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Pest survey card on *Xylella fastidiosa*

European Food Safety Authority (EFSA),
Sybren Vos, Melanie Camilleri, Makrina Diakaki, Elena Lázaro, Stephen Parnell, Martijn Schenk, Gritta Schrader, Antonio Vicent

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. The purpose of the document is to assist the Member States to plan annual survey activities of quarantine organisms using a statistically sound and risk-based pest survey approach, in line with current international standards. The data requirements for such an activity include the pest distribution, its host range, its biology and risk factors, as well as available detection and identification methods. This document is part of a toolkit that consists of pest-specific documents, such as the pest survey cards, and generic documents relevant for all pests to be surveyed, including the general survey guidelines and statistical software such as RiBESS+.

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Keywords: detection survey, delimiting survey, host plants, *Philaenus spumarius*, plant pest, risk-based surveillance, *Xylella fastidiosa*

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Correspondence: ALPHA@efsa.europa.eu

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Introduction

The information presented in this survey card was summarised from recent publications on *Xylella fastidiosa* including the following documents (i) from EFSA: update of EFSA host plant database (EFSA, 2018); EFSA updated pest categorisation of *Xylella fastidiosa* (EFSA PLH Panel, 2018); EFSA pest risk assessment (EFSA PLH Panel, 2015) and its update (EFSA PLH Panel, 2019a); (ii) from the FAO: International Plant Protection Convention (IPPC) standards on phytosanitary measures ISPM (International Standards for Phytosanitary Measures) 27 (FAO, 2016a), the diagnostic protocol for *X. fastidiosa* (FAO, 2018) and the Guidelines for the prevention, eradication and containment of *X. fastidiosa* in olive-growing areas (Catalano et al., 2019); and, (iii) the European and Mediterranean Plant Protection Organisation (EPPO) diagnostic protocol for *X. fastidiosa* PM 7/24 (4) (EPPO, 2019a).

In addition, for the purpose of clarity, the specific terms used in surveillance in plant health referred to in ISPM 31 (FAO, 2016b) and in ISPM 5 (FAO, 2019) are included in the glossary of this document, alongside the corresponding terms used in the survey card.

The objective of this pest survey card is to provide the relevant biological and epidemiological information that is needed to prepare the surveys in the EU Member States (MSs). The focus of the pest survey card is to assist the MSs with the design of their annual surveys of the territory, understood as the annual detection surveys that are performed to substantiate the pest freedom (see Glossary) statements or to confirm the pest status. Nevertheless, the information and rationales provided are also relevant for the preparation of delimiting surveys in the event of a first positive finding (see Glossary for definitions of detection and delimiting surveys).

This document is part of a toolkit that has been developed to assist and support the MSs with planning a statistically sound and risk-based pest survey approach in line with the IPPC guidelines for surveillance (FAO, 2016c). The toolkit consists of pest specific documents and more general documents relevant for all pests to be surveyed:

i. Pest-specific documents

  a. The pest survey card on *X. fastidiosa*[1]

  b. *Xylella fastidiosa* pest survey guidelines

ii. General documents:

  a. The general survey guidelines

  b. The RiBESS+ manual available online[2]

  c. The statistical tools RiBESS+ and SAMPELATOR which are available online[3] with open access after registration.

[1] The content of this EFSA Supporting Publication is reproduced as a live document available at https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489 where it will be updated whenever new relevant information becomes available.
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://websso-efsa.openanalytics.eu/auth/realms/efsa/protocol/openid-connect/auth?response_type=code&client_id=shiny-efsa&redirect_uri=https%3A%2F%2Fshiny-efsa.openanalytics.eu%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce-237f192a72d5&login=true&scope=openid

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Xylella fastidiosa* Wells et al., 1987

Class: Gammaproteobacteria, **Order:** Xanthomonadales, **Family**: Xanthomonadaceae, **Genus**: *Xylella*, **Species**: *Xylella fastidiosa*

Common names of the diseases caused by *X. fastidiosa* in English: alfalfa dwarf, Pierce's disease of grapevine, peach phony rickettsia, plum leaf scald, citrus variegated chlorosis, olive quick decline syndrome, leaf scorch (almond, elm, oak, oleander, American sycamore, mulberry and maple).

Subspecies: Although, only the subspecies *fastidiosa* and *multiplex* are currently officially considered as valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB) (Bull et al., 2012), six different subspecies of *X. fastidiosa* have been proposed (Schaad et al., 2004) as described in EFSA PLH Panel (2018), namely:

- *X. fastidiosa* subsp. *fastidiosa*
- *X. fastidiosa* subsp. *multiplex*
- *X. fastidiosa* subsp. *pauca*
- *X. fastidiosa* subsp. *sandyi*
- *X. fastidiosa* subsp. *tashke*
- *X. fastidiosa* subsp. *morus*

Sequence types (STs): Multilocus sequence typing (Maiden et al. 1998) is a genetic typing methodology that is widely used to characterise *X. fastidiosa* (Scally et al., 2005; Yuan et al., 2010; Elbeaino et al., 2014; Nunney et al., 2014; Denancé et al., 2017, 2019). Different STs have been identified in the different outbreaks in the EU as shown in Figure 1. Open-access[4], curated databases that integrate population sequence data for *X. fastidiosa*, can be found as part of the public databases for molecular typing and microbial genome diversity. New STs are continuously being identified, as seen by the examples of Nunney et al., (2019) and Saponari et al., (2019).

In conclusion, *X. fastidiosa* is a well-described (Wells et al., 1987) and clearly identifiable bacterium (Su et al., 2016). The detection strategies in the annual surveys that are currently performed in the EU MSs primarily aim to detect *X. fastidiosa* at the species level. Following a positive detection, *X. fastidiosa* subspecies and ST assignment is required in order to determine the appropriate survey strategy for the delimitation given that that host range differs between subspecies (Chatterjee et al., 2008) and between STs (EFSA, 2018; EFSA PLH Panel, 2018). The host range reported in the literature for each subspecies and each ST is available in EFSA's updated host plant database. It is, however, important to underline the fact that recombination is a major element in the evolution of *X. fastidiosa*, occurring within short time frames and being associated with new host–associations (Coletta-Filho et al., 2017).

[4] Public databases for molecular typing and microbial genome diversity, in particular the *Xylella fastidiosa* MLST website (https://pubmlst.org/xfastidiosa/) sited at the University of Oxford (Jolley et al., 2004)

Vector taxonomy: *Xylella fastidiosa* is a xylem-inhabiting bacterium which is spread by xylem sap-feeding insects. The vector insects belong to the order Hemiptera, suborder Auchenorrhyncha, infraorder Cicadomorpha (= Clypeorrhyncha) (Redak et al., 2004), superfamilies Cicadoidea, Cercopoidea and Membracoidea. All Cicadoidea (cicadas) and Cercopoidea - such as the Aphrophoridae family, known as froghoppers and spittlebugs - are considered as xylem fluid feeders. Within the superfamily Membracoidea, only the insects belonging to the subfamily Cicadellinae (known as sharpshooters) are xylem fluid feeders. Only these insects have been shown to be vectors of *X. fastidiosa* (EFSA PLH Panel, 2015, 2018).

Native Aphrophoridae species to the EU are known to transmit the bacterium, in particular *Philaenus spumarius, P. italosignus and Neophilaenus campestris*. From these, *P. spumarius* is currently considered the most important vector since it is the only one proven so far to effectively transmit the bacterium in natural conditions in the EU (see Section 1.3.2.).

1.2. EU pest regulatory status

The EU legislation relevant for *X. fastidiosa* is updated in the Official Journal of the European Union and also available on the website of the European Commission[5]. *Xylella fastidiosa* is known to occur in the EU, it is a quarantine pest and is regulated in the EU as a harmful organism under Annex IAII of Council Directive 2000/29/EC[6]. The bacterium is also addressed under other Annexes[7,8,9].

With regards to the insect vectors of *X. fastidiosa*, Annex IAI prohibits the introduction of the *Cicadellidae* (non-European) known to be the vector of Pierce's disease as harmful organisms, such as (a) *Carneocephala fulgida* Nottingham (*Xyphon fulgidum* Nottingham – new valid name), (b) *Draeculacephala minerva* Ball, and (c) *Graphocephala atropunctata* (Signoret).

The introduction into the EU of some known host plants of *X. fastidiosa* is prohibited under Annex IIIA, which concerns plants, plant products and other materials, the introduction of which shall be prohibited in all MSs. This includes *Citrus*, *Fortunella*, *Poncirus* and their hybrids from third countries, *Vitis* from third countries other than Switzerland, and *Prunus* originating from non-European countries, other than dormant plants free from leaves, flowers and fruit from Mediterranean countries, Australia, New Zealand, Canada and the continental states of the USA.

In addition, following the 2013 outbreak in olive trees in the south of Italy, emergency measures on *X. fastidiosa* were introduced to prevent further introduction and its spread within the EU and these are laid down in Implementing Decision 2015/789/EU[10]. This Decision is amended regularly based on experience and new scientific knowledge.

[5] https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa_en
[6] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1–112. Consolidated version of 01/04/2018.
[7] In Annex IIAI as Citrus variegated chlorosis on plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds (Consolidated version of 01/04/2018).
[8] In Annex IAI as Peach phony rickettsia (Consolidated version of 01/04/2018).
[9] In Annex IVAI as Peach phony rickettsia with special requirements for plants for planting of *Prunus* L., (Consolidated version of 01/04/2018).
[10] Commission Implementing Decision (EU) 2015/789 of 18 May 2015 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.) (notified under document C(2015) 3415) (OJ L 125, 21.5.2015, p. 36)

Furthermore, in 2015, the European Commission prepared Guidelines for the survey of *X. fastidiosa* (European Commission, 2015).

1.3. Pest distribution

Xylella fastidiosa being a vector-borne bacterium, this section addresses the global and EU distribution of both the bacterium and its vectors.

1.3.1. *Xylella fastidiosa* distribution

In the EU, since *X. fastidiosa* was first detected in olive trees in Southern Italy in 2013, the bacterium has been reported in France (in Corsica and the Provence Alpes Cotes d'Azur region), in Spain (in the Balearic Islands, in Madrid and Comunitat Valenciana - province of Alicante), in Italy (Apulia and Tuscany) and more recently in Portugal (Porto). The situation of the reported outbreaks in the EU can be monitored by consulting the EUROPHYT Outbreak database[11].

Figure 1 maps the EU distribution of *X. fastidiosa* according to the outbreak reports of the different subspecies and STs (updated from EFSA PLH Panel, 2018).

Xylella fastidiosa originates in the Americas. Outside the Americas (Figure 2), the species has been reported on almond and grapevine in Iran (Amanifar et al., 2014). Many other reports are unconfirmed (EPPO, 2019b; EFSA, 2018). The bacterium causing pear leaf scorch disease in Taiwan first identified as *X. fastidiosa* (Su et al., 2013) was later reported as being from a new species *X. taiwanensis* (Su et al., 2016).

[11] https://ec.europa.eu/food/plant/plant_health_biosecurity/harmful_organism_outbreaks_en

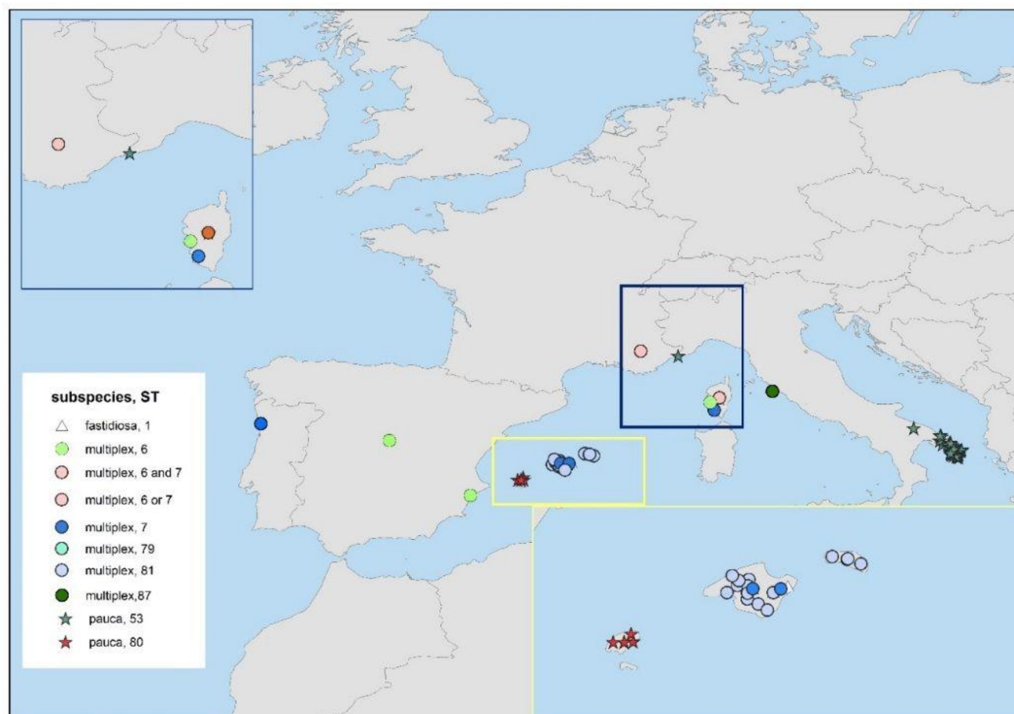

Figure 1: Distribution of the different *Xylella fastidiosa* subspecies and sequence types reported in the EU. Data points are extracted from a literature search and current EU outbreak reports (updated from EFSA PLH Panel, 2018)

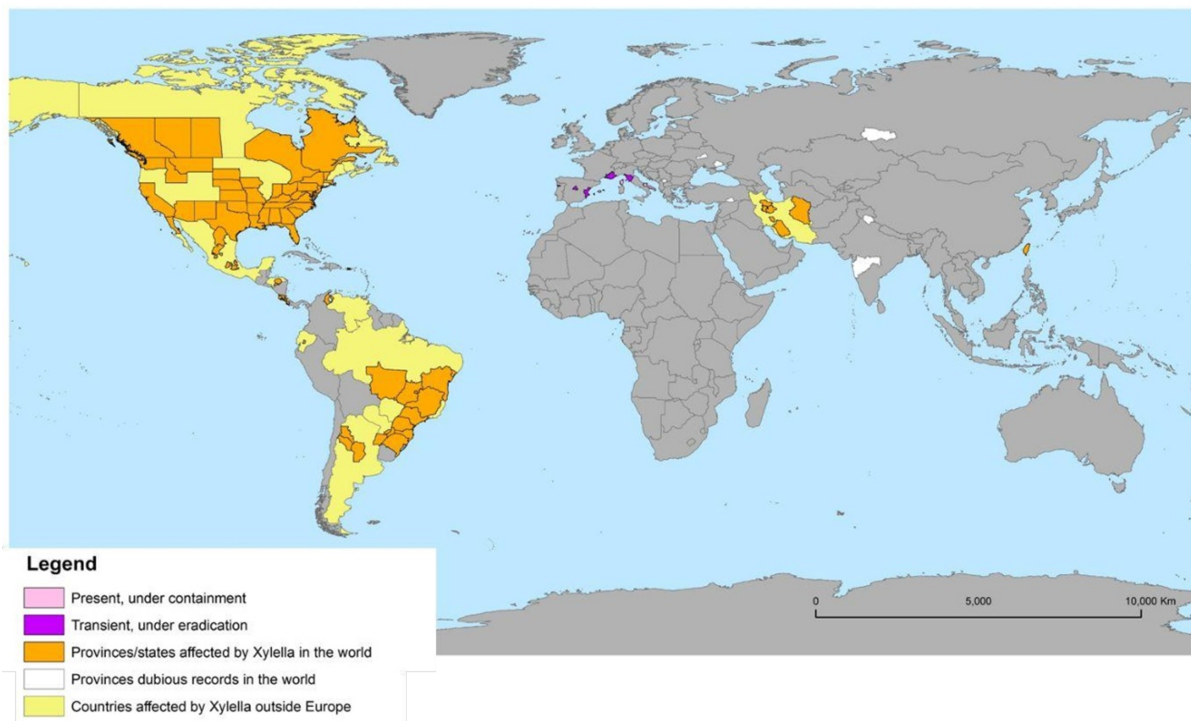

Figure 2: World distribution of *Xylella fastidiosa* and pest status in the EU member states (Updated from EFSA PLH Panel, 2018)

1.3.2. *Xylella fastidiosa* vector distribution in the EU

EFSA PLH Panel (2018) maps the species richness of spittlebugs, sharpshooters and cicadas in Europe. It can be concluded that potential vectors – xylem sap feeders - of *X. fastidiosa* are available throughout the EU territory.

The non-EU Cicadomorpha vectors of *X. fastidiosa* are instead discussed in EFSA PLH Panel (2019b): approximately 30,000 potential vector species belong to this group of insects, among which, 49 are confirmed vectors (including the invasive species *Homalodisca vitripennis*, the glassy-winged sharpshooter). Since potential and confirmed vector species of the bacterium are native and available throughout the EU, this survey card will not address the surveillance of the vectors not known to be present in the EU.

A list of European putative vectors of *X. fastidiosa* is available in the table in Appendix C of EFSA PLH Panel (2015). This table lists the vector species of *X. fastidiosa* specifying their distribution in the EU as well as their host range and ranks their competency as vectors of the bacterium. More recent and updated results are presented by Di Serio et al. (2019). The authors present - in Annex 3 of the latter publication - the results of a systematic review summarising the data and information on biology, phenology and control of vectors and potential vectors of *X. fastidiosa*.

As discussed in EFSA PLH Panel (2018), following the discovery of *X. fastidiosa* in the EU, attempts to identify vectors have been made, although final data on vector competence are so far published only for *X. fastidiosa* subspecies *pauca* ST53 in olive groves in the Apulia region, where the spittlebug *P. spumarius* is the main vector. Saponari et al. (2014) first transmitted *X. fastidiosa* to periwinkle using infected field-collected *P. spumarius* adults. The olive-to-olive transmission was achieved by Cornara et al. (2017a) under controlled conditions with acquisition of healthy *P. spumarius* adults on infected olives and inoculation to self-rooted olive plants. In the same experiments, *P. spumarius* transmitted *X. fastidiosa* to periwinkle following acquisition from different infected plant species (olive, cherry, almond, *Polygala*), but *N. campestris* failed to transmit *X. fastidiosa* under the same experimental conditions. In a more recent study, *P. italosignus* and *N. campestris* transmitted ST53 to olive and *Polygala myrtifolia* (Cavalieri et al., 2018).

Although no vector transmission tests results have been published so far for *X. fastidiosa* isolates from other EU outbreaks, *P. spumarius* specimens from Corsica in France, (Cruaud et al., 2018) and *P. spumarius* and *N. campestris* specimens from Alicante in Spain (EFSA PLH Panel, 2018) were found contaminated with *X. fastidiosa*.

Despite these knowledge gaps, in consideration of its involvement in the different outbreaks in the EU, and considering its proven competence to transmit the bacterium, *P. spumarius* is today the most important vector to take into account in surveillance in the EU. However, in the absence or low abundance of *P. spumarius*, an area should not be excluded from the survey as other potential vectors might be present. Vector sampling should be tailored to each case following understanding of the specific pathosystem involved and the most up to date knowledge produced by ongoing research programmes (POnTE, 2019; XF-Actors, 2019).

1.4. Life cycle

Diseases caused by *X. fastidiosa* result from the interaction between the bacterium, host plants, including reservoir hosts, insect vectors and environmental conditions (EFSA, 2018; Chatterjee et al., 2008).

The time frame for the survey activity is not addressed here as it will vary across the EU depending on the climate conditions. The ideal timing of surveillance activities depends on the phenology of the plant species to be surveyed and the synchronism with the infectious stages of the insect vectors.

1.4.1. *Xylella fastidiosa* and host plant interaction

Systemic colonisation of the plants by *X. fastidiosa* occurs via a complex pattern, which depends on the host species and the genotype of the pathogen (EFSA PLH Panel, 2015, 2018). Bacterial cells can move systemically through the xylem vessels of infected susceptible plants. In some host plants, however, their presence may remain restricted to parts of the plant (Purcell and Saunders, 1999). The time period between inoculation and the appearance of symptoms in a given plant (incubation period) is highly variable and ranges from a few months to years, depending on the *X. fastidiosa* genotype, the host species, the physiological stage (age) of the plant and growing conditions (EFSA PLH Panel, 2018, 2019a). Symptom expression is usually linked to the occlusion of xylem vessels, hence, symptoms of *X. fastidiosa* often resemble those caused by water stress. In some cases, the infection results in rapid death of the host plant (Purcell and Saunders, 1999; Martelli et al., 2016). On the other hand, some plant species may not even express any symptoms, which may also depend on the growing conditions (EFSA PLH Panel, 2015, 2018, 2019a).

1.4.2. *Xylella fastidiosa* and its vector interaction

<u>*Xylella fastidiosa* acquisition and detection surveys</u>

Considering that the aim of a detection survey is to detect the bacterium, all insects that could acquire the bacterium are potentially relevant to be monitored. The bacterium acquisition capacity of the vectors will determine their relevance to the survey. In both systemic and local infections, *X. fastidiosa* can be acquired by xylem-feeding insect vectors (both nymphs and adults) (Hill and Purcell, 1995, 1997; Purcell and Saunders, 1999; EFSA PLH Panel 2015, 2018 and 2019a). Therefore, detection surveys should focus on monitoring the xylem sap feeder insect species.

Although vectoring of *X. fastidiosa* by phloem sap feeding insect species has not been proven so far, it is however possible that phloem sap feeding insects may also occasionally ingest xylem sap of infected plants (EFSA PLH Panel, 2019b), and therefore might be positive when tested for *X. fastidiosa*. In Apulia, Elbeaino et al. (2014) reported positive results for field specimens of the phloem feeder *Euscelis lineolatus* when tested for *X. fastidiosa*. Hence, such species should not be disregarded in a detection survey when they are particularly abundant in an area.

Xylella fastidiosa transmission and delimiting surveys

After a first positive finding of the bacterium in an area, the information on spread becomes a very important factor to consider in order to delimit the area where the pest is circulating. The spread of the bacterium depends on the competence of the infected insect to transmit it. Successful transmission results from three consecutive events: ingestion of bacterial cells from a source plant, attachment and retention of bacteria in the foregut of the vector, followed by detachment and inoculation into a new host (Almeida et al., 2005). Although *X. fastidiosa* transmission is restricted to xylem sap-feeding insects with piercing-sucking mouthparts, insect transmission of *X. fastidiosa* is known for its lack of species specificity (Damsteegt et al., 2006). Therefore, all xylem sap-feeding insects are considered as potential vectors (Frazier, 1944; Purcell, 1989; Almeida et al., 2005). The bacterium is transmitted in a persistent manner, and there is no latency period following acquisition (Almeida et al., 2005). Bacteria do not systemically infect the insect body (Purcell and Finlay, 1979) and adhere to the precibarium and the cibarium (parts of the foregut). This implies that vectors lose infectivity when moulting, as the foregut is of ectodermal origin and is renewed. Therefore, newly emerged adults must again acquire *X. fastidiosa* to become infectious. The bacterium is not transovarially transmitted to the offspring of infected vectors (Freitag, 1951). The number of bacterial cells per insect is low, but very few live bacterial cells are sufficient for successful transmission (Hill and Purcell, 1995, 1997). Due to their mobility, adult insects are usually responsible for *X. fastidiosa* spread. They are more likely to become infected than nymphs, because they are more likely to have fed on multiple plants, including woody hosts (nymphs only feed on herbaceous hosts). Moreover, adult insects have a relatively long life and persistence of infection, so they are the most suitable targets for surveillance. Regarding the nymphs, several of them can aggregate and share the same spittle mass, and several spittle masses can be found on the same plant. Adults generally appear from the dried-up spittle in spring and live until autumn. It is not recommended thus to target the nymphs in a survey programme for *X. fastidiosa*.

Transmission efficiency varies substantially depending on the insect species, host plant and *X. fastidiosa* genotype (Redak et al., 2004; Lopes et al., 2010; Almeida, 2016). Vector species can have different transmission efficiencies depending on host plant species, or even when feeding on different tissues or phenological stages of the same host plant (Lopes et al., 2010; Daugherty et al., 2011). In addition, transmission efficiency is influenced by temperature (Daugherty and Almeida, 2009). Temperature has an effect on pathogen multiplication in source plants (Feil and Purcell, 2001), on the pathogen multiplication in vectors (Dohm et al., 2002), on the successful establishment of the pathogen in the new host (Chu and Volety, 1997), and on the vector behaviour (e.g. feeding rate (Su and Mulla, 2001), probing behaviour (Sylvester, 1964), or movement (Vail and Smith, 2002).

Philaenus spumarius

So far, only *P. spumarius* (meadow spittlebug) has been identified as an effective vector of *X. fastidiosa* in the EU under natural conditions (Saponari et al., 2014, Cornara et al., 2017b). It is a highly polyphagous, univoltine species. The host range of the nymphs varies from that of the adult insects. The nymphs prefer tender plant parts and herbaceous plants. In particular they are found on plants of the Asteraceae, Fabaceae, and Apiaceae families (Di Serio et al., 2019; Dongiovanni et al., 2018). These plant species are often growing as the crop under cover, the field hedges and in natural and semi-natural areas. The adult insects, by contrast, rather feed on woody plant species. The adult insect is polyphagous and its preference for one plant species or another depends on the vegetation growing in the area. In addition, the distribution of meadow spittlebugs mainly depends on the

distribution of suitable host plants, which often occur in an aggregated pattern (Biedermann, 2002). More details on the host range of adult insects are reported in Section 1.5.

Seasonal movement of adults from the herbaceous vegetation of olive groves to the canopy of olives and other evergreen or deciduous trees and shrubs has been observed in northern and southern Italy in late spring and early summer (Di Serio et al., 2019; Cornara et al., 2017a; Bodino et al., 2017). This movement can also be observed when the grass cover persists over the summer, and thus is probably not caused only by the herbaceous hosts drying out. At the end of summer and beginning of autumn, adult females return to herbaceous vegetation for oviposition (Cornara et al., 2018). Such seasonal patterns and host-shifting may vary depending on the climatic conditions in the survey area. One example of the life cycle of *P. spumarius* is illustrated in Figure 3.

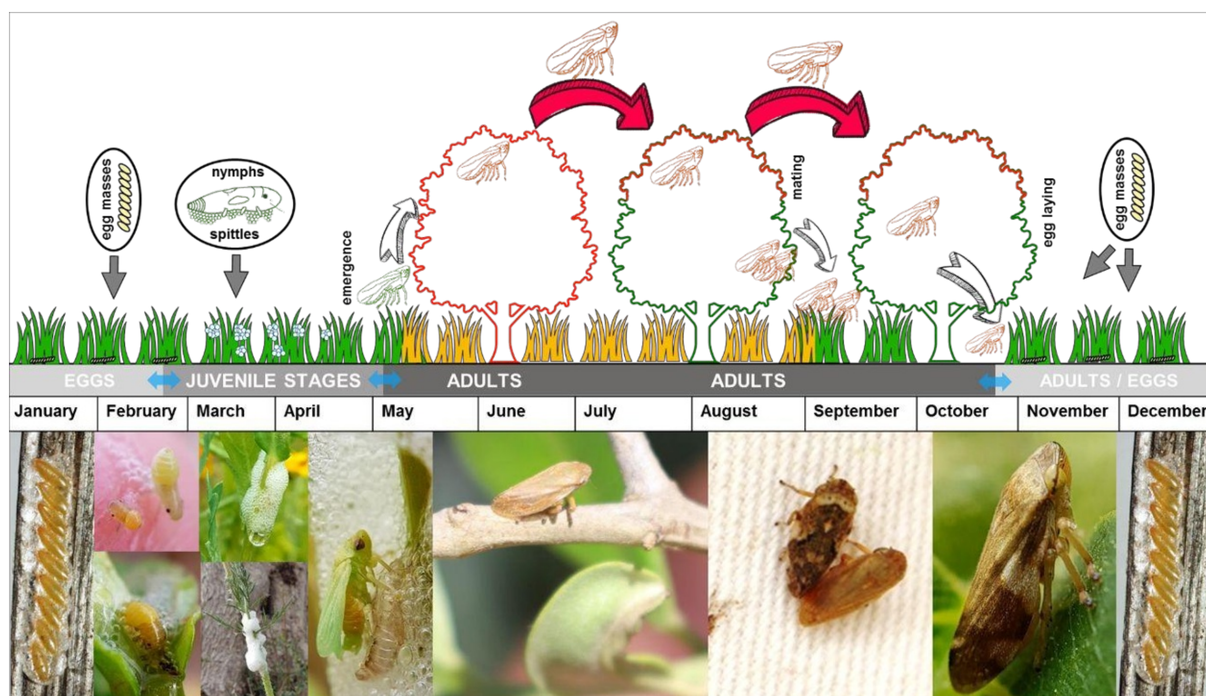

Figure 3: Life cycle of *Philaenus spumarius* in the Apulia region in Italy. Newly emerged adults (green insect) acquire *Xylella fastidiosa* when feeding on an infected host (red tree or yellow grass). The infected vector can then transmit the bacterium to healthy hosts (green), which will then in turn become infected. Eggs do not carry the vector since transovarial transmission is not possible. Nymphs can also become infected although lose infectivity when moulting (Graphic designed and developed by Infoxylella®, revised by D. Bosco, V. Cavalieri and E. Dongiovanni - Photographs by A. Coti and V. Cavalieri)

Climatic conditions in different regions in the EU can affect the life cycle of this spittlebug, thus the timing of vector surveillance should be adjusted accordingly. In Apulia (southern Italy), the emergence of *P. spumarius* adults usually around the end of April and beginning of May, with a high population abundance in late spring to early summer and movement from herbaceous plants to olive trees is observed. Although adult vectors could be collected from May onwards, surveillance of adult vectors in Apulia (Italy) should take place later during the summer period to increase the chances of finding infected insects (Figure 3). In several warm and dry areas of the Mediterranean Basin, aestivation of adults has been observed (Cruaud et al., 2018) and therefore should be taken into consideration for the collection of the samples as during this period it is very difficult to capture the

insects. The dates for adult collection also vary from year to year in the same location. For example, in Corsica in 2016, adults only emerged in early June, and were very difficult to collect in summer, but huge adult populations reappeared in early October following the first rainfall (Cruaud et al., 2018). In the Mediterranean climate, adult insects are abundant soon after emergence and then later after aestivation in October. In the Netherlands, *P. spumarius* can be detected from June onwards to October (Noordijk et al., 2019), with a peak in the adult population in June and July. In the context of the surveillance for *X. fastidiosa,* when the insect samples are collected late in the season, the insects might have fed on multiple host plants and the chances to be infected are higher, should the bacterium be present.

In the context of the surveillance activities, *P. spumarius* is the best candidate for monitoring in the EU. Cruaud et al. (2018) indicate that vector monitoring and testing is an essential component for early detection of *X. fastidiosa*. The authors indicate that for achieving reliable results, they used in their study highly sensitive methods, such as the nested PCR approach. However, further investigation is needed to better characterise the vector population dynamics, ecology and feeding behaviour which will allow better targeting of the surveys in time and space. Until such information is available the detection survey should be designed to capture *P. spumarius*, but it is worth considering that testing for the presence of *X. fastidiosa* of any sharpshooters, cicadas and spittlebugs that are caught along with *P. spumarius* takes relatively few extra resources. Insects from the same species could be pooled for reducing the samples size for laboratory testing (see Section 2.3.2.).

1.5. Host range and main hosts

Since *X. fastidiosa* is a polyphagous pest, the aim of this section is to characterise the host plant population targeted by the survey activity. The hosts included in the target population have to be chosen with caution since the statement of pest freedom made based on surveillance activities will only be valid for this target population.

EFSA host plant database[12]

Information about host plant species and the *X. fastidiosa* subspecies able to infect them can be found in the EFSA *Xylella* host plant database (EFSA, 2018). This is a comprehensive global database of all host plant species in which the pathogen has been detected and reported. A total of 563 host plant species have so far been reported in the scientific literature (EFSA, 2018). From these, 312 were confirmed using at least two different molecular tests for the detection of *X. fastidiosa*. From these, 192 specific species were found to have been infected in natural conditions. The reports indicate the susceptibility of the host species at the *X. fastidiosa* level, at the *X. fastidiosa* subspecies level or at multilocus ST level, depending on data available in the cited literature. It has to be noted that the number of reports of pest-host associations, do not necessarily reflect their epidemiological relevance, because the research and reporting interests may also be influenced by the economic and/or social importance of a crop.

[12] The raw data and related metadata of EFSA (2018) are published in Zenodo in the EFSA Knowledge Junction community (available online https://doi.org/10.5281/zenodo.1339344 Accessed on 21/05/2019 note that new versions may be published and the latest one should always be consulted). Interactive reports are available online at: https://www.efsa.europa.eu/en/microstrategy/xylella (accessed on 07/06/2019)

The composition of the target population in terms of host plant species may vary depending on the type of survey to be developed and are addressed in this section under the paragraph "Annual detection surveys in the MSs". It will only include species belonging to the above-mentioned list of 192 species, since there is high certainty regarding their host status.

Host plant categories

Host plants species for *X. fastidiosa* can be found in various environments and, thus, for the purposes of surveillance, four categories of host plants are proposed. The categories are:

- Host plants in agricultural areas, with a particular interest in permanent crops of economic importance.
- Host plants in natural or semi-natural areas, with a particular interest in shrubs and herbaceous vegetation. Semi-natural areas are populated by Mediterranean and sub-Mediterranean evergreen sclerophyllous bush and shrub plants (also known as maquis, garrigue, matorral and phrygana *sensu lato*), and plants growing in broadleaved evergreen forests that are in the stages of re-colonisation or degradation (e.g. rosemary, milkwort, oleander and lavender).
- Ornamental host plants in nurseries or gardens (e.g. rosemary, milkwort, oleander and lavender).
- Forestry and shade tree host plants, with a particular interest in broadleaved species, species growing in nurseries, mixed forests, in parks or along roads (e.g. oak, elm, maple and plane trees).

Identifying the hosts of *X. fastidiosa* for each host plant category can be a helpful approach to structuring the target population in homogeneous groups of host plants and better tailor the survey to each category of hosts in each MS. However, in consideration of the limited knowledge on the host specificity of the *X. fastidiosa* subspecies and ST, the survey design should address all four categories, especially for the annual detection surveys. Moreover, within each category of host, the survey should target the main potential host plants and should not focus on one host plant alone, since doing so comes with the risk of missing infections in other plant species.

Annual detection surveys in the MSs

In the case of the annual detection surveys in the MSs (the major scope of this survey card), the objective is determining the presence or absence of *X. fastidiosa* at species level, therefore disregarding subspecies and ST in the survey area. This implies that a large number of host plant species should be included in the target population, as not to exclude any *X. fastidiosa* subspecies or ST. Listed below are some rationales that can assist the MSs to define the target population of the detection surveys in their specific situations.

i. Main host plant species as per 2015/789/EU

Under Decision 2015/789/EU, several host plants are considered by the European Commission to pose a greater risk. These are *Coffea* spp., *Lavandula dentata* L., *Nerium oleander* L., *Olea europaea* L., *P. myrtifolia* L. and *Prunus dulcis* (Mill.) D.A. Webb. From these, *Coffea* spp. are not grown under field conditions in the EU, although imported as ornamentals, and will be further discussed in regard to the pathway of introduction which end point could constitute for a location of higher risk (see Section 1.8.). The species *L. dentata*, *N. oleander*, *O. europaea*, *P. myrtifolia* and *P. dulcis* are considered to be good candidates for inclusion in the target population.

ii. Host plant species reported in different phytosanitary crises

Important phytosanitary crises have been reported in Brazil on citrus species, in the USA on grapevine and in the EU on olive and almond trees. Therefore, in consideration of the important impacts reported on these crops, the target population should include citrus, grapevine, almond and olive trees.

iii. Plant species able to host multiple *X. fastidiosa* subspecies

The aim of the survey is to detect *X. fastidiosa* irrespective of the subspecies and, thus, when possible, it should include plant species that are able to host multiple *X. fastidiosa* subspecies, ensuring that the host plant species surveyed do not exclude any *X. fastidiosa* subspecies from the surveillance. As good surveillance practice, plant species able to host multiple subspecies of *X. fastidiosa* should, therefore, be included in the target population of the detection survey when these species are growing in the survey area. Table 1 presents the plant species able to host three different subspecies of *X. fastidiosa*. This is the maximum number of subspecies currently reported for a single host plant species. More details can be found in Annex A, where also the plant species that have been reported to host two or less *X. fastidiosa* subspecies are presented. The Annex A is an extract of the currently reported host plants of *X. fastidiosa* in the literature as per EFSA (2018).

Table 1: Plant species susceptible to at least three subspecies of *Xylella fastidiosa*, for inclusion in the target population for surveillance (extracted from EFSA, 2018). Note that plant species reported to be susceptible to *X. fastidiosa* subsp. *morus* or *X. fastidiosa* subsp. *tashke* do not host multiple *X. fastidiosa* subspecies

Host plant species	**Number of records in the literature for each *Xylella fastidiosa* subspecies**[a]			
	fastidiosa	***multiplex***	***pauca***	***sandyi***
Prunus dulcis	20	21	6	
Polygala myrtifolia	2	55	5	
Spartium junceum	2	5	1	
Nerium oleander	1		9	23
Rhamnus alaternus	1	1	1	
Rosmarinus officinalis	1	4	2	

[a] Literature screening only included cases where infection was natural and where two different molecular methods were used for the identification of the bacterium in plant tissue (EFSA, 2018).

iv. Host plant species preferred by the vector *Philaenus spumarius*

The adult insects are the most relevant life stage for the sampling and testing as explained in Section 1.4. Therefore, it is important to identify their preferred host plants. However, in humid areas adults could feed on Asteraceae and Fabaceae all over the summer, similar to the nymphs. In Mediterranean dry conditions, the adult insect will prefer woody hosts.

Since nitrogen-fixing plants are attractive to spittlebugs (Thomson, 2004, Di Serio et al., 2019), they should be considered for the target population. Such hosts may also play an important role in disease spread. Another set of host plant species of interest is linked to the bacterial acquisition rate, which varies according to the plant species. According to Cornara et al. (2017a), in Apulia, *P. spumarius* adults acquired *X. fastidiosa* from several host plant species in the field, with the highest rates from *O. europaea*, *P. myrtifolia* and *Acacia saligna* Labill. (acacia). In Mediterranean conditions, in particular for Liguria in Italy, Di Serio et al. (2019) indicate that the alternative woody host plants preferred by adult insects are *Quercus* spp. and *Pistacia* spp. In the Apulian sites of their study, the spittlebug adults were more frequently collected on *Myrtus* spp., *Pistacia* spp. and *Phillyrea* spp. Cruaud et al. (2018) indicate that in Corsica, adult *P. spumarius* were mainly collected from *Cistus monspeliensis*, to a lesser extent in grasses and clover and that the insect rarely switches to woody plants. Adult *P. spumarius* have been reported in central Spain mainly on plants of *Juniperus*, *Quercus*, *Lavandula*, *Thymus* and *Avena* (Morente et al., 2018).

From the above listed hosts of *P. spumarius*, no plant species of the genus *Pistacia*, *Thymus* and *Avena* have been reported as host plants of *X. fastidiosa.*

In addition, water stress of the plants seems to influence the host preference of the adult insects as they feed on the less stressed plants and on the tender tissues of the woody plants.

The interaction between *P. spumarius* and its host plants seems to vary depending on the ecosystem and the climatic conditions. This suggests that host preference of *P. spumarius* cannot be defined at EU level and should be tailored by each MS.

Delimiting surveys in the Member States

After a positive finding, the survey aims to delimit the area where the pest is contained and in this context, the target population will include mainly the host plants that are relevant for that specific area and the specific infectious agent (*X. fastidiosa* subspecies or STs). In addition to the above-mentioned rationales (with the exception of rationale iii), those below can be used to inform the choice of the host species for inclusion in the target population of a delimiting survey.

v. Host plant species and duration of the median asymptomatic period

Considering the long and variable duration of the incubation period (i.e. from infection to symptom expression) of *X. fastidiosa* in host plants and even in some cases the presence of asymptomatic hosts, detection surveys cannot solely be based on visual examination of host plant species. In addition, the symptoms when expressed are not specific enough and could be confused with water stress. The absence of symptoms does not indicate the absence of infection, which is why surveillance activities should systematically include sampling and laboratory testing. Nonetheless, when *Xylella*-like symptoms are observed, the samples should be preferably taken from the symptomatic plant parts as they have a higher chance of being infected. After a first positive finding during a delimiting or a monitoring survey, when the subspecies of *X. fastidiosa* has been confirmed, it would be a good practice to include host plants in the target population that have a short asymptomatic period.

EFSA PLH Panel (2019a, Appendix J) presents a comparative analysis of the duration of the asymptomatic period for a few combinations of host plant species - *X. fastidiosa* subspecies. Table 2 presents the corresponding median time after infection until symptoms are visible for each host-

subspecies combination for the following host plant species: grape (*V. vinifera* and *V. rotundifolia*), almond (*P. dulcis*), sweet orange (*Citrus sinensis*), olive (*O. europaea*), elm (*Ulmus americana*) plane (*Platanus occidentalis*), pink periwinkle (*Catharanthus roseus*), blueberry (*Vaccinium corymbosum*) and mulberry (*Morus rubra* and *M. alba*). Further details are available in EFSA PLH Panel (2019a). Since these data are derived from relatively few papers (35 papers and 124 studies) and refer to specific host plant species, the time to symptom expression should not be extrapolated to other host/subspecies combinations or be generalised to the host plant genus level. Additionally, the studies reviewed were all reporting results of experiments done with relatively young plants with artificial inoculation under controlled conditions and, thus, might not have the same results in field conditions.

Table 2: Median duration of the asymptomatic period for different host plants and *Xylella fastidiosa* subspecies derived from survival analysis (EFSA PLH Panel, 2019a)

Host plant species	Median time to symptom expression (days) for each *Xylella fastidiosa* subspecies		
	fastidiosa	***multiplex***	***pauca***
Almond (*Prunus dulcis*)	105	116	
Grapevine (*Vitis rotundifolia* and *Vitis vinifera*)	52		
Olive (*Olea europaea*)			390
Ornamental (*Catharanthus roseus*)			30
Shade trees (*Platanus occidentalis* and *Ulmus americana*)		330	
Sweet Orange (*Citrus sinensis*)			160

vi. Host plant species serving as reservoirs for *Xylella fastidiosa*

Some host plant species, such as common grasses might act as reservoirs for the bacterium (Lopes et al., 2003). Reservoir plants are selected by female insect vectors for oviposition, suggesting that depending on the time period, nymphs and spittle can be found on them. Depending on the vector species, different grass species may be preferred. The study by Purcell and Saunders (1999) suggests that the reservoir plants to be prioritised differ depending on the environment in which they are present. For instance, when studying the vineyards of northern California, important permanent reservoir species include California blackberry (*Rubus ursinus*), French broom (*Genista monspessulana*) and periwinkle (*Vinca major*), as seen by data regarding the multiplication, systemic movement and overwintering survival of *X. fastidiosa* on these hosts. The identification of reservoir-acting host species is only relevant at local level.

Summary of host plant selection

Some host plant species are in line with multiple rationales as indicated above and could therefore be systematically included in the target population for annual detection survey in a MS as they cover the host plant categories agricultural areas, nurseries/gardens and natural/semi-natural areas. Namely, *C. sinensis*, *V. vinifera*, *P. dulcis*, *O. europaea*, *N. oleander, P. myrtifolia, Lavandula angustifolia, L. dentata* and *Rosmarinus officinalis.* With regards to forestry and shade tree host plants, very little information is available on the potential introduction of *X. fastidiosa* through species for plants for planting. Monitoring this category of hosts in the EU might be particularly relevant for trees from the genera *Quercus*, *Ulmus*, *Acer* and *Platanus*, since these have been reported to be infected by *X. fastidiosa* outside the EU in numerous occasions. Additionally, some of these genera represent a large percentage of forestry plants in various EU MSs. Further details on the susceptible hosts are available in the EFSA host plant database (EFSA, 2018). An Excel file (Annex A) is provided together with this pest survey card for assisting the MSs in the selection of the host plant species for the survey activity taking into account the objectives of the survey and the different rationales presented. The Appendix A (at the end of this document) provides an explanation on the use of the Excel file (Annex A).

1.6. Environmental suitability

Host plants and vectors of *X. fastidiosa* are present throughout the EU and would not be limiting factors for the establishment of the bacterium.

As shown in Figure 1 and Figure 2, *X. fastidiosa* has been reported across a wide range of climatic zones in tropical countries and subtropical areas (e.g. Brazil, Costa Rica and southern California) and also in more temperate or even continental climate regions (e.g. British Columbia, southern Ontario and Saskatchewan in Canada, the north-eastern regions of the USA and Argentina). In the EU, it has been reported in southern Apulia and in the Argentario promontory in Italy, on the island of Corsica and in the Provence-Alpes-Côte d’Azur region in France, as well as in Alicante and Madrid in mainland Spain and on the Balearic Islands. The pathogen was also recently reported in northern Portugal.

Most of the EU territory, except for some restricted high-altitude areas and northern parts of Scandinavia, consists of climate types where the pathogen has been reported to be present elsewhere in the world (EFSA PLH Panel, 2019a). However, assessments on the climatic suitability of *X. fastidiosa* using ensemble species distribution modelling methods (Naimi and Araújo, 2016) have indicated that southern areas of the EU are at higher risk than other areas (EFSA PLH Panel, 2019a) (Figure 4). EFSA PLH Panel (2019a) also presents the results obtained for different *X. fastidiosa* subspecies and shows that subsp. *multiplex* could become established further north in Europe than other subspecies.

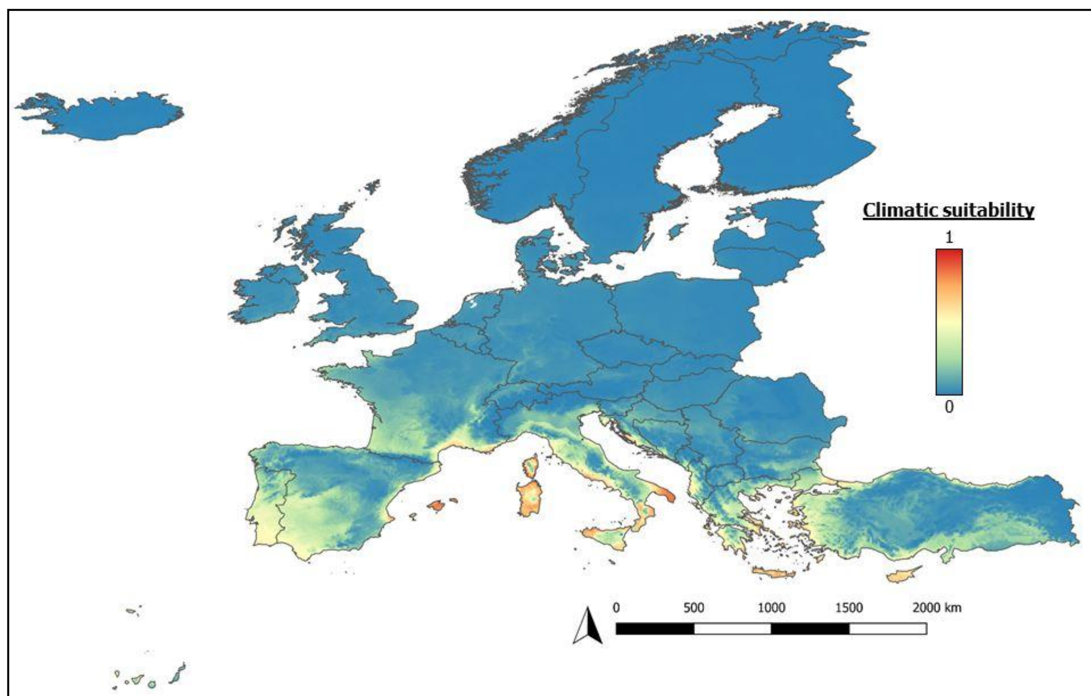

Figure 4: Estimated climatic suitability map for *Xylella fastidiosa* according to species distribution ensemble modelling (EFSA PLH Panel, 2019a)

1.7. Spread capacity

Detailed information regarding a pest's spread capacity is fundamental when preparing a survey. When designing a survey, a pest's spread capacity will define the key distances to consider around risk locations and potential entry points. These distances will determine the areas to survey and consequently the corresponding surveillance efforts. In the case of a first positive finding of a pest, the information on the spread capacity is essential to decide on the extent of the area to delimit. The pest's spread rate will result from the combination of the natural dispersal means and the human-assisted ones. Several factors will influence this spread rate, in particular the mobility and biological characteristics of the pest/vector association, the abundance of the pest/vector and the availability of host plants. As reported in EFSA PLH Panel (2019a), the spread of *X. fastidiosa* has been addressed using a short-range spread model, mainly related to the natural dispersal of the vector, and a long-range spread model that also includes human-assisted movement.

Estimates of short-range spread of *X. fastidiosa* are needed for determining the areas around risk locations (see Section 1.8.) in detection and delimiting surveys.

Natural dispersal of the bacterium itself is negligible and the movement by flight of infected insect vectors is the main mean of natural spread. Regarding *P. spumarius,* little is currently known and more information would be needed for optimising the resources used for survey activities.

Human activities can facilitate the spread of the bacterium through the movement of infected plant material (EFSA PLH Panel, 2015). This is exacerbated by the long incubation (i.e. asymptomatic) period of the disease which allows infected plants to remain undetected for long periods. In addition, the vectors could be transported across long distances by vehicles, as hitchhikers (EFSA PLH Panel,

2015). Long-range movement of spittlebugs could also occur through passive movement by wind (Wiegert, 1964; Halkka et al., 1971; EFSA PLH Panel, 2019a). However, these events of long distance spread of infected vectors followed by successful transmission of the bacterium are thought to be rare events. In Apulia, isolated outbreaks in olive orchards have been discovered up to 30 km from known infected areas (EFSA PLH Panel, 2018), which reflects an example of long-distance dispersal. It is likely that human-assisted dispersal influences long range spread of outbreaks, as well as much further movements that could introduce the bacterium into new provinces or MSs. More work is needed to quantify the nature and magnitude of human-assisted spread (EFSA PLH Panel, 2019a).

Epidemiological data from the Apulian monitoring service indicate that spread of *X. fastidiosa* occurs via a combination of short- and long-range dispersal which results in a clustered disease distribution. In EFSA PLH Panel (2019a; Table A.5), an epidemiological model for long-range spread was used, estimating a median short distance spread of approximately 150 m per year and a long-distance spread with a median of approximately 10 km per year. These results are similar to the ones observed in the Apulian territory.

However, new data suggests that the natural dispersal distances of the vector may currently be underestimated (EFSA PLH Panel, 2019a). In the EFSA risk assessment, a different model was used for simulating the short range spread of *X. fastidiosa* and was configurated in an homogeneous olive orchard characterised by a regular planting distance, assuming *P. spumarius* is the only competent vector of the bacterium. Figure 5 shows the results of simulations performed using this short-range spread model under four different scenarios. The results reflect an average situation in an homogenous olive orchard with data and information mainly from Italy and may not be representative for the dispersal capacity of other insect vectors in other environments with different host plants, *X. fastidiosa* subspecies and climate conditions. For example, temperature is known to have a strong impact on the dispersal capacity of insects. A detailed description of the scenarios is provided in EFSA PLH Panel (2019a).

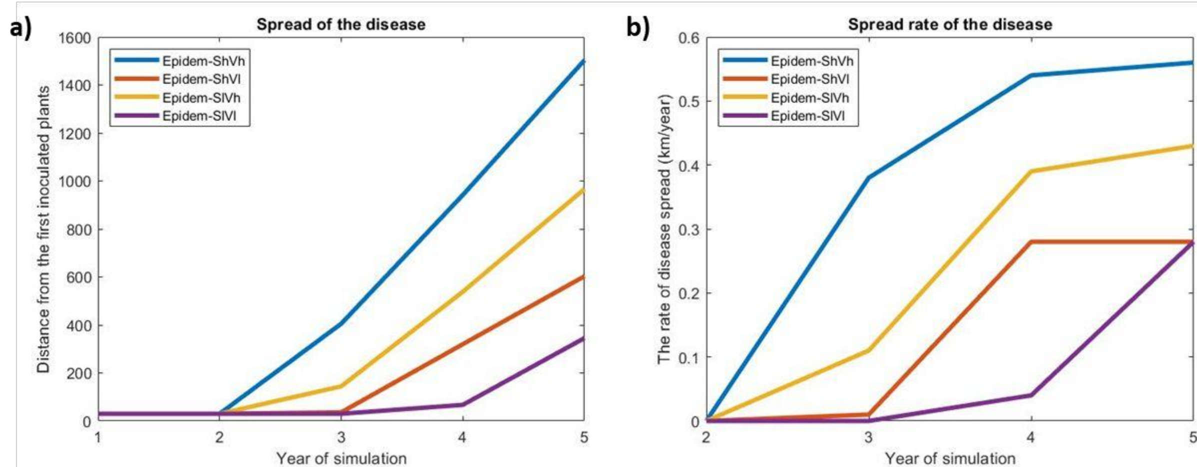

Figure 5: Short-range spread of the disease caused by *Xylella fastidiosa* measured in terms of (a) maximum distance (m) of the infected plants from the first inoculated plants and (b) rate of spread (km/year) in the four scenarios. The four scenarios are 1) Epidem-ShVh: high plant susceptibility and high vector population density; 2) Epidem-ShVl: high plant susceptibility and low vector population density; 3) Epidem-SlVh: low plant susceptibility and high vector population density; 4) Epidem-SlVl: low plant susceptibility and low vector population density (EFSA PLH Panel, 2019a)

The short-spread model (EFSA PLH Panel, 2019a) included only short-range natural dispersal of *P. spumarius* and no long-range movements due to human activity or strong winds. In the initial phase of disease spread (0–2 years), the rate of increase is negligible but accelerates after the second year. For example, it was shown that under a scenario of high plant susceptibility and high vector population density, these spread rates lead to maximum spread distances (distance from the first inoculated plants) of up to 1.5 km in 5 years (EFSA PLH Panel, 2019a). After year 5, the rate of spread of the disease is 0.65 km per year (EFSA PLH Panel, 2019a). However, when vector population density was low, after year 5, the rate of disease spread was significantly lower, namely 0.28 km per year.

Conclusion on spread

It is necessary to tailor the disease spread information to the local situation before it can be used in the design of the surveys. For example, one needs to take into account vector density, host plant density and susceptibility in an area, landscape fragmentation, and the time of the year of the survey. The spread models used in EFSA PLH Panel (2019a) might be useful for such tailoring. The long-range spread model estimated a median short distance spread of approximately 150 m per year and a long-distance spread with a median of approximately 10 km per year. The short range spread model (which only considers local spread) estimated that in a scenario of high plant susceptibility and high vector population density, the natural spread rate of the disease is approx. 1.5 km after 5 years, with an acceleration of the spread after the first two years following a new infection (EFSA PLH Panel, 2019a). In other situations, there could be different spread rates, for example, areas with lower density of host plants would experience slower dispersal of the disease, whereas, regions where there is a greater risk of human assisted or wind driven movement of vectors the rates of spread could be higher.

1.8. Risk factor identification

The identification of the risk factors and their relative risk estimation is essential for performing a risk-based survey. It needs to be tailored to the situation of each EU MS. 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 are those that have more than one level of risk for the target population. The risk factors that will be considered for the surveys need to be characterised by their relative risk and the proportion of the overall plant population on which they apply. This section presents two examples of risk factors that can be integrated in the development of a risk-based surveys.

Example 1: host plants of multiple *Xylella fastidiosa* subspecies

As described in Section 1.5 (iii. Plant species able to host multiple *X. fastidiosa* subspecies) for selection of host plants in the target population, certain plant species are able to host multiple *X. fastidiosa* subspecies. The ones that can host three *X. fastidiosa* subspecies have a higher probability to be found infected than the plants that can host two. These in turn have a higher probability to be found infected than the plants that can host only one *X. fastidiosa* subspecies. Thus, the epidemiological units (see Glossary) that include plants that can host the three *X. fastidiosa* subspecies can be attributed a higher relative risk than the other ones. The estimation of the relative

risks could be based on the number of reports available in the literature that can be extracted from the EFSA host plant database (EFSA, 2018).

Example 2: Spread of *Xylella fastidiosa*

The spread capacity of the pest and in particular the distances the pest could spread per year are needed to define the areas to focus on in the context of the annual detection surveys. Also, these distances are important to integrate in the strategy for delimiting surveys after a first positive finding in an area that was already targeted by the annual detection surveys in the previous years. Instead, in the situation of a monitoring survey in an area where the pest has already been circulating for several years, the yearly spread rates estimations are not informative for delimiting an area where the pest is contained. The latter case is not addressed by this survey card.

For the identification of the risk areas, it is necessary to first identify the risk activities that could contribute to the introduction or the spread of *X. fastidiosa*. These activities should be connected to specific locations that are then called 'risk locations'. In consideration of the spread capacity of the pest and the availability of host plants, risk areas around these locations can then be defined.

Risk activities

Long-distance spread of *X. fastidiosa* occurs through the movement of infected propagating material (e.g. budwood, rootstock seedlings and budded trees including ornamental plants) (EFSA PLH Panel, 2015) confirmed by the interceptions of diseased plant consignments on entry to the EU. The trade, movement, import and preparation of plant propagating material can be considered as risk activities. The unintentional movement of infected insect vectors associated with the movement of plant material from areas where *X. fastidiosa* is already present into currently pest free areas is also addressed by this risk activity. It is to be noted that trade of host plant commodities from areas where the pest is known to occur is subject to special requirements in the EU (see Section 1.2.). However, the asymptomatic period in certain host plants does limit the effectiveness of the import inspections based on symptom observations. It is therefore important to focus surveillance efforts on activities along these pathways that could potentially result in the introduction and spread of the bacterium.

Touristic routes such as vehicle and boat transportations from areas where the pest occurs to suitable areas for the bacterium to become established could also be used for risk-based surveys. This would include purposeful movement of plant material by citizens, especially by plant collectors.

In addition, activities in urban areas such as an increased movement of people and purchasing/movement of plants (planting in residential/urban gardens) should not be disregarded in the survey. The neglected and abandoned fields and orchards in rural areas, are also considered to be a risk, since these locations are likely to be absent from any pest monitoring activities.

The most relevant risk activities to be considered in the case of *X. fastidiosa* are (i) production and handling of plants for planting (excluding seeds) and (ii) their transport, with a special focus on the relevant host plants retained for the surveillance activity in the MS.

Risk locations

Within the EU, nurseries and garden centres that handle imported host plants from areas where the pest occurs can be considered as risk locations as they have a higher probability of being infested with

X. fastidiosa than the areas where the host plants grow naturally or are in agricultural schemes. These locations need to be geo-localised to be able to target the surveys. The nurseries themselves are already subject to obligatory regular official examinations under Council Directive 2000/29/EC Article 6 paragraph 5. This is especially the case for nurseries that import plant material of host plants. It might also be necessary to consider the host plant materials that might be introduced to the EU illegally.

The type of locations corresponding to the risk activities regarding *X. fastidiosa* are summarised in Table 3. Locations may be assigned to different relative risk levels depending on traits such as handling plant material from infested areas or being found in an area of dense production involving host plant species.

For the estimation of the relative risks corresponding to the risk locations, the interception data for *X. fastidiosa* can be taken into account in terms of the origins, commodity, trade volumes, end use, final destination, and the known hosts of the detected *X. fastidiosa* STs or subspecies. For example, frequent interceptions of infected *Coffea* plants are reported in EUROPHYT. This pathway could be considered to be a higher risk, thus the destination locations of these imported consignments could be classified as high risk locations. Other host plant species, on which *X. fastidiosa* has been intercepted include: *Pelargonium* x *hortorum*, *Juglans*, *Rubus fruticosus*, *R. idaeus* and *O. europaea*. Despite the low number of interceptions on these species in comparison with *Coffea* plants, trade volumes for each commodity may influence the order of priority to be considered.

Table 3: Risk activities and corresponding risk locations relevant for surveillance of *Xylella fastidiosa* in all EU Member States

Risk activity	Risk locations
Production, storage and handling of host plants for planting	- Nurseries and garden centres cultivating storing ornamental plants, crop plants or treelings for planting
Transport of propagating material	- Stops along main roads and railways (e.g. truck parking lots) for routes connected to infested areas - Airports and harbours with movement from infested countries or areas
Tourism	- Host crops, gardens parks in the vicinity of touristic sites

Risk area

The risk areas can be defined as the epidemiological units contiguous to the risk locations. The definition of risk areas around a certain risk location takes into consideration the spread capacity of the vector and the availability of host plants. Based on the indicative distance values for the yearly spread of *P. spumarius*, different risk areas can be defined.

- In the case of a detection survey i.e. where no positive case has yet been reported, the objective of the survey is to substantiate pest freedom or to detect the bacterium, should the pest be present. The smaller the risk areas, the higher the number of risk areas that can be surveyed for the same level of surveillance efforts. For *X. fastidiosa* which is capable of long-range jumps, it is important to cover a large number of risk locations. Assuming that in a suitable environment an

infected host plant remains persistently infected and that competent vectors are present, the radius from the risk location where it is most likely to find the pest should be approximately 150 m based on the above mentioned long-range spread model estimation (EFSA PLH Panel, 2019a, Table A.5.).

- In the case of a delimiting survey i.e. a first positive finding has been reported, the first action should be to trace-back the introduction site of the pest (risk location). The survey distances (from the risk location) depend on the period since the last detection survey (and thus the maximum time available for pest entry and onward spread). In the delimiting survey, the strategy being to determine the smallest area where the pest is contained, the recommended approach is to survey concentric circles around the risk location from the periphery to the inside of the risk area until the risk location itself. As shown in Figure 5, the results of the short-range spread modelling indicate that these distances vary over time:

    - In the first and second years of introduction of the bacterium, the spread model shows that spread is negligible (Figure 5) (EFSA PLH Panel, 2019a). However, a precautionary distance of 150 m per year can be considered for the first two years. This distance results from the long-range spread model fitting of the Apulian monitoring data (EFSA PLH Panel, 2019a, Table A.5.).

    - From years 3 to 5, the short-range spread model shows distances of up to 1500 m following the introduction of *X. fastidiosa* (Figure 5, EFSA PLH Panel 2019a).

- Different bands or areas can be defined around a risk location considering the time since the last detection survey was conducted in the site as shown in Table 4. The delimiting activity would start from the outer band to the inside. For example, in the year 1 scenario the delimiting survey starts from the periphery of the area at 150 m from the risk location. Instead in the year 2 scenario, a first band is surveyed from 300 m to 150 m from the risk location. If no positives are found in that band, the delimiting survey continues in the inner band from 150 m to the risk location. Similarly, for the scenarios year 2, 3, 4 and 5.

Table 4: Bands to consider around the risk locations for a delimiting survey of *Xylella fastidiosa* depending on the time since the last detection survey was conducted

Years since last detection survey in the site	Distance from risk location
Year 1	0-150m
Year 2	150-300m
Year 3	300-500m
Year 4	500-1000m
Year 5	1000-1500m

Once the area where the pest is circulating has been delimited, a buffer zone should be defined around the infected area. EFSA PLH Panel (2019a) analyses the effectiveness of different width of the buffer zone in containing the disease. The establishment of such area should be based on the biology and ecology of the pest. In EFSA PLH Panel (2019a, Table A.5.) the 95% range of the long-distance spread is from approximately 8 to 20 km with a median of approximately 10 km per year.

2. Detection and identification

The information presented in this pest survey card was summarised from EPPO's PM 7/24 (4) on *X. fastidiosa* (EPPO, 2019a), and a PM7 standard of EPPO that is currently under development, among others, on *P. spumarius*.

Substantiating the pest status of *X. fastidiosa* and early detection of the pest are the main objectives of the annual detection survey. The detection relies on the combination of visual examination, sampling and testing of insect vectors and plant material. This defines the two survey components that will be considered for the survey design: host plants and insect vectors.

2.1. Visual examination

Although *X. fastidiosa* has the ability to cause symptoms, some infected host plants can remain asymptomatic during their lifetime. In addition, the duration of the asymptomatic period varies depending on the host plant species. In any survey, it is therefore essential to systematically sample and test the surveyed plants for *X. fastidiosa*. However, visual examination to detect *Xylella*-like symptoms plays a major role in the sampling procedure. In an infected tree, the bacterium is not evenly distributed and collecting samples on plant parts with *Xylella*-like symptoms can increase the effectiveness of sampling.

2.1.1. Disease symptoms on plants

In time, the progressive establishment and proliferation of *X. fastidiosa* in the xylem system of a plant blocks the transportation of mineral nutrients and water. The resulting symptoms are not specific to this pathogen and may be confused with other biotic stresses or abiotic stresses such as drought. Symptoms include leaf scorching, wilting of the foliage, defoliation, chlorosis or bronzing along the leaf margin, and dwarfing (EPPO, 2019a). In addition, during the long incubation period, infected plants although being asymptomatic, can be infectious (FAO, 2018; EFSA Plant health Panel, 2019a). At the same time, symptoms are variable and their expression depends on combination of plant host and bacterial strain, as well as the environmental conditions in the area and the specific growing conditions of the individual plant and its phenological stage (EPPO, 2019a). Since these factors are highly variable, the presence of *X. fastidiosa* in plants leads to diverse symptoms, varying from asymptomatic infections to complete plant death (EFSA PLH Panel, 2015). Given the high diversity in symptom expression, visual examination of *X. fastidiosa* symptoms is associated with a low specificity.

For more extensive descriptions of the specific symptoms of major diseases caused by *X. fastidiosa* we refer to the EPPO diagnostic standard PM 7/24 (4) (EPPO, 2019a). It is not possible to give a general description of the symptoms of *X. fastidiosa* given their variability. The specific symptoms of a variety

of hosts can be found for example in the EPPO Global Database[13], in POnTE (2019)[14] and ONPV (online) [15].

The main symptoms on plants are summarised below:

- leaf scorching
- wilting of foliage
- stunting and dieback of shoots and twigs
- (premature) defoliation
- chlorosis or bronzing along the leaf margin
- formation of new malformed (asymetric) leaves
- dwarfing

Some examples are given below of symptoms on several of the host plants that were identified as good candidates for inclusion in the target population (Section 1.5.), these include:

- The agricultural hosts *V. vinifera* (Figure 6), *Citrus* (Figure 7), *O. europaea* (Figure 8) and *Prunus* sp. (Figure 9).
- Hosts in semi-natural areas (e.g. woody shrubs growing in Mediterranean areas), which are also ornamental hosts, namely *N. oleander* (Figure 10) and *P. myrtifolia* (Figure 11).
- Forestry hosts, such as *Quercus* (Figure 12) and *Platanus* (Figure 13).

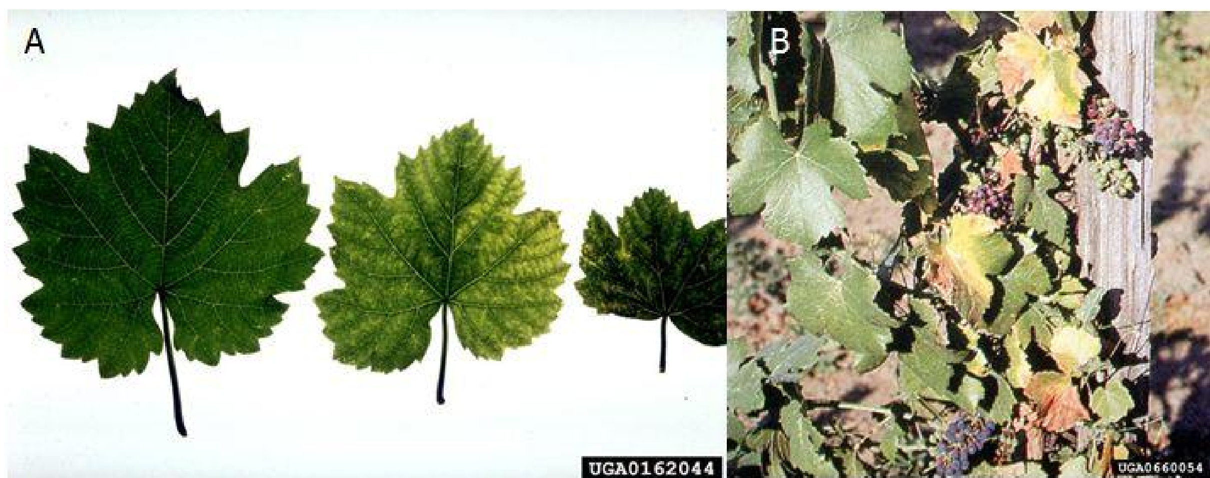

Figure 6: Symptoms of *Xylella fastidiosa* on *Vitis vinifera* L. (A) (Source: Alex. H. Purcell, University of California – Berkeley, Bugwood.org); (B) (Source: ENSA-Montpellier, École nationale supérieure agronomique de Montpellier, Bugwood.org)

[13] EPPO Global database available online at: https://gd.eppo.int /taxon/XYLEFA/photos
[14] POnTE (2019) and its webpage on symptoms of *Xylella* available online at: https://www.ponteproject.eu/category/symptom-xylella/).
[15] https://agriculture.gouv.fr/sites/minagri/files/xylella_fastidiosa_symptomes_et_risques_de_confusions_biotiques_et_abiotiques_dgal-1.pdf

Figure 7: Symptoms of citrus variegated chlorosis. (A) Fruit and leaf symptoms: CVC affected fruit and leaves on the left and unaffected fruit and leaves on the right; (B) Regular size fruit on the left, CVC affected fruit on right; (C) Yellow discolorations in the leaves of CVC infected orange tree (Source: Alexander Purcell, University of California, Bugwood.org)

Figure 8: Symptoms of *Xylella fastidiosa* on *Olea europaea* L. (A) Leaves; (B) Branches; (C) Tree canopy

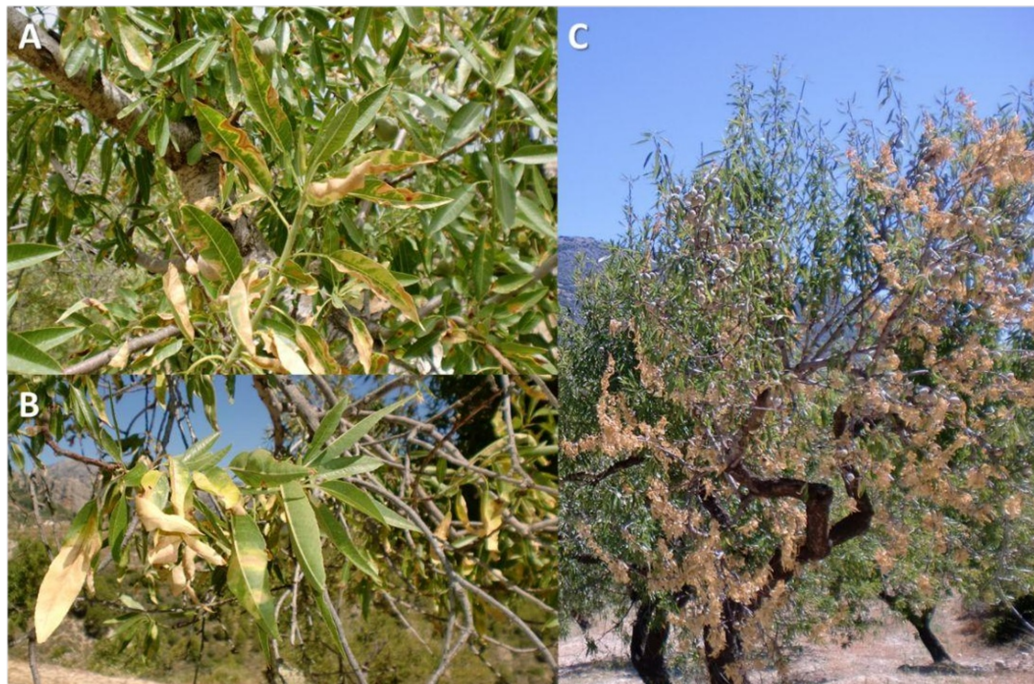

Figure 9: Symptoms of *Xylella fastidiosa* on *Prunus* sp. (A), (B) Almond leaves showing marginal and apical scars of golden colour with the chlorotic zone preceding the scald; (C) infected tree with dried out branches

Figure 10: Symptoms of *Xylella fastidiosa* on *Nerium oleander* L. (A) Initial marginal leaf chlorosis; (B) Leaf necrosis; (C) Desiccation and decline visible on whole tree (Source: Donato Boscia, CNR - Institute for Sustainable Plant Protection, UOS, Bari, Italy)

Figure 11: Symptoms of *Xylella fastidiosa* on *Polygala myrtifolia* L. (A) Leaf scorch and desiccation starting at the tip, on *X. fastidiosa* inoculated plants grown in greenhouse (B) Entire branches die-back and plant turns pale brown (C) Scorch progression to entire leaves (Source: Donato Boscia, CNR - Institute for Sustainable Plant Protection, UOS, Bari, Italy)

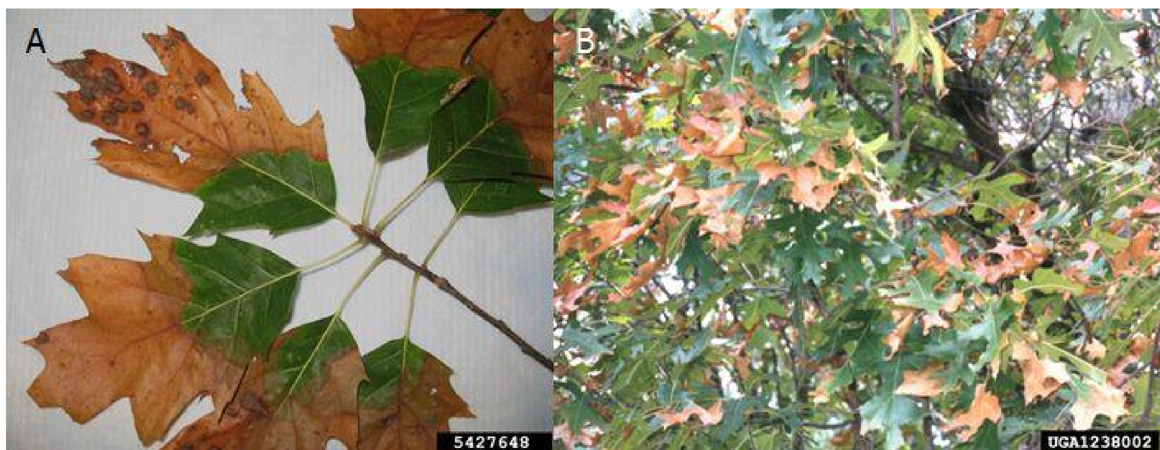

Figure 12: Symptoms of *Xylella fastidiosa* on *Quercus sp*. L. (A) (Source: Nancy Gregory, University of Delaware, Bugwood.org); (B) (Source: Randy Cyr, Greentree, Bugwood.org)

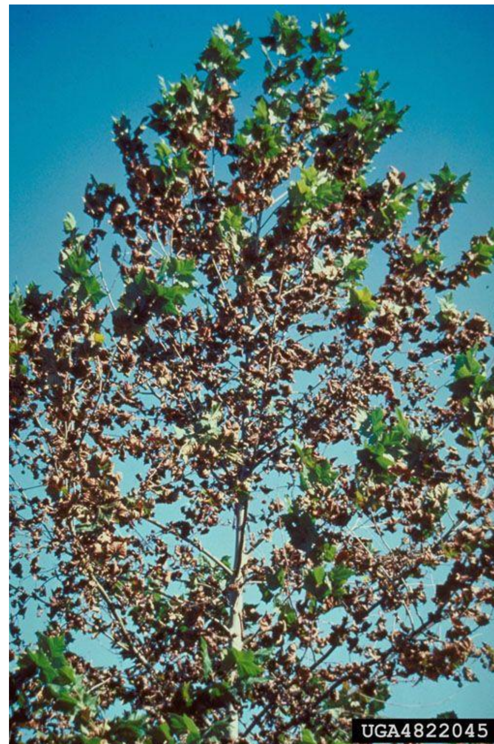

Figure 13: Symptoms of *Xylella fastidiosa* on *Platanus occidentalis* L. (Source: Edward L. Barnard, Florida Department of Agriculture and Consumer Services, Bugwood.org)

Risk of misidentification

Other biotic or abiotic factors (other pathogens, environmental stresses, water deficiencies, salinity, air pollutants, nutritional problems, sunburn etc.) can cause comparable symptoms in host plants of *X. fastidiosa*. ONPV (online)[16] present illustrations of symptoms that can be confused with *Xylella* like symptoms on different host species.

2.1.2. Vectors

Morphological identification of the vector insects to the species level in the field is not recommended and one needs to collect specimens and take these to the laboratory for proper identification. However, trained inspectors could eventually identify the most common xylem sap-feeder insect species in the field. Morphological identification of vector species requires examination of adult specimens. Keys to the identification of Auchenorrhyncha at the genus level are available (such as Ossiannilsson, 1981; Dietrich, 2005; Biedermann and Niedringhaus, 2009; Stewart and Bantock, 2012; Purcell et al, 2014; Germain, 2016). *Xylella fastidiosa* is not pathogenic to the vectors, so no symptoms can be observed on vectors carrying this bacterium.

Although *N. campestris* (Figure 14) has been confirmed as a vector of *X. fastidiosa* subsp. *pauca* ST53 to olive plants under experimental conditions (Cavalieri et al., 2018), the main vector known so far in

[16]https://agriculture.gouv.fr/sites/minagri/files/xylella_fastidiosa_symptomes_et_risques_de_confusions_biotiques_et_abiotiques_dgal-1.pdf

the EU is *P. spumarius* (Figure 15). *Philaenus spumarius* is highly polyphagous, and consequently nymphs and adults can be detected on various plants, in habitats that provide sufficient humidity such as meadows, abandoned fields, waste grounds, roadsides, banks of streams, hayfields, marshlands, parks, gardens and cultivated fields (Yurtsever, 2000). *Philaenus spumarius* exhibits balanced polymorphism of dorsal colour and pattern variation (Rodrigues et al., 2016) (Figure 15). Sixteen adult colour phenotypes are known to occur in natural populations (Yurtsever, 2000). The frequency and occurrence of the colour phenotypes vary among populations (Quartau and Borges,1997; Yurtsever, 2000).

Figure 14: Adult *Neophilaenus campestris* (Source: Joe Botting, Yorks (June 2008))

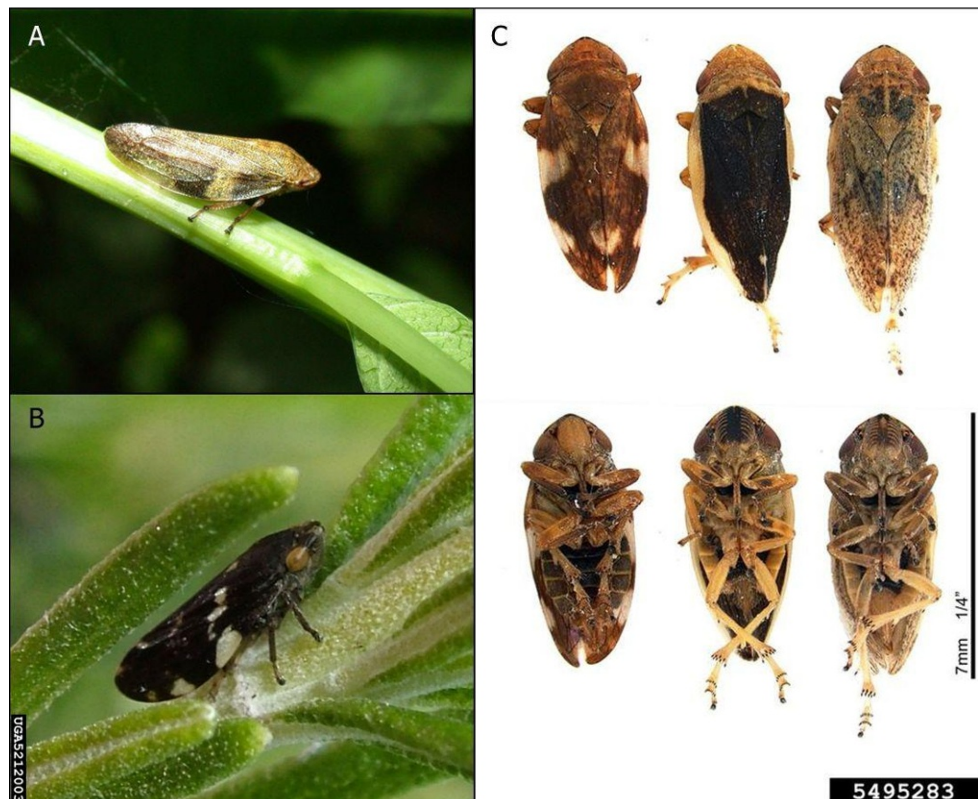

Figure 15: Polymorphism of adults of *Philaenus spumarius*, vector of *Xylella fastidiosa* (A) (Source: Steve McWilliam, shutterstock.com); (B) (Source: Cheryl Moorehead, bugwood.org); (C) (Source: Emilie Bess, USDA APHIS PPQ, Bugwood.org)

The most straightforward method for spotting the presence of *P. spumarius* is by searching for spittle (Figure 16). Nymphs begin forming the spittle as soon as they start feeding on plants. The spittle of the last three instars is easily spotted, while the first instar nymphs tend to settle on the basal part of the host plant, making their detection more difficult (McEvoy, 1986). Note that although the presence of spittle indicates the presence of *P. spumarius*, the sampling procedure is focused on adult insects (see Section 2.2.2.).

Figure 16: Nymphs and spittle of *Philaenus spumarius* (Image: Tomasz Klejdysz, shutterstock.com)

2.2. Sampling

In order to better use the resources for the annual detection surveys of the quarantine pests, the field visits and sample collections are optimised per crop. However, in surveying for *X. fastidiosa*, multiple hosts have to be inspected and sampled over many different environments. This implies that the pest-specific survey activities, such as sample collection, need to be distributed over time and space for optimisation purposes.

2.2.1. Sampling plant material

Although experimental data on sampling is still limited, especially for asymptomatic plant material, the EPPO diagnostic standard PM 7/24 (4) (EPPO, 2019a) provides guidelines on sampling, based on current practices in the EU, in order to maximise the probability of *X. fastidiosa* detection. These guidelines include sampling plant material (both symptomatic and asymptomatic), as well as sampling vectors. Based on recent experimental data on sampling (XF-Actors, 2019), EPPO (2019a) provides guidance on sampling for the following plant species: *O. europaea*, *N. oleander*, *P. myrtifolia*, *Lavandula* spp., *Prunus* spp. and *Coffea* spp.

Samples for the laboratory should be composed of branches or cuttings with leaves attached (EPPO, 2019a). The timing of the sampling is an important factor to take into consideration. The exact timing

of the optimal sampling period depends on the plant species and area. The current EU guidelines require sampling of young branches of leaves during the summer time, also aiming at rapid removal of infected plants should these be detected. The sample should include mature leaves and sampling young growing shoots briefly after emergence should be avoided because the bacteria could go undetected in the new season's flush (EPPO, 2019a). For small plants the entire plant can be sent to the laboratory. Guidance on the number of branches to be collected from (a)symptomatic plants is provided in the diagnostic protocol for *X. fastidiosa* (Section 3.2.1.2 in EPPO, 2019a). When testing individual plants, sample sizes should consist of:

- for symptomatic plants, branches that are representative for the observed symptoms and contain at least 10–25 leaves, depending on leaf size;
- for asymptomatic plants, at least 4-10 branches that are representative of the entire aerial part of the plant, when testing individual plants, depending on the host and plant size.

However, for a detection survey, one will usually sample multiple plants and test a composite sample from the survey site. There are two potential approaches to collecting such composite samples; either by pooling in the field or pooling in the laboratory. When pooling in the laboratory, branches from multiple trees will be collected in the field, and a number of leaves (including their petioles) per branch will then be taken from multiple branches and combined into a single sample. The number of leaves per sample depends on the weight of the leaves. When pooling in the field, the composite sample will be produced on site, again by taking a number of leaves from branches of multiple trees. The disadvantage of the latter procedure is that one cannot retest a new sample without revisiting the field. This would be relevant in the case of a positive detection and when one can determine which tree was positive from the remaining leaves of the (stored) branches in the laboratory without having to collect new branches from the trees that were originally sampled. Examples of recommendations for composite samples are given in PM 7/24 (4) (EPPO, 2019a; Loconsole et al., 2018). For example, for olive the laboratory sample may consists of up to 20 gram of leaf petioles, corresponding to about 800-900 leaf petioles, while four leaf petioles are recommended per plant, meaning testing up to 200-225 plants in a single pooled sample. For *P.myrtifolia* the laboratory sample may consist of up to 20 g of shoots (1.5-2 cm in size), corresponding to about 250 shoots, while two shoots are recommended per plant, meaning testing up to 125 plants in a single pooled sample. It is important to establish a validated protocol for testing a particular plant species prior to the actual survey. The number of examined plants and survey sites which are required to achieve the predefined confidence levels, will depend on the method sensitivity and diagnostic sensitivity. The diagnostic sensitivity is highly dependent on the plant matrix, and one thus need to know (e.g. from literature) or establish this number when designing the survey. This will be further addressed in Section 2.3.3.

2.2.2. Sampling vectors

In an area where *X. fastidiosa* is present, Cornara et al. (2017b) show that the number of PCR-positive *P. spumarius* on each plant was positively correlated with the plant infection status, which reinforces the potential of vector sampling to detect the bacterium in a given area. Currently, sweep nets are commonly used to collect adult insects of *P. spumarius* (Cruaud et al., 2018; Cornara et al., 2018). However, Purcell et al. (1994) pointed out that sweep nets are not effective for sampling insects from the tree canopy, in contrast with its high efficacy on ground cover. Existing trapping methods were proven to be less effective than sweep nets, including minicage (biocenometers), pitfall traps, sticky traps, aerial suction traps, beat tray and tanglefoot bands (Weaver and King, 1954;

Lavigne, 1959; Wilson and Shade, 1967; Novotny, 1992; Pavan, 2000; Bleicher, et al., 2010). Purcell et al. (1994) suggested that a combination of sampling methods provides a more accurate estimation of abundance and movement of insects; such data are important for understanding the role of a vector in disease spread (Purcell et al. 1994; Irwin and Ruesink 1986; Cornara, 2017a). Ongoing research is focusing on sampling of vectors, in particular regarding chemical signals and plant volatiles to enhance trapping efficiency (POnTE, 2019; XF-Actors, 2019).

For surveillance, sweep netting is preferred to yellow sticky traps. The insects have to be tested in the laboratory and sweeping nets ensure that the insects are in a good condition. The success of the diagnostics relies on the quality and freshness of the sample. The sample is preserved better when using sweep nets compared to yellow sticky traps. If yellow sticky traps are used, they should not be left in the field for more than two weeks. Di Serio et al. (2019) describe a procedure for collecting potential vectors[17].

Sampling for vectors should preferably be done from late spring to early autumn (EPPO, 2019a). To maximise the likelihood of detection, insect samples should be collected when the adults are abundant in the field and after they have fed on multiple hosts at the end of the summer or after aestivation. Adult insects must be killed by placing them in a vial filled with 70% ethanol, which is then tightly sealed. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20°C or −80°C, with or without ethanol.

2.3. Pest detection and laboratory testing

2.3.1. Testing plant material

Xylella fastidiosa can be detected in the laboratory by several validated molecular and serological tests. In addition, molecular tests are available for subspecies assignment of *X. fastidiosa*. The EPPO diagnostic standard PM 7/24 (4) (EPPO, 2019a) addresses detection and subspecies assignment of *X. fastidiosa* in different plant matrices. For a positive detection to be considered valid, a minimum of two positive screening tests should be undertaken for plant samples (symptomatic and asymptomatic). These tests should either differ in the underlying biological principles or in the genomic sequence they target. For testing symptomatic plants from a known outbreak area or a buffer zone around an outbreak, a single test including serological tests (e.g. ELISA) may be considered sufficient. The PM 7/24 (4) standard includes, e.g., ELISA (Sherald and Lei, 1991) as a serological method, while the described molecular methods for detection and identification of *X. fastidiosa* include conventional PCR (Minsavage et al., 1994), several real-time PCRs (Harper et al., 2010, erratum 2013; Li et al., 2013; Francis et al., 2006; Ouyang et al., 2013) and loop-mediated isothermal amplification (LAMP) (Yaseen et al., 2015). Serological methods such as ELISA can be used for detection of *X. fastidiosa* in areas where its prevalence is high and large numbers of samples need to be handled, but molecular methods are recommended for detection in pest-free areas and buffer zones given their higher sensitivity.

[17] Also see the EFSA Xylella Tutorial on "How to collect data on *Philaenus spumarius* (spittlebug)" at https://www.youtube.com/watch?v=Rjh7FFQCtg8

2.3.2. Testing vectors

When needed, nymphs and adults of collected vectors can be identified by molecular identification based on conventional PCR on the COI gene, followed by Sanger sequencing analysis. A protocol is described in PM 7/129 on 'DNA barcoding as an identification tool for a number of regulated pests' (EPPO, 2016). Moreover, EPPO is currently developing a diagnostic protocol for the detection and identification of *P. spumarius, N. campestris* and *P. italosignus.* The described identification methods include conventional and real-time PCR.

For detection of *X. fastidiosa* in vectors, as well as its subspecies assignment, we refer to the EPPO diagnostic standard PM 7/24 (4) (EPPO, 2019a). Molecular tests are preferred for detection in vectors, given that serological tests are not sensitive enough to detect the bacterium in vector samples. Despite the proliferation of *X. fastidiosa* in the foregut of the insect vector, it is usually present in low numbers (FAO, 2018; Purcell et al., 2014). Therefore, real-time PCR (Harper et al., 2010, erratum 2013) is recommended for detecting of *X. fastidiosa* in vector samples. It is important to note that due to the small number of bacteria in vector samples, identification of *X. fastidiosa* in these samples can only be done at species level and not at ST level.

In order to reduce the number of samples to be tested in the laboratory, it is possible to pool the samples of the same species. For small vectors (e.g. *Philaenus*) 1–5 heads can be pooled, while for large vectors (e.g. *Cicada orni* or *Aphrophora* spp.) a single insect's head should be used (EPPO, 2019a). If a vector sample tests positive, one then needs to identify any infected host plants by tracing back to the areas where the positive vectors were caught. If, on the other hand, a vector sample tests negative, this could be a false negative. This could occur even when using molecular methods for identification. The rate of false negatives determines the method sensitivity.

2.3.3. Method sensitivity

To perform a statistically based sample size calculation, it is necessary to determine the overall method sensitivity. This is the probability that a truly positive epidemiological unit that is inspected will be detected and confirmed as positive. This should be viewed separately for the two potential survey components (insect vectors and host plant material).

The method sensitivity of the plant component can be broken down to several levels, namely the probability that a truly positive leaf tests positive in the laboratory (diagnostic sensitivity), the probability of selecting a positive leaf from a truly positive plant, and the probability of selecting a positive plant from a truly positive epidemiological unit. If *X. fastidiosa* expressed consistent and typical symptoms, the probability of selecting positive plants and positive (symptomatic) leaves would be high. However, it is known, that *X. fastidiosa* can occur asymptomatically for a prolonged period of time and may even remain asymptomatic depending on the host and growing conditions. As such, the probability of selecting positive plants and positive leaves generally will be a function of the total number of leaves per epidemiological unit being collected and tested. This does not mean that visual examination should not be performed, because it will aid in choosing a better sample when symptoms are present and will then increase the method sensitivity beyond the sensitivity used for the calculations when designing the survey.

The method sensitivity of the vector component has two levels, being the probability that a truly positive vector tests positive in the laboratory (diagnostic sensitivity) and the probability of catching a

positive vector within a truly positive epidemiological unit. Since *X. fastidiosa* does not give symptoms in the vector, the probability of catching a positive vector is a function of the total number of vectors per epidemiological unit being caught and tested. This is somewhat complicated by the fact that, unlike when sampling leaves, one does not know beforehand how many vectors will be caught, while estimating population densities of vector species might also be challenging.

The diagnostic sensitivity is defined as the probability of obtaining a positive test result on a truly infected sample. For several serological and molecular tests for *X. fastidiosa*, the diagnostic sensitivity has been determined for particular host species, mostly hosts of economic importance and with previous findings (E.g. olive, *P. myrtifolia* and *Citrus*). Data on diagnostic sensitivity are made available in the EPPO database of validation data for diagnostic tests (EPPO, 2018). For example, a diagnostic sensitivity of 100% is obtained (meaning all tests on truly infected samples are positive) when four leaf petioles of infected olive leaves are pooled with 20 g (= 800-900 pieces) of leaf petioles from healthy trees, followed by the CTAB method for DNA extraction and the real-time PCR method of Harper et al. (2010, erratum 2013), as a molecular test. Note that the recommendations for preparing a composite sample for olive (see Section 2.2.1.) are based on this data; sampling four leaf petioles from 200 trees, one infected tree would still give a positive test result when all 800 leaf petioles are pooled into a single sample.

The choice of methods to use for detection of *X. fastidiosa* also depends on the available equipment and the host species. For example, using the QuickPick Plant DNA kit and the KingFisher for DNA extraction on spiked olive petioles and the same real-time PCR method of Harper et al. (2010, erratum 2013), resulted in a diagnostic sensitivity of 67%. When the same method was applied to petioles of *Vitis vinifera* the diagnostic sensitivity was 94%. This underlines the importance of generating validation data for each specific host and laboratory method when designing the survey. One remaining knowledge gap is data on the probability of selecting a positive leaf from a truly positive plant in the case of an asymptomatic infection.

Note that although isolation of *X. fastidiosa* on artificial media is challenging, isolation must be performed for new hosts and in the case of a first detection in an area (EPPO, 2019a). When isolation of *X. fastidiosa* is successful, the subspecies can then be determined using molecular tests. Providing that bacterial titres are high enough, the subspecies – or even ST – of the pathogen can also be determined directly on plant samples using next-generation sequencing (Bonants et al., 2019). In the event of a finding on a new host, it is also advisable to perform pathogenicity tests and to prove Koch's postulates (EPPO, 2019a; EFSA, 2018).

2.4. Other methods for detection of *Xylella fastidiosa*

Recent developments in remote sensing techniques and hyperspectral imaging methods show that these methods are becoming more reliable for the early detection of infected trees, particularly for the detection of the pre-visual symptoms (Kumar et al., 2012; Li et al., 2014). In Zarco-Tejada et al. (2018), airborne imaging spectroscopy and thermography revealed the presence of *X. fastidiosa* in olive trees prior to symptom expression. This method - when further developed and validated in other areas - could be used for prioritising the sampling sites, particularly in the context of monitoring and delimiting surveys of the disease following outbreaks.

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 MS. The size of the defined target population and its structure in terms of number of the epidemiological units need to be known. Table 5 shows an example of these definitions.

The pest survey card provides information and suggestions to guide the survey designer in the preparation of a survey. The aim of this preparatory phase is to compile all the data requirements that are needed to develop a risk-based and statistically sound survey design, the details of which are presented in a separate document, 'The specific guidelines for the survey of *X. fastidiosa*'.

Usually, surveys are designed at the level of a host plant species and not of a pest, suggesting that the presence or absence of multiple pests is to be evaluated while visiting a target population, typically comprising of one or a few host plant species. This would optimise field inspections since they are organised per crop visit and not by pest. However, due to the vast host range of *X. fastidiosa*, surveillance efforts cannot be focused on one or a few host plant species. Consequently, each MS is required to make an evidence-based prioritisation of the host plant species under consideration for its surveillance activities. To design a survey for an area of interest (for example, a MS), it is necessary to specify the size, structure and geographic distribution of the target population. As the annual detection surveys of *X. fastidiosa* are performed at the MS level in the EU, these target population characteristics have to be collected by the MSs. The information below is provided to help in defining the target population and prioritise the host plant species that will comprise it.

Table 5: Example of definitions of the target population, epidemiological unit and inspection unit in an agricultural area

	Definition	**Unit**
Target population	Areas in a MS with agricultural hosts, forestry hosts, ornamental hosts and hosts in natural and semi-natural areas	Total number of hectares
Epidemiological units	Hectares in a MS with at least one host plant	Hectare
Inspection units (host)	Host plant materials: plants, leaves and leaf petioles	Number of plants, trees, leaves and leaf petioles
Inspection units (vector)	Sweep net contents after single session or sessions around the same tree	Number of insects

To design a survey on *X. fastidiosa* the following steps will generally be necessary:

1/ Determine the type of survey based on its objectives. For *X. fastidiosa*, the type of survey will depend on the pest status in the area of interest. The objective could be to substantiate pest freedom, to delimit an outbreak area following an infection or to determine the pest prevalence. The next steps deal with the example of substantiating pest freedom.

2/ Define the target population and the epidemiological unit. When determining the target population for surveillance of *X. fastidiosa*, one needs to select the host plants that are relevant for the survey area. For example, the target population could be all hectares in a MS where *P. dulcis* hosts are grown. The epidemiological unit would then be a single hectare where *P. dulcis* is grown. 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. For *X. fastidiosa*, it might be necessary to include multiple components in the survey design (i.e. host plants and vectors).

3/ Determine the size of the target population. This would be the number of single hectares in a MS where the host of interest is grown.

4/ Determine the inspection unit. In the case of an almond orchard, for example, the inspection unit is a single almond tree.

5/ Determine the number of inspection units per epidemiological unit. In the case of an almond orchard, this is the average number of almond trees per epidemiological unit.

6/ Develop a sampling procedure within the epidemiological units and determine the method sensitivity. For example, when examining *P. dulcis* trees, a representative number of trees should be examined by taking a representative number of samples. One can use RiBESS+ to calculate how many inspection units need to be examined or sampled when using predefined prevalence level (e.g. 1%) to obtain a particular method sensitivity. This method sensitivity is in turn needed to calculate the number of inspections sites (Step 8). Note that a larger number of inspected units will result in a higher method sensitivity, but this will be more laborious per site. However, a higher method sensitivity will result in a lower number of inspection sites in the calculations for Step 8. Vice versa, a low number of inspected units per site will result in low method sensitivity, and consequently a higher number of sites to be visited. In the end, this will need to be balanced.

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 into 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 sample size. One can use RiBESS+ to calculate how many epidemiological units need to be surveyed in order to achieve a predefined confidence level (e.g. 95%) and a predefined prevalence level (e.g. 1%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. This will for example, result in the number of hectares that one needs to survey in a MS in order to state with 95% confidence that the prevalence of *X. fastidiosa* in *P. dulcis* will be at 1% or below.

9/ Summarise and evaluate. At this stage, one needs 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, or the survey design should be adjusted, necessitating one to go back to Step 2 (adjusting the number of components) or Step 6 (when rebalancing method sensitivity and sample size).

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

11/ Select the survey sites from the list of available locations.

12/ Consider which data are needed and how these data will be reported.

13/ Develop or update the specific instructions for the inspector.

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Glossary

TERM	DEFINITION*
Buffer zone	An area surrounding or adjacent to an area officially delimited for phytosanitary purposes in order to minimize 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	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).
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 if pests are present (ISPM 5: FAO, 2019).
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).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016a).
Expert knowledge Elicitation	is a systematic, documented and reviewable process to retrieve expert judgements from a group of experts in the form of a probability distribution (EFSA guidance on EKE, 2014).
Epidemiological unit	It is a homogeneous area where the interactions between the pest, the

analogous to the term ***lot*** *used in 'Methodologies for sampling of consignments (ISPM 31: FAO 2016b*)	host plants and the abiotic and biotic factors and conditions would result into 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, on 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.
Host plant	It is part of plants for planting that have been found to be susceptible to *X. fastidiosa* in the Union territory and, thus, belong to the list of genera and species of the Commission database.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016a).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2019). In the context of surveillance for *X. fastidiosa*, inspection is to include sampling and laboratory testing irrelevantly of whether *Xylella*-like symptoms are observed in the field or not.
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, pest vectors that will be scrutinised for identifying and detecting 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 authorized 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 diagnostic sensitivity (DSe) 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 epidemiological unit that is inspected will be detected and confirmed as positive.
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).
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 (Cameron et al., 2014).
RiBESS+	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 (Cameron et al., 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 to 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 should the pest be present.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
Sampling effectiveness	The probability to select infected leaves from an infected plant. The effectiveness of vector sampling using the sweeping method is defined as the probability of capturing a *P. spumarius* in a field where the insect is present.
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005). The sample size also refers to the number of inspection units to be collected for further testing within a selected epidemiological unit of site.

Specified plant	A plant species known to be susceptible to *X. fastidiosa*. The list of specified plants includes all plants considered as host plants, (the latest updated 'specified plants' list is provided in Decision 2017/2352/EU) in addition to all plants for planting that are mentioned in Annex I of the EU Decision 2015/789. Thus, it refers to cases reported across the world.
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 examinations, other than visual, to determine if pests are present or to identify pests (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 (DSp) 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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