

Updated pest categorisation of Xylella fastidiosa

Michael Jeger, David Caffier, Thierry T. Candresse, Elisavet Chatzivassiliou, Katharina Dehnen-schmutz, Gianni Gilioli, Jean-claude Grégoire, Josep Anton Jaques Miret, Alan Macleod, Maria Navajas Navarro, et al.

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Updated pest categorisation of Xylella fastidiosa

EFSA Panel on Plant Health (EFSA PLH Panel), Michael Jeger, David Caffier, Thierry Candresse, Elisavet Chatzivassiliou, Katharina Dehnen-Schmutz, Gianni Gilioli, Jean-Claude Grégoire, Josep Anton Jaques Miret, Alan MacLeod, Maria Navajas Navarro, Björn Niere, Stephen Parnell, Roel Potting, Trond Rafoss, Vittorio Rossi, Gregor Urek, Ariena Van Bruggen, Wopke Van der Werf, Jonathan West, Stephan Winter, Rodrigo Almeida, Domenico Bosco, Marie-Agnès Jacques, Blanca Landa, Alexander Purcell, Maria Saponari, Ewelina Czwienczek, Alice Delbianco, Giuseppe Stancanelli, and Claude Bragard

Abstract

Following a request from the European Commission, the EFSA Plant Health Panel updated its pest categorisation of Xvlella fastidiosa, previously delivered as part of the pest risk assessment published in 2015. X. fastidiosa is a Gram-negative bacterium, responsible for various plant diseases, including Pierce's disease, phony peach disease, citrus variegated chlorosis, olive quick decline syndrome, almond leaf scorch and various other leaf scorch diseases. The pathogen is endemic in the Americas and is present in Iran. In the EU, it is reported in southern Apulia in Italy, on the island of Corsica and in the Provence-Alpes-Côte d'Azur region in France, as well as in the Autonomous region of Madrid, the province of Alicante and the Balearic Islands in Spain. The reported status is 'transient, under eradication', except for the Balearic Islands, Corsica and southern of Apulia, where the status is 'present with a restricted distribution, under containment'. The pathogen is regulated under Council Directive 2000/29/EC and through emergency measures under Decision (EU) 2015/789 (as amended Decision (EU) 2017/2352). The pest could enter the EU via host plants for planting and via infectious insect vectors. The host range includes hundreds of host species listed in the EFSA host plant database. In the EU, host plants are widely distributed and climatic conditions are favourable for its establishment. X. fastidiosa can spread by movement of host plants for planting and infectious insect vectors. X. fastidiosa is known to cause severe direct damage to major crops including almonds, citrus, grapevines, olives, stone fruits and also forest trees, landscape and ornamental trees, with high impacts. The criteria assessed by the Panel for consideration as a potential Union quarantine pest are met (the pathogen is present in the EU, but it has a restricted distribution and is under official control). X. fastidiosa is not considered as a regulated nonquarantine pest (RNQP) as the pathogen may spread also via insect vector transmission.

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Keywords: European Union, pest risk, plant health, plant pest, quarantine, leaf scorch, citrus variegated chlorosis, Pierce's disease, olive quick decline syndrome

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Editorial: Explanatory editorial corrections were carried out: i) for the Figure 2, where the European NUTS2 region centroids of PACA and Corsica in France, Apulia region in Italy have been differentiated from the other points and surrounded by black square for clarity; ii) Annex D Interceptions: 'Country of origin' reported by Denancé et al. (2017) in 2015 was replaced by 'Latin American countries'.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Council Directive 2000/29/EC¹ on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community establishes the present European Union plant health regime. The Directive lays down the phytosanitary provisions and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union. In the Directive's 2000/29/EC annexes, the list of harmful organisms (pests) whose introduction into or spread within the Union is prohibited, is detailed together with specific requirements for import or internal movement.

Following the evaluation of the plant health regime, the new basic plant health law, Regulation (EU) 2016/2031² on protective measures against pests of plants, was adopted on 26 October 2016 and will apply from 14 December 2019 onwards, repealing Directive 2000/29/EC. In line with the principles of the above mentioned legislation and the follow-up work of the secondary legislation for the listing of EU regulated pests, EFSA is requested to provide pest categorisations of the harmful organisms included in the annexes of Directive 2000/29/EC, in the cases where recent pest risk assessment/pest categorisation is not available.

1.1.2. Terms of reference

EFSA is requested, pursuant to Article 22(5.b) and Article 29(1) of Regulation (EC) No 178/2002³, to provide scientific opinion in the field of plant health.

EFSA is requested to prepare and deliver a pest categorisation (step 1 analysis) for each of the regulated pests included in the appendices of the annex to this mandate. The methodology and template of pest categorisation have already been developed in past mandates for the organisms listed in Annex II Part A Section II of Directive 2000/29/EC. The same methodology and outcome is expected for this work as well.

The list of the harmful organisms included in the annex to this mandate comprises 133 harmful organisms or groups. A pest categorisation is expected for these 133 pests or groups and the delivery of the work would be stepwise at regular intervals through the year as detailed below. First priority covers the harmful organisms included in Appendix 1, comprising pests from Annex II Part A Section I and Annex II Part B of Directive 2000/29/EC. The delivery of all pest categorisations for the pests included in Appendix 1 is June 2018. The second priority is the pests included in Appendix 2, comprising the group of *Cicadellidae* (non-EU) known to be vector of Pierce's disease (caused by *Xylella fastidiosa*), the group of *Tephritidae* (non-EU), the group of potato viruses and virus-like organisms, the group of viruses and virus-like organisms of *Cydonia* Mill., *Fragaria* L., *Malus* Mill., *Prunus* L., *Pyrus* L., *Ribes* L., *Rubus* L. and *Vitis* L. and the group of *Margarodes* (non-EU species). The delivery of all pest categorisations for the pests of the pests included in Appendix 2 is end 2019. The pests included in Appendix 3 cover pests of Annex I part A section I and all pests categorisations should be delivered by end 2020.

For the above mentioned groups, each covering a large number of pests, the pest categorisation will be performed for the group and not the individual harmful organisms listed under "such as" notation in the Annexes of the Directive 2000/29/EC. The criteria to be taken particularly under consideration for these cases, is the analysis of host pest combination, investigation of pathways, the damages occurring and the relevant impact. Finally, as indicated in the text above, all references to 'non-European' should be avoided and replaced by 'non-EU' and refer to all territories with exception of the Union territories as defined in Article 1 point 3 of Regulation (EU) 2016/2031

The list of harmful organisms for which pest categorisation is requested is provided below in Sections 1.1.2.1, 1.1.2.2 and 1.1.2.3. Such list includes *Xylella fastidiosa*, as the causal agent of diseases such as the "Citrus variegated chlorosis" and the "Peach phony rickettsia" as listed in the

¹ 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 169/1, 10.7.2000, p. 1–112.

² Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants. OJ L 317, 23.11.2016, p. 4–104.

³ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31/1, 1.2.2002, p. 1–24.



Annexes of Directive 2000/29/EC. With mandate ARES (2017) 6346828 - 22/12/2017, the European Commission has requested that the pest categorisation of *Xylella fastidiosa* should be jointly delivered by June 2018 together with the update of the *Xylella* host plants database, in order to provide a common and comprehensive base for the follow-up development of an update of the pest risk assessment for *X. fastidiosa* by March 2019.

1.1.2.1. Terms of Reference: Appendix 1

List of harmful organisms for which pest categorisation is requested. The list below follows the annexes of Directive 2000/29/EC.

Annex IIAI

(a) Insects, mites and nematodes, at all stages of their development

Aleurocantus spp. Anthonomus bisignifer (Schenkling) Anthonomus signatus (Say) Aschistonyx eppoi Inouye Carposina niponensis Walsingham Enarmonia packardi (Zeller) Enarmonia prunivora Walsh Grapholita inopinata Heinrich Hishomonus phycitis Leucaspis japonica Ckll. Listronotus bonariensis (Kuschel)

(b) Bacteria

Citrus variegated chlorosis *Erwinia stewartii* (Smith) Dye

(c) Fungi

Alternaria alternata (Fr.) Keissler (non-EU pathogenic	Elsinoe spp. Bitanc. and Jenk. Mendes
isolates)	Fusarium oxysporum f. sp. albedinis (Kilian and
Anisogramma anomala (Peck) E. Müller	Maire) Gordon
<i>Apiosporina morbosa</i> (Schwein.) v. Arx	Guignardia piricola (Nosa) Yamamoto
Ceratocystis virescens (Davidson) Moreau	Puccinia pittieriana Hennings
<i>Cercoseptoria pini-densiflorae</i> (Hori and Nambu) Deighton	Stegophora ulmea (Schweinitz: Fries) Sydow & Sydow
Cercospora angolensis Carv. and Mendes	Venturia nashicola Tanaka and Yamamoto
(d) Viene and viene like anomiome	

(d) Virus and virus-like organisms

Beet curly top virus (non-EU isolates) Black raspberry latent virus Blight and blight-like Cadang-Cadang viroid Citrus tristeza virus (non-EU isolates) Leprosis Little cherry pathogen (non- EU isolates) Naturally spreading psorosis Palm lethal yellowing mycoplasm Satsuma dwarf virus Tatter leaf virus Witches' broom (MLO)

Numonia pyrivorella (Matsumura)

Scrobipalpopsis solanivora Povolny

Tachypterellus quadrigibbus Say

Pissodes spp. (non-EU)

Scirtothrips aurantii Faure *Scirtothrips* citri (Moultex)

Scolytidae spp. (non-EU)

Toxoptera citricida Kirk.

Unaspis citri Comstock

Oligonychus perditus Pritchard and Baker

Xanthomonas campestris pv. *oryzae* (Ishiyama) Dye and pv. *oryzicola* (Fang. et al.) Dye



Annex IIB

(a) Insect mites and nematodes, at all stages of their development

Anthonomus grandis (Boh.) Cephalcia lariciphila (Klug) Dendroctonus micans Kugelan Gilphinia hercyniae (Hartig) Gonipterus scutellatus Gyll. Ips amitinus Eichhof *Ips cembrae* Heer *Ips duplicatus* Sahlberg *Ips sexdentatus* Börner *Ips typographus* Heer *Sternochetus mangiferae* Fabricius

(b) Bacteria

Curtobacterium flaccumfaciens pv. flaccumfaciens (Hedges) Collins and Jones

(c) Fungi

Glomerella gossypii Edgerton *Gremmeniella abietina* (Lag.) Morelet Hypoxylon mammatum (Wahl.) J. Miller

1.1.2.2. Terms of Reference: Appendix 2

List of harmful organisms for which pest categorisation is requested per group. The list below follows the categorisation included in the annexes of Directive 2000/29/EC.

<u>Annex IAI</u>

(a) Insects, mites and nematodes, at all stages of their development

Group of Cicadellidae (non-EU) known to be vector of Pierce's disease (caused by *Xylella fastidiosa*), such as:

- 1) Carneocephala fulgida Nottingham
- 2) Draeculacephala minerva Ball

Group of Tephritidae (non-EU) such as:

- 1) Anastrepha fraterculus (Wiedemann)
- 2) Anastrepha ludens (Loew)
- 3) Anastrepha obliqua Macquart
- 4) Anastrepha suspensa (Loew)
- 5) Dacus ciliatus Loew
- 6) Dacus curcurbitae Coquillet
- 7) Dacus dorsalis Hendel
- 8) Dacus tryoni (Froggatt)
- 9) Dacus tsuneonis Miyake
- 10) Dacus zonatus Saund.
- 11) Epochra canadensis (Loew)

(c) Viruses and virus-like organisms

Group of potato viruses and virus-like organisms such as:

- 1) Andean potato latent virus
- 2) Andean potato mottle virus
- 3) Arracacha virus B, oca strain

- 3) Graphocephala atropunctata (Signoret)
- 12) Pardalaspis cyanescens Bezzi
- 13) Pardalaspis quinaria Bezzi
- 14) Pterandrus rosa (Karsch)
- 15) Rhacochlaena japonica Ito
- 16) Rhagoletis completa Cresson
- 17) Rhagoletis fausta (Osten-Sacken)
- 18) Rhagoletis indifferens Curran
- 19) Rhagoletis mendax Curran
- 20) Rhagoletis pomonella Walsh
- 21) Rhagoletis suavis (Loew)

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- 5) Potato virus T
- non-EU isolates of potato viruses A, M, S, V, X and Y (including Yo, Yn and Yc) and Potato leafroll virus



Group of viruses and virus-like organisms of Cydonia Mill., Fragaria L., Malus Mill., Prunus L., Pyrus L., Ribes L., Rubus L. and Vitis L., such as:

- 1) Blueberry leaf mottle virus
- 2) Cherry rasp leaf virus (American)
- 3) Peach mosaic virus (American)
- 4) Peach phony rickettsia
- 5) Peach rosette mosaic virus
- 6) Peach rosette mycoplasm
- 7) Peach X-disease mycoplasm

- 8) Peach yellows mycoplasm
- 9) Plum line pattern virus (American)
- 10) Raspberry leaf curl virus (American)
- 11) Strawberry witches' broom mycoplasma
- 12) Non-EU viruses and virus-like organisms of *Cydonia Mill., Fragaria L., Malus Mill., Prunus L., Pyrus L., Ribes L., Rubus L.* and *Vitis L.*

Annex IIAI

(a) Insects, mites and nematodes, at all stages of their development

Group of Margarodes (non-EU species) such as:

1) Margarodes vitis (Phillipi)

3) Margarodes prieskaensis Jakubski

2) *Margarodes vredendalensis* de Klerk

1.1.2.3. Terms of Reference: Appendix 3

List of harmful organisms for which pest categorisation is requested. The list below follows the annexes of Directive 2000/29/EC.

Annex IAI

(a) Insects, mites and nematodes, at all stages of their development

Acleris spp. (non-EU) Longidorus diadecturus Eveleigh and Allen Amauromyza maculosa (Malloch) Monochamus spp. (non-EU) Anomala orientalis Waterhouse Myndus crudus Van Duzee Arrhenodes minutus Drury Nacobbus aberrans (Thorne) Thorne and Allen Choristoneura spp. (non-EU) Naupactus leucoloma Boheman Conotrachelus nenuphar (Herbst) *Premnotrypes* spp. (non-EU) Dendrolimus sibiricus Tschetverikov Pseudopityophthorus minutissimus (Zimmermann) Diabrotica barberi Smith and Lawrence Pseudopityophthorus pruinosus (Eichhoff) Diabrotica undecimpunctata howardi Barber Scaphoideus luteolus (Van Duzee) Diabrotica undecimpunctata undecimpunctata Spodoptera eridania (Cramer) Mannerheim Spodoptera frugiperda (Smith) Diabrotica virgifera zeae Krysan & Smith Spodoptera litura (Fabricus) Diaphorina citri Kuway Thrips palmi Karny Heliothis zea (Boddie) Xiphinema americanum Cobb sensu lato (non-EU Hirschmanniella spp., other than Hirschmanniella populations) gracilis (de Man) Luc and Goodey Xiphinema californicum Lamberti and Bleve-Zacheo Liriomyza sativae Blanchard (b) Fungi

Ceratocystis fagacearum (Bretz) Hunt *Chrysomyxa arctostaphyli* Dietel *Cronartium* spp. (non-EU) *Endocronartium* spp. (non-EU) *Guignardia laricina* (Saw.) Yamamoto and Ito *Gymnosporangium* spp. (non-EU) *Inonotus weirii* (Murril) Kotlaba and Pouzar *Melampsora farlowii* (Arthur) Davis



Mycosphaerella larici-leptolepis Ito et al. *Mycosphaerella populorum* G. E. Thompson *Phoma andina* Turkensteen *Phyllosticta solitaria* Ell. and Ev. Septoria lycopersici Speg. var. malagutii Ciccarone and Boerema Thecaphora solani Barrus Trechispora brinkmannii (Bresad.) Rogers

(c) Viruses and virus-like organisms

Tobacco ringspot virus Tomato ringspot virus Bean golden mosaic virus Cowpea mild mottle virus Lettuce infectious yellows virus Pepper mild tigré virus Squash leaf curl virus Euphorbia mosaic virus Florida tomato virus

(d) Parasitic plants

Arceuthobium spp. (non-EU)

Annex IAII

(a) Insects, mites and nematodes, at all stages of their development

Meloidogyne fallax Karssen	Rhizoecus hibisci Kawai and Takagi
Popillia japonica Newman	

(b) Bacteria

Clavibacter michiganensis (Smith) Davis et al. ssp. *Ralstonia solanacearum* (Smith) Yabuuchi et al. *sepedonicus* (Spieckermann and Kotthoff) Davis et al.

(c) Fungi

Melampsora medusae Thümen

Synchytrium endobioticum (Schilbersky) Percival

Annex I B

(a) Insects, mites and nematodes, at all stages of their development

Leptinotarsa decemlineata Say

Liriomyza bryoniae (Kaltenbach)

(b) Viruses and virus-like organisms

Beet necrotic yellow vein virus

1.2. Interpretation of the terms of reference

This pest categorisation focuses on the species *Xylella fastidiosa*, including all its subspecies known so far, as well as questions relating to its European insect vectors and provide an update of the previous pest categorisation included in Section 3.1 of EFSA PLH Panel (2015a). This categorisation will not include the newly described species *Xylella taiwanensis* (Su et al., 2016) nor the non-European insect vectors of *X. fastidiosa*, which will be addressed in a different pest categorisation.

1.3. Additional information

As this pest categorisation updates the information provided in Section 3.1 of the pest risk assessment published by EFSA in 2015 (EFSA PLH Panel, 2015a), parts of this previous opinion are therefore used throughout this document, and cited in quotation marks.

2. Data and methodologies

2.1. Data

2.1.1. Literature search

A literature search on *X. fastidiosa* was conducted both in 2017 and 2018. The final search was made in the Web of Science bibliographic database on 16.03.2018 in 'All databases' includes: all subscribed databases like: BIOSIS Citation Index, CABI: CAB Abstracts, Chinese Science Citation Database, Current Contents Connect, Data Citation Index, FSTA (the food science resource), KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index, SciELO Citation Index, Zoological records etc. The key word used in this search was simply '*Xylella'* in order to retrieve as many updated references as possible published between 2015 and 2018. There were no language limits in this search. We obtained around 480 records and after removal of the duplicates, we ended up with 460 records. Some of the references were eliminated, because *Xylella* was not the main topic of the study and *Xylella taiwanensis* studies were not taken into account in the present pest categorisation.

A separate search was conducted for the update of the EFSA *Xylella* host plant database and it will be published in a separate scientific report in the following month. *Xylella* host plant database was first time initiated in 2013 (EFSA, 2013) and the first list of host plant species of *X. fastidiosa* was based on the University of Berkeley online list. In 2015, EFSA published the full pest risk assessment (EFSA PLH Panel, 2015a) with a long appendix, showing the *X. fastidiosa* host plant database, which was updated in 2016 (EFSA, 2016) and since that time EFSA is requested to maintain a regularly updated *Xylella* (including all species, also *X. taiwanensis*) host plant database.

2.1.2. Database search

Pest information on host(s) and its distribution was obtained from the European and Mediterranean Plan Protection Organization (EPPO) Global Database (EPPO, 2018) and compared with the *Xylella* host plant database (EFSA, 2018) and relevant publications.

Data on the importation of commodity types that could potentially provide a pathway for the pest to enter the EU and on hosts grown in the EU were obtained from Eurostat (Statistical Office of the European Union) and from the ISEFOR database (2017 update).

The Europhyt database was consulted for pest-specific notifications on interceptions and outbreaks. Europhyt is a web-based network run by the Directorate General for Health and Food Safety (DG SANTÉ) of the European Commission, and is a subproject of PHYSAN (Phyto-Sanitary Controls) specifically concerned with plant health information. The Europhyt database manages notifications of interceptions of plants or plant products that do not comply with EU legislation, as well as notifications of plant pests detected in the territory of the Member States (MS) and the phytosanitary measures taken to eradicate or avoid their spread.

2.2. Methodologies

The Panel performed the pest categorisation for *X. fastidiosa*, following the guiding principles and steps presented in the EFSA guidance on the harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and as defined in the International Standard for Phytosanitary Measures No 11 (FAO, 2013) and No 21 (FAO, 2004).

In accordance with the guidance of a harmonised framework for pest risk assessment in the EU (EFSA PLH Panel, 2010), this work was initiated following an evaluation of the EU plant health regime. Therefore, to facilitate the decision-making process, in the conclusions of the pest categorisation, the Panel explicitly addresses each criterion for a Union quarantine pest and for a Union regulated nonquarantine pest (RNQP) in accordance with Regulation (EU) 2016/2031 on protective measures against pests of plants, and includes additional information required in accordance with the specific terms of reference received by the European Commission. In addition, for each conclusion, the Panel provides a short description of its associated uncertainty.

Table 1 presents the Regulation (EU) 2016/2031 pest categorisation criteria on which the Panel bases its conclusions. All relevant criteria have to be met for the pest to potentially qualify either as a quarantine pest or as a RNQP. If one of the criteria is not met, the pest will not qualify. A pest that does not qualify as a quarantine pest may still qualify as a RNQP that needs to be addressed in

the opinion. For the pests regulated in the protected zones only, the scope of the categorisation is the territory of the protected zone; thus, the criteria refer to the protected zone instead of the EU territory.

It should be noted that the Panel's conclusions are formulated respecting its remit and particularly with regard to the principle of separation between risk assessment and risk management (EFSA founding regulation (EU) No 178/2002); therefore, instead of determining whether the pest is likely to have an unacceptable impact, the Panel will present a summary of the observed pest impacts. Economic impacts are expressed in terms of yield and quality losses and not in monetary terms, whereas addressing social impacts is outside the remit of the Panel, in agreement with EFSA guidance on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010).

Table 1: Pest categorisation criteria under evaluation, as defined in Regulation (EU) 2016/2031 on protective measures against pests of plants (the number of the relevant sections of the pest categorisation is shown in brackets in the first column

Criterion of pest categorisation	Criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Criterion in Regulation (EU) 2016/2031 regarding protected zone quarantine pest (Articles 32–35)	Criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest
Identity of the pest (Section 3.1)	Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?	Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?	Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?
Absence/ presence of the pest in the EU territory (Section 3.2)	Is the pest present in the EU territory? If present, is the pest widely distributed within the EU? Describe the pest distribution briefly!	Is the pest present in the EU territory? If not, it cannot be a protected zone quarantine organism.	Is the pest present in the EU territory? If not, it cannot be a regulated non-quarantine pest. (A regulated non-quarantine pest must be present in the risk assessment area).
Regulatory status (Section 3.3)	If the pest is present in the EU but not widely distributed in the risk assessment area, it should be under official control or expected to be under official control in the near future	The protected zone system aligns with the pest free area system under the International Plant Protection Convention (IPPC). The pest satisfies the IPPC definition of a quarantine pest that is not present in the risk assessment area (i.e. protected zone)	Is the pest regulated as a quarantine pest? If currently regulated as a quarantine pest, are there grounds to consider its status could be revoked?
Pest potential for entry, establishment and spread in EU territory (Section 3.4)	Is the pest able to enter into, become established in, and spread within, the EU territory? If yes, briefly list the pathways!	Is the pest able to enter into, become established in, and spread within, the protected zone areas? Is entry by natural spread from EU areas where the pest is present possible?	Is spread mainly via specific plants for planting, rather than via natural spread or via movement of plant products or other objects? Clearly state if plants for planting is the main pathway!
Potential for consequences in EU territory (Section 3.5)	Would the pests' introduction have an economic or environmental impact on the EU territory?	Would the pests' introduction have an economic or environmental impact on the protected zone areas?	Does the presence of the pest on plants for planting have an economic impact, as regards the intended use of those plants for planting?



Criterion of pest categorisation	Criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Criterion in Regulation (EU) 2016/2031 regarding protected zone quarantine pest (Articles 32–35)	Criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest
Available measures (Section 3.6)	Are there measures available to prevent the entry into, establishment within or spread of the pest within the EU such that the risk becomes mitigated?	Are there measures available to prevent the entry into, establishment within or spread of the pest within the protected zone areas such that the risk becomes mitigated? Is it possible to eradicate the pest in a restricted area within 24 months (or a period longer than 24 months where the biology of the organism so justifies) after the presence of the pest was confirmed in the protected zone?	Are there measures available to prevent pest presence on plants for planting such that the risk becomes mitigated?
Conclusion of pest categorisation (Section 4)	A statement as to whether (1) all criteria assessed by EFSA above for consideration as a potential quarantine pest were met and (2) if not, which one(s) were not met	A statement as to whether (1) all criteria assessed by EFSA above for consideration as potential protected zone quarantine pest were met, and (2) if not, which one(s) were not met	A statement as to whether (1) all criteria assessed by EFSA above for consideration as a potential regulated non-quarantine pest were met, and (2) if not, which one(s) were not met

The Panel will not indicate in its conclusions of the pest categorisation whether to continue the risk assessment process, but following the agreed two-step approach, will continue only if requested by the risk managers. However, during the categorisation process, experts may identify key elements and knowledge gaps that could contribute significant uncertainty to a future assessment of risk. It would be useful to identify and highlight such gaps so that potential future requests can specifically target the major elements of uncertainty, perhaps suggesting specific scenarios to examine.

3. Pest categorisation

3.1. Identity and biology of the pest

3.1.1. Identity and taxonomy

Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?

Yes, the identity of the pest is well established.

The bacterium *X. fastidiosa* is responsible for several major transmissible plant diseases: alfalfa dwarf, Pierce's disease of grapevine, phony peach disease, plum leaf scald, citrus variegated chlorosis disease, olive quick decline syndrome and several leaf scorches recorded on almond, elm, oak, oleander, American sycamore, mulberry and maple.

X. fastidiosa is a gammaproteobacterium in the family Xanthomonadaceae. The scientific name for the bacterium is *X. fastidiosa* Wells et al., 1987.

3.1.2. Biology of the pest

X. fastidiosa is a xylem-inhabiting bacterium. It is transmitted by xylem sap-feeding insects, and causes major plant diseases. These diseases are characterised by symptoms often similar to those caused by water stress. Many host plants remain symptomless while infected by the bacterium, and may serve as reservoirs in the environment (Hopkins and Purcell, 2002), while for others, the infection produces rapid death (Purcell and Saunders, 1999; Martelli et al., 2016). Colonisation patterns are complex and depend on the host plant and the pathogen genotype (EFSA PLH Panel, 2015a).

Generally, when plants are susceptible, the bacteria move systemically through the xylem vessels and are accessible for acquisition by xylem-feeding piercing-sucking insect vectors after a variable length of time, according to the plant species (Hill and Purcell, 1995a,b, 1997). Symptoms are usually linked to the occlusion of xylem vessels. Alternatively, the bacterium may also stay locally in some host plants, but still it may be acquired by insect vectors (Purcell and Saunders, 1999). The time lapse between inoculation and symptom appearance in plant is highly variable according to the plant species and age (generally shorter in herbaceous vs. woody hosts) and it is ranging from few months (e.g. a minimum of 3 months following artificial inoculation – pinprick stem inoculation – of *X. fastidiosa* to young *Citrus* seedlings, as indicated by Lopes et al., 2005) to more than 1 year (e.g. 12–14 months following artificial inoculation of *X. fastidiosa* to young olive seedlings, according to Saponari et al., 2016). The possibility that a non-systemic host with only localised infection could still contribute to the spread of the disease to other plants, as shown previously (Hill and Purcell, 1995a,b, 1997), was discussed in the EFSA pest risk assessment (EFSA PLH Panel, 2015a) as well as in the other statements on the susceptibility of various plant species to *X. fastidiosa* strain CoDiRO (EFSA PLH Panel, 2015b, 2016a).

Diseases caused by *X. fastidiosa* are usually the outcome of a complex interaction between the bacterium, host plants, including reservoirs and alternative ones, insect vectors and environmental conditions.

X. fastidiosa is exclusively transmitted by xylem sap-feeding insects belonging to the order Hemiptera, suborder Auchenorrhyncha – Cicadomorpha (= Clypeorrhyncha) (Redak et al., 2004). The bacteria are transmitted in a persistent manner, but there is no latency period following acquisition (Almeida et al., 2005). Vectors (both nymphs and adults) acquire the bacteria by feeding in the xylem and can inoculate the pathogen to healthy plants immediately after acquisition. Bacteria are restricted to the alimentary canal and do not systemically infect the insect body. They adhere to and multiply in the precibarium and the cibarium (parts of the foregut). This implies that vectors lose infectivity with moulting, as the foregut is of ectodermal origin and is renewed with moulting. Therefore, newly emerged adults must feed again on an infected plant to become infectious and spread X. fastidiosa. Once infected, adult vectors can transmit the bacterium during their whole lifetime, as multiplies and persists in the vector foregut (Almeida et al., 2005). The bacterium is not transovarially transmitted to the progeny of the vector (Freitag, 1951). Winged adults, because of their high mobility and of their persistent infection, are mostly responsible for X. fastidiosa spread. Since the bacterium is restricted to the foregut (Purcell and Finlay, 1979), the number of bacterial cells per insect is low. But very few live bacterial cells in the vector's foregut are required for transmission (Hill and Purcell, 1995a,b). Therefore, a sensitive diagnostic tool, such as polymerase chain reaction (PCR), is needed to detect the presence of X. fastidiosa in the insect vectors. Enzyme-linked immunosorbent assay (ELISA) is not sensitive enough to detect X. fastidiosa in the vector insects. Importantly, even PCR (or qPCR and other related methods) have so far not been shown to provide robust results in insects. In fact, according to Cornara et al. (2016), due to the insufficient sensitivity of PCR on insects, the relationship between test plant infection status and spittlebug infection status (as determined by PCR) is not strong. However, the same paper indicates that vector transmission to plants and X. fastidiosa population size in the vector (as determined by qPCR) are significantly correlated. A more sensitive nested PCR technique has recently been proposed (Cruaud et al., 2018), but has not yet been applied in transmission experiments to see whether the transmission and pathogen detection in the vector are significantly correlated.

Although *X. fastidiosa* transmission is restricted to xylem sap-feeding insects with piercing-sucking mouthparts, insect transmission of *X. fastidiosa* is known to lack specificity. Therefore, all xylem sap-feeding insects are considered vectors, which assumption has not so far been disproven (Frazier, 1944; Purcell, 1989; Almeida et al., 2005). However, transmission efficiency varies substantially depending on insect species, host plants and *X. fastidiosa* genotype (Redak et al., 2004; Lopes et al., 2010; Almeida, 2016).

As reported in the previous EFSA opinion (EFSA PLH Panel, 2015a), 'the ecology of *X. fastidiosa* diseases is the outcome of complex biotic and abiotic interactions. Although general insights from one disease system are useful for another, ecological parameters are not necessarily transferable'.

'Despite the fact that *X. fastidiosa* has a notoriously large alternative host plant range, the epidemiological importance of its hosts varies. The spring spread of *X. fastidiosa* from host plants in riparian habitats (i.e. along creeks/rivers) into vineyards in coastal areas of northern California is well established (Purcell, 1974). Although there is vector spread of *X. fastidiosa* from grapevine to grapevine in late summer and autumn, only the spring spread from alternative hosts to the grapevine is of epidemiological importance, because freezing winter temperatures may eliminate *X. fastidiosa*



from grapevines inoculated after spring months (reviewed in Hopkins and Purcell, 2002). A similar scenario occurs in the Central Valley of California (USA), where insect vectors move to vineyards for brief flights from alfalfa fields, but there is no further spread from grapevine to grapevine (Purcell and Frazier, 1985). Few Pierce's diseased grapevines in regions without freezing winter temperatures recover. The opposite scenario occurs with citrus variegated chlorosis in Brazil. In that case, *X. fastidiosa* is also known to colonise a wide range of weeds associated with citrus orchards (Lopes et al., 2005), but disease spreads primarily from citrus to citrus (Laranjeira et al., 1998). Alternative hosts, in this case, may be important for maintenance of the pathogen in the environment, and provide a habitat for insect vectors, but their epidemiological impact is deemed to be low'.

Similarly, epidemics of Pierce's disease of grapevines in California, USA, may also have distinct characteristics if vector species are different. In northern coastal California, spread is driven by adult *Graphocephala atropunctata* leafhoppers that overwinter in riparian areas adjacent to vineyards. In spring they migrate to vineyards and infect vines, leading to a disease distribution radiating from the overwintering habitat of vectors. After the introduction of the invasive species *Homalodisca vitripennis* to southern California, Pierce's disease epidemics had devastating consequences for vineyards in Temecula Valley, where entire vineyards were symptomatic (i.e. no edge effect). In this case, insect vectors overwintered on adjacent citrus plants, reaching extremely large populations (one to two million per hectare) (Coviella et al., 2006) that were subsequently-distributed throughout vineyards (Perring et al., 2001), leading to an extensive disease spread.

In the Americas, most diseases caused by *X. fastidiosa* are vectored by sharpshooter leafhoppers. In Europe, spittlebugs are much more abundant and diverse than sharpshooter leafhoppers, but not much is known about their biology, ecology and role as vectors. In addition, agricultural practices and environmental conditions, including the landscape and climate, are extremely variable in the EU (EFSA PLH Panel, 2015a).

After the detection of *X. fastidiosa* in Europe in October 2013, two large research projects <u>POnTE</u> and <u>XF-ACTORS</u> were funded by EU Horizon 2020 programme to investigate the biology and the control of *X. fastidiosa* in Europe. The first results of these projects, and of transnational, national and EFSA funded projects have been discussed at the European conference on *Xylella fastidiosa*⁴ held in Palma de Mallorca in November 2017. The overall conclusions of the conference were that the control of *X. fastidiosa* is complex and need to be tailored to each case, understanding the pathosystem (pathogen, vectors, host, environment); progress has been made on *Xylella* research in Europe in recent years but there is still a long way to go and research is still needed, particularly to better understand the ecology and epidemiology of *X. fastidiosa* in the EU (see Section 3.2.2).

3.1.3. Intraspecific diversity

3.1.3.1. Subspecies of *X. fastidiosa*

Subspecies have been delineated within the *X. fastidiosa* species, based on their in-between values of DNA–DNA hybridisation (Schaad et al., 2004), sequence differences of 2% or more at synonymous sites (Schuenzel et al., 2005), and distinct 16S rRNA gene and 16S-23S rRNA spacer sequences (Hernandez-Martinez et al., 2006; Su et al., 2012), or based on multilocus sequence typing (MLST) (Nunney et al., 2014). New genomic data will soon probably provide additional information at the infra-species level.

At least six different subspecies of *X. fastidiosa* have been proposed (Schaad et al., 2004), but only the subspecies *fastidiosa* and *multiplex* are officially considered so far as valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB) (Bull et al., 2012).

X. fastidiosa subsp. *fastidiosa* causes Pierce's disease of grapevine (Nunney et al., 2010), and has been found in a wide range of perennial plants, shrubs and trees (EFSA, 2018). The subspecies *fastidiosa* is more diverse in Central America (EFSA PLH Panel, 2015a); it has been suggested that its presence in the USA is the consequence of an introduction (Nunney et al., 2010).

X. fastidiosa subsp. *multiplex* is linked to leaf scorch diseases of trees. 'The subspecies *multiplex* appears, so far, to have the widest host range in terms of plant species expressing disease symptoms (Nunney et al., 2013). It is subdivided into various subgroups, which are mostly associated with specific host plants (Nunney et al., 2013). The presence of the subspecies *multiplex* in Brazil is considered to be the result of an introduction from the USA associated with plums (Nunes et al., 2003;

⁴ https://www.efsa.europa.eu/en/events/event/171113



Almeida et al., 2008; Nunney et al., 2012b). Nunney et al. (2012b) raised the hypothesis of a recent inter-subspecies recombination between the sympatric *X. fastidiosa* subsp. *pauca* and subsp. *multiplex* in South America to explain why host plants such as citrus or coffee, which have been cultivated there for about two hundred fifty years, have been reported as affected for only the last twenty-five years (EFSA PLH Panel, 2015a)'.

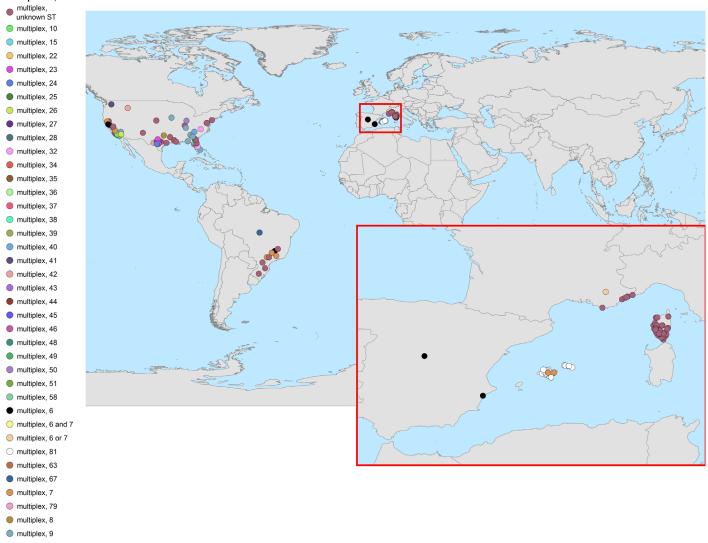
X. fastidiosa subsp. *pauca* causes citrus variegated chlorosis in *Citrus* spp., but is also found in coffee plants. This subspecies is also associated with olives trees in Argentina (Haelterman et al., 2015), Brazil (Coletta-Filho et al., 2016) and Italy (Elbeaino et al., 2014). Isolates within ssp. *pauca* causing citrus variegated chlorosis in Brazil are reasonably well characterised. (Nunney et al., 2012a).

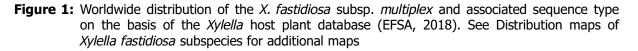
X. fastidiosa subsp. *sandyi* is responsible for oleander leaf scorch, but also associated with *Jacaranda* spp., Daylily and Magnolia (Schuenzel et al., 2005; Hernandez-Martinez et al., 2007).

Additionally, there are two other proposed subspecies, *X. fastidiosa* subsp. *tashke* (Randall et al., 2009), in association with leaf scorch of *Chitalpa tashkentensis*, and *X. fastidiosa* subsp. *morus* (Nunney et al., 2014), causing leaf scorch of red mulberry (*Morus rubra*). These last two subspecies were only rarely reported (EFSA PLH Panel, 2015a; EFSA, 2018).

Based on the *Xylella* host plant database (EFSA, 2018), the distribution of each subspecies has been mapped (Figure 1 and Distribution maps in Distribution maps of *Xylella fastidiosa* subspecies), and updated from the previous EFSA PLH Panel (2015a).

Legend subspecies, MLST





Subspecies have been generally helpful to infer about the general biology of isolates. For example, isolates collected from symptomatic grapevines in California fall within subspecies *fastidiosa*, while those collected from almond trees fall within subspecies *fastidiosa* and *multiplex* (Almeida and Purcell, 2003). The isolates collected from almonds that belong to subspecies *fastidiosa* are capable of causing disease in grapevines and almond trees, while those belonging to subspecies *multiplex* cause disease only in almonds (EFSA PLH Panel, 2015a). It has been suggested that each *X. fastidiosa* subspecies has a largely non-overlapping set of symptomatic host plants (Nunney et al., 2013), although there is evidence of additional host specialisation within subspecies (Sanderlin, 2017). However, different *X. fastidiosa* subspecies may be found in the same host plant, for example *Polygala myrtifolia*, coffee plants, and almond trees (see European STs world-wide in Annex A of this opinion, EFSA *Xylella* host plant database (EFSA, 2018)).

3.1.3.2. Multilocus sequence types

Multilocus sequence typing (Maiden et al., 1998) is largely accepted as a very useful genetic typing methodology. This method is based on the sequence of a set of seven housekeeping genes (for *Xylella*, the sirohaem synthase gene (*cysG*), glutamate symport protein gene (*gltT*), DNA polymerase III holoenzyme chi subunit gene (*holC*), 2-isopropylmalate synthase gene (*leuA*), ABS transporter sugar permease gene (*malF*), NADH ubiquinone oxidoreductase NQ012 subunit gene (*nuoL*) and ubiquinol cytochrome C oxidoreductase gene (*petC*) – see *X. fastidiosa* MLST databases. Each sequence of a given housekeeping gene is assigned to a distinct allele. For a given *Xylella* isolate, the alleles at each of the seven genes define the sequence type (ST). MLST has been widely used to characterise *X. fastidiosa* (Scally et al., 2005; Yuan et al., 2010; Elbeaino et al., 2014; Nunney et al., 2014; Denance et al., 2017). The method (Yuan et al., 2010) is listed among the validated tests for the identification of *X. fastidiosa* and the determination of the subspecies.

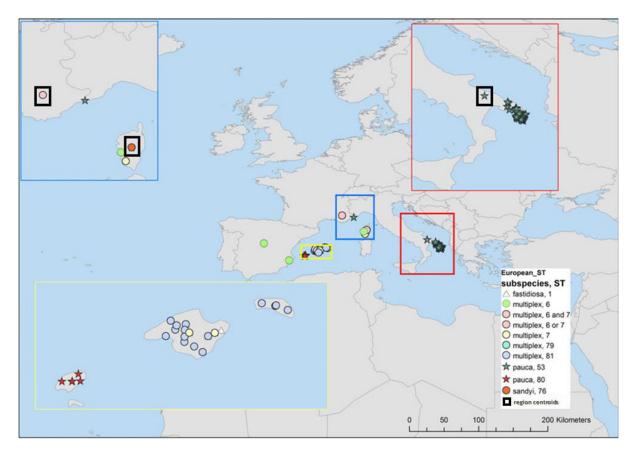


Figure 2: Distribution of the different *X. fastidiosa* subspecies and ST's reported in the European territory. Data originated from the literature search and current EU outbreaks (Black squares in magnification boxes in PACA and Corsica regions of France and Apulia in Italy, differentiate European NUTS2 region centroids from the other points)

'MLST allows for the grouping of genotypes that are biologically distinct within the various *X. fastidiosa* subspecies. For example, within subspecies *pauca*, there are biologically and genetically distinct genotypes that cause disease in citrus and coffee (Almeida et al., 2008). In this specific case, there is no cross-infection (Almeida et al., 2008).... Although genotyping allows for genetic and phenotypic inference, biological (e.g. experimental cross-infection assays) and epidemiological studies (surveys that type field isolates) are [nevertheless] important to determine the phenotypic characteristics of individual isolates' (EFSA PLH Panel, 2015a).

To date, there are up to 81 different recorded types worldwide (EFSA, 2018). Up to eight different ST's are recorded in the EU, belonging to the subspecies *fastidiosa* (ST1), *multiplex* (ST6, 7, 79, 81), *pauca* (53, 80) and *sandyi* (76) (Figure 2).

3.1.3.3. Comparative genomics

Although MLST provides a robust typing method to discriminate different isolates, its discriminative power is limited by the set of genes used and by the possibility of recombination. Whole genome sequences analyses can provide additional information that is useful from phylogenetic and taxonomic points of view. Currently, only a limited number of whole-genome sequences are publicly available of the subspecies *fastidiosa*, *sandyi*, *multiplex*, *pauca* and *morus* (Simpson et al., 2000; Van et al., 2003; Schuenzel et al., 2005; Chen et al., 2010), but many more are expected to be available in the very near future. In a publication on comparative genomics of *X. fastidiosa*, Marcelletti and Scortichini (2016) suggested that subsp. *sandyi* and *morus* may potentially be included within the subsp. *fastidiosa*. Additional genomic sequence data confirm clades supporting the three main *X. fastidiosa* subspecies: *fastidiosa*, *multiplex*, and *pauca*, *morus* and *sandyi* appear to be intermediate between *fastidiosa* and *multiplex*; a topic that requires being further studied (Almeida, personal communication).

'The robustness of infra-subspecies data, especially in the context of the host plant–pathogen genotype associations, is still being assessed by the scientific community and is currently considered as weak because the available data are limited (Yuan et al., 2010; Almeida and Retchless, 2013)' (EFSA PLH Panel, 2015a). The link between the host and *Xylella* genotype is not yet fully understood. Also, the importance of homologous recombination on the evolution of *X. fastidiosa* needs to be underlined. Recombination is a major element in *X. fastidiosa* evolution, occurring within short time frames and being associated with new host-pathogen associations (Coletta-Filho et al., 2017). This also explains why this pest categorisation addresses the *X. fastidiosa* as a species rather than individual subspecies.

3.1.4. Detection and identification of the pest

Are detection and identification methods available for the pest? Yes

Although detection and identification methods are available for the pest, EFSA PLH Panel (2015a) noted that 'The symptoms associated with the presence of *X. fastidiosa* in plants vary from asymptomatic associations to plant death, due to the large number of different host affected by the bacteria, pathogen diversity, and partly because of the wide range of climatic conditions in areas where the pathogen is found'.

'Most host plants infected with *X. fastidiosa* do not express any symptom. Symptoms often consist of a rapid drying of leaf margins, with scorched leaves. The different names given to the disease illustrate this heterogeneity of symptoms: Pierce's disease of grapevines, alfalfa dwarf, almond leaf scorch, phony peach disease, plum leaf scald, citrus variegated chlorosis or leaf scorch of elms, coffee, oak, sycamore and oleander (Figure 3)' (EFSA PLH Panel, 2015a). EPPO (2018) provides extensive and detailed information of symptoms on major diseases caused by *X. fastidiosa*.



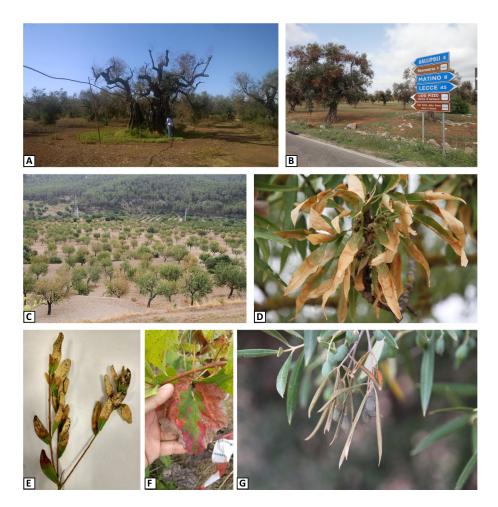


Figure 3: *X. fastidiosa* infected symptomatic plants recorded in the European outbreaks: (A) and (B) olive trees in Apulia, Italy; (C) and (D) almonds in the Balearic Islands (Spain), (E) *Polygala myrtifolia* in Spain, (F) grapevine in Spain, (G) wild olive in Spain (pictures from Italy: courtesy of Donato Boscia, Institute for Sustainable Plant Protection; National Research Council of Italy, from Spain: courtesy of Juan A. Navas-Cortés; Instituto de Agricultura Sostenible and Conselleria de Medi Ambient, Agricultura i Pesca, Direcció General d'Agricultura i Ramaderia, Government of the Balearic Islands, Spain)

'The reliable detection and identification of *X. fastidiosa* is very important not only because of its quarantine status, but also because the different genotypes are markedly different in host range and, therefore, in terms of plant disease significance. Another reason is that *X. fastidiosa* infects a wide range of host plant species asymptomatically. Symptom development depends on host plant species–*X. fastidiosa* genotype (Almeida and Purcell, 2003) and is usually correlated with high bacterial populations within plants (Hill and Purcell, 1995a,b; Newman et al., 2003). Because bacterial populations within plants are also correlated with the efficiency pathogen acquisition efficiency by its insect vectors (Hill and Purcell, 1997), plant species infected with low populations of *X. fastidiosa* may serve as an inefficient reservoir for vectors to acquire the bacterium (Almeida et al., 2005) (EFSA PLH Panel, 2015a)'.

Whenever possible, isolation and achieving Koch's postulates is considered to be the 'gold standard' (EFSA, 2016b). Both EPPO and IPPC recommend isolation and pathogenicity tests in critical situations. Nevertheless, isolation of the bacteria is very difficult, even from symptomatic hosts (EPPO, 2018).

EPPO recently revisited its standards on diagnostic protocol for *X. fastidiosa* (PM 7/24(3)) (EPPO, 2018), following a thorough review of the literature and current practices in the EU. Briefly, two different procedures are proposed for the detection of the pathogen in plants and the insect vectors. For plant samples, at least two positive screening tests, based on different biological principles or targeting different parts of the genome, are required for a positive detection. Isolation is also recommended, followed by subsequent tests (including a pathogenicity test in critical cases) and subspecies determination. Attempts to assign a subspecies by molecular tests may also be performed.



For the detection of *X. fastidiosa* in insects, the proposed methodology is simpler, with at least two methods based on different biological principles or targeting different parts of the genome. Subspecies determination is considered by EPPO as optional. Bacteria are usually present at lower concentrations in insect vectors, rendering isolation and detection more difficult. Recently, Cruaud et al. (2018) proposed a nested PCR for the detection of *X. fastidiosa* in insects, in order to overcome the insufficient sensitivity of conventional and qPCR.

Several methods have been used to identify *X. fastidiosa* directly in petiole or stem cross-sections, including serologically based methods such as dot-immunobinding assay (EPPO, 2004), dot tissue blot immunobinding assay (Djelouah et al., 2014), ELISA (Sherald and Lei, 1991), membrane entrapment immunofluorescence (Hartung et al., 1994), immunofluorescence (Carbajal et al., 2004) or Western blot (Lee et al., 1992). Such methods are sometimes considered less sensitive than the isolation approach (French et al., 1978; Sherald and Lei, 1991). Those methods could also lead to false-negative or -positive detections.

Numerous PCR-based methods have been proposed for *X. fastidiosa* detection, with different objectives, including general detection and quarantine purposes (EFSA PLH Panel, 2015a). Here, we list only the molecular tests most commonly used in the EU, the conventional PCR. A database of validated tests for the identification of *X. fastidiosa* and its subspecies, as referred to in article 3(2) of Commission implementing decision (EU) 2015/789 is available, listing conventional PCR proposed by Minsavage et al. (1994) or real-time PCR based on Francis et al. (2006) or Harper et al., 2010 (and erratum 2013), loop isothermal amplification (LAMP) by Harper et al., (2010 and erratum 2013), ELISA or immunofluorescence, for screening and identification tests in demarcated areas and sites of production referred to in article 9(8) of the EU decision. For other areas or sites of production, real-time PCR or LAMP as proposed by Harper et al., (2010 and erratum 2013) is listed. The real-time PCR methods proposed by Li et al., 2013 and Ouyang et al., 2013 are also listed by EPPO (2018). Proficiency test and performance studies are available, in the framework of XF-ACTORS, PONTE and PROMODE (EPPO, 2018).

For the identification of the subspecies, the method proposed is the MLST analysis (Yuan et al., 2010). PCR (Hernandez-Martinez et al., 2006; Pooler and Hartung, 1995) is still also accepted, despite their more limited use. The method proposed by Hernandez-Martinez et al. (2006) is targeting the three subspecies *fastidiosa*, *multiplex* and *sandyi* while the one proposed by Pooler and Hartung (1995) targets only the subspecies *pauca*. The data provided by comparative genomics as well as the current discussions on the subspecies status stress the usefulness of using a multi-gene strategy for the identification at infraspecies level.

3.2. Pest distribution

3.2.1. Pest distribution outside the EU

The distribution of *X. fastidiosa* has already been documented in the EFSA PLH Panel (2015a): the pest has been reported in North America, Central America and Caribbean, South America, Asia and Europe. A new detailed global distribution map has been compiled based on the EFSA host plant database (EFSA, 2018) and is shown in Figure 4. Additional information on the different STs (sequence types) found in the EU is also provided (Figure 2).



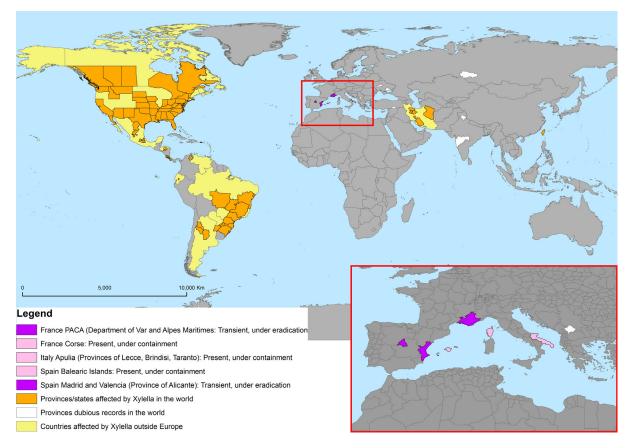


Figure 4: Global distribution map based on the EFSA *Xylella* host plant database 2018 (literature data 1900–2018) and current notifications (excluding interceptions)

3.2.2. Pest distribution in the EU

Is the pest present in the EU territory? If present, is the pest widely distributed within the EU?

Yes, the pest is present in the EU territory. It is currently reported in South of Apulia (Italy), in Corsica and in the Departments of Var and Alpes Maritimes in the Provence-Alpes-Côte d'Azur (PACA) region (France) and in the Autonomous region of Madrid, in the province of Alicante in Valencia and the Balearic Islands (Spain). It has limited distribution within the EU. In agreement with the Decision (EU) 2015/789, demarcated areas have been established in the Union territory. Reported status is 'transient, under eradication', except for the Balearic Islands (Spain), Corsica (France) and Southern Apulia (Italy) where the status is 'present with a restricted distribution, under containment'.

In the European territory, the pest status is considered as 'transient, under eradication' process, except for the South of Apulia (Italy), Corsica (France) and Balearic Islands (Spain), where the current status is 'present with a restricted distribution'. Additionally, *X. fastidiosa* subsp. *fastidiosa* ST1 was also found in 2015 in Saxony, Germany, at one location in isolated potted plants of *Nerium oleander*, *Rosmarinus*, *Streptocarpus* hybrid and *Erysimum* hybrid kept in greenhouse. According to EU emergency measures, a demarcated area was established in Saxony and Thuringia and placed under surveillance, but it was then lifted since March 2018 because of the eradication of the local infestation (Europhyt Outbreak (C) N°-946).

The current distribution of *X. fastidiosa* in the EU based on the *Xylella* host plant database (EFSA, 2018) is shown in Figure 4. The situation in France, Italy and Spain is briefly described here, focusing on the host plants, *X. fastidiosa* subspecies and sequence types and potential insect vectors. The combined host plant table is provided in Annex C.

3.2.2.1. Current situation in France

Several cases of *X. fastidiosa* infections in imported plants or plant material, involving mainly coffee plants have been reported in France from 2012 to 2015 (Jacques et al., 2016; Denance et al., 2017; EPPO Global database). The first detection of *X. fastidiosa* in natural settings in France was reported in July 2015 from Corsica (Chauvel et al., 2015; EPPO Global database). Then, foci were detected in the Provence-Alpes-Côte-d'Azur (PACA) region, continental France. A case of contamination of an apple tree (*Malus domestica*) and a peach tree (*Prunus cerasifera*) in continental France, and a holm oak (*Quercus ilex*) in Corsica were detected but not further confirmed (Denance et al., 2017).

3.2.2.1.1. Corsica

In July 2015, France notified the presence of *X. fastidiosa* in Corsica, novel foci of infected plants were continuously reported as investigations progressed. In October 2017, a total of 350 foci were identified following the analysis of more than 15 thousands samples (Direction régionale de l'alime ntation, de l'agriculture et de la forêt de Corse Le ministère de l'agriculture en region). *X. fastidiosa*-infected plants were detected with a higher prevalence along the coast of southern Corsica, but some foci were also detected at higher altitudes and in northern Corsica. Since December 14, 2017, the entire territory of Corsica was declared as area under containment by Commission Implementing Decision (EU) 2017/2352.

The list of *X. fastidiosa*-infected plant species enlarges with up to 36 species declared host of *X. fastidiosa* in Corsica (see Annex C). It should, however, be noticed that a few other species (for instance fig and holm oak) were also found infected but were not officially confirmed (Denance et al., 2017). Apart from the non-native *P. myrtifolia*, most host species so far recorded in Corsica are species that are indigenous and common to Mediterranean scrubland. No olive tree (*Olea europaea*) or holm oak (*Q. ilex*) was confirmed contaminated. However, non-official analyses have reported some *X. fastidiosa*-positive cases in such plants (Denance et al., 2017). Strawberry tree (*Arbutus unedo*), citrus (*Citrus* spp.), eucalyptus (*Eucalyptus* spp.) and grapevines (*Vitis* spp.) have been tested but no infection has so far been detected. Apart from ornamentals, almond is the other crop species affected by the bacterium in Corsica.

Most infections in Corsica are associated with *X. fastidiosa* subsp. *multiplex* ST6 and ST7 (Denance et al., 2017; http://draaf.corse.agriculture.gouv.fr/Xylella-fastidiosa-en-Corse). The genome sequences of the first isolated strains from Corsica revealed that these strains are close to but not identical to their US relatives, the strains Dixon and Griffin-1, respectively. Mobile elements such as plasmid and phage sequences differed between US and Corsican strains. In few foci other STs (ST53, ST76 and ST 79, one focus each) were detected following direct typing of DNA extracted from plant material, but strains were not isolated (Denancé et al., in preparation). The role of *X. fastidiosa* subsp. *multiplex* ST6 and ST7 in leaf scorching of *P. myrtifolia* has been demonstrated following the fulfilment of Koch's postulates (Denancé et al., in preparation).

A large range of insects that could be considered as potential vectors of *X. fastidiosa* has been observed in Corsica (Cruaud et al., 2018). The list includes *Philaenus spumarius*, known as the major vector of *X. fastidiosa* in olive groves in Italy. No transmission tests have been carried out in Corsica so far, but molecular analyses confirmed that an average of 20% of the specimens were with *X. fastidiosa* (Cruaud et al., 2018). The prevalence of contaminated specimens is, however, highly variable depending on the period of the year and location; the bacterial load is assumed to be low in contaminated *P. spumarius*. Partial typings of *X. fastidiosa* in *P. spumarius* revealed occurrence of alleles that were not yet observed in plants, indicating that insects may feed on plants that are not sampled or that they efficiently inject only part of the bacterial population they harbour. Among other insects sampled in Corsica and analysed for *X. fastidiosa* contamination, yet no other species, including *Neophilaenus campestris*, tested positive (Françoise Poliakoff, personal communication). Complete typing of two *X. fastidiosa* contaminated specimens of *P. spumarius* from Corsica resulted in *X. fastidiosa* subsp. *multiplex* ST7. Partial typing of other specimens revealed the potential presence in *P. spumarius* in Corsica of *X. fastidiosa* subsp. *fastidiosa* strains based on *holC_1* and *gltT_1. P. spumarius* contaminated by *X. fastidiosa* carrying *holC_3* were also detected (Cruaud et al., 2018).

3.2.2.1.2. Provence-Alpes-Côte d'Azur

Following the discovery of *X. fastidiosa* in Corsica, *X. fastidiosa*-infected *P. myrtifolia* plants were detected in Nice, in the PACA. Other cases of *X. fastidiosa*-infected plants were then reported the region mostly from urban and semiurban areas. In December 2017, 54 foci representing 116



X. fastidiosa-infected plants out more than five thousands analysed samples were reported. Up to six different plant species have been reported as infected in the area. Again, most of these infected species are indigenous or introduced ornamentals.

X. fastidiosa subsp. *multiplex* ST6 and ST7 have been found in most foci. Only one focus of *P. myrtifolia* plants was detected in Menton, infected by *X. fastidiosa* subsp. *pauca* ST53 (Denance et al., 2017). As the strains occurring in these plants were not isolated, no comparison with the strain from Apulia could be made.

3.2.2.2. Current situation in Italy (southern Apulia)

In October 2013, Italy notified the presence of *X. fastidiosa* in Southern Apulia (Lecce Province), associated with quick decline symptoms in olive trees (*O. europaea*) and leaf scorch in oleander and almond (EFSA, 2013; Saponari et al., 2013). From this first foci, covering an area of ca 8000 ha, additional scattered outbreaks were discovered in the province of Lecce.

The current demarcation of the infected and buffer areas in Apulia is reported in the official website – Emergenza *Xylella* – of the Region Apulia.

Analysis of the data collected in the monitoring programs from 2014 to 2017 shows the intensive sampling effort conducted by the Plant Health Authorities in Apulia and the pathogen spread in the region. Spread of the disease over time is represented in the dynamic map that can be found at XF-ACTORS PROJECT.

Axenic cultures of the bacterium and genomic studies have unravelled important genetic and epidemiological information. MLST and preliminary comparative genomic analyses of the draft genome of an olive-infecting strain (Loconsole et al., 2016; Giampetruzzi et al., 2017a) showed the genetic relatedness of the Apulian and X. fastidiosa subsp. pauca isolates. Using the MLST approach, all the Apulian isolates were found to harbour a single, unique and novel ST, identified as ST53 (Loconsole et al., 2016). Concomitantly in 2014, this genotype (ST53) was reported in Costa Rica (Nunney et al., 2014). Genomic analyses and evolutionary studies based on the draft genomes of isolates recovered in both countries have further confirmed that, within the subspecies pauca, the Apulian and Costa Rican isolates form a compact phylotype in a clade divergent from the South American pauca isolates (Giampetruzzi et al., 2017b). The clustering and distinctiveness of the ST53 isolates support the hypothesis of their common origin, and the limited genetic diversity among these isolates suggests this is a relatively new emerging clade within subspecies *pauca*. The low genetic variability detected upon comparative analysis of the draft genomes of more than 40 isolates recently recovered from diseased olive trees located in different municipalities of the Apulia, provide evidence of the recent introduction of X. fastidiosa in this region. Thus, the overall results of several genetic studies conclusively support (i) the Central American link of the Apulian strain causing disease in olive trees in southern Italy, and that (ii) the current epidemic is the consequence of a recent and single introduction (Sicard et al., 2017).

With regard to the distribution and spread of infections at field level, multiyear surveys in selected olive orchards have clearly shown that the infections progress rapidly and generally following an 'aggregated patterns', which indicates that secondary infections occur from the infected primary sources (Montes-Borrego et al., 2017). The rapid progression of infections in the olive orchards is also accompanied by symptom severity. Although symptoms (scattered branch dieback) may vary depending on tree age, cultivar and agricultural practices, the canopies of the affected trees generally completely desiccate within 2–4 years.

Koch's postulates with the Apulian isolate have been fulfilled for olives, *P. myrtifolia* and *N. oleander* (Saponari et al., 2017). This study also provided experimental evidence for differential responses to the infections among olive cultivars. In agreement with previous studies (Giampetruzzi et al., 2016), inoculated plants of cultivar Leccino had lower bacterial titres and symptom scores. Similarly, in the same study, plants of the cultivar Frantoio and Coratina showed lower intensity of shoot dieback compared to the susceptible cultivar Cellina di Nardò.

Olive, being widely cultivated in the area concerned, is by far the most commonly infected and severely affected host in all the Apulian outbreaks (www.emergenzaxylella.it). However, searches for susceptible plant species using PCR identified a list of 31 plant species (see Annex C) naturally infected with the ST53 isolate. Among these hosts, the majority showed typical leaf-scorching symptoms, while very few species (*Rhamnus alaternus, Myoporum insulare, Westringia glabra*) were symptomless or highly symptomatic with severe dieback and desiccation (*N. oleander, Acacia saligna, P. myrtifolia*)

3.2.2.3. Current situation in Spain

3.2.2.3.1. Balearic Islands

In 2016, Spain notified the presence of *X. fastidiosa* subsp. *fastidiosa* on cherry trees in Majorca. Plants of *Polygala myrtifolia* at the same location were also found infected by *X. fastidiosa* subsp. *fastidiosa* and *X. fastidiosa* subsp. *multiplex* (Olmo et al., 2017). After this first detection, new foci were continuously reported on the island. Both *X. fastidiosa* subspecies are widespread throughout the entire island. Subsequently, *X. fastidiosa* subsp. *multiplex* was also reported in Menorca and *X. fastidiosa* subsp. *pauca* in Ibiza. Up to May 2018, a total of 691 positive plants out of 5,176 samples analysed were identified in different foci in Majorca (415 positives), Menorca (115 positives) and Ibiza (161 positives) (see Annex C – for the host plant species). No cases of *X. fastidiosa* infection have been reported in Formentera. Due to the widespread distribution of the pest, the whole territory of the Balearic Islands was declared as area under containment by Commission Implementing Decision (EU) 2017/2352.

In Majorca, *X. fastidiosa* subsp. *fastidiosa* ST1, and *X. fastidiosa* subsp. *multiplex* ST81 (similar to ST6 in all MLST alleles but with a single-nucleotide polymorphism in cysG 3 allele) and ST7 were identified. In Menorca, only *X. fastidiosa* subsp. *multiplex* ST81 was identified, and in Ibiza, ST80 of *X. fastidiosa* subsp. *pauca* (not previously described) was identified. Some strains of *X. fastidiosa* subsp. *fastidiosa* ST1 and *X. fastidiosa* subsp. *multiplex* ST81 were isolated from various hosts and the genome sequencing of some isolates is under way.

Currently, 18 host species including cultivated, ornamental and landscape plant species have been found infected by the subspecies *fastidiosa, multiplex* and *pauca* of *X. fastidiosa,* with wild olive, almond, cultivated olives, grapes and figs with higher number of positives and higher sampling effort, in that order.

Various insect species belonging to Aphrophoridae that could be considered as potential vectors of *X. fastidiosa* have been captured in Majorca. Nymphs of aphrophorids were observed during March-April on eight herbaceous plant species, mainly on *Reichardia picroides*, *Crepis vesicaria* and *Sonchus oleraceus* (Asteraceae) and *Foeniculum vulgare* (Apiaceae), on olive, citrus and almond orchard ground covers. The potential insect vector species identified are *N. campestris*, *Neophilaenus lineatus* and *P. spumarius* and *N. campestris* being the most abundant. So far, none of the species have proved to be infected by *X. fastidiosa*.

3.2.2.3.2. Alicante

In 2017, Spain notified the presence of *X. fastidiosa* in the Alicante province, Valencian Community. Soon after, the extensive surveys and analyses of 11,784 samples from the entire Valencian Community revealed 209 samples infected from 178 almond orchards in 27 municipalities in the Alicante province up to February 2018. The demarcated area comprises about 87,814 ha and covers 57 municipalities. So far, only almond trees have been found infected in the affected area. All plants analysed were infected by *X. fastidiosa* subsp. *multiplex* ST6. Strains of *X. fastidiosa* subsp. *multiplex* ST6 have been isolated from different almond plots and the genome sequencing is underway.

A range of insects (> 2,000 individuals) that considered as potential vectors of *X. fastidiosa* have been captured in the area. These specimen insects belong to the Aphrophoridae (88%), Issidae (0.65%) and Cicadellidae (10.2%) families. From all specimens analysed, 75% are *Neophilaenus* spp. (mainly *N. campestris*), and 15% *Philaenus* spp. (mainly *P. spumarius*). Molecular analyses of 327 individuals captured within or nearby infected plots revealed the presence of *X. fastidiosa* in *P. spumarius* (average 27%) and in *N. campestris* (1,2%). Sequencing of the seven MLST genes of some *P. spumarius* specimens confirmed their contamination by *X. fastidiosa* subsp. *multiplex* ST6. No transmission tests have been performed so far in Alicante.

3.2.2.3.3. Madrid

In 2018, Spain, notified the presence of *X. fastidiosa* in an olive tree (cv Picual) located in the Autonomous Region of Madrid in open field. The plant initially analysed was infected by *X. fastidiosa* subsp. *multiplex* ST6. The demarcated area currently comprises 8,171,28 ha and affects four municipalities.



3.3. Regulatory status

3.3.1. Council Directive 2000/29/EC

X. fastidiosa is listed in Council Directive 2000/29/EC (Table 2). Details are presented in Table 2, as *Xylella* is listed in the Directive under different synonyms.

Table 2: X. fastidiosa in Council Directive 2000/29/EC

1.	Xylella fastidiosa (Well and Raju)		
(d)	Viruses and virus-like organisms		
5.	Viruses and virus-like organisms of <i>Cydonia</i> Mill., <i>Fragaria</i> L., <i>Malus</i> Mill., <i>Prunus</i> L., <i>Pyrus</i> L., <i>Ribes</i> L., <i>Rubus</i> L. and <i>Vitis</i> L., such as:		
(d)	Peach phony rickettsia		
Annex II, Part A	Harmful organisms whose introduction into, and spread within, all member states shall be banned if they are present on certain plants or plant products		
Section I	Harmful organisms not known to occur in the community and relevant for the entire community		
(b)	Bacteria		
	Species	Subject of contamination	
1.	Citrus variegated chlorosis	Plants of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids, other than fruit and seeds	
Annex IV, Part A	Special requirements which must be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within all member states		
Section I	I Plants, plant products and other objects originating outside the community		
	Plants, plant products and other objects	Special requirements	
23.2	Peach phony rickettsia	(b) no symptoms of diseases caused by the relevant harmful organisms have been observed on plants at the place of production or on susceptible plants in its immediate vicinity, since the beginning of the last three complete cycles of vegetation.	

The introduction into the EU of some known host plants is prohibited. This includes (*Citrus*, *Fortunella*, *Poncirus* and their hybrids, other than fruit and seeds, *Vitis* other than plants originating in third countries (see Annex III, Part A, of Directive 2000/29/EC) and *Prunus*, originating from non-European countries), with the exception of dormant *Prunus* plants (free from leaves, flowers and fruit) from Mediterranean countries, Australia, New Zealand, Canada and the continental states of the USA (see Annex III, part A, of Directive 2000/29/EC).

3.3.2. EU emergency measures

Decision (EU) 2015/789 as amended by Decision (EU) 2017/2352

A. Regulated plant species

Two different categories of plant species are regulated (https://ec.europa.eu/food/plant/plant_hea lth_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en):

- the <u>host plants</u>, i.e. plants for planting, other than seeds, belonging to the genera and species listed in the Commission database of host plants susceptible to *X. fastidiosa* (and its subspecies) in EU territory.
- the <u>specified host plants</u>, which means host plants and all plants for planting, other than seeds, belonging to those listed in Annex I of <u>Decision (EU)</u> 2015/789 and found infected worldwide.

B. Control measures to prevent spread within the Union:

• **Establishment of demarcated areas**, as soon as the presence of *X. fastidiosa* is confirmed. An 'infected zone' and a 'buffer zone' are delineated. The 'infected zone' shall include all plants known to be infected, all plants showing symptoms indicating possible infection, and all other plants liable to be infected due to their close proximity to infected plants, or common source of



production, if known, with infected plants, or plants grown from them. The 'buffer zone' shall be of a width of at least 10 km for outbreaks subject to containment measures, while 5 km for outbreaks subject to eradication measures and 1 km for isolated outbreaks where no natural spreading occurred and eradication measures have been immediately taken.

The eradication measures apply to any official detection of *X. fastidiosa* in the Union territory, except where containment measures are applied (the Balearic Islands, Corsica and southern Apulia).

- **Eradication measures**: Within the *infected zone*, all *host plants* or the *host plants* of the *Xylella* subspecies concerned (e.g. *pauca, fastidiosa, multiplex*) located within the 100 m radius around the infected plants should be removed, irrespective of their health status. Prior to the removal, appropriate phytosanitary treatments should be applied against the vector in order to avoid further dispersal. All *specified plants* (non-host plants) within the 100 m radius have to be sampled and tested for the presence of the bacterium. Within the buffer zone, intensive surveillance should be carried out consisting of visual inspections, sampling and testing of symptomatic plants. That surveillance shall take place in a grid of 100 m × 100 m squares in the first km of the buffer zone adjacent to the infected zone, with surveillance in a grid of 1 km × 1 km in the rest of the buffer zone.
- **Containment measures**: Within the infected zone, lighter provisions apply, consisting of intensive surveillance and immediate removal of at least the infected plants. These measures should be implemented, where applicable, at least within the last 20 km strip of the infected zone adjacent to the buffer zone, as well as around the production sites authorised to move specified plants out of the demarcated area (e.g. nurseries, garden centres) and around sites with high cultural, scientific and social value. Within the buffer zone, the same provisions as the ones presented in the eradication measures apply. As regards Corsica and the Balearic Islands, there are no provisions for buffer zones as the infected zones are surrounded by the sea.
- **Movement of plants within and out of the demarcated areas**: Strict requirements for the movement out of the demarcated areas and from infected zones into their respective buffer zones for the 'specified plants';

C. Control measures to prevent introduction into the Union

- Imports of the *Coffea* plant for planting prohibited from Costa Rica and Honduras.
- Imports of specified regulated plants: imports of the 'specified plants' (more than 200 plant species and 35 genera) from infected third countries are only possible if the plants are grown under protected conditions and, prior to their export and on entry into the EU, are inspected, sampled and tested for the absence of the bacterium.
- Importation from pest-free countries or pest-free areas only possible if the Commission has officially been informed about the health status of these areas.

3.4. Entry, establishment and spread in the EU

3.4.1. Host range

EFSA periodically updates the *Xylella* host plant database. The extraction table presented in Annex A summarises the 'host range' of *X. fastidiosa* based on peer-reviewed literature from the EFSA *Xylella* host plant database (EFSA, 2018). The list is based on hosts reported in the current literature in association with *X. fastidiosa* EFSA host plant database (EFSA, 2018). A major question raised in the previous EFSA risk assessment (2015) was the way *X. fastidiosa* would be able to infect indigenous European host plants. In an attempt to provide an overview of the current situation in the EU, a table of the current reported host plants and of the associated STs is given (Annex A). This clearly shows that indigenous European plant species are hosts for *X. fastidiosa*. Further, the different subspecies and sequence types sometimes share common hosts, like *Asparagus, Cystus, Ficus carica, Fraxinus angustifolia, O. europaea, Prunus* sp., *P. myrtifolia, Rosmarinus officinalis, Westringia fruticosa*, but sometimes infect some specific host plant species (Annex A).

Also to be considered are the EU official list of the 'host plants', i.e. plants for planting, other than seeds, belonging to the genera and species listed in the Commission database of host plants susceptible to *X. fastidiosa* (and its subspecies) in the Union territory, and the list of 'specified plants', i.e. host plants and all plants for planting, other than seeds, belonging to the listed annex I of Decision (EU) 2015/789 (as amended) and found infected worldwide (see Section 3.3.2).



'Host plants of economic importance (i.e. crops and certain ornamentals) known to be susceptible to disease caused by this bacterium are thus listed. Additionally, it is important to stress that Koch's postulates have not necessarily been fully fulfilled for each of the host– *X. fastidiosa* subspecies-sequence type combination (EFSA, 2016b)'.

3.4.2. Entry

Is the pest able to enter EU territory?

Yes, the pest has already entered the EU. Major pathways are i) plants for planting and ii) insect vectors (both on their own and as hitchhikers).

In the previous EFSA PLH Panel (2015a), seven pathways were analysed including plants for planting infected with *X. fastidiosa*, plants or plant material imported for research or breeding purposes, seeds, fruits, cut flowers and ornamental foliage infected with *X. fastidiosa*, detached wood and infectious insect vectors.

Both the **plants for planting infected with** *X. fastidiosa* (including the plants or plant material imported for research or breeding purposes) and the **infectious insect vectors** are considered to be major pathways, while the others were considered as unlikely or very unlikely with high uncertainties (EFSA PLH Panel, 2015a). Since 2014 and until April 2018, there were 51 records of interception of *X. fastidiosa* in plants for planting in the Europhyt database. There are four records of Cicadellidae interceptions since 2004, whether these were potential vectors of *Xylella* (subfamily Cicadellinae) is not known.

When the information about the country of origin is available (only 58% of the interceptions), it shows that most recorded cases concern coffee plants imported from Costa Rica and Honduras. Reports of interceptions from *Coffea* sp. have been linked with *X. fastidiosa* ST53, 72, 73, 74, 75, 76 and 77 (Annex D; Bergsma Vlami et al., 2015, 2017; Jacques et al., 2016; Loconsole et al., 2016; Denance et al., 2017).

Following emergency measures and the restriction to importation of coffee plants, fewer interceptions have been notified. A commercial lot of *Mandevilla sanderi* from Brazil, another of *Pelargonium* x *Hortorum* from Mexico and lots of *Juglans* sp. and *Rubus fruticosus* from the USA were also intercepted. Information on the subspecies or sequence type (ST) from these interceptions is often not available. Some of these interceptions have nevertheless been described as subspecies *pauca* or *sandyi* (Loconsole et al., 2016). However, the plant for planting pathway is partially closed for some plant species due to the existing legislation (see section 3.3.2).

In 2018, the Regional Government of the Autonomous Community of Andalusia, Spain, reported the first detection of *X. fastidiosa* in *P. myrtifolia* in mainland Spain. This interception occurred in the municipality of El Ejido, Almería province, in three *Polygala* plants (see Figure 5) growing within an insect-proof net greenhouse in a nursery. Infected plants showed a generalised light chlorosis and not the typical symptoms of the bacterium on *P. myrtifolia*. *X. fastidiosa* subspecies identification is still pending. Traceability of the *Polygala* lots present in the greenhouse and surveys of host plants outside the greenhouse within a 5-km radius are ongoing, including a nearby almond plot and several greenhouses growing different vegetables. The sampling that brought this discovery is part of the intensive surveys carried out throughout Spain since 2014.



Figure 5: General overview of the insect-proof net greenhouse where *Polygala myrtifolia* plants were found infected by *X. fastidiosa* (left) and *P. myrtifolia* plants showing generalised light chlorosis (right). (Source: Consejeria Consejería de Agricultura, Pesca y Desarrollo Rural, Government of Andalusia, Spain)

There are uncertainties due to the limited number of specific studies related to the other analysed pathways, which were considered by the EFSA PLH Panel as unlikely (seed, fruit, cut flowers and ornamental foliage infected with *X. fastidiosa*) or very unlikely (detached wood) (EFSA PLH Panel, 2015a).

3.4.3. Establishment

Is the pest able to become established in EU territory?

Yes, the pathogen already established and spread in some EU regions.

As host plants and suitable habitats exist in the risk assessment area, and as vectors are known to occur, there is a potential for establishment and spread of *X. fastidiosa* (EFSA PLH Panel, 2015a). The outbreaks in southern Italy, Corsica in France and the Balearic Islands in Spain (see Section 3.2.2) shows that the pathogen, following entry, can establish and spread.

3.4.3.1. Distribution of main host plants in the EU

Many host plant species are indigenous or are cultivated in the EU, with many hosts of economic importance, including grapevine, citrus, almond, plum and peach trees and native or planted trees such as elm, oak, or sycamore (EFSA PLH Panel, 2015a). If there is still uncertainty with regard to the potential host range of *X. fastidiosa* in the European flora as a range of European wild plant species may never have met the bacterium and it is not known whether they would be hosts, symptomatic or asymptomatic (EFSA, 2013), the number of host plants recorded in Europe has largely increased following surveys (see Section 3.4.1). The environmental conditions found in the risk assessment area are suitable for survival, multiplication and spread of both *X. fastidiosa* and its vectors. Tropical, subtropical and Mediterranean climates appear to be particularly favourable for *X. fastidiosa* persistence and disease outbreaks (Purcell, 1997), although *X. fastidiosa* is also encountered in cooler climates, as shown by reports in Canada and New Jersey (EFSA PLH Panel, 2015a).

If there is still uncertainty with regard to the potential host range of *X. fastidiosa* in the European flora as a range of European wild plant species may never have met the bacterium and it is not known whether they would be hosts, symptomatic or asymptomatic (EFSA, 2013), the number of host plants recorded in Europe has largely increased following surveys (see Section 3.2.2).

Considering the great variety of climatic zones where the pathogen is well established, ranging from temperate to tropical zones, it is very likely that the pathogen will find suitable climatic conditions in the EU. Establishment and spread would be likely, especially in the southern part of the risk assessment area, including the Mediterranean coast, as the Mediterranean climate (Köppen–Geiger



climate group Csa and Csb) also occurs in California, where three *X. fastidiosa* subspecies have been detected so far (EFSA PLH Panel, 2015a). The recent establishment of *X. fastidiosa* in Apulia, Italy, Corsica in France and in the Balearic Islands in Spain confirms this statement.

Several approaches have been used to infer the suitability of climatic zones for X. fastidiosa, primarily the subspecies fastidiosa in the USA. Purcell and Feil (2001) used isotherms of January temperatures for zones where Pierce's disease has a severe (4.5° C), occasional (1.7° C) or rare (-1.1° C) impact on grapes. Hoddle (2004) used CLIMEX to produce maps of potential distribution for X. fastidiosa and its vector H. vitripennis, based on data from Feil and Purcell (2001) and Feil (2001). The optimum in vitro growth temperature for the bacteria X. fastidiosa subsp. fastidiosa is 28°C, and the bacterium did not grow in vitro at 12°C (Feil and Purcell, 2001). Anas et al. (2008) used the number of winter days with temperatures below -12.2°C or -9.4°C to predict areas at risk of Pierce's disease and the effect of warming on disease severity. These temperature parameters have also been used for creating a NAPPFAST map for X. fastidiosa in the USA (Engle and Magarey, 2008 in EFSA PLH Panel, 2015a). More recently, Bosso et al. (2016) applied a Maxent model to detect and predict the current and future potential distribution of X. fastidiosa in the Mediterranean basin. In a recent study, Godefroid et al. (2018) estimated the potential distribution of X. fastidiosa in Europe, based on different subspecies or types. They conclude that X. fastidiosa subsp. multiplex might pose a threat to most of Europe under current and future climate conditions, while Mediterranean coastal areas of Spain, Greece, Italy and France, the Atlantic coastal areas of France, Portugal and Spain and the south-western regions of Spain and the lowlands in southern Italy are most threatened by, additionally to subsp. multiplex, subsp. fastidiosa and pauca.

'In grapevines, plants may recover from infections during winter. Plants systemically infected, with or without symptoms, may not be infected by X. fastidiosa in the following years' (EFSA PLH Panel, 2015a). This is a very well reported phenomenon in grapevines (Feil and Purcell, 2001); on the west coast of the USA, it limits the northern spread of Pierce's disease (Hopkins and Purcell, 2002). Although the recovery mechanism remains unknown, low winter temperatures increase the rate of recovery (Purcell, 1980). In the field, recovery happens more often when infections occur in the summer or autumn than during the spring (Feil and Purcell, 2001; Feil et al., 2003). It should be noted that winter recovery has been demonstrated for grapevines infected with X. fastidiosa subsp. fastidiosa, and that most of the research on the topic has been conducted in California. Winter recovery has also been shown for Prunus (Ledbetter et al., 2009)' (EFSA PLH Panel, 2015a). Nevertheless, the presence in the Washington DC area of trees chronically infected with isolates of X. fastidiosa subsp. multiplex highlights the fact that this bacterium can survive at higher latitudes. Henneberger et al. (2004) pointed out also that the bacteria were able to overwinter in sycamore trees at relatively low air temperatures $(-5^{\circ}C)$, probably being protected in the roots. Similarly, X. fastidiosa is reported to survive severe winter conditions $(-28^{\circ}C)$ in almond in Iran. A key question is certainly how winter temperatures may affect establishment in northern Europe.

3.4.4. Spread

Is the pest able to spread within the EU territory following establishment? How?

Yes, spread within the EU territory following establishment is likely, via plants for planting and insect vectors (both on their own and as hitchhikers).

RNQPs: Is spread mainly via specific plants for planting, rather than via natural spread or via movement of plant products or other objects?

No, spread occurs via plants for planting and insect vectors (both on their own and as hitchhikers)

3.4.4.1. Vectors and their distribution in the EU

According to EFSA PLH Panel (2015a), '*X. fastidiosa* is exclusively transmitted by xylem sap-feeding insects (order Hemiptera, suborder Auchenorrhyncha - Cicadomorpha: Redak et al., 2004). They have sucking mouthparts (mandibular and maxillary stylets) that allow them to reach the xylem of their host plants, from which they ingest sap and egest saliva. Owing to the very poor nutritional value of xylem fluid, xylem fluid feeders ingest large amounts of sap and produce large amounts of honeydew. They are generally not direct pests unless present at very high population levels. Within the Cicadomorpha, the three superfamilies, Cercopoidea, Cicadoidea and Membracoidea, include xylem fluid-feeding groups but, whereas all Cercopoidea (known as spittlebugs or froghoppers) and Cicadoidea (cicadas) are regarded as

xylem fluid feeders, the superfamily Membracoidea includes a single xylem fluid-feeding subfamily, the Cicadellinae (known as sharpshooters). Only these three groups of "specialists" in xylem fluid feeding have been shown to be vectors of *X. fastidiosa*. Some phloem sap feeders also feed marginally to the xylem, however tests for *X. fastidiosa* transmission capacity on one of these species were negative (Purcell, 1980). Spittlebugs, cicadas and sharpshooters are heterometabolous insects that develop through egg, five nymphal stages and adult (winged) stage. Nymphs of cicadas and of spittlebugs of the family Cercopidae are subterranean root feeders, whereas nymphs of spittlebugs of the family Aphrophoridae and the subfamily of Cicadellinae develop on aboveground plant. All adults feed and live on the aerial parts of host plants (Ossiannilsson, 1981; Tremblay, 1995; Redak et al., 2004) (EFSA PLH Panel, 2015a)'.

In Europe, only a few species of sharpshooters (Cicadellidae, subfamily Cicadellinae) are present (Wilson et al., 2009), although one species, *Cicadella viridis*, is widespread, common and locally abundant in humid areas. Differently, quite a high number of spittlebug species (Cercopoidea: Aphrophoridae and Cercopidae), are present (de Jong, 2013). Among these, the meadow spittlebug, *Philaenus spumarius* (Figure 7) is the most common species. It is present in a very wide geographical area, colonises different ecological niches and is locally very abundant.

3.4.4.1.1. Identifying vectors

Although screening for vector species is largely based on PCR analyses for the identification of *X. fastidiosa* in the head of field-collected insects, the final evidence of transmission competence must be based on transmission experiments. According to EFSA PLH Panel (2015a), 'Although it is expected that all sharpshooters and spittlebug species are vectors of *X. fastidiosa* (Frazier, 1944; Purcell, 1989; Almeida et al., 2005), it is important to demonstrate that species not formally identified as vectors can transmit the bacterium from plant to plant. In addition to identifying new vector species, studies should go further and provide information on the efficiency of the transmission process, so that the epidemiological relevance of newly identified species can be better put in context. This is important because, as previously demonstrated (Lopes et al., 2010; Daugherty et al., 2011), vector species may have very different transmission efficiencies depending on host plant species, or even by feeding on different tissues of the same host plant. Lastly, it is imperative to understand that detection of a pathogen within a putative vector is by no means evidence that a species is a vector; plant-to-plant transmission experiments are the only way to prove that a species is a vector'.

Furthermore, a positive transmission to a given test plant does not necessarily imply that the vector can transmit the pathogen to other plants known to be host.

3.4.4.1.2. Geographical distribution and population abundance of European vectors and potential vectors

Although some species of spittlebugs and sharpshooters are ubiquitous and present in most, if not all, European countries, some others have a more restricted area of distribution or, due to their low population levels or to their narrow ecological niches, are known from only few countries/geographical areas. The xylem-sap feeder species richness in European countries is summarised in Figure 6A, that only includes sharpshooters and spittlebugs, the two insect groups regarded as confirmed X. fastidiosa vectors, and in Figure 6B, which includes all the xylem-sap feeder species (spittlebugs, sharpshooters, cicadas). It is clear that, wherever in Europe the bacterium is eventually present or introduced, indigenous xylem-sap feeder insects are present and may act as vectors. Species richness of potential vectors, including cicadas is relatively homogenous over the EU, although the number of reported species of spittlebugs and sharpshooters is higher in Western, than Eastern Europe. The possibility that this difference in species number between western and eastern EU is due to less intense surveys for the presence of spittlebugs and sharpshooters in Eastern Europe cannot be excluded. When considering all the xylem-sap feeders, including cicadas, species richness is higher in the Mediterranean area. However, due to the widespread presence of several species of potential vectors all over the EU, it is important to obtain information on the population level, rather than on the absolute number of species. Since xylem-sap feeders were not regarded as a pest until the identification of X. fastidiosa in the EU, accurate estimates of population abundance of European spittlebugs are not available, although some data come from scattered field observations. Aphrophora salicina can be quite abundant on Salix cinerea, with up to one spittle mass (containing 2-8 nymphs) per two branchlets (Badmin, 2006). Both Aphrophora alni and A. salicina can be abundant on S. cordata (Czerniakowski, 2005). N. lineatus can be abundant on grasses (Novotny, 1987; Eyre et al., 2001), while N. campestris was relatively common, but not dominant, in the olive agroecosystems of Apulia (Ben Moussa et al., 2016). According to the literature, P. spumarius is clearly the dominant spittlebug species in Europe, in different geographical areas and in different ecosystems/



crops, including the olive groves of Apulia (Saponari et al., 2014; Ben Moussa et al., 2016; Cornara et al., 2017), vineyards (Braccini and Pavan, 2000; Pavan, 2006; Kunz et al., 2010), grasslands (Eyre et al., 2001), and strawberry fields in Finland and Norway (Raatikainen and Vasarainen, 1970; Taksdal, 1977). Detailed data on population abundance of *P. spumarius* in Mediterranean olive groves of Northern and Southern Italy are in the study carried out in the frame of EFSA procurement on 'Collection of data and information on biology and control of vectors of Xylella fastidiosa'. In this study, the site with the highest *P. spumarius* population had an average of 21 (with a peak of 49) nymphs per m^2 in 2016 and an average of 30 (with a peak of 68) nymphs per m^2 in 2017. In the same site, the adult population peaked at about 0.7–1.5 adults per sweep in the olive canopy (and at about 1.3–1.7 adults per sweep on other woody hosts) in 2016 and 2017, respectively. P. spumarius has a very wide distribution and, besides the Palearctic regions, it is also present in the Nearctic Region. In the United States, very high population densities of this species were reported in alfalfa and strawberry. Wiegert (1964) observed peak densities of 1,280 nymphs/m² and 466 adults/m² in alfalfa fields of Michigan, while Zajac and Wilson (1984) reported densities close to 1,000 nymphs/m² on strawberry. However, these population peaks were followed by steady decreases of the populations, likely due to the two concurrent factors, mortality and emigration.

Among European sharpshooters, *Cicadella viridis* is by far the most common and abundant species and, although few data on its population densities are available, it has been reported that in wet meadows with *Juncus* spp. and *Holcus* spp., densities of about 1,800 eggs and first instar nymphs and of almost 400 adults per m² can occur (Tay, 1972). Unlike *P. spumarius, C. viridis*, besides being very abundant on herbaceous hosts, is almost absent on the woody ones (Pavan, 2006). Moreover, the species is hydrophilic and therefore absent in dry environments, such as most of the olive groves and vineyards in the Mediterranean area. The other species of sharpshooters, such as *Graphocephala fennahi* and *Evacanthus interruptus*, seem to have a scattered or very limited distribution and low population densities.



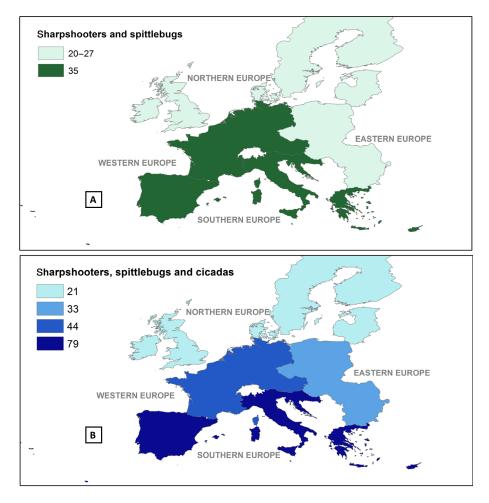


Figure 6: Species richness of spittlebugs, sharpshooters and cicadas in different regions⁵ of Europe according to Fauna Europea (de Jong et al., 2014) (accessed 30.1.2018). (A) Sum of sharpshooters and spittlebugs in the EU 28; (B) Sum of different species belonging to spittlebugs, sharpshooters, cicadas in the EU28

3.4.4.1.3. Biology of European vectors and potential vectors

A list of potential vectors of X. fastidiosa in Europe, gathering all the sharpshooters and spittlebugs was drawn from the Fauna Europaea database (de Jong, 2013) and is reported in Annex C of the EFSA PLH Panel (2015a). The large majority of these species shares two biological features, they are polyphagous and univoltine. Among sharpshooters, only Cicadella lasiocarpae and Anoterostemma ivanoffi have a restricted host range on Carex and Juncus, respectively, while the nymphs of G. fennahi are associated with Rhododendron but the adults may visit several other woody hosts. Among only A. salicina and A. pectoralis (mainly associated with Salix spp.) and spittlebuas, Philaenus italosignus, P. maghresignus and P. signatus (whose nymphs develop on Asphodelus spp.) have a narrow host range. Although most of the species are polyphagous, several of them have a relatively restricted ecological niche, as they live only in humid areas (e.g. Cicadella viridis, Aphrophora similis) or they are associated with grasses only (e.g. Evacanthus acuminatus, Evacanthus interruptus). As for the spittlebugs, the most prominent biological feature, which can have important consequences for their role or potential role as X. fastidiosa vectors, is the association of nymphal stages with herbaceous plants, while the adults tend to colonise many different woody hosts, including Quercus, Salix, Ulmus, Betula and woody crops such as olive, grapevine, stone and pome fruits. Haematoloma dorsata and, to a lesser extent, Neophilaenus spp. adults feed on conifers. Host-shifting

⁵ The regions potentially at risk in the European Union: Northern EU: Lithuania, Denmark, Latvia, Ireland, Finland, Estonia, Sweden, United Kingdom; Southern EU: Spain, Greece, Malta, Italy, Croatia, Slovenia, Portugal, Cyprus; Western EU: Belgium, Netherlands, Luxembourg, France, Germany, Austria; Eastern EU: Hungary, Poland, Czech Republic, Bulgaria, Slovakia, Romania.

behaviour is also well known for spittlebug adults that move from herbaceous to woody hosts soon after reaching adulthood and are back to herbaceous plants at the end of the season when they search for oviposition sites. *P. spumarius*, the meadow spittlebug, is a univoltine, highly polyphagous species that develops as nymph on many herbaceous dicotyledonous species (monocotyledonous are rarely exploited as host plants), mainly within the families Asteraceae and Fabaceae. Adults feed on herbaceous and woody hosts such as oak (evergreen and deciduous), myrtle, lentisk, almond, grapevine, olive, peach, etc. Females undergo an ovarian parapause and start maturing eggs only from late August onwards, depending on the latitude (Witsack, 1973; Cornara et al., 2018). The meadow spittlebug overwinters as eggs, laid on stubble, basal dry leaves, and the dead parts of plants; most of the eggs are laid close to the ground between two apposed surfaces (Cornara et al., 2018). Data on the fecundity of *P. spumarius* are sometimes conflicting, ranging from 22 (Wiegert, 1964) to 350–400 eggs per female (Yurtsever, 2000). On the other hand, data from Witsack (1973) and the EFSA funded project on 'Collection of data and information on biology and control of vectors of *Xylella fastidiosa*' (IPSP-CNR, technical report, 2017) are quite consistent and provide estimates in the range of 100 eggs per female, although a single female can lay more than 300 eggs. For a comprehensive review on *P. spumarius*, see Cornara et al. (2018).

Among sharpshooters, *C. viridis* is the dominant species, especially in wet or humid meadows or grasslands, but also in the herbaceous cover of some vineyards (Pavan, 2006). *C. viridis* prefers gramineous plants for feeding and *Juncus* for oviposition. It has 1–3 generations per year, depending on the latitude and altitude (Frediani, 1955; Arzone, 1972; Tay, 1972; Pavan and Gambon, 2004). The females lay summer eggs mostly on *Juncus, Ranunculus, Agropyron* and *Erigeron*, while overwintering ones are laid mainly on *Juncus, Alnus, Fraxinus, Rosa* (Arzone, 1972; Tay, 1972) and *Rosa* (Freidani, 1955). A fecundity of about 35–60 eggs per female has been estimated for this species by Tay (1972) and Arzone (1972). According to Frediani (1955), the fecundity is higher, at least 100 eggs per female.

As for flight activity and dispersal, little information is available for the European vectors and potential vectors. Although we know that both sharpshooters and spittlebug adults actively move to plants different from those hosting nymphs, no estimation of this active dispersal is available for European sharpshooters. In North America few authors provided estimates of *P. spumarius* dispersal capability. While nymphs can only move between neighbouring herbaceous plants, adults can fly, and be transported, over longer distances. Conflicting results on the dispersal capability of *P. spumarius* adults have been published. Halkka et al. (1971) suggested that active flights were limited to 40–80 m, while Putman (1953) noted massive long-distance movements that could be interpreted as a migration phenomenon. It is worth noting that this latter paper simply describes visual observations and no measurements of dispersal were done. In Italy, some mark-release-recapture experiments are ongoing, and preliminary results indicate that spittlebugs were recaptured within a 100 m radius of the release points (Plazio et al., 2017).





Figure 7: *Philaenus spumarius* at different life stages in Italy (A) Egg mass, (B) Nymph, (C) adult (pictures: courtesy of Vincenzo Cavalieri and Nicola Bodino, IPSP-CNR)

The *X. fastidiosa* transmission ability is so far proven, both under field and laboratory conditions, only for *P. spumarius*. This species acts as a vector of the strain infecting olives in the Apulia region of Italy (Saponari et al., 2014). *P. italosignus* (Figure 8) and *N. campestris* (Figure 9) also proved to be competent vectors when allowed to feed on infected source plants and then transferred to healthy ones under laboratory conditions (Cavalieri et al., 2018). In California, *P. spumarius* proved to be a vector of *X. fastidiosa* to grape (Severin, 1950; Cornara et al., 2017) and to almond (Purcell, 1980).

3.4.4.1.4. Known vectors for EU outbreaks/sequence types

С

According to EFSA PLH Panel (2015a), 'At a minimum, the identification of new vector species involves the confinement of field-collected insects on uninfected plants for an inoculation access period of 96 hours. After the inoculation access period (IAP), plants should be sprayed with appropriate pesticides and maintained in an insect-free greenhouse for later detection of the bacterium. This test determines only whether or not an insect is already contaminated by bacteria and is able to transmit to a given plant species. Negative results do not imply that the species is not a vector'.

Once an insect species has been identified as a vector following the above mentioned procedure, a systematic testing to determine vector status under controlled conditions (including controlled acquisition) is advisable. To this purpose, according to EFSA PLH Panel (2015a), the following protocol is suggested: 'Insects from a healthy colony should be confined to *X. fastidiosa* – infected plants (or plant tissue) for an acquisition access period (AAP) of 96 h and subsequently transferred to uninfected plants for a 96-h IAP. In this way, source plants suitable for *X. fastidiosa* acquisition by a given potential vector are identified. Vector status may be investigated with any host plant species. However, bacterial isolates present in each region should be used for this work, i.e. genetic resolution to at least the subspecies level'. If putative vectors' survival on host plants is minimal, shorter acquisition access periods can be used in attempts to verify their vector status.

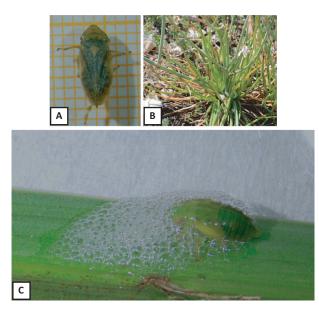


Figure 8: *Philaenus italosignus* at different life stages in Italy (A) Adult, (B) Spittles of *Philaenus italosignus* on *Asphodelus* sp., (C) Nymph in foam (pictures: courtesy of Vincenzo Cavalieri and Nicola Bodino, IPSP-CNR)

Following the discovery of *X. fastidiosa* in the EU, some attempts to identify vectors have been made, although final data on vector competence are available only for the CoDIRO strain of *X. fastidiosa* (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. Cornara et al. (2016) confirmed the role of *P. spumarius* and transmitted *X. fastidiosa* to olive, oleander and periwinkle plants using spittlebugs collected in heavily infected olive groves. Finally, the olive-to-olive transmission was achieved under fully controlled conditions with acquisition of healthy *P. spumarius* adults on infected olives and inoculation to self-rooted olive plants (Cornara et al., 2017). In the same experiments, *P. spumarius* transmitted *X. fastidiosa* to periwinkle following acquisition from different infected plant species (olive, cherry, almond, *Polygala*). *N. campestris* failed to transmit *X. fastidiosa* under the same experimental conditions.



Figure 9: Neophilaenus campestris at different life stages in Italy (A) Foam close to the ground on grasses, (B) Nymph, (C) Adult (pictures: courtesy of Vincenzo Cavalieri and Nicola Bodino, IPSP- CNR)

As mentioned above, *P. italosignus* and *N. campestris* transmitted ST53 to olive and *Polygala myrtifolia* (Cavalieri et al., 2018). The role of *P. italosignus* is probably negligible, as it has never been found in the infected demarcated area of Apulia and immatures are strictly associated with *Asphodelus* spp. *N. campestris* likely is of limited importance for the spread of *X. fastidiosa* in Apulia, because it has no preference for olives and therefore has little chance to acquire *X. fastidiosa* from and transmit it to olives. However, the presence of different vector species in different host plants and



ecological niches can increase the range of *X. fastidiosa* natural reservoirs in the environment that, in their turn, can serve as a source of inoculum for the crops.

In Corsica, outbreaks of *X. fastidiosa* are associated with ST6 and ST7 and, although no transmission tests have been carried out yet, these STs were identified in insects and molecular analyses confirmed that an average of 20% of the *P. spumarius* specimens were contaminated (Cruaud et al., 2018). These findings strongly suggest the role of *P. spumarius* in the spread of these STs of *X. fastidiosa* subsp. *multiplex*, although the final evidence should come from transmission experiments. In Spain, a number of different STs has been identified in the Balearic Islands (belonging to the subspecies *multiplex*, *fastidiosa* and *pauca*) and in Alicante (ST6 of subspecies *multiplex*). However, so far no transmission tests have been done and preliminary data on *X. fastidiosa* genetic characterisation from *P. spumarius* and *N. campestris* collected in Alicante (see Section 3.2.2.3 Current situation in Spain) confirm the presence of *X. fastidiosa* subsp. *multiplex* in this spittlebug species.

Characterisation of the sequence types detected in vectors is challenging, due to the very low number of bacterial cells in the insects that generally hamper successful MLST analyses (Cruaud et al., 2018; EPPO, 2018). Therefore, no published information at the ST levels is so far available for *X. fastidiosa* – positive insects in the EU.

3.4.4.2. Spread by natural means

The only route of natural spread of *X. fastidiosa* is by insect vectors, mainly sharpshooters and spittlebugs. In Europe, the major role of spittlebugs has been emphasised (EFSA PLH Panel, 2015a), because of the presence of few sharpshooter species and the wide distribution and abundance of spittlebugs, namely *Philaenus* and *Neophilaenus* spp. Transmission is rapid because there is no latency period. Therefore, the vector can transmit the bacterium immediately after acquisition. Moreover, the pathogen persists and multiplies in the foregut of the adult vectors, which can remain infectious throughout their lifespan (Almeida et al., 2005). Since the bacterium is lost with moulting and nymphs have a very limited mobility, only adults are regarded as being responsible for *X. fastidiosa* spread. The potential vector species in the EU are listed in Annex C of the EFSA PLH Panel (2015a).

Dispersal seems to be primarily limited by the short-range flight of spittlebugs, whose active flights probably do not exceed a range of 100 m (Weaver and King, 1954; Halkka et al., 1971; Plazio et al., 2017). Although Putman (1953) noticed 'long-distance flights' in Ontario, no data were provided to support this statement and, so far, in Europe no migration phenomena have ben observed. The active dispersal of sharpshooters seems to be in the same range (Blackmer et al., 2004). Passive flights of spittlebugs through the wind are also possible (Wiegert, 1964; Halkka et al., 1971) and may allow for the dispersal of infected insects over longer distances. Finally, whose active flights it is worth noting that, according to EFSA PLH Panel (2015a), the density and pattern of host plants in the landscape will have a significant influence on spread (Plantegenest et al., 2007), particularly on short- and mediumrange vector dispersal from plant to plant. In general, landscapes characterised by areas of contiguous hosts at high densities will be more conducive to spread. This concept has been applied to the case of ST53 strain of olive in the Apulia region (Strona et al., 2017), leading to the conclusion that X. fastidiosa will persist in the area. A mathematical model for the spread of this disease in Apulia has been produced (White et al., 2017) showing how the width of a control zone (established just outside the infected zone), together with the intensity of surveillance in this control zone, may delay the spread of the disease. According to Bosso et al. (2016), the potential distribution under current condition comprises Portugal, Spain, Italy, Corsica, Albania, Montenegro, Greece and Turkey as well as all countries of northern Africa and the Middle East. X. fastidiosa is not predicted to change its distribution in the Mediterranean basin in response to climate change.

3.4.4.3. Spread by human assistance

Transportation of infected plant material is generally an effective means of long-distance dispersal (EFSA PLH Panel, 2015a). Vegetative propagation through grafting is widely used for most long-lived perennial *X. fastidiosa* hosts; transportation of live plant tissue is a common practice in the various agricultural industries affected by this pathogen, eventually increasing its geographic distribution (Almeida et al., 2014). Two factors are considered as important in the initial spread: (1) the long incubation period required for symptom expression and (2) the fact that the bacterium can be transmitted from plant material taken from infected but as yet asymptomatic plants used for grafting. The ban of marketing propagative material produced in the infected zones or the production of such a material under vector-proof screen houses may limit this route of *X. fastidiosa* spread.



Inadvertent transportation of vectors in vehicles should also be considered, as this has been observed for *P. spumarius* in the infected area of Apulia (see Figure 12 in EFSA PLH Panel, 2015a) and is considered to be the cause of the Oria outbreak, approximately 30 km away from the infected area at that time (see Section 3.2.2.2.). Spread by vehicles may occur via the general public by car or by the transport of agricultural vehicles with infected plant material and vectors. Measures aimed at suppressing insect populations (e.g. mechanical removal of weeds and insecticide applications), required by Decision (EU) 2015/789, can effectively contribute to reduce the probability of hitchhiking infectious vectors, thus avoiding long-distance spread of the pathogen.

In the currently affected zones of the risk assessment area, spread by human assistance could also be increased by commercial practices such as the direct retail selling of small potted cuttings and the important ferryboat traffic between Apulia and Greece, Corsica and mainland France and Italy, Balearic Islands and mainland Spain. However, the movement ban of specified plants out of any demarcated area and official checks required at the control measures taken in ferry ports aim at limiting such a risk.

Human-assisted spread would result in stratified dispersal, with one long-distance component allowing the colonisation of new areas; sometimes very far from the area of origin, followed by local colonisation of these newly reached spots by a diffusion process depending on autonomous local spread of the vectors (EFSA PLH Panel, 2015a).

Since *X. fastidiosa* distribution in the EU is very likely the result of repeated and independent introductions, and the time of these introductions is debated, the only example of a 'recent' introduction followed by a clear and documented spread is the one of ST53 associated with the quick olive decline syndrome of olive in the Apulia region of Italy. In this area, following the identification of the pathogen in the autumn of 2013, regular and intensive surveys have been carried out up to now. The rapid spread of the olive disease over time is represented in the dynamic map that can be found at XF-ACTORS PROJECT noting that this spread pattern is not representative of other *Xylella* host plant association, as each specific *X. fastidiosa* genotype/host plant/vector association is highly peculiar and strongly influenced by the local environmental conditions, as well as by the efficacy of eradication and containment measures put in place, so that spread patterns of *Xylella* diseases are very difficult to predict.

3.5. Impacts

Would the pests' introduction have an economic or environmental impact in the EU?

Yes, in countries where it occurs, *X. fastidiosa* is known to cause severe direct damage to important crops such as almonds, citrus, grapevines, olives and stone fruits and also to forest trees and landscape and ornamental trees.

RNQPs: Does the presence of the pest on plants for planting have an economic impact, as regards the intended use of those plants for planting?

Yes the pest has impact on plants for planting

X. fastidiosa has already had an economic impact in the EU, on olive trees as well as on almond and, cherry trees. The bacterium has also already been recorded on grapevines.

In countries where *X. fastidiosa* occurs, it causes severe direct damage to important crops such as grapevine, citrus and stone fruits and also to forest trees and landscape and ornamental trees. It also has indirect economic impact in areas producing plants for planting, as plants exports from areas where the disease is known to occur may be forbidden (EFSA PLH Panel, 2015a). Thus, *X. fastidiosa* is considered to be a serious thread for agriculture, the environment and the economy (IPPC factsheet, 2017).

Historically, Pierce's disease caused by *X. fastidiosa* was responsible for an outbreak in California in the 1880s with the destruction of more than 16,000 ha of grapes (Goodwin and Purcell, 1997). Major outbreaks were also reported in the 1930s and 1940s. In 1999, the disease re-emerged after the introduction of the glassy winged sharpshooter, *H. vitripennis*, and affected 25% of the 1,200 ha of vineyards in Riverside County, California (EFSA PLH Panel, 2015a). According to Tumber et al. (2014), federal, state and local governments and industry, together, spent nearly US\$544 million dollars in the 1999–2010 period. The current annual cost of Pierce's disease in California is estimated around 104 million US\$/year, in terms of disease prevention measures and vine losses (Blua et al., 1999).

Less recognised are the economic impacts of regions where susceptible crops could be grown if not for diseases caused by *X. fastidiosa*, for example grapes cannot be grown because of this pathogen in the southeastern states near the Gulf of Mexico.



Since its discovery in Brazil in 1987, it is estimated that citrus variegated chlorosis is responsible for the removal of more than 100 millions of citrus trees. The current annual cost of control measures is approximated around 120 million US\$/year (IPPC, 2017). However, according to Fundecitrus, plant health management policies, which included among other measures the removal of infected trees, led to a better situation in recent years, with about 3.0% of infected plants in 2016 compared with 43.8% in 2004 (FUNDECITRUS BRAZIL). The recent introduction in Apulia, Italy, has heavily impacted a large area of olive groves, the infected area exceeding 5,000 km² and including 1–3 millions of olive trees (Signorile, 2018). Luvisi et al. (2017) estimated ca 115 € per dead olive tree while increase in management cost was assessed around 31%. Besides the agricultural and economic impact, olive quick decline disease also affected symbolic, centennial trees, considered of inestimable social, historical and cultural importance (IPPC, 2017). More importantly, the whole landscape has changed dramatically in just a few years, from one dominated by old, sometimes monumental trees, to another with dead trees that remind onlookers of what has been lost. The impact of changes to the landscape and ecosystem services is not easy to determine (Almeida, in preparation).

Ornamental plants are also affected. In the USA, oleander is planted along the sides of roads and in private gardens: losses on Californian highways alone have been estimated to amount to US\$125 million (Henry et al., 1997). In the Mediterranean basin, oleander is used as ornamental plant but is also very common in the wild. In New Jersey, bacterial leaf scorch was estimated to affect 35% of the street and landscape oaks, with both aesthetic and economic consequences (Gould et al., 2004). Although reported more frequently since 1980, the impact of *X. fastidiosa* in forests is more difficult to assess owing to a general lack of data (Sinclair and Lyon, 2005) in (EFSA PLH Panel, 2015a).

Besides ornamental and garden plants that may be affected as shown recently in southern France, the question of how *X. fastidiosa* may affect the scrubland Mediterranean vegetation also called 'maquis' is yet to be answered.

Indirect losses can be linked not only to limitations in trade of plants for planting following the finding of *X. fastidiosa*, but also the effect of *X. fastidiosa* on the landscape.

3.6. Availability and limits of mitigation measures

Are there measures available to prevent the entry into, establishment within or spread of the pest within the EU such that the risk becomes mitigated?

Yes, there are measures available to prevent entry into, establishment within or spread of the pest within the EU territory, like the emergency measures adopted by the EU.

RNQPs: Are there measures available to prevent pest presence on plants for planting such that the risk becomes mitigated?

Yes, among which screen house production of plants for planting, the thermotherapy of dormant plants, certification of plant propagation material and control of insect vectors.

3.6.1. Phytosanitary measures

X. fastidiosa is currently regulated in the EU as a quarantine organism under Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. Additionally, emergency measures have been implemented since February 2014 and updated several times. Current emergency measures, applicable to all EU Member States, are laid down in Decision (EU) 2015/789 and amended in several occasions by (see Section 3.3.2).

Two different categories of plant species are regulated:

- **Host plants:** i.e. plants for planting, other than seeds, belonging to the genera and species listed in the Commission database of host plants susceptible to *X. fastidiosa* in the Union territory, as having been found to be susceptible in the EU to *X. fastidiosa* and its subspecies.
- Specified plants: i.e. host plants and all plants for planting, other than seeds, belonging to the genera or species listed in Annex I of Decision (EU) 2015/789 (as amended by Decision (EU) 2017/2352) and found infected worldwide.

Besides official control measures, survey activities have been reinforced. Guidelines for the survey of *X. fastidiosa* in the Union territory have been published (European Commission guidelines for the survey



of *Xylella fastidiosa*, 2015). Efforts have been made towards public awareness of the disease and the risk of inadvertently moving plants from demarcated areas. 'Strategies for preventing the spread from areas where the pathogen is present and for the control of an outbreak should focus on the two main pathways (plants for planting and infectious insects in plant consignments) and be based on an integrated system approach, combining, when applicable, the most effective options (e.g. removal of plants, control of vectors, establishment of pest-free areas, intensive surveillance, certification, screen house production, testing for plant propagation material, preparation, treatment and inspection of consignments for the pathway of the infectious vectors in plant consignments' (EFSA PLH Panel, 2015a).

3.6.1.1. Biological or technical factors limiting the feasibility and effectiveness of measures to prevent the entry, establishment and spread of the pest

There are biological and technical factors that greatly limit the effectiveness of measures aimed at preventing entry, establishment and spread of *X. fastidiosa*. Among the most important ones are, the frequent asymptomatic association of the bacterium with the plant, the very wide range of host plant species and the ubiquitous presence of insect vectors and potential vectors in both the exporting and recipient countries. Moreover, considering the wide range of host plant species, there is a large trade volume of potentially infected plants. Finally, plants for planting material originate from numerous exporting countries where *X. fastidiosa* is present.

3.6.2. Pest control methods

There is no single control method for *X. fastidiosa*. Control should be implemented with regards to each specific situation, in an integrated manner and with an area-wide management approach. Control methods were listed extensively in the previous EFSA risk assessment (EFSA PLH Panel, 2015a). Besides the surveillance, eradication and containment measures already that are in place through the current emergency measures in place for all EU Member States, the currently used control methods are briefly listed below:

- Host plant resistance
- Plant removal
- Screen house production of plants for planting, heat treatment of dormant plant material
- Certification
- Control of the insect vectors on both weeds and crops.

Host plant resistance. Following the introduction and spread of *X. fastidiosa* in the Apulian olive groves, at least two olive cultivars, Leccino and FS-17, have shown traits of tolerance to the pathogen. The tolerance is expressed by significantly reduced symptoms development, when compared with highly sensitive cultivars (i.e. Ogliarola salentina and Cellina di Nardò) growing under a very high pressure of inoculum inside the infected zone, and by a lower amount of bacterial cells in infected tissues (Baù et al., 2017; Boscia et al., 2017a,b; IPSP-CNR technical report, 2017). Some of the genes putatively involved in the tolerant response to *X. fastidiosa* in the Leccino cultivar have been identified following a comparative transcriptomic analysis (Giampetruzzi et al., 2016), while others are currently under investigation (Saldarelli, personal communication). These findings, together with further studies aiming at investigating the molecular basis of the host response and pathways modulating different defence responses might open the possibility of breeding for resistance or tolerance.

Conventional breeding in California that produced wine grape varieties with strong resistance required decades to produce but the first grapes varieties resistant to *X. fastidiosa* subsp. *fastidiosa* are now being released (Walker and Tenscher, 2016; Walker et al., 2016).

Plant removal. Roguing infected plants, and those surrounding them, that are likely in the incubation phase of the infection process, may be an effective control method as it suppresses the sources of inoculum for vectors in the environment (although the uncertainty is high due to the long incubation period). This measure may be more effective for reducing secondary spread (within the crop), as in olive groves in the Salento area, rather than primary spread due to incoming infected insects from outside the crop.

In a containment approach, where eradication is no longer feasible, severe pruning of infected, symptomatic plants has been indicated by some authors as a possible way to suppress disease symptoms in the plants (see EFSA PLH Panel, 2015a). Pruning of sweet orange trees in Brazil was reported to reduce the symptoms of citrus variegated chlorosis and eliminate infection, but only in very specific conditions at the very beginning of symptom development (Amaral et al., 1994), but this is the



only case reported in literature (EFSA PLH Panel, 2015a). A recent paper (Daugherty et al., 2018) point out that severe pruning of infected grapevines has limited efficacy for managing Pierce's Disease. Severe pruning of infected olive trees has been applied within the infected area in Apulia, but this measure seems not preventing that disease symptoms re-appear.

Screen house production of plants for planting has been demonstrated to be very effective in the case of CVC and has been compulsory in Sao Paulo State of Brazil since 2003 (Gonçalves et al., 2011; EFSA PLH Panel, 2015a).

Hot treatment of dormant plant material (see the opinions EFSA PLH Panel, 2015a risk assessment and EFSA PLH Panel, 2015c titled 'Hot water treatment of *Vitis* sp. for *Xylella fastidiosa'* for details describing the assessment of Hot Water Treatment on *Vitis* sp. planting material and assessing its efficacy in the elimination of *X. fastidiosa*

Certification (see EFSA PLH Panel, 2015a; EPPO protocols, 2018)

Control of insect vectors can be achieved by soil tilling in spring to kill the nymphs on the herbaceous plants (Regione Puglia, 2016) and by the application of insecticides on crops against the adults. Using both methods has a multiplier rather than additive effect and when combined with attempts to lower the percentage of insects infectious with *X. fastidiosa* can lower the number of infections by vectors. A very recent paper summarises the results of different insecticide application trials conducted in recent years in Apulia against *P. spumarius*. Under those experimental conditions, synthetic pyrethroids and neonicotinoids⁶ showed the highest efficacy, neonicotinoids being more persistent (Dongiovanni et al., 2018).

3.7. Uncertainty

- Uncertainty on the taxonomic status of some subspecies of *X. fastidiosa* (e.g. subsp. *morus* and subsp. *sandyi*).
- The dynamics of *X. fastidiosa* diseases in the new context of European ecosystems and geographic areas remains to be understood.
- Uncertainty about the actual host range of subspecies and sequence types.
- Taking into account the potential asymptomatic association of the bacteria with plants and the fact that the presence of *X. fastidiosa* can remain undetected for long periods, there is uncertainty about the current distribution of the bacteria worldwide, especially in areas where surveys intensity is low.
- The thermotherapy was only assessed on the grapevine and a limited number of host plants; The precise extent of the economic and environmental impacts.

4. Conclusions

X. fastidiosa meets the criteria assessed by EFSA for consideration as a Union quarantine pest. The identity of the pest is clearly established. *X. fastidiosa* is present in EU territory, either under eradication or containment in demarcated areas, but with limited distribution. There is no doubt about the economic consequences of its presence in EU territory. Spread within EU territory following establishment is likely, via plants for planting and insect vectors (both on their own and as hitchhikers) (Table 3).

⁶ Further restrictions on the use of imidacloprid, clothianidin and thiamethoxam were adopted by the European Commission under Regulations (EU) 2018/783-785.



Table 3:The Panel's conclusions on the pest categorisation criteria defined in Regulation (EU)2016/2031 on protective measures against pests of plants (the number of the relevant sections of the pest categorisation is shown in brackets in the first column)

sections of the pest eategonsation is shown in brackets in the first country								
Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest	Key uncertainties					
Identity of the pest (Section 3.1)	Yes, the identity of the pest is well established	Yes, the identity of the pest is well established	The identity of the pest is well established at the species level, yet there is uncertainty with the status of some subspecies of <i>X. fastidiosa.</i> Taxonomic status of various subspecies is subject to modification with novel accumulation of data through whole genome sequences					
Absence/ presence of the pest in EU territory (Section 3.2)	Yes, the pest is present in EU territory. It is currently reported in Italy (southern Apulia), in France (Corsica Island and the Provence- Alpes-Côte d'Azur region) and in Spain (Madrid region, the Alicante province and the Balearic Islands). In agreement with Decision (EU) 2015/789, demarcated areas have been established in EU territory. Reported status is 'transient, under eradication', except in the Balearic Islands (Spain), Corsica (France) and southern Apulia (Italy) where the status is 'present with a restricted distribution, under containment'	Yes, the pest is present in EU territory. Reported status is 'transient, under eradication', except in the Balearic Islands (Spain), Corsica (France) and southern Apulia (Italy) where the status is 'present with a restricted distribution, under containment'	Uncertainty about the current distribution of <i>X. fastidiosa</i>					
Regulatory status (Section 3.3)	Reported status is transient, under eradication, except in the Balearic Islands (Spain), Corsica (France) and southern Apulia (Italy) where the status is present with a restricted distribution, under containment	Yes, the pest is regulated as a quarantine pest. Reported status is transient, under eradication, except in the Balearic Islands (Spain), Corsica (France) and southern Apulia (Italy) where the status is present with a restricted distribution, under containment						
Pest potential for entry, establishment and spread in the EU territory (Section 3.4)	Yes, the pest has already entered the EU. Spread within EU territory following establishment is likely, via plants for planting and insect vectors (both on their own and as hitchhikers)	No, spread occurs via plants for planting and insect vectors (both on their own and as hitchhikers)	The dynamics of <i>X.</i> <i>fastidiosa</i> diseases in the new context of European ecosystems and geographic areas remains to be understood					



Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest	Key uncertainties
Potential for consequences in the EU territory (Section 3.5)	Yes, in countries where it occurs, <i>X. fastidiosa</i> is known to cause severe direct damage to important crops such as grapevines, citrus, olives and stone fruits and also to forest trees, landscape and ornamental trees	Yes the pest on plants for planting has an economic impact	The extent of the economic and environmental impact
Available measures (Section 3.6)	Yes, measures are available. Emergency measures have been implemented in Europe since February 2014 and updated several times	Yes, among which screen house production of plants for planting, thermotherapy of dormant plants, certification and control of insect vectors	Thermotherapy only assessed on grapevine and pecan walnut
Conclusion on pest categorisation (Section 4)	Yes, all the criteria are met for consideration as a potential quarantine pest	No, <i>X. fastidiosa</i> is currently regulated as a quarantine pest. It is not considered as a RNQP as once entered, the pathogen may not only spread via plants for planting but also by insect vector transmission	
Aspects of assessment to focus on/ scenarios to address in future if appropriate			

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Abbreviations

DG SANTÉDirectorate General for Health and Food SafetyEPPOEuropean and Mediterranean Plant Protection Organizati
EPPO European and Mediterranean Plant Protection Organizati
FAO Food and Agriculture Organization
IAP inoculation access period
IPPC International Plant Protection Convention
ISPP-CTPPB International Society of Plant Pathology Committee on the
Taxonomy of Plant Pathogenic Bacteria
LAMP loop isothermal amplification
MLST multilocus sequence typing
MS Member State
PCR polymerase chain reaction
PLH EFSA Panel on Plant Health
RNQP regulated non-quarantine pest
ST sequence type
TFEU Treaty on the Functioning of the European Union
ToR Terms of Reference



Annex A – Host plants reported worldwide of the multilocus sequence type (ST) of *Xylella fastidiosa* found in Europe

Plant species	Subspecies	ST	Location	Country	
Acacia dealbata	acia dealbata multiplex ST6 and/or ST7		Corsica	France	
Acacia saligna	multiplex	ST81	Majorca	Spain	
Acacia saligna	pauca	ST53	Lecce province	Italy	
Acacia sp.	multiplex	ST81	Majorca	Spain	
Acacia sp.	, pauca	ST80	Ibiza	Spain	
Acer pseudoplatanus	multiplex	ST6 and/or ST7	Corsica	France	
Acer sp.	fastidiosa	ST1	Alameda (CA)	United States of America	
Anthyllis hermanniae	multiplex	ST6 and/or ST7	Corsica	France	
Artemisia arborescens	multiplex	ST6 and/or ST7	Corsica	France	
Asparagus acutifolius	multiplex	ST6 and/or ST7	Corsica	France	
Asparagus acutifolius	pauca	ST53	Lecce province	Italy	
Asparagus acutifolius	multiplex	ST6 and/or ST7	Corsica	France	
Calicotome spinosa	fastidiosa	ST1	Majorca	Spain	
Calicotome villosa	multiplex	ST6 and/or ST7	Corsica	France	
Catharanthus roseus	pauca	ST53	Lecce province	Italy	
Cercis occidentalis	fastidiosa	ST1	Riverside (CA)	United States of America	
Cercis siliquastrum	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France	
Chenopodium album	pauca	ST53	Lecce province	Italy	
Cistus creticus	multiplex	ST6 and/or ST7	Corsica	France	
Cistus creticus	pauca	ST53	Lecce province	Italy	
Cistus monspeliensis	fastidiosa	ST1	Majorca	Spain	
Cistus monspeliensis	multiplex	ST6 and/or ST7*	Corsica	France	
, Cistus salviifolius	, multiplex	ST6 and/or ST7	Corsica	France	
Cistus sp.	, multiplex	unknown	Corsica	France	
Citrus sinensis	fastidiosa	ST1	Polk Co. (FL)	United States of America	
Coffea arabica	pauca	ST53	San Jose Province	Costa Rica	
Coffea arabica	unknown	ST76	Costa Rica	Costa Rica	
Coronilla valentina	multiplex	ST6 and/or ST7	Corsica	France	
<i>Coronilla valentina</i> subsp. <i>glauca</i>	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France	
Cytisus scoparius	multiplex	ST6 and/or ST7	Corsica	France	
<i>Cytisus</i> sp.	, multiplex	ST6 and/or ST7	Corsica	France	
Cytisus villosus	multiplex	ST6 and/or ST7	Corsica	France	
, Dodonaea viscosa	, pauca	ST53	Lecce province	Italy	
Eremophila maculata	, pauca	ST53	Lecce province	Italy	
Erigeron bonariensis	, pauca	ST53	Lecce province	Italy	
Erigeron sumatrensis	pauca	ST53	Lecce province	Italy	
Euphorbia terracina	pauca	ST53	Lecce province	Italy	
Euryops chrysanthemoides	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France	
, Ficus carica	multiplex	ST81	Majorca	Spain	
Ficus carica	multiplex	ST81	Menorca	Spain	
Fraxinus angustifolia	multiplex	ST81	Majorca	Spain	
Genista corsica	multiplex	ST6 and/or ST7	Corsica	France	
Genista ephedroides	multiplex	ST6 and/or ST7	Corsica	France	



Plant species	Subspecies	ST	Location	Country	
Genista lucida	fastidiosa	ST1	Majorca	Spain	
Genista x spachiana	multiplex	ST6 and/or ST7	Corsica	France	
<i>Genista</i> sp.	multiplex	unknown	Corsica	France	
Grevillea juniperina	, pauca	ST53	Lecce province	Italy	
Hebe sp.	multiplex	ST6 and/or ST7	Corsica	France	
Hebe sp.	, pauca	ST53	Lecce province	Italy	
Helichrysum italicum	multiplex	ST6 and/or ST7	Corsica	France	
Helichrysum italicum	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France	
Heliotropium	pauca	ST53	Lecce province	Italy	
europaeum					
Juglans regia	fastidiosa	ST1	Majorca	Spain	
Laurus nobilis	pauca	ST53	Lecce province	Italy	
Lavandula angustifolia	multiplex	ST6 and/or ST7	Corsica	France	
Lavandula angustifolia	pauca	ST53	Lecce province	Italy	
Lavandula dentata	multiplex	ST81	Majorca	Spain	
Lavandula dentata	pauca	ST80	Ibiza	Spain	
Lavandula dentata	multiplex	ST6 and/or ST7	Corsica	France	
<i>Lavandula</i> sp.	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France	
Lavandula sp.	multiplex	ST6 and/or ST7	Corsica	France	
<i>Lavandula</i> sp.	unknown	unknown	Ibiza	Spain	
Lavandula stoechas	multiplex	ST6 and ST7	Corsica	France	
Lavandula stoechas	pauca	ST53	Lecce province	Italy	
Lavandula x heterophylla	multiplex	ST6 and/or ST7	Corsica	France	
Lavandula x intermedia	multiplex	ST6 and/or ST7	Corsica	France	
Lavandula x intermedia	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France	
Medicago sativa	fastidiosa	ST1	California (CA)	United States of America	
Medicago sativa	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France	
Metrosideros excelsa	multiplex	ST6 and/or ST7	Corsica	France	
<i>Metrosideros</i> sp.	fastidiosa	ST1	Orange (CA)	United States of America	
Myoporum insulare	pauca	ST53	Lecce province	Italy	
Myrtus communis	multiplex	ST6 and/or ST7	Corsica	France	
Myrtus communis	pauca	ST53	Lecce province	Italy	
Nerium oleander	pauca	ST53	San Jose province	Costa Rica	
Nerium oleander	раиса	ST53	Lecce province	Italy	
Nerium oleander	unknown	unknown	Ibiza	Spain	
Nerium oleander	unknown	unknown	Majorca	Spain	
Olea europaea	multiplex	ST7	Riverside (CA)	United States of America	
Olea europaea	multiplex	ST81	Majorca	Spain	
Olea europaea	multiplex	ST81	Menorca	Spain	
Olea europaea	pauca	ST53	Brindisi province	Italy	
Olea europaea	pauca	ST53	Lecce province	Italy	
Olea europaea	pauca	ST80	Ibiza	Spain	
Olea europaea	multiplex	ST6	Community of Madrid	Spain	
Olea europaea subsp. sylvestris	multiplex	ST81	Majorca	Spain	
, Olea europaea subsp. sylvestris	multiplex	ST81	Menorca	Spain	



Plant species	Subspecies	ST	Location	Country
<i>Olea europaea</i> subsp. <i>sylvestris</i>	pauca	ST80	Ibiza	Spain
<i>Olea</i> sp.	multiplex	ST7	Los Angeles (CA)	United States of America
<i>Olea</i> sp.	pauca	ST53	Lecce province	Italy
Pelargonium fragrans	pauca	ST53	Lecce province	Italy
Pelargonium graveolens	multiplex	ST6 and/or ST7	Corsica	France
Pelargonium sp.	multiplex	ST6 and/or ST7	Corsica	France
Periwinkle (common name)	pauca	ST53	Lecce province	Italy
Phagnalon saxatile	multiplex	ST6 and/or ST7	Corsica	France
Phillyrea latifolia	pauca	ST53	Lecce province	Italy
Pluchea odorata	fastidiosa	ST1	Riverside (CA)	United States of America
Polygala myrtifolia	fastidiosa	ST1	Majorca	Spain
Polygala myrtifolia	multiplex	ST6 and/or ST7	Corsica	France
Polygala myrtifolia	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France
Polygala myrtifolia	multiplex	ST7	Majorca	Spain
Polygala myrtifolia	multiplex	ST79	Corsica	France
Polygala myrtifolia	multiplex	ST81	Majorca	Spain
Polygala myrtifolia	multiplex	ST81	Menorca	Spain
Polygala myrtifolia	pauca	ST53	Provence-Alpes-Côte d'Azur	France
Polygala myrtifolia	pauca	ST53	Lecce province	Italy
Polygala myrtifolia	pauca	ST80	Ibiza	Spain
Polygala myrtifolia	sandyi	ST76	Corsica	France
<i>Polygala</i> sp.	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France
Polygala x dalmaisiana	multiplex	unknown	Corsica	France
Polygala x grandiflora nana	multiplex	unknown	Corsica	France
Prunus avium	fastidiosa	ST1	Majorca	Spain
Prunus avium	fastidiosa	ST1	San Bernardino (CA)	United States of America
Prunus avium	pauca	ST53	Lecce province	Italy
Prunus avium	multiplex	unknown	Provence-Alpes-Côte d'Azur	France
Prunus cerasifera	multiplex	ST6 and/or ST7	Corsica	France
Prunus cerasifera	multiplex	unknown	Provence-Alpes-Côte d'Azur	France
Prunus cerasus	multiplex	unknown	Provence-Alpes-Côte d'Azur	France
Prunus domestica	multiplex	ST81	Majorca	Spain
Prunus dulcis	fastidiosa	ST1	Majorca	Spain
Prunus dulcis	fastidiosa	ST1	California	United States of America
Prunus dulcis	fastidiosa	ST1	Fresno (CA)	United States of America
Prunus dulcis	fastidiosa	ST1	Kern County (CA)	United States of America
Prunus dulcis	fastidiosa	ST1	Riverside (CA)	United States of America
Prunus dulcis	fastidiosa	ST1	San Bernardino (CA)	United States of America
Prunus dulcis	fastidiosa	ST1	San Joaquin Valley (CA)	United States of America



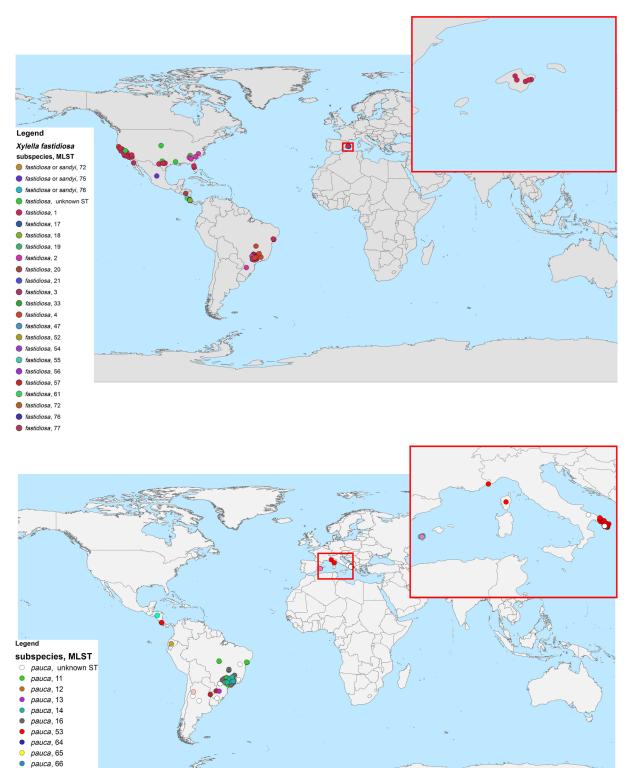
Plant species	Subspecies	ST	Location	Country
Prunus dulcis	fastidiosa	ST1	Stanislaus (CA)	United States of America
Prunus dulcis	fastidiosa	ST1	Tulare (CA)	United States of America
Prunus dulcis	multiplex	ST6	Alicante province	Spain
Prunus dulcis	multiplex	ST6	Solano (CA)	United States of America
Prunus dulcis	multiplex	ST6 and/or ST7	Corsica	France
Prunus dulcis	multiplex	ST7	Kern County (CA)	United States of America
Prunus dulcis	multiplex	ST81	Majorca	Spain
Prunus dulcis	multiplex	ST81	Menorca	Spain
Prunus dulcis	pauca	ST53	Lecce province	Italy
Prunus dulcis	multiplex	ST6	Riverside (CA)	United States of America
Prunus dulcis	multiplex	ST6	San Joaquin Valley (CA)	United States of America
Prunus dulcis	multiplex	ST6	Solano (CA)	United States of America
Prunus dulcis	multiplex	ST7	Glenn County (CA)	United States of America
Prunus dulcis	multiplex	ST7	Kern County (CA)	United States of America
Prunus dulcis	multiplex	ST7	Majorca	Spain
Prunus dulcis	pauca	ST80	Ibiza	Spain
Prunus persica	pauca	ST53	Corsica	France
Prunus sp.	multiplex	ST7	Kern County (CA)	United States of America
Quercus ilex	pauca	ST53	Corsica	France
Quercus suber	multiplex	ST6 and/or ST7	Corsica	France
Rhamnus alaternus	fastidiosa	ST1	Majorca	Spain
Rhamnus alaternus	pauca	ST53	Lecce province	Italy
Rhamnus alaternus	multiplex	ST81	Menorca	Spain
Rosa canina	multiplex	ST6 and/or ST7	Corsica	France
<i>Rosa</i> sp.	multiplex	unknown	Corsica	France
Rosmarinus officinalis	multiplex	ST6 and/or ST7	Corsica	France
Rosmarinus officinalis	multiplex	ST81	Menorca	Spain
Rosmarinus officinalis	pauca	ST53	Lecce province	Italy
Rosmarinus officinalis	multiplex	ST81	Majorca	Spain
Rosmarinus officinalis	multiplex	ST80	Ibiza	Spain
Salvia mellifera	multiplex	ST7	Riverside (CA)	United States of America
Sambucus canadensis	fastidiosa	ST1	Leesburg (FL)	United States of America
Spartium junceum	fastidiosa	ST1	Riverside (CA)	United States of America
Spartium junceum	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France
Spartium junceum	multiplex	ST6 and/or ST7	Corsica	France
Spartium junceum	pauca	ST53	Lecce province	Italy
Spartium sp.	, multiplex	unknown	Corsica	France
Vinca minor	pauca	ST53	Lecce province	Italy



Plant species	Subspecies	ST	Location	Country
Vitis aestivalis	fastidiosa	ST1	Val Verde (TX)	United States of America
Vitis girdiana	fastidiosa	ST1	Riverside (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Baja California (MX)	Mexico
<i>Vitis</i> sp.	fastidiosa	ST1	Majorca	Spain
<i>Vitis</i> sp.	fastidiosa	ST1	Alameda (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	California (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Florida (FL)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Georgia (GA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Napa Co. (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Riverside (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	San Joaquin Valley (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	San Luis Obispo (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Santa Barbara(CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Santa Cruz (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Tulare (CA)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Val Verde (TX)	United States of America
<i>Vitis</i> sp.	fastidiosa	ST1	Ventura (CA)	United States of America
Vitis vinifera	fastidiosa	ST1	Majorca	Spain
Vitis vinifera	fastidiosa	ST1	Blanco Co. (TX)	United States of America
Vitis vinifera	fastidiosa	ST1	Gillespie (TX)	United States of America
Vitis vinifera	fastidiosa	ST1	Napa Co. (CA)	United States of America
Vitis vinifera	fastidiosa	ST1	Riverside (CA)	United States of America
Vitis vinifera	fastidiosa	ST1	San Bernardino (CA)	United States of America
Vitis vinifera	fastidiosa	ST1	Travis (TX)	United States of America
Westringia fruticosa	multiplex	ST6 and/or ST7	Provence-Alpes-Côte d'Azur	France
Westringia fruticosa	pauca	ST53	Lecce province	Italy
Westringia glabra	pauca	ST53	Lecce province	Italy

Grey highlights: non-European countries. *: mixed infection.





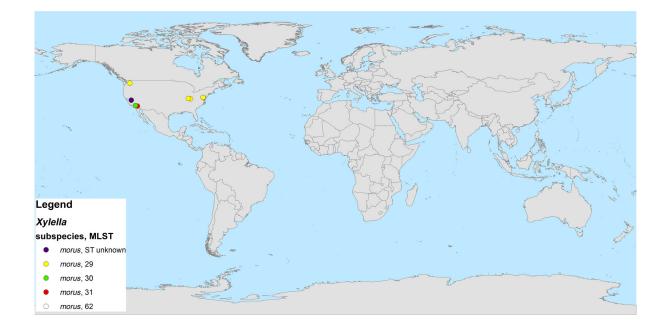
Annex B – Distribution maps of Xylella fastidiosa subspecies

pauca, 68
 pauca, 69
 pauca, 70
 pauca, 71
 pauca, 73 and 53
 pauca, 74
 pauca, 78
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 pauca, 80
 pauca, to determine

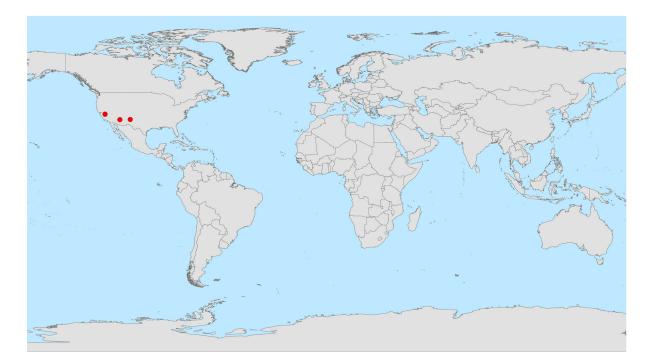




sandyi, 76







Legend subspecies, MLST • tashke, unknown ST

Annex C – List of the host plants in France, Italy and Spain

	Frar	ice	Italy	Spain					
Hosts	Corsica	PACA	Southern Apulia	Alicante	Balearic Islands (Ibiza)	Balearic Islands (Majorca)	Balearic Islands (Menorca)	Madrid Province	
Acacia dealbata	ST6–ST7								
Acacia saligna		ST6–ST7	ST53			ST81			
Acacia sp.					ST80	ST81			
Acer pseudoplatanus	ST6–ST7								
Anthyllis hermanniae	ST6–ST7								
Artemisia arborescens	ST6–ST7								
Asparagus acutifolius	ST6–ST7		ST53						
Calicotome spinosa						ST1			
Calicotome villosa	ST6–ST7								
Catharanthus sp.			ST53						
Cercis siliquastrum		ST6–ST7							
Chenopodium album			ST53						
Cistus creticus	ST6–ST7		ST53						
Cistus monspeliensis	ST6–ST7					ST1			
Cistus salviifolius	ST6–ST7								
Coronilla glauca		ST6–ST7							
Coronilla valentina	ST6–ST7								
Cytisus scoparius	ST6–ST7								
<i>Cytisus</i> sp.	ST6–ST7								
Cytisus villosus	ST6–ST7								
Dodonaea viscosa			ST53						
Eremophila maculata			ST53						
Erigeron bonariensis			ST53						
Erigeron sumatrensis			ST53						
Euphorbia terracina			ST53						
Euryops chrysanthemoides		ST6–ST7							
Ficus carica						ST81	ST81		
Fraxinus angustifolia						ST81			



Hosts	France		Italy	Spain					
	Corsica	PACA	Southern Apulia	Alicante	Balearic Islands (Ibiza)	Balearic Islands (Majorca)	Balearic Islands (Menorca)	Madrid Province	
Genista corsica	ST6–ST7								
Genista ephedroides	ST6–ST7								
Genista lucida						ST1			
Genista x spachiana (syn. Cytisus racemosus)	ST6–ST7								
Grevillea juniperina			ST53						
Hebe sp.	ST6–ST7		ST53						
Helichrysum italicum	ST6–ST7								
Heliotropium europaeum			ST53						
Juglans regia						ST1			
Laurus nobilis			ST53						
Lavandula angustifolia	ST6–ST7		ST53						
Lav-ula dentata	ST6–ST7				ST80	ST81			
Lav-ula stoechas	ST6–ST7		ST53						
<i>Lav-ula</i> x allardii (syn. <i>Lav-ula</i> x heterophylla)	ST6–ST7								
Lav-ula x intermedia	ST6–ST7	ST6–ST7							
Lav-ula sp.	ST6–ST7	ST6–ST7							
Metrosideros excelsa	ST6–ST7								
Medicago sativa		ST6–ST7							
Myoporum insulare			ST53						
Myrtus communis	ST6–ST7		ST53						
Nerium oleander			ST53		Pending assignment	Pending assignment			
Olea europaea			ST53		ST80	ST81	ST81	ST6	
Olea europaea var. sylvestris					ST80	ST81	ST81		
Pelargonium x fragrans			ST53						
Pelargonium graveolens	ST6–ST7								
Pelargonium sp.	ST6–ST7								
Phagnalon saxatile	ST6–ST7								
Phillyrea latifolia			ST53						



	Franc	France		Spain					
Hosts	Corsica	PACA	Southern Apulia	Alicante	Balearic Islands (Ibiza)	Balearic Islands (Majorca)	Balearic Islands (Menorca)	Madrid Province	
Polygala myrtifolia	ST6, ST7, ST76, ST79	ST6, ST7, ST53	ST53		ST80	ST1, ST7, ST81	Pending assignment		
Prunus avium			ST53			ST1			
Prunus cerasifera	ST6–ST7								
Prunus cerasus		ST6–ST7							
Prunus domestica						ST81			
Prunus dulcis	ST6–ST7		ST53	ST6	ST80	ST1, ST7, ST81	ST81		
Quercus ilex	ST53								
Quercus suber	ST6–ST7								
Rhamnus alaternus			ST53			ST1	ST81		
Rosa canina	ST6–ST7								
Rosmarinus officinalis	ST6–ST7		ST53		ST80	ST81	ST81		
Spartium junceum	ST6–ST7	ST6–ST7	ST53						
Vinca sp.			ST53						
Vitis vinifera						ST1			
Westringia fruticose		ST6–ST7	ST53						
Westringia glabra			ST53						



Annex D – Interceptions of commodities of *Xylella fastidiosa* from the third countries

Year	Country of origin	Plant	Number	ST	Reference/Source
Unknown	Costa Rica	Coffea arabica	1	ST76	Loconsole et al. (2016)
Unknown	Costa Rica	Coffea arabica	1	ST72	Loconsole et al. (2016)
Unknown	Costa Rica	Coffea arabica	1	ST72	Loconsole et al. (2016)
Unknown	Unknown	Coffea arabica	1	ST73	Loconsole et al. (2016)
2012	Ecuador	Coffea arabica	1	ST74	Jacques et al. (2016)
2012	Ecuador	Coffea arabica	1	ST74	Jacques et al. (2016)
2012	Mexico	Coffea canephora	1	ST75	Jacques et al. (2016)
2014	Costa Rica	Coffea arabica	1	NA	Bergsma Vlami et al. (2015)
2014	Honduras	Coffea arabica	1	NA	Bergsma Vlami et al. (2015)
2014	Costa Rica	Coffea arabica	1	ST72	Bergsma Vlami et al. (2017)
2014	Costa Rica	Coffea arabica	1	ST76	Bergsma Vlami et al. (2017)
2014	Costa Rica	Coffea arabica	1	ST76	Bergsma Vlami et al. (2017)
2014	Costa Rica	Coffea arabica	1	ST53	Bergsma Vlami et al. (2017)
2014	Costa Rica	Coffea arabica	1	ST77	Bergsma Vlami et al. (2017)
2014	Costa Rica	Coffea arabica	1	ST73 and ST53	Bergsma Vlami et al. (2017)
2014	Costa Rica	Coffea arabica	1	NA	Bergsma Vlami et al. (2017)
2014	Honduras	Coffea arabica	1	NA	Bergsma Vlami et al. (2017)
2015	Latin American countries	<i>Coffea</i> sp.	1	NA	Denance et al. (2017)
2015	Latin American countries	<i>Coffea</i> sp.	1	ST72	Denance et al. (2017)
2015	Latin American countries	<i>Coffea</i> sp.	1	ST76	Denance et al. (2017)
2015	Latin American countries	<i>Coffea</i> sp.	1	St53	Denance et al. (2017)
2015	Unknown	Coffea sp.	7	NA	Europhyt access: 12/6/2018
2015	Unknown	Coffea arabica	13	NA	Europhyt access: 12/6/2018
2015	Brazil	Mandevilla sanderi	1	NA	Europhyt access: 12/6/2018
2015	Costa Rica	Coffea arabica	5	NA	Europhyt access: 12/6/2018
2015	Costa Rica	Coffea sp.	7	NA	Europhyt access: 12/6/2018
2015	Honduras	Coffea arabica	7	NA	Europhyt access: 12/6/2018
2015	Honduras	Coffea sp.	1	NA	Europhyt access: 12/6/2018
2016	Unknown	<i>Coffea</i> sp.	1	NA	Europhyt access: 12/6/2018
2016	Mexico	Pelargonium x hortorum	1	NA	Europhyt access: 12/6/2018
2016	Netherlands	Coffea arabica	3	NA	Europhyt access: 12/6/2018
2017	USA	Juglans	1	NA	Europhyt access: 12/6/2018
2018	USA	Rubus fruticosus	1	NA	Europhyt access: 12/6/2018
2018	USA	Rubus idaeus	1	NA	Europhyt access: 12/6/2018

Grey highlights: Information from the literature.



Annex E – List of figures and tables

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