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Guidelines for the survey of *Xylella fastidiosa* (Wells et al.) in the Union territory

1 INTRODUCTION

Xylella fastidiosa, one of the most harmful plant pathogenic bacteria worldwide, is subject to a regular survey activity within the Union territory. In this respect, in accordance with Article 3 of Decision (EU) 2015/789¹, Member States conduct annual surveys on a list of regulated plants (also known as 'specified plants') for the presence of *X. fastidiosa*.

These general survey activities consist of visual examinations and, in the case of any suspicion of infection, collection of samples and testing. They are carried out at appropriate times of the year, taking into account available scientific and technical evidence, the biology of the pest, its vectors, the presence and biology of the specified plants, as well as any appropriate information, concerning the presence of *X. fastidiosa*. For demarcated areas, where the pest is known to be present, different survey provisions apply.

At the same time, according to Article 14 of Decision (EU) 2015/789, Member States shall by 31 December of each year communicate to the Commission and to the other Member States a survey plan with the measures, including the scheduled time period of each measure, to be taken in the following year.

Therefore, in order to assist Member States in the drawing up of this survey plan, the following guidelines have been prepared with the following objectives:

- Ensure the highest possible level of early detection of outbreaks of *X. fastidiosa* in the Union territory;
- Harmonise survey activities across the Union, taking into consideration the level of Member States' specificities as regards risk factors.

As proposed outcome, the guidelines aim to:

- Identify key elements to be taken into consideration during the national assessment of the risk factors;
- Outline plant species of particular importance for the potential spread of *X. fastidiosa* in the EU;
- Achieve a common approach about the methodology used by Member States for visual surveys and laboratory detection of *X. fastidiosa*, including its subspecies and strains;

¹ Commission Implementing Decision (EU) 2015/789 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.).

- Outline means for obtaining information about suspected cases of *X. fastidiosa* in addition to official surveys.

2 IDENTIFICATION OF LOCATIONS AT MOST RISK (SENSITIVE AREAS, RISKY SPOTS) FOR THE SURVEY

2.1 Human assisted spread pathways and risky points of introduction in an area

2.1.1 *Chance of establishment due to environmental conditions (climate suitability, open air/greenhouse)*

Member States shall identify the locations at most risk taking into consideration the climatic and environmental conditions, common production and growing practices in their territory, as well as the available information on the world-wide distribution of *X. fastidiosa*².

2.1.2 *Existing trade patterns*

Factors to be considered, in relation to the major entry pathways³:

- Third Countries - the risk is different and related to the pest status of the country - widespread, pest free areas, reliability of Third Country declarations according to Article 16 of Decision 2015/789/EU⁴;
- EU internal trade in relation (directly or not) to demarcated areas;
- Volume of trade;
- Seasonality of trade;
- Plant species traded;
- Potential presence of vectors associated to trade.

2.1.3 *Traffic ways (in proximity of demarcated areas only)*

Factors to be considered:

- Roads;
- Airports;
- Ferry Ports.

2.1.4 *Nurseries*

Factors to be considered:

- Imported plants;
- Origin of the stock materials;
- Sites where mother plants are grown;

² See section 3.1.2.1, EFSA PLH Panel (EFSA Panel on Plant Health), 2015. Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal 2015;13(1):3989, 262 pp., doi:10.2903/j.efsa.2015.3989.

³ According to EFSA PLH Panel (2015) major pathways are 1) plants for planting (excluded seeds) and 2) infectious vectors on plant consignments.

⁴ http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/index_en.htm

- Ready for sale stocks;
- Open production fields;
- Protected environment production sites.

2.1.5 Sites where plants originating in risky areas are grown or kept

Factors to be considered:

- Recently established plantations, such as commercial orchards, parks and landscape developments;
- Retailers of plants for planting (nursery stock);
- Garden centres;
- Markets (based on the assessment of their trade practice);
- Plant collections (based on the assessment of the risk of plant import and plant exchange they are involved).

2.2 Human assisted spread by vectors

Hitch-hiking insects on different transport means (cars, lorries, ferries) may transfer the bacterium for long distances to new areas, out of the demarcated areas. Each Member State should identify the risk of this type of spread individually, particularly considering entry points with high traffic or trade (see 2.1.2 and 2.1.3).

2.3 Awareness raising campaigns

Information submitted in accordance with Article 2 of Decision 2015/789/EU about any suspected presence of *X. fastidiosa* should be considered as factor for the selection of the survey sites. Awareness campaigns have been proved to be useful tools for detecting new outbreaks.

Member States should, therefore, make information available to the general public, travellers, professional and international transport operators concerning the threat of *X. fastidiosa* for the Union territory. Information sheets and pest alerts have proved to be useful in communicating with industry professionals and the wider public, along with a mechanism to report and be able to follow up on suspected findings.

Training of professional operators in symptom recognition and those involved in conducting official surveys of land or crops for other purposes has also helped to identify additional outbreaks. At the same time, citizen initiatives may also generate additional suspect reports for areas in the wider environment which may not be surveyed for plant health purposes (e.g. non-professionals have allowed detecting *X. fastidiosa* on plants of *Polygala myrtifolia* in the French territory).

Early reporting of a suspected presence of *X. fastidiosa*, both in open field or protected conditions, increases the effectiveness of any control strategy and prevents further spread of the bacterium in the rest of the Union territory.

3 PRIORITISATION OF THE SPECIFIED PLANTS TO BE SURVEYED

3.1 Priority plants in relations to the different subspecies and strains of *X. fastidiosa* currently causing outbreaks and/or frequently intercepted in the EU

In general trees, shrubs or perennial host plant species can be assumed having a higher risk for introduction and spread of *X. fastidiosa* because of:

- a higher chance of propagating vegetatively asymptomatic infected plants;
- strict certification and testing systems are not applied to all species; and
- a longer life cycle increasing the chance of successful transfer by insect vectors, when plants are grown outdoors⁵.

Therefore, vegetatively propagated trees, shrubs or perennial host plants should be prioritised in surveys, especially if they grow outdoors, for example:

- *Olea europea*⁶;
- *Nerium oleander*⁷;
- *Polygala myrtifolia*⁸;
- *Prunus* sp., and where possible, *P. dulcis*⁹, *P. avium*¹⁰, *P. cerasifera*¹¹.

However, given the high number of intercepted plants recently reported in the Union territory, particular attention should be also given to:

- *Coffea* sp.¹².

The list of priority plants may be further expanded once the updated version of the EFSA database¹³ of host plants of *X. fastidiosa* will be made available (Jan-Feb 2016).

3.2 Indicator plants

The definition of indicator plants may be of help in maximising the efficiency of detecting *X. fastidiosa* in the Union territory. Such plant species may be selected on the basis of the following criteria:

- Well expressed and clearly identifiable symptoms, at an early stage, under EU climatic conditions;

⁵ EFSA (European Food Safety Authority), 2015. Categorisation of plants for planting, excluding seeds, according to the risk of introduction of *Xylella fastidiosa*. EFSA Journal 2015;13(3):4061, 31 pp. doi:10.2903/j.efsa.2015.4061. Available at:

http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4061.pdf

⁶ Host of *X. fastidiosa* subsp. *pauca* (CoDIRO) in Apulia and *X. fastidiosa* subsp. *multiplex* in the Americas.

⁷ Host of *X. fastidiosa* subsp. *pauca* (CoDIRO) in Apulia, as well as subsp. *fastidiosa* and subsp. *sandyi* in the Americas.

⁸ Host of *X. fastidiosa* subsp. *pauca* (CoDIRO) in Apulia and *X. fastidiosa* subsp. *multiplex* in France.

⁹ Host of *X. fastidiosa* subsp. *pauca* in Apulia, as well as *X. fastidiosa* subsp. *fastidiosa* and *X. fastidiosa* subsp. *multiplex* in the America.

¹⁰ Host of *X. fastidiosa* subsp. *pauca* in Apulia and *X. fastidiosa* subsp. *fastidiosa* in the America.

¹¹ Host of *X. fastidiosa* subsp. *multiplex* in France and in the Americas.

¹² Host of *X. fastidiosa* subsp. *fastidiosa*, *X. fastidiosa* subsp. *Pauca* and *X. fastidiosa* subsp. *sandyi*.

¹³ Current version of the EFSA database of *X. fastidiosa* host plants can be downloaded at <http://www.efsa.europa.eu/en/efsajournal/pub/4061>

- Susceptible for a range of subspecies and strains

The selection of the indicator plants may be requested to EFSA on the basis of above listed criteria.

4 SURVEY/SAMPLING IN A RISKY LOCATION

4.1 Plant species to be selected

Individual assessment is necessary case by case by Member State in relation to the identified risk factors (see chapter 2.1.5).

Plant species have to be selected on the basis of Annex I of Decision 2015/789/EU. Preliminary reasoning on the selection of the plant species is to be provided in the survey plan, and confirmed in the final report.

4.2 Appropriate number of visual checks, samples to be taken

It has to be noted that symptoms depend on specific host and *X. fastidiosa* strain combinations. Generally, symptoms include leaf scorch, defoliation, chlorosis, withering and desiccation of branches, and dwarfing. However, these can be confused with other biotic or abiotic causes (environmental stresses, water deficiencies, salt, air pollutants, nutritional problems, etc.).

The number of visual checks has to be defined in proportion to the existing risk identified by the Member State. Details on the sampling strategy have to be provided on a preliminary basis in the survey plan and later confirmed in the final report.

4.3 Appropriate period for the survey

Survey and sampling period depend mainly on the host plants and environment conditions. There are no scientific evidences supporting direct correlations between *X. fastidiosa* strain and sampling period.

4.3.1 Visual checks

- a) Open fields – annual and perennial plant species should be inspected during the vegetative season (not during the dormancy stage), with the optimal period being from late spring to early autumn;
- b) Protected environment sites – may be checked all year around;

Nurseries/garden centres – symptoms are not always readily apparent on nursery stocks, and thus periodic inspections of the site should be performed according to the production cycles. Fields and facilities used to grow the mother stock plants should be inspected prior to the harvest of the propagating materials, preferably from late spring through early autumn. Important to note that tree nursery stock and propagation material of fruit plants is normally traded during the winter season, while garden and patio plants are traded from February till the end of June.

As regards specifically *Polygala myrtifolia* and in reference to chapter 3.1, it is important to note that based on French experience, positive cases have been found with highest level of occurrence on plants planted in open fields for more than two years, and located in residential areas and in maquis shrubland, rather than in sites of resellers under protected environment.

- c) Sites receiving multiple consignments may require more than one inspection annually, based on the assessment of risks.

4.3.2 Sampling for laboratory testing

Sampling for laboratory testing should be made at the same time as visual checks. The sampling period is in general advised to be in the vegetation period.

- **Symptomatic plants**

For the majority of the symptomatic hosts, sampling should be when symptoms are more expressed, from late spring to early autumn.

Based on the experience gained in the outbreaks identified in Italy and France, the following aspects should be considered:

- a) For *Polygala* spp., sampling can be performed from late spring to early autumn;
- b) For *Olea europea* and *Nerium oleander*, observations conducted in Italy (Apulia region) indicated that:
 - withering, desiccation and leaf scorching symptoms associated to *X. fastidiosa* infections are more strongly expressed in summer, although persistent during the entire year;
 - in some cases, symptoms were, however, also observed during winter at the start of the new vegetation;
 - the bacterium could be detected throughout the year by using serological and molecular tests, although these latest have proven to have higher level of sensitivity and reliability;
 - isolation of the bacterium in axenic culture failed from the field-collected samples after the high temperature (> 38 °C) recorded during the summer season.
- c) For deciduous plant species (e.g. *Prunus* spp.), in Italy (Apulia region), symptoms were consistently recorded, together with a detectable bacterium concentration, in leaves collected during summer. Important to highlight that asymptomatic leaves collected earlier in the vegetation period from the same trees tested negative.

- **Asymptomatic plants**

Sampling is theoretically possible during the entire vegetation period. However, sampling after warm periods (e.g. late summer-early autumn) increases the probability for an accurate bacterial detection.

The selection of the plant species and the number of samples per survey site depends on the Member States' risk assessment: targeted sampling is particularly advised in absence of symptoms. However, priority should be given to symptomatic plants, where present.

- **Vectors**

Member States may decide to sample and test insect vectors for the presence of *X. fastidiosa* outside of demarcated areas based on their risk assessment, and taking into account the occurrence of the potential insect vectors¹⁴ in their territory.

They may share information about the used sampling technique with other Member States.

Net sweeping method is usually used for sampling adult specimen of *Philaenus spumarius*. Specimen may be collected directly from host plants or from the ground vegetation.

4.4 Repetition frequency

- The repetition frequency for risky locations should be defined based on the appropriate risk factors;
- For open fields, in pest free areas, where only asymptomatic plants have been detected during the visual check, sampling once per year in the optimal period (see chapter 4.3.2) is sufficient;
- For nurseries and sites with protected environment, the sampling frequency should relate to the number of production cycles;

5 SAMPLING OF A PLANT

5.1 Appropriate sampling period per species

See chapter 4.3.2.

5.2 Repetition frequency

If symptoms are observed, the sampling should be repeated in different periods, depending on the type and phenology of the plants.

5.3 How to take the sample

The samples should consist of:

- mature tissues (branches and leaves), collection of shoot portions in active growth should be avoided;
- mature leaves with petioles and woody twigs for perennial plant species;
- stem and the mature leaves in the case of herbaceous species, where possible.

These types of tissues could ensure the highest possible level of detection of the target pathogen. In general, it is recommended to follow the general principles of the good practice for sampling of plants and plant parts for laboratory analysis (e.g. registration of the spot and the sample, disinfection of the tools). Appropriate tools have to be provided for the sampling, e.g. in the case of large trees telescopic pruners are necessary.

5.3.1 From a symptomatic plant

Take the twigs, branches or the stems with symptomatic leaves, or close to the symptoms, avoiding dead tissues or sections of the plants at an advanced stage of the symptom expression. Guidelines related to appropriate samples to be acquired from symptomatic plants

¹⁴ See Appendix C, EFSA PLH Panel (EFSA Panel on Plant Health), 2015. Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal 2015;13(1):3989, 262 pp., doi:10.2903/j.efsa.2015.3989.

for the diagnosis of *X. fastidiosa* will be provided in the new EPPO diagnostic protocol for *X. fastidiosa*¹⁵.

5.3.2 From an asymptomatic plant

In the case of trees or shrubs, each sample should consist of twigs, harbouring mature leaves, collected from different parts around the canopy of each plant.

For olives in Italy (Apulia region), with trees at the early stages of infection, samples should be collected from the medium upper part of the canopy in order to increase the possibility of the early detection of the infection.

If necessary, dormant plants can be sampled by taking mature branches (e.g. woody cuttings), from which the xylem tissue is recovered and processed for *X. fastidiosa* detection.

Guidelines related to appropriate samples to be acquired from asymptomatic plants for the diagnosis of *X. fastidiosa* in the laboratory will be provided in the new EPPO diagnostic protocol for *X. fastidiosa*¹⁵.

5.4 How to preserve, transport samples

Samples should be:

- shaken to ensure that no vectors are moved with the plant materials (e.g. adults vector will fly away, when the leaves, twigs are shaken). It is important to check that the sample does not contain any adult or juveniles of the vector species;
- put in closed container (e.g. plastic bag, etc.);
- kept at cool temperatures avoiding to expose samples for prolonged periods to sun or hot temperatures;
- transferred to the diagnostic laboratory as soon as possible, before the plant tissues deteriorate.

In the laboratory, samples should be stored at 4 – 10 °C, and general principles of the good laboratory practices for analysis plant samples for bacterial infection should be followed.

6 DIAGNOSTICS

Diagnostics should be executed in accordance with the new EPPO diagnostic protocol for *X. fastidiosa*¹⁵.

¹⁵ The draft version will be available for Member States' experts in February 2016.