Clematis vitalba*: when infected, it may show yellowing symptoms or be symptomless; it is present in all European countries, and widespread in France, Germany, Italy, the United Kingdom and former Yugoslavia (CABI 2016).

Conclusion on host range and main hosts

Flavescence dorée phytoplasma's host range, in addition to *Vitis* spp., includes *Ailanthus altissima*, *Alnus* spp. and *Clematis vitalba*. However, the host of major economic importance remains grapevine.

Detection surveys to substantiate freedom from FDp could concentrate on commercial and productive vineyards and nurseries. In case of outbreak, delimiting and buffer zone surveys should extend to the wild and American *Vitis* spp. plants in abandoned vineyards, in wild areas or in backyards and gardens that could be reservoirs of FDp and potential source of primary infections. If *Scaphoideus titanus* presence is confirmed in an outbreak area, then the delimiting survey could be extended to *Ailanthus altissima*, *Alnus* spp., *Clematis vitalba* and FDp's occasional vectors (*Dictyophara europaea*, *Orientus ishidae*, *Phlogotettix cyclops*) in order to also consider the spread and transfer of FDp from those reservoirs to productive vineyards.

1.6. Environmental suitability

Flavescence dorée phytoplasma is expected to infect and survive in grapevines wherever they grow (Figure 1; EFSA, 2019). Its rate of multiplication in the plant and latency period in the vector are influenced by climatic conditions and in particular by temperature (Galetto et al., 2011; Salar et al., 2013). The EFSA PLH Panel (2016), following CLIMEX simulations, indicated a limited likelihood that *S. titanus* could become established in some areas of southern Spain, Crete and Cyprus, where dry, warm conditions prevail, whereas the establishment of the vector in the northern Member States is limited by host availability (see Figure 4 of EFSA PLH Panel, 2016).

Conclusion on environmental suitability

Given that *Vitis* spp. are the most relevant hosts of FDp, their availability is therefore the main limiting factor for the establishment and spread of FDp. The whole European grapevine-growing area can be considered suitable for FDp to become established and spread further if the phytoplasma and its main vector, *Scaphoideus titanus*, are present.

1.7. Spread capacity

Natural spread

The natural spread of infectious *Scaphoideus titanus* is considered the main means of spread for FDp. Epidemic growth rates within vineyards are influenced by weather conditions: FDp grows faster at 25–26°C than 20–22°C (EFSA PLH Panel, 2014). The latency period between phytoplasma acquisition by *S. titanus* and transmission to new host plants is also temperature-dependent. Furthermore, prolonged warmer summers favour *S. titanus* establishment while dry conditions limit it.

The natural spread rate is not considered to be very high for a series of reasons linked to the vector's biology:

i) *Scaphoideus titanus* is not a strong flier: about 20–40 m (Lessio and Alma, 2004; Beanland et al., 2006) with a less frequent long-range dispersal of up to 300 m (Lessio et al., 2014);
ii) some authors assume that its limited capacity to fly high would reduce the contribution of passive dispersal by strong winds (Steffek et al., 2007; Zeisner, 2008);
iii) FDp-infected *S. titanus* survive for a shorter period than non-infected *S. titanus*, limiting its capacity to spread FDp.

The spatial extent of the natural spread of FDp depends on a number of factors including the initial size of the introduced population, vector presence and host densities (including alternative hosts), and the time since introduction.

In a recent assessment conducted by EFSA (2019), the maximum distance expected to be covered in one year by FDp disease was estimated at 44 m (with a 95% uncertainty range of 1–1,300 m), with *S. titanus* being the main limiting factor on disease progression.

Flavescence dorée phytoplasma acquisition efficiency by *S. titanus* is strongly influenced by grapevine genotype, being higher on more susceptible varieties: disease diffusion correlates better with vector acquisition efficiency than with FDp load in source grapevines (Galetto et al., 2016).

Human-assisted spread

The human-assisted movement of grapevine planting material of *Vitis* spp. is one of the two mechanisms for long-distance spread of FDp, together with the spread of infectious vectors (EFSA PLH Panel, 2016). The vegetative multiplication of grapevine and the trade of plants for planting can spread the disease even in the absence of the vector (EFSA PLH Panel, 2016). However, the local spread of FDp is hampered in the absence of the vector and the dispersal of FDp at destination, at local level, requires the contribution of *S. titanus*.

Flavescence dorée phytoplasma cannot be transmitted mechanically by pruning activity but can be transmitted by grafting, albeit with only limited efficiency (Osler et al., 2002; Credi et al., 2012).

Another expression of human-assisted spread is the dispersal of *S. titanus* by hitchhiking, for example, on agricultural machinery (e.g. those used for mechanical summer pruning). This mechanism is expected to influence only the short-distance spread of the disease.

Conclusion on spread capacity

The two main spread mechanisms of flavescence dorée phytoplasma are the movement of its vector *Scaphoideus titanus* and the movement of infected plants for planting within the EU. The insect can spread the disease by hitchhiking and by active flight.

The yearly spread rate of the disease was estimated at 44 m, ranging from 1 to 1,300 m per year (95% uncertainty range). These are the spread values that could be used for defining the extent of detection and delimiting surveys.

1.8. Risk factor identification

Identification of risk factors and their relative risk estimation are essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest in the area of interest. The risk factors that are relevant for surveillance need to be characterised by their relative risk (should have more than one level of risk for the target population) and the proportion of the overall target population to which they apply. The identification of risk factors needs to be tailored to the situation in each Member State. This section presents examples of risk factors for FDp and *Scaphoideus titanus* and is not necessarily exhaustive.

To identify the risk areas, it is first necessary to identify the activities that could contribute to the introduction or spread of FDp. These activities should then be connected to specific locations. Risk areas can be defined around these locations, bearing in mind that their size depends on the spread capacity of the target pests and the availability of host plants around these locations (Table 1). For a

detection survey, a risk area of about 50 m wide around the possible risk locations can be defined. In the event of a new FDp finding in an infected plant, in the presence of *S. titanus*, a delimiting survey should be conducted starting 1.3 km from the finding. Delimiting surveys should be extended to wild and ornamental *Vitis* spp. plants. Around the infested zone, a buffer zone of at least 1.3 km wide is recommended.

Example 1: Infected plant propagation material

The main trade of grapevine plants for planting in the EU originate from Member States where FDp is present. National measures in Croatia, France, Hungary, Italy and Portugal impose mandatory hot-water treatments in nurseries for rootstocks, scions or grafted cuttings and, depending on the country, they might be specific to some areas or, in some cases, the nursery activity is forbidden if located within FDp outbreak areas (Fanjul, 2017). However, FDp outbreaks reported in the EU indicate that the application of phytosanitary measures cannot always prevent the dispersal of FDp (Steffek et al., 2007), in particular in infected rootstocks in nurseries, as they are often asymptomatic (EFSA PLH Panel, 2016).

Being a principal mechanism for the spread of FDp (EFSA PLH Panel, 2016), the movement and trade within the EU of *Vitis* plant propagating material is therefore considered to be a risk activity when originating from those Member States where FDp is already present. The locations handling these plants are mainly grapevine nurseries and new plantations.

Example 2: Land management

The presence of American vines left from the rootstocks of abandoned vines and other *Vitis* spp. growing in the wild provide a reservoir for both the pathogen and the vector, possibly with a higher proportion of infected vectors than in the contiguous commercial vineyards (Ripamonti et al., 2020). Furthermore, insecticide applications are forbidden in natural areas and abandoned vineyards and these areas might be difficult to access with machinery (Pavan et al., 2012). In those areas, *S. titanus* population levels can be higher than treated vineyards. For these reasons, some regions provide protocols for handling and containing wild vines (e.g. Camerano and Terzuolo, 2015) to avoid the spread of *S. titanus*. Furthermore, in north-western Italy, a higher proportion of infected insects was recorded in the wild area than in the vineyard (Lessio et al., 2007; Ripamonti et al., 2020). As *S. titanus* has been shown to move from untreated to treated vineyards (Pavan et al., 2012), in the presence of FDp infection this can represent a real risk factor.

Table 1: Example of risk activities and corresponding risk locations relevant for the surveillance of flavescence dorée phytoplasma and *Scaphoideus titanus*

Risk activity	Risk locations	Risk areas
Exchange of propagation material (e.g. cuttings, plants, etc.) of *Vitis* spp.	Grapevine nurseries and new plantations	Surroundings of vineyards, parks and gardens with *Vitis* spp. plants within a radius of at least 44 m of the risk locations (for detection surveys)
Abandoned vineyards	Wild vine plants in the proximity of productive vineyards	
Historical findings	Eradicated outbreak areas and locations where positive inspections were performed	

Example 3: Vector presence

For FDp to spread among *Vitis* spp. plants, *S. titanus* must be present. The areas where the vector is present can already be prioritised for FDp detection surveys. In addition, different levels of risk can be defined based on information on the vector density in the vineyards, if available. In these areas the surveys could be risk–based, focusing the inspections on the fields with higher vector density. For example, if the density of the vector's population increases substantially from one year to the other, a

more intensive sampling could be required, not only in the vineyard but also in the neighbouring fields and among the wild plants at the field edges.

When *S. titanus* is not present in a grapevine-growing area, FDp can still be introduced through infected plant propagation material. New plantations of vineyards should be targeted as a priority by the survey as the probability of infection in old plantations is low in the absence of the vector. In areas free of *S. titanus*, the survey of adults only, by using yellow sticky traps, is advised in order to confirm the absence of the vector.

2. Detection, sampling and identification

2.1. Detection

2.1.1. Visual examination

The goal of the visual examination is to detect both the symptoms of the disease and the presence of the vector nymphs.

Flavescence dorée phytoplasma

At plant level, the symptoms of FDp appear after a prolonged latency, usually the year after the infection.

They are visible throughout the growing season, from the spring (stunted sprouting) till post-harvest (lack of cane lignification). The maximum symptom expression is observed in summer (July and August). A characteristic symptom of FDp that should be observed is the lack of cane lignification.

CABI (2019) provides a list of symptoms that can be observed in the different parts of the plant. In addition, Fanjul (2017) illustrates spring and summer symptoms on different cultivars based on the experience and evidence collected in Piemonte region (Italy) and Istria region (Croatia).

The symptoms of FDp-infected plants could be one or a combination of the following:

- Roots: reduced root system.
- Shoots: symptoms can be limited to a single or a few shoots on the plant (Figure 6); stunted sprouting, shortened internodes, gummosis, presence of black pustules, lack of lignification (Figure 7).
- Leaves: abnormal colour, forms (in particular, leaf-roll downwards, blade thickening with cracking if folded), patterns, necrotic intervein lamina (EPPO, 2016), reduced size, premature leaf fall (Figures 7 and 8).
- Growing point: dieback.
- Inflorescences: blight, necrosis, fall, shedding.
- Fruit: complete absence of grape bunches, reduced size, lower sugar content and higher acidity (EPPO, 2016), mummification (Figure 7).
- Whole plant: death, dieback.

Figure 6: Leaves of grapevine infected with flavescence dorée phytoplasma, showing rolling and reddening of the blade on a single shoot of the plant (Source: Federico Bondaz, EPPO Global Database)

Figure 7: Examples of different symptoms of flavescence dorée phytoplasma on leaves, shoots and fruit (Source: Institute for Sustainable Plant Protection, CNR, Turin)

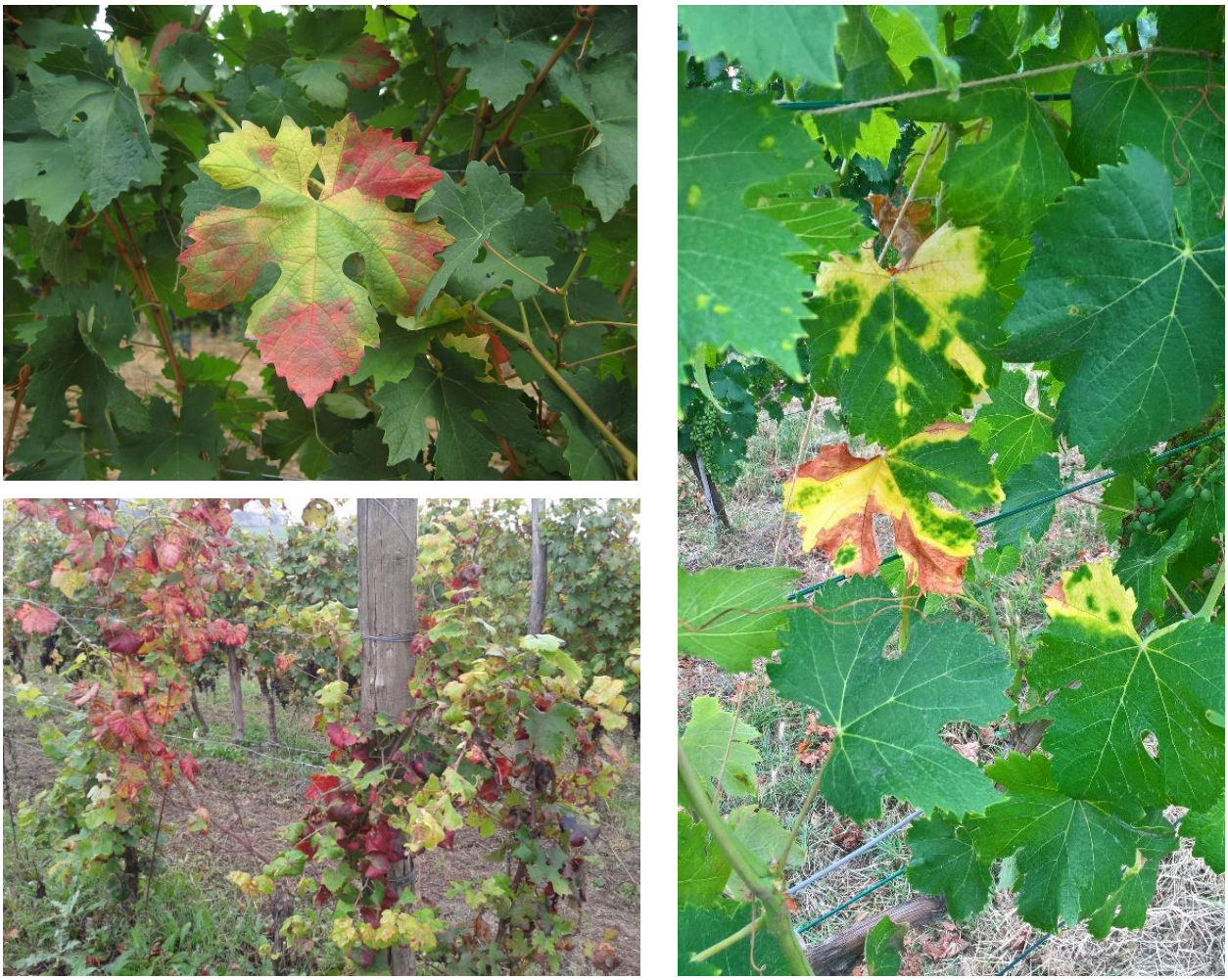

Figure 8: Reddening and yellowing of grapevine leaves infected with flavescence dorée phytoplasma (Source: Institute for Sustainable Plant Protection, CNR, Turin)

Symptoms, particularly when mild, can be difficult to identify, due to the diversity of their expression. The possibility of detecting symptoms is even more difficult in abandoned vineyards and wild *Vitis* spp. plants, which are often difficult to access. Moreover, wild *Vitis* spp. can be found in wide areas, which is an additional challenge for the surveys. Furthermore, the absence of symptoms on infected rootstocks affects both feasibility and effectiveness of the surveillance for the detection of all infected plants in nurseries (EFSA PLH Panel, 2016).

The susceptibility of the plants to FDp infection is also genotype-dependent: Eveillard et al. (2016) compared 28 *Vitis* genotypes including grapevine cultivars, rootstocks and wild species, proving that even wild rootstocks may be highly susceptible to FDp in their natural environment.

Risks of misidentification

Late in the season, the symptoms can be confused with viral infections that cause downward leaf roll or with senescence showing leaf reddening or yellowing.

Some of the symptoms are similar to other phytoplasmas and misidentifications could occur mainly with bois noir of grapevine and palatinate grapevine yellows, which are most common in European vineyards and are associated with the *Ca*. Phytoplasma solani and 16S-rV group, respectively (Maixner et al., 2000; Angelini et al., 2001; Quaglino et al., 2013). A high local incidence of some of these diseases could also mask early FDp outbreaks. Other important diseases with similar symptoms are the grapevine yellows that affect Croatian, Italian, Australian and American grapevines, associated with aster yellows group phytoplasmas (CABI, 2019). Symptoms due to other causal agents can also

be misidentified, e.g. grapevine leaf roll-associated viruses, stunted shoot growth caused by eriophyid bud mites, discoloured leaves caused by late frost events, and physiological disorders (Fanjul, 2017).

All the above-mentioned aspects indicate the need for trained and skilled inspectors to conduct visual examinations of FDp-like symptoms and to confirm the suspected infections by laboratory identification using molecular methods.

At the vineyard level, the symptoms usually appear aggregated in the field. Frequently an edge effect can be observed, as a result of the primary spread of FDp-infected vectors from outside the vineyard. The spread of infected vectors, from vine to vine is limited by regular insecticide application (Figure 9).

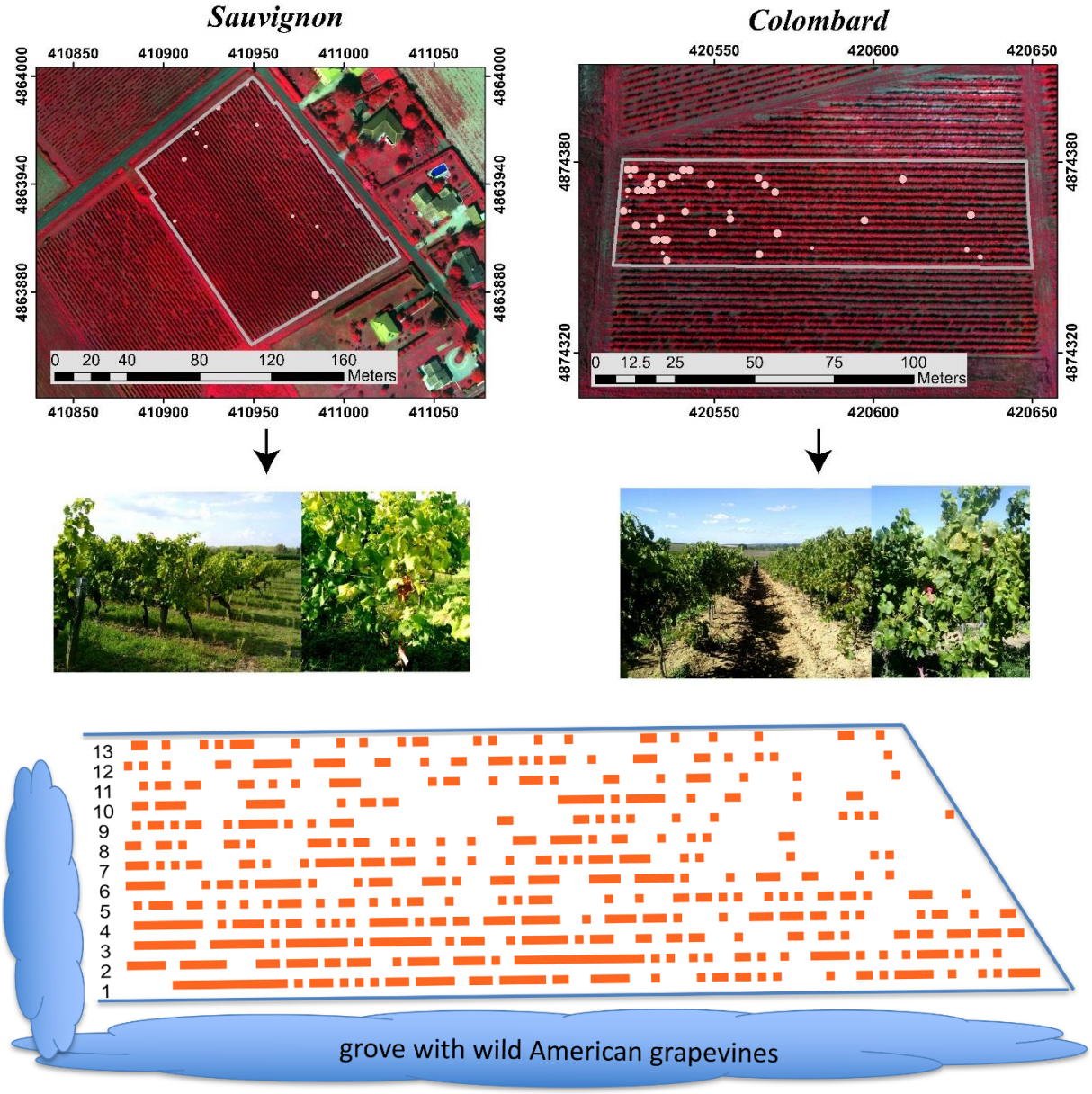

Figure 9: Upper figure: examples of the distribution of plants infected with flavescence dorée phytoplasma in two vineyards (Sauvignon and Colombard) with UAV images in false colours on top, localising all infected grapevines (the size of the pink circles varies according to the disease severity) and real pictures of the symptom expression in the plants underneath (Source: Albetis et al., 2017; courtesy of CESBIO and Ecole d'Ingénieurs de PURPAN). Lower figure: scheme of an infected vineyard: spatial distribution of symptomatic grapevines and potential sources of infected *S. titanus*, with edge effect (Source: Pavan et al., 2012; courtesy of DI4A, Università di Udine)

Remote sensing is potentially an alternative method for identifying the presence and effective prevalence of FDp in vineyards, although the operational mapping technique using UAV-based imagery is still at an experimental stage (Albetis et al., 2017, 2019).

Scaphoideus titanus

Different techniques for detecting the insect can be used depending on the life stages (Figure 10) and the aim of the survey (Rigamonti et al., 2016):

i) visual examination of leaves for nymph density estimates, supporting insect distribution studies, sampling plan design (could also be used for canopy infestation model development);

ii) beating tray and sticky traps to detect the presence in the plant canopy of adults and/or nymphs (could also be used to validate short- and long-term phenology models).

Eggs: The eggs are about 1 mm long, reniform, laterally compressed, transparent, change to yellow with time, and show the red eyes of the embryo in the final stage. The nodes on the bark of 2-year-old, or older, canes (cordons) are the preferred sites for laying the eggs (Bagnoli and Gargani, 2011) and the eggs are therefore very hard to find by visual examination in the field.

Nymphs: Surveys for nymphs are generally conducted in June when later instar nymphs, which are larger, can be more easily identified. They can be found on the basal buds (suckers) and basal leaves on the underside of the leaf at the beginning of the season. The observations should be conducted in the early morning, limiting the movement of leaves: if disturbed, *S. titanus* nymphs tend to jump away, a typical behaviour that makes them distinguishable from other leafhopper species nymphs such as *Empoasca vitis* or *Zygina rhamni* (which move in a zig-zag manner or laterally on the leaf surface when disturbed) (Prezman, 2017). Another characteristic which helps to distinguish *S. titanus* nymphs from e.g. *E. vitis* or *Metcalfa pruinosa* are the two rhomboid black spots on the abdomen (Alma et al., 2016).

Adults: Adult females are larger (5.5–5.8 mm) than males (4.8–5 mm) and have three brown transverse bands at the vertex level while males only have one (Schvester et al., 1962; Vidano 1964). They are monitored using yellow sticky traps during the flying period (Figure 11). Commonly, 5–6 traps per hectare are used, but this may vary depending on plant density, proximity to other vineyards, etc. In Italy a minimum of three traps per hectare plus one extra trap per each additional hectare is recommended in the vineyards. At the beginning of July, each single trap should be positioned on the branches at the level of maximum canopy development, avoiding the trap being hidden by leaves. The traps should be replaced every 10–14 days until the middle of October (Lessio et al., 2011). The traps can be placed by the growers if properly instructed. Yellow sticky traps are not specific and attract a high number of insects, but *S. titanus* are not difficult to recognise, given their limited body size. *Scaphoideus titanus* adults could be confused with other species living in vineyards; Alma et al. (2016) provide information on the main morphological and biological differences between *S. titanus*, *O. ishidae*, *Anoplotettix fuscovenosus*, *P. cyclops* and *Platymetopius major*.

Figure 10: *Scaphoideus titanus*. Clockwise from top left: eggs, young nymph, fifth instar nymph, adult on grapevine leaves. The images are not to the same scale (Source: DISAFA Entomology, University of Turin)

The presence of *S. titanus* is always monitored in nurseries and is very frequent in commercial vineyards, where targeted pesticide applications might be performed. The intensity of the inspections depends on the country and area under surveillance (EFSA PLH Panel, 2016). Inspections are focused on production areas with no particular consideration of the presence of *S. titanus* on wild, abandoned or ornamental *Vitis* spp. plants.

Figure 11: Adults of *Scaphoideus titanus* captured on a yellow sticky trap (Source: DISAFA Entomology, University of Turin)

All the above-mentioned aspects indicate the need for trained and skilled inspectors to conduct visual examinations of FDp-like symptoms and support further confirmation of suspected infections by laboratory identification using molecular methods in certain circumstances.

Conclusions for detection methods

Visual examination of flavescence dorée phytoplasma requires skilled inspectors as it is complicated by many factors: the cultivar-dependent symptom expression, even the absence of symptoms on rootstocks, the possible risk of misidentification and the difficult access to wild and abandoned *Vitis* spp.

For detecting *Scaphoideus titanus*, the visual examination must be performed at the right time of year, the underside of the leaves should be checked in the early morning for the nymphs and yellow sticky traps are recommended for the adults. Inspection of wild and abandoned *Vitis* spp. plants growing in the proximity of commercial vineyards should also be considered.

2.2. Sampling

Flavescence dorée phytoplasma

According to EPPO diagnostic protocol PM 7/079 (2016), sampling should be performed from July to October: symptomatic leaves without necrotic areas or signs of other pests should be selected, for a total of 20 leaves per plant, pooling a maximum of five plants together. Asymptomatic leaves can be selected too, but in this case there are some limitations on the choice of laboratory test (see Section 2.3.1.). Furthermore, it should be remembered that phytoplasmas show unequal distribution in the

hosts and seasonal variability in concentration, where low titres of FDp are likely to support the plant tolerance to the infection (Pasquini et al., 2014).

Flavescence dorée phytoplasma symptoms are likely to appear the year after infection, or even later (Osler et al., 2002), but after this first year of symptom expression, a spontaneous and cultivar-dependent symptom remission could also occur, leaving the plant symptomless and without detectable FDp (Morone et al., 2007; Galetto et al., 2014). During the year, FDp can be detected in the plant as early as the flowering season, reaching the maximum titre in early summer (Roggia et al., 2014). It is advisable to avoid sampling after grape harvest due to the high Taq polymerase inhibitor present in the leaves (Pasquini et al., 2014).

A sample from a single shoot with symptoms can provide a reliable and representative evaluation of FDp titre for the whole plant. Therefore, the collection of leaves from one shoot per plant could be an acceptable sampling protocol, particularly in the presence of symptoms (Roggia et al., 2014). The best leaves to be selected are those at the proximal part of the cane (Pasquini et al., 2014).

Prezelj et al. (2013), in addition to the widely used leaf veins, also indicate berries as a similarly reliable tissue for detection, plus canes in February and flowers and tendrils in May. Early testing in the year following a new outbreak could help, for example, in selecting the infected plants to be destroyed before the disease starts spreading.

DNA extraction is recommended from fresh material. If that is not possible, petioles and midribs should be dissected from freshly picked leaves and stored at -20°C until the DNA is extracted. Once in laboratory, vein leaf separation from the lamina is the preliminary operation, in order to select the phloem tissue, where the phytoplasma can be found.

Scaphoideus titanus

Eggs, nymphs and adults of *S. titanus* all show an aggregated distribution pattern. Consequently, at low population levels, the numerical sample size for estimating the nymphal density will be very high (Lessio and Alma, 2006).

Nymphs are usually monitored by direct counting, applying sequential sampling plans (Lessio and Alma, 2006). The method, the template and an example of a completed sampling sheet can be found on the official website of Piemonte region[8]. Samplings of nymphs are aimed at: i) estimating population density; and ii) identifying the best moment for the application of insecticides (when fifth instar nymphs are present, in order to avoid the emergence of adults).

Scaphoideus titanus adults are more likely to be infected late in the growing season although adults collected throughout the season are suitable for FDp detection (Lessio et al., 2009; Ripamonti et al., 2020).

An adaptive management approach requires the selection of the most suitable sampling plan in order to limit the frequency of sampling operations: Rigamonti et al. (2016), for example, show how, under specific agricultural conditions, a sequential enumerative sampling plan can be extended to other vineyards with a similar canopy architecture without knowing their specific infestation levels, thus requiring a smaller effort to estimate population density than with enumerative sampling. It is worth noting that in the areas where both FDp and *S. titanus* are known to occur, although sampling of the vector may provide very useful information on its population level, control of the vector population is compulsory and depends on the population levels.

[8] Metodologie per il rilievo del livello di popolazione di *Scaphoideus titanus.* Annex of 'Lotte obbligatorie - Flavescenza dorata'. Available online: https://www.regione.piemonte.it/web/sites/default/files/media/documenti/2019-05/Metodo%20Monitoraggio%20Scaphoideo.pdf

Conclusions for sampling

Samples for testing the presence of flavescence dorée phytoplasma should be taken in the summer, before harvest.

Plant tissues should be sampled from the symptomatic parts of the plant, whenever possible. In particular, symptomatic shoots from the proximal part of the cane should be targeted.

Sampling of vectors should follow the most suitable plan and method in line with the aim of the vector surveys.

2.3. Identification

2.3.1. Laboratory testing

Laboratory testing should be performed on host plant tissue as well as vectors to diagnose pathogen presence.

Testing plant material

When symptoms are identified, molecular diagnosis can be performed: the number of molecular tests depends on the local situation. Rootstocks are frequently asymptomatic, so only molecular detection can reveal the presence of FDp (EFSA PLH Panel, 2016).

EPPO (2016) provides a detailed diagnostic protocol for FDp, where the following molecular methods are proposed:

- LAMP (loop-mediated isothermal amplification): suitable for preliminary screening, also on-site, only for symptomatic samples originating from areas where FDp is known to occur. Positive samples require confirmation with one of the next two methods.
- Real-time PCR or nested PCR: should be the first choice with symptomatic samples originating from areas where FDp is not known to occur or with asymptomatic samples.

Loiseau (2015) provides the results of interlaboratory trials comparing seven molecular protocols for the detection of FDp in grapevine. Generally, real-time PCR showed higher diagnostic sensitivity and diagnostic specificity than conventional PCR; in particular, real-time PCR protocols by Hren et al. (2007), Pelletier et al. (2009) and under-patent oligonucleotides had a diagnostic sensitivity and a diagnostic specificity higher than 90%.

Kogovšek et al. (2016) proved that LAMP assays developed and optimised for rapid laboratory and on-site FDp detection on symptomatic samples are highly specific, rapid (as they do not require any DNA extraction) and as effective as qPCR. Later, a test performance study conducted by Mehle et al. (2017) indicated that the accuracy of the LAMP test for FDp is over 98%.

Testing vectors

Correctly identified vectors should be preserved in pure ethanol or stored at -20°C for PCR analysis. Unlike for FDp in plants, no standard protocols have been established on the molecular analysis to be conducted on vector samples. Some examples of the protocols mentioned by authors are, for DNA extraction, Gatineau et al. (2001) or Marzachì et al. (1998), while for real-time PCR assays Pelletier et al. (2009) or Angelini et al. (2007). For conventional PCR assays, 16S phytoplasma universal primer pairs followed by nested group-specific PCR described by EPPO (2016, Appendix 3) or Clair et al. (2003) can be used.

Conclusion for pest identification

Molecular methods are available for the identification of FDp both in host plants and vectors. LAMP is suggested for preliminary screenings of symptomatic plant material and real-time/nested PCR is always necessary in the case of asymptomatic samples.

3. Key elements for survey design

Based on the analyses of the information on the pest–host plant system, the different units that are needed to design the survey have to be defined and tailored to the situation in each Member State. The size of the defined target population and its structure in terms of the number of epidemiological units need to be known.

When several pests have to be surveyed in the same vineyard (EPPO, 2018), it is recommended that the same epidemiological and inspection units are used for each pest in order to optimise field inspections since they are organised per crop visit and not by pest. This would maximise the efficiency in the use of resources allocated to the survey programme. Table 2 shows an example of these definitions.

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for flavescence dorée phytoplasma and *Scaphoideus titanus*

	Definition
Target population	*Vitis* plants in a Member State
Epidemiological unit	A homogeneous area that contains at least one individual host plant (e.g. commercial vineyard, rootstock nursery, hectare, NUTS area)
Inspection unit	A single host plant or a sticky trap

To design a survey on FDp and *S. titanus* the general guidelines provide further details on the following steps that will generally be necessary:

1/ Determine the type of survey based on its objectives. For FDp and *S. titanus*, the type of survey will depend on the pest status (according to ISPM No. 8, FAO, 2017) in the area of interest. The objective could be to substantiate pest freedom, to delimit an outbreak area following an infection/infestation or to determine pest prevalence. The next steps deal with the example of substantiating pest freedom.

The overall confidence level and design prevalence of the survey have to be decided by the risk managers before designing the surveys as they reflect the acceptable level of the risk of infestation of the host plants by FDp. The general guidelines for pest surveillance provide further details on the choice of these values and the related consequences in terms of survey design.

2/ Define the target population and its size. When determining the target population for surveillance of FDp and *S. titanus*, the host plants that are relevant for the survey area have to be selected. The size of the target population should be determined. For example, the target population could be all host trees in a Member State.

3/ Define the epidemiological units. The epidemiological units should be single homogeneous areas that each contain at least one individual host plant. The epidemiological unit could be an extended area (e.g. NUTS2 or NUTS3 region), a vineyard or even a hectare or even a smaller unit. The larger the epidemiological unit, the larger the assumption on homogeneity is. Note that it is recommended that the survey parameters are harmonised among the different pests affecting the same host plants in order to optimise field inspections, which are generally organised per crop visit and not by pest.

4/ Determine the inspection unit. For FDp these could be the individual *Vitis* spp. plants, while for *S. titanus* adults they are the sticky traps.

5/ Determine the number of inspection units per epidemiological unit.

6/ Implement the inspections and, if appropriate, the sampling, following the procedures suggested by the competent authorities, within the epidemiological units and estimate the method effectiveness in order to determine the overall method sensitivity (sampling effectiveness x diagnostic sensitivity). A representative number of plants should be examined and if there are suspicious symptoms they should be sampled and tested. RiBESS+ can be used to calculate how many inspection units need to be examined or sampled when using a predefined prevalence level (e.g. 1%) to obtain a particular confidence level. This confidence level is in turn needed to calculate the number sites to be inspected (Step 8). Note that the more units are inspected the higher the confidence will be. The competent authorities need to align the survey efforts with the resources available.

7/ Define the risk factors. A risk factor affects the probability of a pest being present or detected in a specific portion of the target population. It may not always be possible to identify or include a risk factor in the survey design. Risk factors can only be included when both the relative risk and the proportion of the overall plant population to which they apply are known or can be reliably estimated.

8/ Determine the number of epidemiological units to survey. RiBESS+ can be used to determine the number of epidemiological units to survey in order to achieve the objectives of the survey set at Step 1 in terms of confidence level (e.g. 95%) and design prevalence (e.g. 1%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. As a result, considering for example fields where host plants are present, the number of vineyards that need to be surveyed for both pathogen and vector are estimated for a Member State in order to state with 95% confidence that the prevalence of FDp will be at 1% or below.

9/ Summarise and evaluate the survey design. At this stage, it is necessary to evaluate whether the above steps have resulted in a survey design that matches the available resources, meaning that a feasible number of inspections can be performed within an acceptable time frame per inspection, and resulting in a feasible number of samples. If not, available resources should be adjusted. This adjustment would result in a modified survey design using different input parameters of the statistical tool RiBESS+ (e.g. varying the number of components, method sensitivity etc.).

10/ Integrate the pest-based survey into a crop-based survey (optional).

11/ Allocate the calculated survey effort. In the survey area, the output of RiBESS+ should be allocated proportionally to the host plant population or to the number of epidemiological units. In addition, the survey size should be selected from the list of available locations.

12/ Data collection and survey reporting. Consider which data are needed and how these data will be reported together with the related assumptions.

13/ Plan, develop or update the specific instructions for the inspector. These activities are not addressed by EFSA and fall within the remit of the competent national authorities.

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General glossary for pest survey

Term	Definition*
Buffer zone	An area surrounding or adjacent to an area officially delimited for phytosanitary purposes in order to minimise the probability of spread of the target pest into or out of the delimited area, and subject to phytosanitary or other control measures, if appropriate (ISPM 5: FAO, 2019).
Component (of a survey)	A component is a survey entity which can be distinguished based on its target population, the detection method (e.g. visual examination, laboratory testing, trapping) and the inspection unit (e.g. vectors, branches, twigs, leaves, fruits). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components.
Confidence	The sensitivity of the survey is a measure of reliability of the survey procedure (Montgomery and Runger, 2010). The term **confidence level** is used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b).
Delimiting survey	Survey conducted to establish the boundaries of an area considered to be infested by, or free from, a pest (ISPM 5: FAO, 2019).
Design prevalence *analogous to the term **level of detection** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence. In 'freedom from pest' approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the 'design prevalence'. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence (EFSA, 2018).
Detection survey	Survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2019).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016a).
Epidemiological unit *analogous to the term **lot** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b*)	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same epidemiology should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a geographical area. They are the units of interest to which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).

Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Expert knowledge elicitation	A systematic, documented and reviewable process to retrieve expert judgements from a group of experts in the form of a probability distribution (EFSA, 2014).
Host plant	A host plant is a plant species belonging to the host range on which the pest could find shelter, feed or subsist at least for a period of time.
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (ISPM 5: FAO, 2019). This definition is limited to array of host plants species and does not include the commodities other than plants or plant parts.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016a).
Infected versus infested	Infected is used when a pathogen is referred to in relation to its hosts (e.g. the trees are infected by the bacterium). Infested is used when an insect is referred to in relation to its hosts (e.g. the trees are infested by beetles). Infested is used when the pest is mentioned in relation to an area (e.g. an infested zone).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine whether pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2019).
Inspection unit *analogous to* ***sample unit*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b*)	The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place (EFSA, 2018).
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2019).
Method sensitivity *analogous to the term* ***efficacy of detection*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method sensitivity (MeSe) is defined as the probability that a truly positive host tests positive. It has two components: the sampling effectiveness (i.e. probability of selecting infested plant parts from an infested plant) and the diagnostic sensitivity (characterised by the visual inspection and/or laboratory test used in the identification process). The diagnostic sensitivity is the probability that a truly positive epidemiological unit will result positive and is related to the analytical sensitivity. It corresponds to the probability that a truly

	positive inspection unit or sample will be detected and confirmed as positive. The sampling effectiveness depends on the ability of the inspector to successfully choose the infested plant parts in a host plant. It is directly linked to the sampling procedure itself and on the training of the inspectors to recognise the symptomatology of the pest. Furthermore, symptom expressions are dependent, among other factors, on the weather conditions as well as on the physiological stage of the host plant when the sample is taken.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2019).
Pest freedom	Pest freedom can be defined, for a given target population, in a statistical framework, as the confidence of freedom from a certain pest against a pre-set design prevalence (threshold of concern).
Population size	The estimation of the number of the plants in the region to be surveyed (EFSA, 2018).
Prevalence *analogous to the term* **incidence (of a pest)** *defined in the 'Glossary of phytosanitary terms' (ISPM 5: FAO 2019)*	Pest prevalence is the fraction of infested units in the total population of host plants. Pest incidence is the proportion or number of units in which a pest is present in a sample, consignment, field or other defined population (ISPM 5: FAO 2019)
Relative risk	The ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group (Dohoo et al., 2010).
Representative sample	A sample that describes very well the characteristics of the target population (FAO, 2014).
RiBESS+	Risk-based surveillance systems. This is an online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of freedom from disease. Free access to the software with prior user registration is available at https://shiny-efsa.openanalytics.eu/
Risk assessment	Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2019).
Risk factor	A factor that may be involved in causing the disease (FAO, 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared with a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas, where the highest probabilities

	exist to find the pest.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
SAMPELATOR	Sample size calculator. This is an online application that implements statistical methods to estimate the sample size for pest prevalence estimation surveys. Free access to the software with prior user registration is available at https://shiny-efsa.openanalytics.eu/
Sample size	The sample size refers to the output of the statistical tools for survey design (RiBESS+ and SAMPELATOR). 'A well-chosen sample will contain most of the information about a particular population parameter but the relation between the sample and the population must be such as to allow true inferences to be made about a population from that sample.' (BMJ, online). The survey sample consists of the required number of 'inspection units' or samples thereof to be examined and/or tested in the survey to retrieve sufficient information on the pest presence or prevalence in the total population. In the case of risk-based surveys, the sample size is calculated on the basis of statistical principles that integrate risk factors. If the examination for pest presence is performed by laboratory testing, at least one sample is taken from each inspection unit. These samples will undergo relevant laboratory testing.
Sampling effectiveness	For plants, it is the probability of selecting infested plant parts from an infested plant. For vectors, it is the effectiveness of the method to capture a positive vector when it is present in the survey area. For soil, it is the effectiveness of selecting a soil sample containing the pest when the pest is present in the survey area.
Specified plant	The plant species known to be susceptible to the pest. For example, for *Phyllosticta citricarpa*, the list of specified plants, which includes host plants and all plants for planting, other than seeds, belonging to the genera or species, can be found in Annex I of Decision (EU) 2015/789.
Survey	An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area (ISPM 5: FAO, 2019).
Target population *analogous to* ***consignment*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are: • definition of the target population: the target population has to be clearly identified;

	• target population size and geographic boundary. (EFSA, 2018)
Test	Official examination of plants, plant products or other regulated articles, other than visual, to determine whether pests are present, identify pests or determine compliance with specific phytosanitary requirements (ISPM 5: FAO, 2019).
Test specificity	The conditional probability of testing negative given that the individual does not have the disease of interest (Dohoo et al., 2010). The test diagnostic specificity is the probability that a truly negative epidemiological unit will give a negative result and is related to the analytical specificity. In freedom from disease it is assumed to be 100%.
Visual examination	The physical examination of plants, plant products, or other regulated articles using the unaided eye, lens, stereoscope or microscope to detect pests or contaminants without testing or processing (ISPM 5: FAO, 2019).

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