2017 First consultation

1 July – 30 September 2017

Compiled comments for Draft diagnostic protocol for *Xylella fastidiosa* (2002-024)

Summary of comments

Name	Summary
EPPO Σ	Finalised by the EPPO Secretariat on behalf of its 51 Member Countries.
European Union	Comments finalised by the European Commission on behalf of the EU and its 28 Member States on 29/09/2017.
Samoa	no further comments
South Africa	No comments from the National Plant Protection Organisation of South Africa.

#	Para	Text	Comment
1	G	(General Comment)	**Cameroon** Ce protocole de diagnostic est très pertinent. Il est détaillé et couvre l'ensemble des étapes pour identifier l'attaque. Devant la sévérité de Xylella, cet outil sera le bienvenu. Compte tenu de la gravité de ce problème, le développement de kits de détection rapide à bas cout devraient aider les pays les moins avancées du point de vue technique ou en équipements de laboratoire à disposer d'un moyen d'analyse *Category : TECHNICAL*
2	G	(General Comment)	**Myanmar** This disease is absent in Myanmar. *Category : SUBSTANTIVE*
3	G	(General Comment)	**Peru** We agree with the DRAFT ANNEX to ISPM 27– Xylella fastidiosa (2004-024) *Category : TECHNICAL*
4	G	(General Comment)	**United States of America** The United States has no comments on this draft standard. *Category : SUBSTANTIVE*
5	G	(General Comment)	**Canada** Canada supports draft annex to ISPM 27 on Xylella fastidiosa. Minor editorial comments presented. A universal change to be made in the draft so the sentence starts with the full genus (Xylella) and not the abbreviation. *Category : SUBSTANTIVE*
6	G	(General Comment)	**European Union** Some restructuring in the section on Detection is proposed to group the parts related to plants and those related to vectors. E.g. Suggestion to move paragraphs 87, 90 and 88 after section

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			3.2.7 which deals with the storage of plant samples. Suggestion to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
7	G	(General Comment)	**Guyana** Guyana has no objection to this Annex *Category : SUBSTANTIVE*
8	G	(General Comment)	**Nicaragua** Nicaragua esta de acuerdo con el desarrollo de este protocolo que permita la identificación de subespecies de X. fastidiosa a través de métodos moleculares. *Category : TECHNICAL*
9	G	(General Comment)	**Nicaragua** Nicaragua propone la inclusión en el protocolo de diagnóstico de PCR, la especificidad en los procedimientos para la identificación de X. fastidiosa subespecie pauca y X. fastidiosa subespecie tashke que afectan los cultivos de café, cítricos, palma africana y plantas ornamentales respectivamente. Agregar en el párrafo 31. *Category : TECHNICAL*
10	G	(General Comment)	**Barbados** Barbados has no objections to this draft annex. *Category : SUBSTANTIVE*
11	G	(General Comment)	**Panama** Solicitar dentro de la norma, opciones para el manejo de inhibidores de PCR, ya que la norma lo menciona frecuentemente. *Category : EDITORIAL*
12	G	(General Comment)	**Panama** El presente anexo de norma, es un instrumento que permite homogenizar el proceso de identificación de la especie Xylella fastidiosa, mas no da, los lineamientos para identificar sub especies o genotipos de la bacteria. *Category : SUBSTANTIVE*
13	G	(General Comment)	**EPPO** Some restructuring in the section on Detection is proposed to group the parts related to plants and those related to vectors. E.g. Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. Category : SUBSTANTIVE (41) France (8 Aug 2017 8:30) *Category : SUBSTANTIVE*
14	G	(General Comment)	**Viet Nam** Vietnam would like to request providing the method for preserving the suspectedly infested Xylella fastidiosa samples/ plant sap (after extraction) in this draft. Because, In international trade, it's necessary to preserve the samples/plant sap (after extraction) after

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			performing a diagnosis for further technical argument/discussion. *Category : SUBSTANTIVE*
15	G	(General Comment)	**Tajikistan** I support the document as it is and I have no comments *Category : SUBSTANTIVE*
16	G	(General Comment)	**Bahamas** The distribution and expansion of X. fastidiosa within recent years and its difficulty to isolate poses a major phytosanitary risk particularly to our citrus industry. The Bahamas supports the adoption of this diagnostic protocol. *Category : SUBSTANTIVE*
17	G	(General Comment)	**PPPO** I do agree with the Draft ISPM. I have no other comments to make. *Category : EDITORIAL*
18	G	(General Comment)	**New Zealand** Have no comments to make on the draft *Category : SUBSTANTIVE*
19	G	(General Comment)	**Thailand** agree with the proposed draft DP for Xylella fastidiosa *Category : SUBSTANTIVE*
20	G	(General Comment)	**Lao People's Democratic Republic** Lao PDR agreed with this drafted ISPM. *Category : SUBSTANTIVE*
21	G	(General Comment)	**Honduras** HONDURAS NO TIENE COMENTARIOS *Category : TECHNICAL*
22	G	(General Comment)	**Lao People's Democratic Republic** Lao PDR has no comment on DRAFT ANNEX to ISPM 27– Xylella fastidiosa (2004-024) *Category : SUBSTANTIVE*
23	G	(General Comment)	**Colombia** El Instituto Colombiano Agropecuario (ICA), como Organización Nacional de Protección Fitosanitaria de Colombia, revisó y analizó el borrador en cuestión, encontrando que el protocolo de diagnóstico propuesto cumple con los requisitos y esta actualizado de acuerdo con la evidencia científica existente. No obstante, teniendo en cuenta las características variables de los síntomas que causa Xylella fastidiosa, se solicita mantener e incluir fotografías de los síntomas más relevantes, las cuales serían de gran utilidad para países que no registran esta plaga. *Category : TECHNICAL*
24	G	(General Comment)	**China** A table (please see the model) contained all the PCR detection methods and their efficiencies should be added as appendix. It could be let technicians know how each method work (target subspecies and sensitivity, etc.) Table The result of PCR detection for subspecies of Xylella

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			fastidiosa Conventional PCR ; Real-time PCR ; LAMP Primer : RST31/33 ; Set A,B,C ; FXYgyr499/ 907 ; XF-F ; XF16S ; XF-F Reference : Minsavage et al. 1994 ; Rodrigues et al. 2003 ; Rodrigues et al. 2003 ; Harper et al. 2010 ; Li et al. 2013 ; Harper et al. 2010 Target sequence rpoD gene ; 16S ; gyrB gene ; rimM gene ; 16S ; rimM gene X. fastidiosa subsp. fastidiosa + + + + N + X. fastidiosa subsp. multiplex N N N + N + X. fastidiosa subsp. sandyi N N N + N + X. fastidiosa subsp. tashke N N N N N + X. fastidiosa subsp. pauca N N N + N + X. fastidiosa subsp. morus N N N N N + X. taiwanensis N N N N N - +, positive; -,negative; N, unkown. *Category : TECHNICAL*
25	G	(General Comment)	**China** In 3.4.3, the PCR method with primer 272-1-int/ 272-2-int (Pooler &Hartung, 1995) is advised to add in Conventional PCR instead of the primer RST31/RST33 (Minsavage et al., 1994). Because some American X. fastidiosa strains from red oak and turkey oak and several strains from grape vines were not detected with this primer RST31/RST33. Reference : PCR assays for the detection of Xylella fastidiosa Review and comparison of published protocols (https://www.eppo.int/MEETINGS/2015_meetings/diag-bact/06_Reisenzein/index.html) . *Category : TECHNICAL*
26	G	(General Comment)	**China** X.fastidiosa is genetically diverse and consists of six sub-species, but the PCR assay designed by Minsavage et al. (1994) is not specific to all the six sub-species, Thus we should note which are positive and negative. The sample will be considered negative if the PCR assay designed by Minsavage et al. (1994) is not specific to some sub-species. *Category : SUBSTANTIVE*
27	G	(General Comment)	**China** Contents of 94 and 101 are the same. It was suggested that footnote 101 should be deleted. *Category : EDITORIAL*
28	G	(General Comment)	**China** 3.2.2 sample collection and 3.2.6 vector sample collection should be together. Contents of 3.2.2 and 3.2.6 are similar, we suggested that should be put together. *Category : EDITORIAL*

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29	G	(General Comment)	**OIRSA** Ask the coordinator of the present normative annex, options for the management of PCR inhibitors, because it frequently mentions. *Category : TECHNICAL*
30	G	(General Comment)	**OIRSA** The present normative annex is an instrument that allows to homogenize the process of identification of the species Xylella fastidiosa, but it does not give the guidelines to identify sub species or genotypes of the bacterium. *Category : SUBSTANTIVE*
31	G	(General Comment)	**Algeria** No Comment *Category : TECHNICAL*
32	G	(General Comment)	**Cuba** El Protocolo de Diagnóstico de Xylella fastidiosa es muy detallado en cuanto a los síntomas en los diferentes hospedantes y las técnicas que se pueden emplear en el diagnóstico, sugerimos que de las otras técnicas de detección serológica que se mencionan como inmunofluorescencia, inmunoblot, etc, se detallen mas las mismas ya que son opciones diferentes a emplear que pueden ser útiles para laboratorios que no empleen comunmente ELISA y no posean lector de la misma. *Category : SUBSTANTIVE*
33	31	Scope of the protocol is for the detection and identification of *X. fastidiosa.* Some discussion on whether the protocol should focus on identification of specific strains of *X. fastidiosa*, e.g. the *X. fastidiosa* citrus variegated chlorosis strains. Some information has been included to enable identification of subspecies.	**Nepal** No any comments. *Category : EDITORIAL*
34	31	Scope of the protocol is for the detection and identification of *X. fastidiosa.* Some discussion on whether the protocol should focus on identification of specific strains of *X. fastidiosa*, e.g. the *X. fastidiosa* citrus variegated chlorosis strains. Some information has been included to enable identification of subspecies.	**Nicaragua** Scope of the protocol is for the detection and identification of X. fastidiosa. Some discussion on whether the protocol should focus on identification of specific strains of X. fastidiosa, e.g. the X. fastidiosa citrus variegated chlorosis strains, X. fastidiosa pauca, X. fastidiosa tashke. Some information has been included to enable identification of subspecies *Category : TECHNICAL*
35	31	Scope of the protocol is for the detection and identification of subspecies of X. fastidiosa~~*X. fastidiosa*~~. Some discussion on whether the protocol should focus on identification of specific strains of *X. fastidiosa*, e.g. the *X. fastidiosa* citrus variegated chlorosis strains. Some information has been included to enable identification of subspecies.	**China** There are six subspecies of X. fastidiosa validated or near validated now, each subspecies was different in genetics and pathogenicity. This protocol only for the species level, we think it should be updated. *Category : SUBSTANTIVE*
36	43	**1. Pest Information**	**Czech Republic** the link does not work in part 1. *Category : TECHNICAL*
37	44	*Xylella fastidiosa* Wells *et al.* (1987) is a xylem-limited bacterium that is the causal agent of many economically important plant diseases of agronomic and horticultural crops such as *Vitis vinifera*, *Prunus domestica*, *Prunus dulcis*, *Citrus sinensis*, *Olea europaea*, *Ulmus* spp. and *Quercus* spp. *X. fastidiosa* has a wide, expanding host range	**European Union** EPPO, 2015: This information is probably not up to date. It is probably wiser to refer to EPPO Global Database https://gd.eppo.int/taxon/XYLEFA This reference may also cover the list of new host plants.

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		and comprehensive lists of susceptible hosts are available at http://www.ec.europe.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm and https://www.efsa.europa.eu/fr/efsajournal/pub/4378. *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2015).	*Category : SUBSTANTIVE*
38	44	*Xylella fastidiosa* Wells *et al.* (1987) is a xylem-limited bacterium that is the causal agent of many economically important plant diseases of agronomic and horticultural crops such as *Vitis vinifera*, *Prunus domestica*, *Prunus dulcis*, *Citrus sinensis*, *Olea europaea*, *Ulmus* spp. and *Quercus* spp. *X. fastidiosa* has a wide, expanding host range and comprehensive lists of susceptible hosts are available at http://www.ec.europe.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm and https://www.efsa.europa.eu/fr/efsajournal/pub/4378. *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2015).	**European Union** The link provided for the EU website is not correct. It should be replaced by https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en . *Category : EDITORIAL*
39	44	*Xylella fastidiosa* Wells *et al.* (1987) is a xylem-limited bacterium ~~that is -~~ the causal agent of many economically important plant diseases of agronomic and horticultural crops such as *Vitis vinifera*, *Prunus domestica*, *Prunus dulcis*, *Citrus sinensis*, *Olea europaea*, *Ulmus* spp. and *Quercus* spp. *X. fastidiosa* has a wide, expanding host range and comprehensive lists of susceptible hosts are available at http://www.ec.europe.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm and https://www.efsa.europa.eu/fr/efsajournal/pub/4378. *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2015).	**Ghana** *Category : EDITORIAL*
40	44	*Xylella fastidiosa* Wells *et al.* (1987) is a xylem-limited bacterium that is the causal agent of many economically important plant diseases of agronomic and horticultural crops such as *Vitis vinifera*, *Prunus domestica*, *Prunus dulcis*, *Citrus sinensis*, *Olea europaea*, *Ulmus* spp. and *Quercus* spp. *X. fastidiosa* has a wide, expanding host range and comprehensive lists of susceptible hosts are available at http://www.ec.europe.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm and https://www.efsa.europa.eu/fr/efsajournal/pub/4378. *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas	**EPPO** The link provided for the EU website is not correct. It shuold be replaced by https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en *Category : EDITORIAL*

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		(Almeida and Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2015).	
41	44	*Xylella fastidiosa* Wells *et al.* (1987) is a xylem-limited bacterium that is the causal agent of many economically important plant diseases of agronomic and horticultural crops such as *Vitis vinifera*, *Prunus domestica*, *Prunus dulcis*, *Citrus sinensis*, *Olea europaea*, *Ulmus* spp. and *Quercus* spp. *X. fastidiosa* has a wide, expanding host range and comprehensive lists of susceptible hosts are available at http://www.ec.europe.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm and https://www.efsa.europa.eu/fr/efsajournal/pub/4378. *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2015).	**EPPO** EPPO, 2015: This information is probably not up to date. It is probably wiser to refer to EPPO Global Database https://gd.eppo.int/taxon/XYLEFA This reference may also cover the list of new host plants. *Category : SUBSTANTIVE*
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43	44	*Xylella fastidiosa* Wells *et al.* (1987) is a xylem-limited bacterium that is the causal agent of many economically important plant diseases of agronomic and horticultural crops such as *Vitis vinifera*, *Prunus domestica*, *Prunus dulcis*, *Citrus sinensis*, *Olea europaea*, *Ulmus* spp. and *Quercus* spp. *X. fastidiosa* has a wide, expanding host range and comprehensive lists of susceptible hosts are available at http://www.ec.europe.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm and https://www.efsa.europa.eu/fr/efsajournal/pub/4378. *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2015).	**Australia** URL doesn't work *Category : EDITORIAL*
44	45	*X. fastidiosa* is genetically diverse and consists of ~~six sub-species~~subspecies. *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Vitis vinifera*, *Prunus dulcis*, *Medicago sativa* and *Acer* spp. (Schuenzel *et al.*,	**European Union** There is reference to six subspecies, however, later on (IPPC & 2 Taxonomic information) the EPPO text has been adopted where only the accepted subspecies are mentioned. It could be considered to omit (in paragraph 1 Pest information) the part of the subspecies.

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		2005). *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Prunus dulcis*, *Prunus persica*, *Quercus* spp. and *Platanus occidentalis*. *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *tashke* has been isolated from the ornamental tree *Chitalpa tashkentensis* (Randall *et al.*, 2009). *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp. Finally, *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* and *Coffea* species, and *Olea europaea*. ~~A different *Xylella* species is associated with pear leaf scorch in Taiwan Province of China (Leu and Su, 1993) and is now classified as *X. taiwanensis* (Su *et al*., 2016). *X. fastidiosa* is also present in Taiwan Province of China on *Vitis vinifera* (Su *et al.*, 2013).~~	*Category : TECHNICAL*
45	45	~~*X.* *Xylella* *fastidiosa* is genetically diverse and consists of six sub-species. *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Vitis vinifera*, *Prunus dulcis*, *Medicago sativa* and *Acer* spp. (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Prunus dulcis*, *Prunus persica*, *Quercus* spp. and *Platanus occidentalis*. *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *tashke* has been isolated from the ornamental tree *Chitalpa tashkentensis* (Randall *et al.*, 2009). *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp. Finally, *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* and *Coffea* species, and *Olea europaea*. A different *Xylella* species is associated with pear leaf scorch in Taiwan Province of China (Leu and Su, 1993) and is now classified as *X. taiwanensis* (Su *et al*., 2016). *X. fastidiosa* is also present in Taiwan Province of China on *Vitis vinifera* (Su *et al.*, 2013).	**Canada** Sentence to start with full genus name and not abbreviation *Category : EDITORIAL*
46	45	*X. fastidiosa* is genetically diverse and consists of ~~six sub-species~~subspecies. *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Vitis vinifera*, *Prunus dulcis*, *Medicago sativa* and *Acer* spp. (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Prunus dulcis*, *Prunus persica*, *Quercus* spp. and *Platanus occidentalis*. *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *tashke* has been isolated from the ornamental tree *Chitalpa tashkentensis* (Randall *et al.*, 2009). *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp. Finally, *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* and *Coffea* species, and *Olea europaea*. ~~A different *Xylella* species is associated with pear leaf scorch in Taiwan Province of China (Leu and Su, 1993) and is now classified as *X. taiwanensis* (Su *et al*., 2016). *X. fastidiosa* is also present in Taiwan Province of China on *Vitis vinifera* (Su *et al.*, 2013).~~	**EPPO** there is reference to six subspecies, however, later on (IPPC & 2 Taxonomic information) the EPPO text has been adopted where only the accepted subspecies are mentioned. It could be considered to omit (in paragraph 1 Pest information) the part of the subspecies. Revised change by France on 8 Aug 2017 8:18 Revised change by France on 8 Aug 2017 8:17 *Category : TECHNICAL*
47	45	*X. fastidiosa* is genetically diverse and consists of six sub-species. *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Vitis vinifera*,	**Egypt** *Category : EDITORIAL*

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		Prunus dulcis, *Medicago sativa* and *Acer* spp. (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Prunus dulcis*, *Prunus persica*, *Quercus* spp. and *Platanus occidentalis*. *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *tashke* has been isolated from the ornamental tree *Chitalpa tashkentensis* (Randall *et al.*, 2009). *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp. Finally, *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* and *Coffea* species, and *Olea europaea.* A different *Xylella* species is associated with pear leaf scorch in Taiwan Province of China (Leu and Su, 1993) and ~~is~~ now classified as *X. taiwanensis* (Su *et al*., 2016). *X. fastidiosa* is also present in Taiwan Province of China on *Vitis vinifera* (Su *et al.*, 2013).	
48	46	*X. fastidiosa* is a Gram-negative, xylem-limited bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, rod-shaped cells, with rounded or tapered ends and numerous irregular ridges or folds on the cell wall surface (Wells *et al*., 1987). The bacterium is inoculated into the water-transporting xylem elements of its host plants by xylem sap-feeding insects. The colonization of the xylem blocks the transport of mineral nutrients and water in the infected plants. Many diseases caused by *X. fastidiosa* are characterized by leaf scorch, defoliation, foliage wilt and a general decline in vigour, but expression of symptoms is heterogeneous, depending on the host plant species, *X. fastidiosa* genotype and the climatic conditions. Many host plants infected with *X. fastidiosa* do not display any symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant systemically, including the roots of infected plants as well as all above-ground plant parts (Aldrich *et al*., 1992; He *et al*., 2000; Li *et al*., 2003). The pathogen overwinters in the xylem of the host plant as well as in weeds. Insect transmission is considered the main factor for *X. fastidiosa* local spread. The vectors belong to the order Hemiptera, sub-order Auchenorrhyncha, ~~and the~~ families of Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee *et al*., 2008), Aphrophoridae and Cicadidae. The transmission of *X. fastidiosa* by insects is persistent. Nymphs and adults are able to acquire the bacteria by feeding on the xylem fluid of an infected plant and then to transmit the pathogen to a healthy host. Once infected, adults can transmit throughout their whole lifetime, as the bacterium multiplies and persists in the vector foregut (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). The movement of infected plants and planting material (e.g, budwood, seedlings) is assumed to be responsible for the long distance spread of the disease and its entry into new areas.	**European Union** 1) For a better distinction with long distance spread (please see last sentence of the paragraph). 2) Unnecessary words. *Category : TECHNICAL*
49	46	~~*X.*~~ *Xylella fastidiosa* is a Gram-negative, xylem-limited bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, rod-shaped cells, with rounded or tapered ends and numerous irregular ridges or folds on the cell wall surface (Wells *et al.*, 1987). The bacterium is inoculated into the water-transporting	**Canada** Sentence to start with full genus name and not abbreviation. *Category : EDITORIAL*

#	Para	Text	Comment
		xylem elements of its host plants by xylem sap-feeding insects. The colonization of the xylem blocks the transport of mineral nutrients and water in the infected plants. Many diseases caused by *X. fastidiosa* are characterized by leaf scorch, defoliation, foliage wilt and a general decline in vigour, but expression of symptoms is heterogeneous, depending on the host plant species, *X. fastidiosa* genotype and the climatic conditions. Many host plants infected with *X. fastidiosa* do not display any symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant systemically, including the roots of infected plants as well as all above-ground plant parts (Aldrich *et al.*, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant as well as in weeds. Insect transmission is considered the main factor for *X. fastidiosa* spread. The vectors belong to the order Hemiptera, sub-order Auchenorrhyncha, and the families of Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee *et al*., 2008), Aphrophoridae and Cicadidae. The transmission of *X. fastidiosa* by insects is persistent. Nymphs and adults are able to acquire the bacteria by feeding on the xylem fluid of an infected plant and then to transmit the pathogen to a healthy host. Once infected, adults can transmit throughout their whole lifetime, as the bacterium multiplies and persists in the vector foregut (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). The movement of infected plants and planting material (e.g, budwood, seedlings) is assumed to be responsible for the long distance spread of the disease and its entry into new areas.	
50	46	*X. fastidiosa* is a Gram-negative, xylem-limited bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, rod-shaped cells, with rounded or tapered ends and numerous irregular ridges or folds on the cell wall surface (Wells *et al*., 1987). The bacterium is inoculated into the water-transporting xylem elements of its host plants by xylem sap-feeding insects. The colonization of the xylem blocks the transport of mineral nutrients and water in the infected plants. Many diseases caused by *X. fastidiosa* are characterized by leaf scorch, defoliation, foliage wilt and a general decline in vigour, but expression of symptoms is heterogeneous, depending on the host plant species, *X. fastidiosa* genotype and the climatic conditions. Many host plants infected with *X. fastidiosa* do not display any symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant systemically, including the roots of infected plants as well as all above-ground plant parts (Aldrich *et al.*, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant as well as in weeds. Insect transmission is considered the main factor for *X. fastidiosa* spread. The vectors belong to the order Hemiptera, sub-order Auchenorrhyncha, and the families of Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee *et al*., 2008), Aphrophoridae and Cicadidae. The transmission of *X. fastidiosa* by insects is persistent. Nymphs and adults	**Ghana** *Category : EDITORIAL*

#	Para	Text	Comment
		are able to acquire the bacteria by feeding on the xylem fluid of an infected plant and then ~~to transmit~~ transmitting the pathogen to a healthy host. Once infected, adults can transmit throughout their whole lifetime, as the bacterium multiplies and persists in the vector foregut (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). The movement of infected plants and planting material (e.g, budwood, seedlings) is assumed to be responsible for the long distance spread of the disease and its entry into new areas.	
51	46	*X. fastidiosa* is a Gram-negative, xylem-limited bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, rod-shaped cells, with rounded or tapered ends and numerous irregular ridges or folds on the cell wall surface (Wells *et al.*, 1987). The bacterium is inoculated into the water-transporting xylem elements of its host plants by xylem sap-feeding insects. The colonization of the xylem blocks the transport of mineral nutrients and water in the infected plants. Many diseases caused by *X. fastidiosa* are characterized by leaf scorch, defoliation, foliage wilt and a general decline in vigour, but expression of symptoms is heterogeneous, depending on the host plant species, *X. fastidiosa* genotype and the climatic conditions. Many host plants infected with *X. fastidiosa* do not display any symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant systemically, including the roots of infected plants as well as all above-ground plant parts (Aldrich *et al.*, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant as well as in weeds. Insect transmission is considered the main factor for *X. fastidiosa* local spread. The vectors belong to the order Hemiptera, sub-order Auchenorrhyncha, ~~and the~~ families of Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al.*, 2004; Chatterjee *et al.*, 2008), Aphrophoridae and Cicadidae. The transmission of *X. fastidiosa* by insects is persistent. Nymphs and adults are able to acquire the bacteria by feeding on the xylem fluid of an infected plant and then to transmit the pathogen to a healthy host. Once infected, adults can transmit throughout their whole lifetime, as the bacterium multiplies and persists in the vector foregut (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). The movement of infected plants and planting material (e.g, budwood, seedlings) is assumed to be responsible for the long distance spread of the disease and its entry into new areas.	**EPPO** Unnecessary words. Revised change by France on 8 Aug 2017 8:21 1 Host plants are not necessarily crop or fruit trees, they can be weeds. So this part of the sentence is not necessary. 2 For a better distinction with long distance spread (please see last sentence of the paragraph). 3 Is the nymph mouth part strong enough to reach the xylem? 4 For completeness, it should be indicated that the bacteria does transmit between the nymph and the adult stage. *Category : TECHNICAL*
52	46	*X. fastidiosa* is a Gram-negative, xylem-limited bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, rod-shaped cells, with rounded or tapered ends and numerous irregular ridges or folds on the cell wall surface (Wells *et al.*, 1987). The bacterium is inoculated into the water-transporting xylem elements of its host plants by xylem sap-feeding insects. The colonization of the xylem blocks the transport of mineral nutrients and water in the infected plants. Many diseases caused by *X. fastidiosa* are characterized by leaf scorch, defoliation, foliage wilt and a general decline in vigour, but expression of symptoms is heterogeneous, depending on	**Egypt** *Category : EDITORIAL*

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		the host plant species, *X. fastidiosa* genotype and the climatic conditions. Many host plants infected with *X. fastidiosa*- shows no sdo not display any symptoms mptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant systemically, including the roots of infected plants as well as all above-ground plant parts plant's shoot and root systems systemically (Aldrich *et al.*, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant as well as in weeds. Insect transmission is considered the main factor for *X. fastidiosa* spread. The vectors belong to the order Hemiptera, sub-orderbelong to the order Hemiptera, sub-order Auchenorrhyncha, and the families of Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee *et al*., 2008), Aphrophoridae and Cicadidae. The transmission of *X. fastidiosa* by insects is persistent. Nymphs and adults are able to acquire the bacteria by feeding on the xylem fluid of an infected plant and then to transmit the pathogen to a other healthy hostplant hosts. Once infected, adults can transmit infection throughout their whole lifetime, as the bacterium multiplies and persists in the vector foregut (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). The movement of infected plants and planting material (e.g, budwood, seedlings) is assumed to be responsible for the long distance spread of the disease and its entry into new areas.	
53	46	*X. fastidiosa* is a Gram-negativeGram-negative bacterium, xylem-limited bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, rod-shaped cells, with rounded or tapered ends and numerous irregular ridges or folds on the cell wall surface (Wells *et al.*, 1987). The bacterium is inoculated into the water-transporting xylem elements of its host plants by xylem sap-feeding insects. The colonization of the xylem blocks the transport of mineral nutrients and water in the infected plants. Many diseases caused by *X. fastidiosa* are characterized by leaf scorch, defoliation, foliage wilt and a general decline in vigour, but expression of symptoms is heterogeneous, depending on the host plant species, *X. fastidiosa* genotype and the climatic conditions. Many host plants infected with *X. fastidiosa* do not display any symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant systemically, including the roots of infected plants as well as all above-ground plant parts (Aldrich *et al.*, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant as well as in weeds. Insect transmission is considered the main factor for *X. fastidiosa* spread. The vectors belong to the order Hemiptera, sub-order Auchenorrhyncha, and the families of Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee *et al*., 2008), Aphrophoridae and Cicadidae. The transmission of *X. fastidiosa* by insects is persistent. Nymphs and adults are able to acquire the bacteria by feeding on the xylem fluid of an infected plant and then to transmit the pathogen to a healthy host. Once infected, adults can transmit throughout their whole lifetime, as the bacterium	**Philippines** *Category : EDITORIAL*

#	Para	Text	Comment
		multiplies and persists in the vector foregut (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). The movement of infected plants and planting material (e.g, budwood, seedlings) is assumed to be responsible for the long distance spread of the disease and its entry into new areas.	
54	48	**Name:** *Xylella fastidiosa* Wells ~~et al.~~*et al.*, 1987	**Egypt** *Category : EDITORIAL*
55	53	**3. Detection**	**Kenya** The term detection may need to be defined both in this document and ISPM 5 where its missing. The term is extensively used in other ISPM documents *Category : SUBSTANTIVE*
56	54	Plants infected with *X. fastidiosa* may be ~~asymptomatic~~ symptomless (Almeida and Purcell, 2003) or the symptoms may be similar to those associated with water stress or physiological disorders. ~~Detection~~ Therefore, detection is ~~therefore~~ based on inspection for symptoms and the use of specific serological and molecular tests.	**Egypt** *Category : EDITORIAL*
57	56	The presence of *X. fastidiosa* can have a broad impact on its host: from causing no symptoms to plant death. Most host plants infected with *X. fastidiosa* do not display any symptoms, while some display symptoms that include leaf scorching, defoliation, chlorosis or bronzing along the leaf margin, and dwarfing. The bronzing may intensify before browning and drying. Symptoms are usually more pronounced in stressed plants (e.g. stressed by high or low temperature, or by drought) and they can vary according to plant species and cultivars and environmental conditions (Janse and Obradovic, 2010; CABI, 2016).	**European Union** Information on symptomatology for the ornamentals (Polygala, Nerium etc) is missing, should be added as in the EPPO protocol. *Category : TECHNICAL*
58	56	The presence of *X. fastidiosa* can have a broad impact on its host: from causing no symptoms to plant death. Most host plants infected with *X. fastidiosa* do not display any symptoms, while some display symptoms that include leaf scorching, defoliation, chlorosis or bronzing along the leaf margin, and dwarfing. The bronzing may intensify before browning and drying. Symptoms are usually more pronounced in stressed plants (e.g. stressed by high or low temperature, or by drought) and they can vary according to plant species and cultivars and environmental conditions (Janse and Obradovic, 2010; CABI, 2016).	**EPPO** Information on symptomatology for the ornamentals (Polygala, Nerium etc) is missing, should be added as in the EPPO protocol. *Category : TECHNICAL*
59	56	The presence of *X. fastidiosa* can have a broad impact on its host: from ~~causing no symptoms~~ symptomless to plant death. Most host plants infected with *X. fastidiosa* do not ~~display~~ show any symptoms, while some display symptoms that include leaf scorching, defoliation, chlorosis or bronzing along the leaf margin, and dwarfing. The bronzing may intensify before browning and drying. Symptoms are usually more pronounced in stressed plants (e.g. stressed by high or low temperature, or by drought) and they can vary according to plant species and cultivars and environmental conditions (Janse and Obradovic, 2010; CABI, 2016).	**Egypt** *Category : EDITORIAL*

#	Para	Text	Comment
60	57	Symptoms can be confused with other biotic (e.g. several fungal diseases) or abiotic causes (environmental stresses, water deficiency, salt, air pollutants, nutritional problems, etc.). Symptoms on various hosts can be seen at https://gd.eppo.int/taxon/XYLEFA/photos and https://nature.berkeley.edu/xylella. Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. Host range can be markedly different between subspecies; however, there is some uncertainty with regards to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subspecies *fastidiosa* not only infects grapes but it also causes alfalfa dwarf and overlaps with ~~*X. fasitidiosa*~~*X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.* 2010). Some examples of the subspecies of *X. fastidiosa* that are linked to the below disease descriptions are provided when widely acknowledged in the current literature. The following descriptions are some of the more characteristic symptoms observed on some key hosts.	**European Union** Better English (not only… but also)? Typo. *Category : EDITORIAL*
61	57	Symptoms can be confused with other biotic (e.g. several fungal diseases) or abiotic causes (environmental stresses, water deficiency, salt, air pollutants, nutritional problems, etc.). Symptoms on various hosts can be seen at https://gd.eppo.int/taxon/XYLEFA/photos and https://nature.berkeley.edu/xylella. Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. Host range can be markedly different between subspecies; however, there is some uncertainty with regards to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subspecies *fastidiosa* not only infects grapes it also causes alfalfa dwarf and overlaps with *X. fasitidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.* 2010). Some examples of the subspecies of *X. fastidiosa* that are linked to the ~~below~~ disease descriptions below, are provided when widely acknowledged in the current literature. The following descriptions are some of the more characteristic symptoms observed on some key hosts.	**Ghana** *Category : EDITORIAL*
62	57	Symptoms can be confused with other biotic (e.g. several fungal diseases) or abiotic causes (environmental stresses, water deficiency, salt, air pollutants, nutritional problems, etc.). Symptoms on various hosts can be seen at https://gd.eppo.int/taxon/XYLEFA/photos and https://nature.berkeley.edu/xylella. Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. Host range can be markedly different between subspecies; however, there is some uncertainty with regards to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subspecies *fastidiosa* not only infects grapes but it also causes alfalfa dwarf and overlaps with ~~*X. fasitidiosa*~~*X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.* 2010). Some examples of the subspecies of *X. fastidiosa* that are linked to the below disease descriptions are provided when widely acknowledged in the current literature. The	**EPPO** Better English (not only… but also)? Typo *Category : EDITORIAL*

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		following descriptions are some of the more characteristic symptoms observed on some key hosts.	
63	57	Symptoms can be confused with other biotic (e.g. several fungal diseases) or abiotic causes (environmental stresses, water deficiency, salt, air pollutants, nutritional problems, etc.). Symptoms on various hosts can be seen at https://gd.eppo.int/taxon/XYLEFA/photos and https://nature.berkeley.edu/xylella. Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. Host range can be markedly different between subspecies; however, there is some uncertainty with regards to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subspecies *fastidiosa* not only infects grapes it also causes alfalfa dwarf and overlaps with *X. fasitidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.* 2010). ~~Some examples of the subspecies~~ The following examples are some of the more characteristic symptoms observed on some key hosts.~~*X. fastidiosa* that are linked to the below disease descriptions are provided when widely acknowledged in the current literature. The following descriptions are some of the more characteristic symptoms observed on some key hosts.~~	**Egypt** *Category : EDITORIAL*
64	61	The first symptoms of citrus variegated chlorosis (CVC) to appear on leaves are mottled variegations, with small chlorotic spots on the upper surface that correspond to small gummy brown spots on the underside of the leaf. Isolates within the *X. fastidiosa* subsp. *pauca* complex have been reported to cause citrus variegated chlorosis ~~(Schaad et al.~~ (Schaad *et al.* 2004; Almeida ~~et al.~~ *et al.* 2008). Symptoms are most obvious on three- to six-year-old trees and mainly on *Citrus sinensis* cultivars. Affected trees show foliar interveinal chlorosis resembling zinc deficiency, but the symptoms are not symmetrical on opposite sides of the leaf. Symptoms of CVC can also be distinguished from zinc chlorosis by the presence of the gummy, brown necrotic regions on the underside of the leaf, which coincide with the chlorosis on the upper leaf surface (CABI, 2016). Sectoring of symptoms in the canopy occurs on newly affected trees. However, the CVC syndrome generally develops throughout the entire canopy on older infected trees. Affected trees are stunted and the canopy has a thin appearance because of defoliation and dieback of twigs and branches. Flowering is abnormal; fruits ripen earlier and do not fill, being much smaller than normal and very firm. The growth rate of affected trees is greatly reduced and twigs and branches may wilt. The plants do not usually die, but the yield and quality of the fruit are severely reduced (Donadio and Moreira, 1998).	**European Union** Italic missing. *Category : EDITORIAL*
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67	65	In three different distant regions around the world (the southern region of the Republic of Italy, the Argentine Republic and the Federative Republic of Brazil), leaf scorching symptoms on *Olea europaea* trees have been associated with *X. fastidiosa* (Saponari *et al.*, 2013; Haelterman *et al.*, 2015; Coletta-Filho *et al.*, 2016). The strains associated with this disease in Italy are a recombinant of alleles within the *X. fastidiosa* ~~subspecies~~ subsp. *pauca* (Loconsole *et al.* 2014). The olive quick decline syndrome is characterized by leaf scorching and randomly distributed desiccation of twigs and small branches, which, in the early stages of the infection, are mainly observed in the upper part of the canopy. Leaf tips and margins turn dark yellow to brown, eventually leading to	**Japan** Editorial *Category : EDITORIAL*

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		desiccation. Over time, symptoms become increasingly severe and extend to the rest of the crown, which acquires a blighted appearance. Desiccated leaves and mummified drupes remain attached to the shoots. Trunks, branches and twigs viewed in cross-section show irregular discoloration of the vascular elements, sapwood and vascular cambium (Nigro *et al.*, 2013). Rapid dieback of shoots, twigs and branches may be followed by death of the entire tree. *X. fastidiosa* has also been detected in young olive trees with leaf scorching and quick decline (EPPO, 2016).	
68	67	The most characteristic symptoms of almond leaf scorching disease are leaf scorching followed by decreased productivity and general decline. Strains of ~~X~~*X. fastidiosa* subsp. ~~*fastidosa*~~*fastidiosa* and subsp. *multiplex* have been reported to cause almond leaf scorch disease (Yuan et al. 2010). In early summer, leaves appear with marginal leaf scorch (brown, necrotic (dead) leaf tissue). Usually, a narrow band of yellow (chlorotic) tissue occurs between the dead tissue and the part of the leaf that is still green, but when the sudden appearance of leaf scorch symptoms is prompted by hot weather the narrow chlorotic band may not develop. As the disease progresses, affected twigs on limbs die back from the tip (Mircetich *et al.*, 1976). Even highly susceptible varieties take many years to die completely, but nut production is severely reduced within a few years in most varieties.	**European Union** Typos. *Category : EDITORIAL*
69	67	The most characteristic symptoms of almond leaf scorching disease are leaf scorching followed by decreased productivity and general decline. Strains of ~~X.~~ *X. fastidiosa* subsp. ~~*fastidosa*~~*fastidiosa* and subsp. *multiplex* have been reported to cause almond leaf scorch disease (Yuan et al. 2010). In early summer, leaves appear with marginal leaf scorch (brown, necrotic (dead) leaf tissue). Usually, a narrow band of yellow (chlorotic) tissue occurs between the dead tissue and the part of the leaf that is still green, but when the sudden appearance of leaf scorch symptoms is prompted by hot weather the narrow chlorotic band may not develop. As the disease progresses, affected twigs on limbs die back from the tip (Mircetich *et al.*, 1976). Even highly susceptible varieties take many years to die completely, but nut production is severely reduced within a few years in most varieties.	**EPPO** Typos *Category : EDITORIAL*
70	67	The most characteristic symptoms of almond leaf scorching disease are leaf scorching followed by decreased productivity and general decline. Strains of ~~X.~~ *X. fastidiosa* subsp. *fastidosa* and subsp. *multiplex* have been reported to cause almond leaf scorch disease (Yuan ~~et al.~~ *et al.* 2010). In early summer, leaves appear with marginal leaf scorch (brown, necrotic (dead) leaf tissue). Usually, a narrow band of yellow (chlorotic) tissue occurs between the dead tissue and the part of the leaf that is still green, but when the sudden appearance of leaf scorch symptoms is prompted by hot weather the narrow chlorotic band may not develop. As the disease progresses, affected twigs on limbs die back from the tip (Mircetich *et al.*, 1976). Even highly susceptible varieties take many	**Thailand** *Category : EDITORIAL*

#	Para	Text	Comment
		years to die completely, but nut production is severely reduced within a few years in most varieties.	
71	77	Samples of necrotic and dead tissue or sections of the plant at an advanced stage of infection are unsuitable for *X. fastidiosa* diagnosis as saprophytes quickly colonize necrotic or dead tissue, interfering with the isolation or detection of the pathogen. ~~Once samples are collected, they should be kept cool and transported to the laboratory as soon as possible. Lower temperatures can reduce sample deterioration. However, *X. fastidiosa* does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate.~~ Samples should be taken from close to the symptoms and preferably consist of stems that have mature symptomatic leaves with petioles and woody twigs. Individual leaves with petioles can also be sampled. The best plant material to test for the presence of *X. fastidiosa* is the leaf petiole and the midrib of mature leaves from either asymptomatic or symptomatic plant material.	**European Union** Suggest to move at the beginning of paragraph 92 which deals with the same idea. Otherwise, move to the end of paragraph 77 (more logical sequence). *Category : SUBSTANTIVE*
72	77	Samples of necrotic and dead tissue or sections of the plant at an advanced stage of infection are unsuitable for *X. fastidiosa* diagnosis as saprophytes quickly colonize necrotic or dead tissue, interfering with the isolation or detection of the pathogen. Once samples are collected, they should be kept cool and transported to the laboratory as soon as possible. Lower temperatures can reduce sample deterioration. However, *X. fastidiosa* does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate. Samples should be taken from close to the symptoms and preferably consist of stems that have mature symptomatic leaves with petioles and woody twigs. Individual leaves with petioles can also be sampled. The best plant material to test for the presence of *X. fastidiosa* is the leaf petiole and the midrib of mature leaves from either asymptomatic or symptomatic plant material.	**European Union** Information on laboratory sample (EPPO & 3.4.1.1 incl Table 1) is missing and could be added as in the EPPO protocol. *Category : TECHNICAL*
73	77	Samples of necrotic and dead tissue or sections of the plant at an advanced stage of infection are unsuitable for *X. fastidiosa* diagnosis as saprophytes quickly colonize necrotic or dead tissue, interfering with the isolation or detection of the pathogen. Once samples are collected, they should be kept cool and transported to the laboratory as soon as possible. Lower temperatures can reduce sample deterioration. However, *X. fastidiosa* does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate. Samples should be taken from close to the symptoms and preferably consist of stems that have mature symptomatic leaves with petioles and woody twigs. Individual leaves with petioles can also be sampled. The best plant material to test for the presence of *X. fastidiosa* is the leaf petiole and the midrib of mature leaves from either asymptomatic or symptomatic plant material.	**EPPO** Information on laboratory sample (EPPO & 3.4.1.1 incl Table 1) is missing and could be added as in the EPPO protocol. *Category : TECHNICAL*

#	Para	Text	Comment
74	77	Samples of necrotic and dead tissue or sections of the plant at an advanced stage of infection are unsuitable for *X. fastidiosa* diagnosis as saprophytes quickly colonize necrotic or dead tissue, interfering with the isolation or detection of the pathogen. ~~Once samples are collected, they should be kept cool and transported to the laboratory as soon as possible. Lower temperatures can reduce sample deterioration. However, *X. fastidiosa* does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate.~~ Samples should be taken from close to the symptoms and preferably consist of stems that have mature symptomatic leaves with petioles and woody twigs. Individual leaves with petioles can also be sampled. The best plant material to test for the presence of *X. fastidiosa* is the leaf petiole and the midrib of mature leaves from either asymptomatic or symptomatic plant material.	**EPPO** Suggest to move at the beginning of paragraph 92 which deals with the same idea. Otherwise, move to the end of paragraph 77 (more logical sequence). *Category : SUBSTANTIVE*
75	77	Samples of necrotic and dead tissue or sections of the plant at an advanced stage of infection are unsuitable for *X. fastidiosa* diagnosis as saprophytes quickly colonize necrotic or dead tissue, interfering with the isolation or detection of the pathogen. Once samples are collected, they should be kept cool and transported to the laboratory as soon as possible. Lower temperatures can reduce sample deterioration. However, *X. fastidiosa* does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate. Samples should be taken from close to the symptoms and preferably consist of stems that have mature symptomatic leaves with petioles and woody twigs. Individual leaves with petioles can also be sampled. The best plant material to test for the presence of *X. fastidiosa* is the leaf petiole and the midrib of mature leaves from either asymptomatic or symptomatic plant material.	**Philippines** Please provide temperature range for sample transport. *Category : SUBSTANTIVE*
76	79	The distribution and concentration of *X. fastidiosa* within the plant can be variable and is dependent upon plant species type, seasonal and environmental factors. To maximize the likelihood of detection, sampling should be performed during the period of active growth of the plants (Hopkins, 1981). This is usually from late spring to autumn in temperate zones.	**Kenya** Sampling after warm periods (e.g. late summer-early autumn) increases the probability for an accurate bacterial detection https://ec.europa.eu/food/sites/food/files/plant/docs/ph_biosec_legis_guidelines_xylella-survey.pdf *Category : TECHNICAL*
77	81	**3.2.2 ~~Sample~~ Plant sample collection**	**European Union** More precise, because sampling of vectors is dealt with in section 3.2.6. *Category : EDITORIAL*
78	81	**3.2.2 Sample collection**	**European Union** A decision-making flow chart for each situation: plant and arthropod samples; would be useful. *Category : TECHNICAL*
79	81	**3.2.2 Sample collection**	**EPPO** A decision-making flow chart for each situation: plant and arthropod samples; would be useful.

#	Para	Text	Comment
			Category : TECHNICAL
80	81	**3.2.2 Plant sSample ample collection**	**EPPO** More precise, because sampling of vectors is dealt with in section 3.2.6. *Category : EDITORIAL*
81	87	**3.2.5** **Sampling of vectors**	**European Union** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
82	87	**3.2.5 Sampling of vectors**	**EPPO** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
83	87	**3.2.5 Sampling of vectors**	**Kenya** Detection of Xylella fastidiosa in insect vectors Should replace "Sampling of vectors" *Category : SUBSTANTIVE*
84	88	Insects collected from the field or from the wild can be analysed by polymerase chain reaction (PCR) to detect *X. fastidiosa*. Enzyme-linked immunosorbent assay (ELISA: see section 3.3) is not sensitive enough, as the bacterium only colonizes the insect foregut where, in spite of its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). On the aphrophorid *Philaenus spumarius*, the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells (Cornara *et al.*, 2016).	**European Union** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
85	88	Insects collected from the field or from the wild can be analysed by polymerase chain reaction (PCR) to detect *X. fastidiosa*. Enzyme-linked immunosorbent assay (ELISA: see section 3.3) is not sensitive enough, as the bacterium only colonizes the insect foregut where, in spite of its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). On the aphrophorid *Philaenus spumarius*, the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells (Cornara *et al.*, 2016).	**EPPO** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
86	88	Insects collected from the field or from the wild can be analysed by polymerase chain reaction (PCR) to detect *X. fastidiosa*. Enzyme-linked immunosorbent assay (ELISA: see section 3.3) is not sensitive enough, as the bacterium only colonizes the insect foregut where, in spite of its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). On the aphrophorid *Philaenus spumarius*, the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells (Cornara *et al.*, 2016).	**Kenya** Paragraph describes detection and not sampling *Category : SUBSTANTIVE*
87	89	**3.2.6** **Vector sample collection**	**European Union** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence.

#	Para	Text	Comment
			Category : SUBSTANTIVE
88	89	~~3.2.6 Vector sample collection~~	**EPPO** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
89	90	~~Adult vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell *et al.*, 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or acetone. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20 °C or −80 °C, or in 95–99% ethanol at −20 °C or −80 °C. Sticky traps can also be stored at −20 °C.~~	**European Union** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
90	90	Adult vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell *et al.*, 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or acetone. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ~~ethanol, or at −20 °C or −80 °C, or in 95–99%~~ ethanol and/or at −20 °C or −80 °C. Sticky traps can also be stored at −20 °C.	**Japan** Editorial *Category : EDITORIAL*
91	90	~~Adult vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell *et al.*, 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or acetone. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20 °C or −80 °C, or in 95–99% ethanol at −20 °C or −80 °C. Sticky traps can also be stored at −20 °C.~~	**EPPO** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : SUBSTANTIVE*
92	90	Adult vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell *et al.*, 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or	**Kenya** with captured insects *Category : TECHNICAL*

#	Para	Text	Comment
		acetone. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20 °C or −80 °C, or in 95–99% ethanol at −20 °C or −80 °C. Sticky traps with captured insects can also be stored at −20 °C.	
93	90	~~Adult~~ vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell *et al.*, 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or acetone. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20 °C or −80 °C, or in 95–99% ethanol at −20 °C or −80 °C. Sticky traps can also be stored at −20 °C.	**Kenya** Delete (adults) *Category : EDITORIAL*
94	91	**3.2.7 ~~Sample~~ Plant sample transport and sample storage in the laboratory**	**European Union** Section 3.2.7 only deals with the storage of plant samples (see end of paragraph 90 for the storage of vector samples). *Category : EDITORIAL*
95	91	**3.2.7 Plant sample transport and ~~Sample~~ storage in the laboratory**	**EPPO** Section 3.2.7 only deals with the storage of plant samples (see end of paragraph 90 for the storage of vector samples). *Category : EDITORIAL*
96	92	Once samples are collected, they should be kept cool and transported to the laboratory as soon as possible. Lower temperatures can reduce sample deterioration. However, X. fastidiosa does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate. Samples should be processed as soon as possible after ~~arrival~~arrival at the laboratory. ~~For~~ However, for isolation (see section 4.1), samples may be kept refrigerated for up to three days. For other tests, samples may be refrigerated for up to one week. **3.2.5 Sampling of vectors** Adult vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell et al., 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or acetone. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20 °C or −80 °C, or in 95–99% ethanol at −20 °C or −80 °C. Sticky traps can also be	**European Union** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. *Category : TECHNICAL*

#	Para	Text	Comment
		stored at −20 °C. Insects collected from the field or from the wild can be analysed by polymerase chain reaction (PCR) to detect X. fastidiosa. Enzyme-linked immunosorbent assay (ELISA: see section 3.3) is not sensitive enough, as the bacterium only colonizes the insect foregut where, in spite of its multiplication, it is generally present at low levels (Purcell et al., 2014). On the aphrophorid Philaenus spumarius, the population size of X. fastidiosa may be limited to fewer than 103 cells (Cornara et al., 2016).	
97	92	Once samples are collected, they should be kept cool and transported to the laboratory as soon as possible. Lower temperatures can reduce sample deterioration. However, X. fastidiosa does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate. Samples should be processed as soon as possible after ~~arrival~~arrival at the laboratory. ~~For~~ However for isolation (see section 4.1), samples may be kept refrigerated for up to three days. For other tests, samples may be refrigerated for up to one week. 3.2.5 Sampling of vectors Adult vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell et al., 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or acetone. Sampling for insects should preferably be done from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20 °C or −80 °C, or in 95–99% ethanol at −20 °C or −80 °C. Sticky traps can also be stored at −20 °C. Insects collected from the field or from the wild can be analysed by polymerase chain reaction (PCR) to detect X. fastidiosa. Enzyme-linked immunosorbent assay (ELISA: see section 3.3) is not sensitive enough, as the bacterium only colonizes the insect foregut where, in spite of its multiplication, it is generally present at low levels (Purcell et al., 2014). On the aphrophorid Philaenus spumarius, the population size of X. fastidiosa may be limited to fewer than 103 cells (Cornara et al., 2016).	**EPPO** Suggest to move paragraphs 87, 90 and 88 after section 3.2.7 which deals with the storage of plant samples. Suggest to put paragraph 90 (storage of insects) before paragraph 88 (analysis of insects) for a more logical sequence. Revised change by France on 24 Sep 2017 7:29 *Category : TECHNICAL*
98	92	Samples should be processed as soon as possible after arrival. For isolation (see section 4.1), samples may be kept refrigerated for up to three days. For other tests, samples may be refrigerated for up to one week.	**Turkey** For molecular or serological detection, mentioning on the possibility of storing the sample longer at -20 or -80oC would be beneficial. *Category : TECHNICAL*
99	92	Samples should be processed as soon as possible after arrival. For isolation (see section 4.1), samples may be kept refrigerated for up to three days. For other tests, samples may be refrigerated for up to one week.	**Philippines** kept refrigerated: indicate the storage temperature 4 deg C or negative -20 deg C? *Category : SUBSTANTIVE*
100	94	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity or reproducibility achieved. The use of names of reagents, chemicals or equipment in these	**European Union** Redundant with footnote 1 (paragraph 101)? If this is the case, delete paragraph 94 or footnote 1.

#	Para	Text	Comment
		diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : EDITORIAL*
101	94	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity ~~or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	**European Union** Better wording (see for example DP Bactrocera dorsalis). *Category : EDITORIAL*
102	94	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity ~~or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	**EPPO** Better wording (see for example DP Bactrocera dorsalis). *Category : EDITORIAL*
103	94	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	**EPPO** Redundant with footnote 1 (paragraph 101)? If this is the case, delete paragraph 94 or footnote 1. *Category : EDITORIAL*
104	94	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity or reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	**Uruguay** Text deleted to avoid repetition with the footnote associated to brand names *Category : TECHNICAL*
105	97	ELISA works well for samples with symptoms and tissue that contains high concentrations of *X. fastidiosa*. The leaf petiole and mid-veins of symptomatic leaves are the best sources of tissue for ELISA. ~~ELISA can also be used on twigs and canes but is unsuitable for use on necrotic or dead tissue.~~The assay can also be used on twigs and canes but is unsuitable for use on necrotic or dead tissue.	**Philippines** *Category : EDITORIAL*
106	98	**3.3.2 Double antibody sandwich ~~ELISA~~ ELISA(DAS-ELISA)**	**Japan** To distingiush between (general) ELISA and DAS-ELISA *Category : TECHNICAL*
107	98	**3.3.2 Double antibody sandwich ELISA**	**Czech Republic** „Agdia" should be instead of „Agdi" *Category : EDITORIAL*
108	99	Positive and negative controls should be included in each test and these are normally	**European Union** The appropriateness of screening tests to be used is not included in

#	Para	Text	Comment
		provided in commercial kits. Positive controls can consist of a reference *X. fastidiosa* strain resuspended in healthy host plant extract (for detection in plant material) or in phosphate-buffered saline (PBS) (for identification of bacterial cultures). Negative controls should consist of healthy host plant extract (for detection in plant material) or a suspension of a non-target bacterial species (for identification of bacterial cultures). For plant materials, the healthy plant extract control should be of the same species or variety to allow for comparison with the test samples and to check for potential background- or cross-reactions.	the IPPC protocol (EPPO & 3.5) and this is very essential. *Category : TECHNICAL*
109	99	Positive and negative controls should be included in each test and these are normally provided in commercial kits. Positive controls can consist of a reference *X. fastidiosa* strain resuspended in healthy host plant extract (for detection in plant material) or in phosphate-buffered saline (PBS) (for identification of bacterial cultures). Negative controls should consist of healthy host plant extract (for detection in plant material) or a suspension of a non-target bacterial species (for identification of bacterial cultures). For plant materials, the healthy plant extract control should be of the same species or variety to allow for comparison with the test samples and to check for potential background- or cross-reactions.	**EPPO** the approprianess of screening tests to be used is not included in the IPPC protocol (EPPO & 3.5) and this is very essential. *Category : TECHNICAL*
110	100	Samples should be processed following the general procedure recommended for the specific serological test being used. In general, plant tissue is macerated in extraction buffer (polyvinylpyrrolidone (PVP)-10, 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2) or in PBS (NaCl, 8 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 12H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) (1:10 w/v) using either a mortar and pestle or a tissue homogenizer (e.g. Polytron[1], Homex[1]) or by pulverising in liquid nitrogen (EPPO, 2016; Loconsole *et al*., 2014). Further information on using ~~ELISA~~ DAS-ELISA to detect plant pathogenic bacteria is available in EPPO (2010).	**Japan** Editorial *Category : EDITORIAL*
111	101	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity ~~and/or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	**European Union** Better wording (see for example DP Bactrocera dorsalis). *Category : EDITORIAL*
112	101	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity ~~and/or~~ and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	**EPPO** Better wording (see for example DP Bactrocera dorsalis). *Category : EDITORIAL*

#	Para	Text	Comment
113	101	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved.~~ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. ~~Laboratory procedures presented in~~ This information is given for the ~~protocols may be adjusted to~~ convenience of users of this protocol and does not constitute an endorsement by the ~~standards~~ CPM of ~~individual laboratories~~the chemical, ~~provided that~~ reagent and/or equipment used. Equivalent products may be used if they ~~are adequately validated~~can be shown to lead to the same results.	**Uruguay** Text deleted to avoid repetition with paragraph 94. Text added according text agreed for footnotes. *Category : TECHNICAL*
114	103	Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], ~~Agdi~~Agdia[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using ELISA is approximately 10^4 colony-forming units (c.f.u.)/ml (Loconsole *et al.*, 2014; EPPO, 2016). The technique is not sensitive enough for use early in the growth season, when no symptoms of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.	**European Union** *Category : EDITORIAL*
115	103	Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], Agdi[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using ELISA is approximately 10^4 colony-forming units (c.f.u.)/ml (Loconsole *et al.*, 2014; EPPO, 2016). The technique is not sensitive enough for use early in the growth season, when no symptoms of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.	**European Union** This protocol provides a sensitivity level for ELISA test, but it should be noted that the detection threshold can be affected by plant species matrices (EPPO, 2016). *Category : TECHNICAL*
116	103	Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], Agdi[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using ~~ELISA~~ DAS-ELISA is approximately 10^4 colony-forming units (c.f.u.)/ml (Loconsole *et al.*, 2014; EPPO, 2016). The technique is not sensitive enough for use early in the growth season, when no symptoms of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.	**Japan** Editorial *Category : EDITORIAL*
117	103	Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], Agdi[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using ELISA is approximately 10^4 colony-forming units (c.f.u.)/ml (Loconsole *et al.*, 2014; EPPO, 2016). The technique is not sensitive enough for use early in the growth season, when no symptoms	**EPPO** This protocol provides a sensitivity level for ELISA test, but it should be noted that the detection threshold can be affected by plant species matrices (EPPO, 2016). *Category : TECHNICAL*

#	Para	Text	Comment
		of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.	
118	103	Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], ~~Agdi~~Agdia[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using ELISA is approximately $10^{~~4~~}$ colony-forming units (c.f.u.)/ml (Loconsole *et al.*, 2014; EPPO, 2016). The technique is not sensitive enough for use early in the growth season, when no symptoms of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.	**EPPO** *Category : EDITORIAL*
119	103	Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], ~~Agdi~~Agdia[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using ELISA is approximately 10^{4} colony-forming units (c.f.u.)/ml (Loconsole *et al.*, 2014; EPPO, 2016). The technique is not sensitive enough for use early in the growth season, when no symptoms of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.	**China** Print error. *Category : EDITORIAL*
120	103	Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], ~~Agdi~~Agdia[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using ELISA is approximately 10^{4} colony-forming units (c.f.u.)/ml (Loconsole *et al.*, 2014; EPPO, 2016). The technique is not sensitive enough for use early in the growth season, when no symptoms of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.	**Singapore** Should be "Agdia" instead of "Agdi". *Category : EDITORIAL*
121	104	The specificity and sensitivity of ~~ELISA~~ DAS-ELISA to detect *X. fastidiosa* on *Olea europaea*, using a kit from Loewe[1], were evaluated by Loconsole *et al.* (2014). Additionally, a test performance study performed at the Institute for Sustainable Plant Protection (Bari, Italy) was conducted on serological kits from Agritest[1], Agdia[1] and Loewe[1]. These studies showed that these kits achieved 100% diagnostic sensitivity and specificity when testing naturally infected samples. The data on the test performance study are available at http://dc.eppo.int/validationlist.php.	**Japan** Editorial *Category : EDITORIAL*
122	107	Once the reaction of the controls has been verified, ~~then~~ the results for each sample are interpreted as follows:	**European Union** Better English? (because the sentence begins with "once"). *Category : EDITORIAL*
123	107	Once the reaction of the controls has been verified, ~~then~~ the results for each sample are interpreted as follows:	**EPPO** Better English? (because the sentence begins with "once"). *Category : EDITORIAL*

#	Para	Text	Comment
124	108	The ELISA is negative if the average absorbance readings of duplicate wells containing tissue macerate is <2× the average absorbance of the negative control wells containing healthy host tissue macerate.	**European Union** In addition to the general statement for thresholds a reference to the instructions for interpretation provided by of kit providers should be added. *Category : TECHNICAL*
125	108	The ELISA is negative if the average absorbance readings of duplicate wells containing tissue macerate is <2× the average absorbance of the negative control wells containing healthy host tissue macerate.	**EPPO** In addition to the general statement for thresholds a reference to the instructions for interpretation provided by of kit providers should be added. *Category : TECHNICAL*
126	110	**3.4 Molecular detection**	**Uruguay** It would be advisable to include a flowchart for the detection and identification of X. fastidiosa in order to clarify the diagnostic process. *Category : TECHNICAL*
127	110	**3.4 Molecular detection**	**Argentina** It would be advisable to include a flowchart for the detection and identification of X. fastidiosa in order to clarify the diagnostic process. *Category : TECHNICAL*
128	111	Various molecular methods have been developed for the detection and identification of *X. fastidiosa* directly on pure cultures, plant tissue and insect vectors (Firraro and Bazzi., 1994; Minsavage *et al*., 1994; Pooler and Hartung 1995; Schaad *et al.*, 2002; Rodrigues *et al.*, 2003; Francis *et al.*, 2006; Harper *et al.*, 2010, erratum 2013; Li *et al.*, 2013; Ouyang *et al.*, 2013). The conventional PCR developed by Minsavage *et al.* (1994) and Rodrigues *et al*. (2003), and two real-time PCR's (Harper *et al.*, 2010, and Li *et al.*, 2013) are described in this protocol for the detection and identification of *X. fastidiosa*.	**European Union** Other alternative incubation conditions can be conducted, e.g. 100°C for 5 min (EPPO, 2016) (last sentence of the paragraph). *Category : TECHNICAL*
129	111	Various molecular methods have been developed for the detection and identification of *X. fastidiosa* directly on pure cultures, plant tissue and insect vectors (Firraro and Bazzi., 1994; Minsavage *et al*., 1994; Pooler and Hartung 1995; Schaad *et al.*, 2002; Rodrigues *et al.*, 2003; Francis *et al.*, 2006; Harper *et al.*, 2010, erratum 2013; Li *et al.*, 2013; Ouyang *et al.*, 2013). The conventional PCR developed by Minsavage *et al.* (1994) and Rodrigues *et al*. (2003), and two real-time PCR's (Harper *et al.*, 2010, and Li *et al.*, 2013) are described in this protocol for the detection and identification of *X. fastidiosa*.	**European Union** The choice for the detection (real time) PCRs among IPPC and EPPO protocols is partly different. Is the choice of IPPC based on validation data and if yes, where can it be found? In Europe the test from Francis et al (2006) is commonly used for confirmation of identifications. However, its limit of specificity (as stated by Harper et al. (2010, erratum 2013)) should be noted: the following strains are not detected OAK0024 L. Nunney, UC Riverside and LIQ0063 L. Nunney, UC Riverside. *Category : TECHNICAL*
130	111	Various molecular methods have been developed for the detection and identification of *X. fastidiosa* directly on pure cultures, plant tissue and insect vectors (Firraro and Bazzi., 1994; Minsavage *et al*., 1994; Pooler and Hartung 1995; Schaad *et al.*, 2002; Rodrigues *et al.*, 2003; Francis *et al.*, 2006; Harper *et al.*, 2010, erratum 2013; Li *et al.*, 2013; Ouyang *et al.*, 2013). The conventional PCR developed by Minsavage *et al.* (1994) and Rodrigues *et al*. (2003), and two real-time PCR's (Harper *et al.*, 2010, and Li *et al.*, 2013) are described in this protocol for the detection and identification of *X. fastidiosa*.	**EPPO** The choice for the detection (real time) PCRs among IPPC and EPPO protocols is partly different. Is the choice of IPPC based on validation data and if yes, where can it be found? In Europe the test from Francis et al (2006) is commonly used for confirmation of identifications. However, its limit of specificity (as stated by Harper et al. (2010, erratum 2013)) should be noted: the following strains are not detected OAK0024 L. Nunney, UC Riverside and LIQ0063 L. Nunney, UC Riverside. *Category : TECHNICAL*

#	Para	Text	Comment
131	111	Various molecular methods have been developed for the detection and identification of *X. fastidiosa* directly on pure cultures, plant tissue and insect vectors (Firraro and Bazzi., 1994; Minsavage *et al*., 1994; Pooler and Hartung 1995; Schaad *et al.*, 2002; Rodrigues *et al.*, 2003; Francis *et al.*, 2006; Harper *et al.*, 2010, erratum 2013; Li *et al.*, 2013; Ouyang *et al.*, 2013). The conventional PCR developed by Minsavage *et al.* (1994) and Rodrigues *et al*. (2003), and two real-time PCR's (Harper *et al.*, 2010, and Li *et al.*, 2013) are described in this protocol for the detection and identification of *X. fastidiosa*.	**EPPO** Other alternative incubation conditions can be conducted, e.g. 100°C for 5 min (EPPO, 2016) (last sentence of the paragraph). *Category : TECHNICAL*
132	112	**3.4.1 Nucleic acid extraction and purification for bacterial colonies and plant material**	**European Union** Due to the heterogeneous distribution of the bacteria into the vessels tissues and to be consistent with the recommendation on the sampling section, the DNA extraction should be conducted on 0,5 to 1g of plant material. The text should be revised accordingly. We have tried to mark these (but to group comments they are often marked as technical). It should be noted in the protocol that other or other similarly DNA extraction kits exist. *Category : SUBSTANTIVE*
133	112	**3.4.1 Nucleic acid extraction and purification for bacterial colonies and plant material**	**EPPO** Due to the heterogeneous distribution of the bacteria into the vessels tissues and to be consistent with the recommendation on the sampling section, the DNA extraction should be conducted on 0,5 to 1g of plant material. The text should be revised accordingly. We have tried to mark these (but to group comments they are often marked as technical) It should be noted in the protocol that other or other similarly DNA extraction kits exist. *Category : SUBSTANTIVE*
134	112	**3.4.1 Nucleic acid extraction and purification for bacterial colonies and plant material**	**Czech Republic** CTAB-based extraction – better to specify that the aquous phase is the upper one *Category : TECHNICAL*
135	112	**3.4.1 DNA extraction from plant materials~~Nucleic acid extraction and purification for bacterial colonies and plant material~~**	**Singapore** The methods mentioned in following para only pertained to extraction of DNA from plant materials. Hence, proposed revision for better title alignment with content. *Category : SUBSTANTIVE*
136	114	*CTAB-based extraction.* 200 mg midrib, petiole or twig tissue is placed into an extraction bag with 5 ml CTAB buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 10 mM ethylenediaminetetraacetic (EDTA); 2% hexadecyltrimethylammonium bromide (CTAB); 3% polyvinylpyrrolidone (PVP-40)) and homogenized using a homogenizer (e.g. Homex1, Polytron1). The homogenate (1 ml) is transferred to a microcentrifuge tube and incubated at 65 °C for 30 min. After cooling, the tube is centrifuged at 16 000 *g* for 5 min. The supernatant (1 ml) is transferred to a new tube and mixed with the same	**European Union** Weight of plant material to be revised. In EPPO protocol (EPPO, 2016), 700µL of supernatant is mixed with 490µL (0,7 volume) of ice-cold isopropanol. *Category : TECHNICAL*

#	Para	Text	Comment
		volume of chloroform:isoamylalcohol (24:1, v/v), vortexed and then centrifuged at 3000 *g* for 15 min. The aqueous layer (c. 750 µl) is carefully transferred to a new tube and mixed with the same volume of ice-cold isopropanol. The suspension is mixed gently and incubated for at least 30 min at −20 °C. After this DNA precipitation step, the suspension is centrifuged at 16 000 *g* for 15 min and the supernatant is then discarded, taking care not to disturb the pellet. The pellet is washed with 1 ml ethanol (70%) by repeating the above centrifugation step. After washing and decanting the supernatant, the pellet is air dried and suspended in 100 µl deoxyribonuclease-free water.	
137	114	*CTAB-based extraction.* 200 mg midrib, petiole or twig tissue is placed into an extraction bag with 5 ml CTAB buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 10 mM ethylenediaminetetraacetic (EDTA); 2% hexadecyltrimethylammonium bromide (CTAB); 3% polyvinylpyrrolidone (PVP-40)) and homogenized using a homogenizer (e.g. Homex1, Polytron1). The homogenate (1 ml) is transferred to a microcentrifuge tube and incubated at 65 °C for 30 min. After cooling, the tube is centrifuged at 16 000 *g* for 5 min. The supernatant (1 ml) is transferred to a new tube and mixed with the same volume of chloroform:isoamylalcohol (24:1, v/v), vortexed and then centrifuged at 3000 *g* for 15 min. The aqueous layer (c. 750 µl) is carefully transferred to a new tube and mixed with the same volume of ice-cold isopropanol. The suspension is mixed gently and incubated for at least 30 min at −20 °C. After this DNA precipitation step, the suspension is centrifuged at 16 000 *g* for 15 min and the supernatant is then discarded, taking care not to disturb the pellet. The pellet is washed with 1 ml ethanol (70%) by repeating the above centrifugation step. After washing and decanting the supernatant, the pellet is air dried and suspended in 100 µl deoxyribonuclease-free water.	**EPPO** Weight of plant material to be revised In EPPO protocol (EPPO, 2016), 700µL of supernatant is mixed with 490µL (0,7 volume) of ice-cold isopropanol *Category : TECHNICAL*
138	115	*DNeasy Plant Mini Kit* (Qiagen)1. DNA is extracted from 200 mg plant tissue (leaf midrib, petiole or twig tissue) and macerated in lysis buffer using homogenizing equipment (e.g. Homex1, Polytron1). Alternatively, plant tissue can be ground to a fine powder in liquid nitrogen prior to extraction. These extracts are then treated according to the manufacturer's instructions.	**European Union** Weight of plant material to be revised. *Category : SUBSTANTIVE*
139	115	*DNeasy Plant Mini Kit* (Qiagen)1. DNA is extracted from 200 mg plant tissue (leaf midrib, petiole or twig tissue) and macerated in lysis buffer using homogenizing equipment (e.g. Homex1, Polytron1). Alternatively, plant tissue can be ground to a fine powder in liquid nitrogen prior to extraction. These extracts are then treated according to the manufacturer's instructions.	**EPPO** Weight of plant material to be revised *Category : SUBSTANTIVE*
140	115	*DNeasy Plant Mini Kit* (Qiagen)1. DNA is extracted from 200 mg plant tissue (leaf	**Australia** According to the manufacturer's instructions, they suggest 100 mg

#	Para	Text	Comment
		midrib, petiole or twig tissue) and macerated in lysis buffer using homogenizing equipment (e.g. Homex1, Polytron1). Alternatively, plant tissue can be ground to a fine powder in liquid nitrogen prior to extraction. These extracts are then treated according to the manufacturer's instructions.	fresh tissue or 20 mg of dry tissue. *Category : TECHNICAL*
141	115	*DNeasy Plant Mini Kit* (Qiagen)1. DNA is extracted from 200 mg plant tissue (leaf midrib, petiole or twig tissue) and macerated in lysis buffer using homogenizing equipment (e.g. Homex1, Polytron1). Alternatively, plant tissue can be ground to a fine powder in liquid nitrogen prior to extraction. These extracts are then treated according to the manufacturer's instructions.	**Turkey** Dneasy Food mericon kit (Qiagen) also to be included *Category : TECHNICAL*
142	116	*QuickPick SML Plant DNA Kit* (Bio-Nobile)1. Plant tissue (200 mg leaf midrib, petiole or twig tissue) is homogenized using any of the available methods (e.g. mechanical grinding with bead mills or with liquid nitrogen, tissue grinder). The plant material should be sufficiently homogenized before starting the purification procedure. Appropriate volumes of Plant DNA Lysis Buffer and proteinase K solution, as specified in the manufacturer's instructions, are added to the plant tissue. The sample is thoroughly vortex-mixed and then incubated at 65 °C for 15–30 min. After the lysis step, DNA purification is performed according to the manufacturer's instructions. Alternatively, a larger sample size can be processed by crushing 0.5–1 g fresh small pieces of midribs, petioles, basal leaf part or twigs in 5 ml sterile water and leaving to soak for 15 min with gentle shaking. The plant extract (250 µl) is centrifuged for 20 min at 20 000 *g*. The pellet is then suspended in 75 µl lysis buffer with 5 µl proteinase K and the manufacturer's instructions followed. This method can be performed either manually or with the KingFisher mL1 (15 samples) or KingFisher Flex1 (96 samples) purification system (Thermo Scientific)1 (validation data available at http://dc.eppo.int/validationlist.php).	**European Union** 1) Weight of plant material to be revised. 2) Performing the method manually is not recommended if the user is not familiar with it because of the risk of cross-contamination between samples is high. Maybe this point should be commented. *Category : TECHNICAL*
143	116	*QuickPick SML Plant DNA Kit* (Bio-Nobile)1. Plant tissue (200 mg leaf midrib, petiole or twig tissue) is homogenized using any of the available methods (e.g. mechanical grinding with bead mills or with liquid nitrogen, tissue grinder). The plant material should be sufficiently homogenized before starting the purification procedure. Appropriate volumes of Plant DNA Lysis Buffer and proteinase K solution, as specified in the manufacturer's instructions, are added to the plant tissue. The sample is thoroughly vortex-mixed and then incubated at 65 °C for 15–30 min. After the lysis step, DNA purification is performed according to the manufacturer's instructions. Alternatively, a larger sample size can be processed by crushing 0.5–1 g fresh small pieces of midribs, petioles, basal leaf part or twigs in 5 ml sterile water and leaving to soak for 15 min with gentle shaking. The plant extract (250 µl) is centrifuged for 20 min	**EPPO** 1 Weight of plant material to be revised 2 Performing the method manually is not recommended if the user is not familiar with it because of the risk of cross-contamination between samples is high. Maybe this point should be commented *Category : TECHNICAL*

#	Para	Text	Comment
		at 20 000 *g*. The pellet is then suspended in 75 µl lysis buffer with 5 µl proteinase K and the manufacturer's instructions followed. This method can be performed either manually or with the KingFisher mL1 (15 samples) or KingFisher Flex1 (96 samples) purification system (Thermo Scientific)1 (validation data available at http://dc.eppo.int/validationlist.php).	
144	118	**3.4.2 DNA Extraction~~Nucleic acid extraction and purification for~~ from insect vectors**	**Singapore** Again, the methods mentioned in following para only pertained to DNA extraction from insect vectors via DNA extraction kits. Hence, proposed change in title to reflect the content. *Category : SUBSTANTIVE*
145	124	This PCR was designed by Minsavage *et al.* (1994) to target part of the *rpoD* gene, producing an amplicon of 733 base pairs (bp). It is widely used in many laboratories for the detection of *X. fastidiosa* in different host plants and vectors. Analytical specificity was validated by Harper *et al.* (2010) with 22 different *X. fastidiosa* strains from 11 different hosts and 12 closely related or host related non-target bacterial strains. In their study, American *X. fastidiosa* strains from red oak and turkey oak and several strains from ~~grape vines~~ grapevines were not detected with this PCR. The analytical sensitivity of the test as stated by Minsavage et al. (1994) and on specific host is 1×10^2 c.f.u./ml. Further validation data on various hosts are available at http://dc.eppo.int/validationlist.php.	**European Union** Typo (for consistency with paragraph 58). This level of sensitivity was established on Vitis vinifera and Prunus persica. The sensitivity is lower on other plants and is documented on the EPPO webpage cited here. *Category : TECHNICAL*
146	124	This PCR was designed by Minsavage *et al.* (1994) to target part of the *rpoD* gene, producing an amplicon of 733 base pairs (bp). It is widely used in many laboratories for the detection of *X. fastidiosa* in different host plants and vectors. Analytical specificity was validated by Harper *et al.* (2010) with 22 different *X. fastidiosa* strains from 11 different hosts and 12 closely related or host related non-target bacterial strains. In their study, American *X. fastidiosa* strains from red oak and turkey oak and several strains from ~~grape vines~~ grapevines were not detected with this PCR. The analytical sensitivity of the test as stated by Minsavage et al. (1994) and on specific host is 1×10^2 c.f.u./ml. Further validation data on various hosts are available at http://dc.eppo.int/validationlist.php.	**EPPO** Typo (for consistency with paragraph 58). This level of sensitivity was established on Vitis vinifera and Prunus persica. The sensitivity is lower on other plants and is documented on the EPPO webpage cited here. *Category : TECHNICAL*
147	140	Primer RST31 (forward)	**European Union** The primer concentration is higher than in Appendix 4 (EPPO). *Category : TECHNICAL*
148	140	Primer RST31 (forward)	**EPPO** the primer concentration is higher than in Appendix 4 (EPPO) *Category : TECHNICAL*
149	144	Taq DNA polymerase (Invitrogen1)	**European Union** The Taq DNA polymerase's concentration is different from the one mentioned in the EPPO protocol and from the original publication. Same comment for primers concentration (see below). *Category : TECHNICAL*

#	Para	Text	Comment
150	144	Taq DNA polymerase (Invitrogen1)	**EPPO** The Taq DNA polymerase's concentration is different from the one mentionned in the EPPO protocol and from the original publication Same comment for primers concentration (see below) *Category : TECHNICAL*
151	166	bp, base pairs; N.A., not applicable~~;~~.	**European Union** Typo. *Category : EDITORIAL*
152	166	bp, base pairs; N.A., not applicable~~;~~.	**EPPO** Typo *Category : EDITORIAL*
153	166	bp, base pairs; N.A., not applicable;.	**Philippines** Check font type consistency *Category : EDITORIAL*
154	167	**3.4.4 Conventional PCR using the primers of Rodrigues *et al.* (2003)**	**Czech Republic** Chap. 3.4.4 - Rodrigues et al., 2003 conventional PCR – unclear if there is any difference among the 16S rDNA primers used (set A,B,C), if they should be used all in three separate reactions or it is enough to chose one set? Both genes should be amplified for positive detection or it is sufficient to test one gene? The given analytical sensitivity corresponds to multiplex PCR but there are only informations for singleplex reactions and missing mutliplex PCR conditions (e.g. anneling tempereture, primer concentrations). *Category : TECHNICAL*
155	168	The PCR based on primers for the 16S ribosomal (r)RNA and *gyrB* genes was developed by Rodrigues *et al.* (2003). The 16S rRNA gene-targeted primers (sets A, B, C), the *gyrB* gene-targeted primers (FXYgyr499 and RXYgyr907) and the multiplex PCR (16SrRNA and *gyrB* primers combined) were evaluated using 30 *X. fastidiosa* strains from different plant hosts and 36 closely related or host related non-target bacterial strains. The analytical sensitivity for the multiplex PCR is approximately 102 c.f.u./ml.	**Kenya** Provide gel specifications that are missing from the document *Category : TECHNICAL*
156	268	Harper *et al.* (2010, erratum 2013) evaluated specificity (analytical specificity) with 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. Only *X. fastidiosa* was detected. *Xylella taiwanensis* from Taiwan Province of China was not detected. The PCR was further validated by Li *et al.* (2013). Diagnostic specificity and sensitivity, as determined using citrus and grape hosts, are 100% (EPPO, 2016). For *Olea europaea* hosts when using CTAB extraction methods, diagnostic specificity is 100% and diagnostic sensitivity is 91% (EPPO, 2016). Further validation data are available at http://dc.eppo.int/validationlist.php. The analytical sensitivity ~~(analytical sensitivity; detection~~ (detection limit) is between 10^2 c.f.u./ml for *Citrus* spp. and *Vitis vinifera* and 10^5 c.f.u./ml for *Olea europaea*.	**European Union** Shorter and clearer ? *Category : EDITORIAL*
157	268	Harper *et al.* (2010, erratum 2013) evaluated specificity (analytical specificity) with 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. Only *X. fastidiosa* was detected. *Xylella taiwanensis* from Taiwan Province of China was not	**EPPO** Shorter and clearer? *Category : EDITORIAL*

#	Para	Text	Comment
		detected. The PCR was further validated by Li *et al.* (2013). Diagnostic specificity and sensitivity, as determined using citrus and grape hosts, are 100% (EPPO, 2016). For *Olea europaea* hosts when using CTAB extraction methods, diagnostic specificity is 100% and diagnostic sensitivity is 91% (EPPO, 2016). Further validation data are available at http://dc.eppo.int/validationlist.php. The analytical sensitivity ~~(analytical sensitivity; detection~~ (detection limit) is between 10^2 c.f.u./ml for *Citrus* spp. and *Vitis vinifera* and 10^5 c.f.u./ml for *Olea europaea*.	
158	272	XF-P (hydrolysis probe): 5′-6-FAM-TCG CAT CCC GTG GCT ~~CAG TCC-BHQ-1-3~~CAGCC-BHQ-1-3′	**China** Based on the original reference (Harper et al., 2010), the sequences of XF-P should be.....CAGCC-BHQ-1-3′ *Category : EDITORIAL*
159	298	94 °C for 2 min	**European Union** Although 94°C for 2 mn is stated in the original publication, when preparing the EPPO protocol EPPO experts agreed on 95°C for 10 mn based on current practices. For Minsavage test, when drafted the protocol we also noted that according to the laboratory there were variations. Could possibilities for variations be indicated in the protocol? *Category : TECHNICAL*
160	298	94 °C for 2 min	**EPPO** Although 94°C for 2 mn is stated in the original publication, when preparing the EPPO protocol EPPO experts agreed on 95°C for 10 mn based on current practices. For Minsavage test, when drafted the protocol we also noted that according to the laboratory there were variations. Could possibilities for variations be indicated in the protocol? *Category : TECHNICAL*
161	307	BSA, bovine serum albumin; N.A., not applicable~~;~~.	**European Union** Typo. *Category : EDITORIAL*
162	307	BSA, bovine serum albumin; N.A., not applicable~~;~~.	**EPPO** Typo *Category : EDITORIAL*
163	311	Li *et al.* (2013) evaluated analytical specificity ~~(analytical specificity)~~ with 77 strains of *X. fastidiosa* from 15 different hosts and 14 non-target bacterial strains. Only *X. fastidiosa* was detected. Diagnostic specificity and sensitivity, as determined using *Citrus* hosts, were both 100%. The analytical sensitivity ~~(analytical sensitivity; detection~~ (detection limit) is between 2 and 10 cells of *X. fastidiosa* per reaction for *Citrus* samples.	**European Union** Shorter and clearer ? *Category : EDITORIAL*
164	311	Li *et al.* (2013) evaluated analytical specificity ~~(analytical specificity)~~ with 77 strains of *X. fastidiosa* from 15 different hosts and 14 non-target bacterial strains. Only *X. fastidiosa* was detected. Diagnostic specificity and sensitivity, as determined using *Citrus* hosts, were both 100%. The analytical sensitivity ~~(analytical sensitivity; detection~~ (detection limit) is between 2 and 10 cells of *X. fastidiosa* per reaction for *Citrus*	**EPPO** Shorter and clearer? *Category : EDITORIAL*

#	Para	Text	Comment
		samples.	
165	350	N.A., not applicable~~;~~.	**European Union** Typo. *Category : EDITORIAL*
166	350	N.A., not applicable~~;~~.	**EPPO** Typo *Category : EDITORIAL*
167	353	A sample will be considered positive if it produces a Ct value of <40, provided the contamination controls are negative.	**European Union** In EPPO Protocols Ct threshold are only provided for situations where the Ct is unusual. For other cases we have the following standard text: Verification of the controls • The PIC and PAC (as well as IC and IPC as applicable) amplification curves should be exponential. • NIC and NAC should give no amplification When these conditions are met: • A test will be considered positive if it produces an exponential amplification curve. • A test will be considered negative, if it does not produce an amplification curve or if it produces a curve which is not exponential. • Additionally for SYBR® Green based real-time PCR tests: the TM value should be as expected. • Tests should be repeated if any contradictory or unclear results are obtained. NIC = Negative isolation control PIC = Positive isolation control NAC = Negative amplification control PAC= Positive amplification control IC= Internal Control IPC= Internal positive controls *Category : TECHNICAL*
168	353	A sample will be considered positive if it produces a Ct value of <40, provided the contamination controls are negative.	**EPPO** In EPPO Protocols Ct threshold are only provided for situations where the Ct is unusual. For other cases we have the following standard text: Verification of the controls • The PIC and PAC (as well as IC and IPC as applicable) amplification curves should be exponential. • NIC and NAC should give no amplification When these conditions are met: • A test will be considered positive if it produces an exponential amplification curve. • A test will be considered negative, if it does not produce an amplification curve or if it produces a curve which is not exponential. • Additionally for SYBR® Green based real-time PCR tests: the TM

#	Para	Text	Comment
			value should be as expected. • Tests should be repeated if any contradictory or unclear results are obtained. NIC = Negative isolation control PIC = Positive isolation control NAC = Negative amplification control PAC= Positive amplification control IC= Internal Control IPC= Internal positive controls *Category : TECHNICAL*
169	356	~~**3.4.7 LAMP[2] using the primers of Harper *et al.* (2010, erratum 2013)**~~	**China** Although LAMP is a good method with high sensitivity, but this method was thought very easy to cross contamination in many labs in China. *Category : TECHNICAL*
170	360	~~Method~~ Analytical specificity ~~(analytical specificity)~~ using hydroxynaphthol blue for endpoint detection is similar to that reported for the real-time PCR (Harper *et al.*, 2010, erratum 2013). In validation, only *X. fastidiosa* was detected among 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. All strains of *X. fastidiosa* were detected~~, except for the *Xylella* strain from Taiwan Province of China~~.	**European Union** Shorter and clearer? Now classified as X. taiwanensis according to end of paragraph 45. *Category : EDITORIAL*
171	360	~~Method~~ Analytical specificity ~~(analytical specificity)~~ using hydroxynaphthol blue for endpoint detection is similar to that reported for the real-time PCR (Harper *et al.*, 2010, erratum 2013). In validation, only *X. fastidiosa* was detected among 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. All strains of *X. fastidiosa* were detected~~, except for the *Xylella* strain from Taiwan Province of China~~.	**EPPO** Now classified as X. taiwanensis according to end of paragraph 45. Shorter and clearer? *Category : EDITORIAL*
172	408	BSA, bovine serum albumin; N.A., not applicable~~;~~.	**European Union** Typo. *Category : EDITORIAL*
173	408	BSA, bovine serum albumin; N.A., not applicable~~;~~.	**EPPO** Typo *Category : EDITORIAL*
174	411	This test is based on the above LAMP primers developed by Harper *et al*. (2010, erratum 2013), and was modified by Yaseen *et al*. (2015). The modifications consist of a simplified extraction method and reduced incubation times. The test is commercially available as ready-to-use kits and is performed in real-time on a specific device or by using a standard real-time thermocycler (e.g. Enbiotech[1], Qualiplante[1], Optigene[1]). The kits should be used as per the manufacturer's instructions. Diagnostic sensitivity and specificity using the Enbiotech[1] and Qualiplante[1] kits have been determined as being between 83% and 92%. The analytical sensitivity of these kits ~~(analytical sensitivity;~~	**European Union** Shorter, clearer ? *Category : EDITORIAL*

#	Para	Text	Comment
		~~detection~~ (detection limit) is between 10^2 and 10^3 c.f.u./ml for citrus, grape and olive. Validation data are available at http://dc.eppo.int/validationlist.php.	
175	411	This test is based on the above LAMP primers developed by Harper *et al*. (2010, erratum 2013), and was modified by Yaseen *et al*. (2015). The modifications consist of a simplified extraction method and reduced incubation times. The test is commercially available as ready-to-use kits and is performed in real-time on a specific device or by using a standard real-time thermocycler (e.g. Enbiotech[1], Qualiplante[1], Optigene[1]). The kits should be used as per the manufacturer's instructions. Diagnostic sensitivity and specificity using the Enbiotech[1] and Qualiplante[1] kits have been determined as being between 83% and 92%. The analytical sensitivity of these kits ~~(analytical sensitivity; detection~~ (detection limit) is between 10^2 and 10^3 c.f.u./ml for citrus, grape and olive. Validation data are available at http://dc.eppo.int/validationlist.php.	**EPPO** Shorter , clearer? *Category : EDITORIAL*
176	417	*Positive nucleic acid control*. This control is used to monitor the efficiency of PCR amplification. Pre-prepared (stored) nucleic acid, whole genomic DNA or a synthetic control (e.g. cloned PCR product) may be used. For this protocol, genomic DNA (50 ng/µl) extracted from either a culture of *X. fastidiosa* or naturally infected tissue is recommended as a positive nucleic acid control.	**Philippines** Very difficult to obtain positive controls (PC). If detection will be carried out by countries with no reported occurrence of the pathogen, where to source the PC is a problem. Are there NPPOs willing to share whole genomic DNA for this purpose? *Category : SUBSTANTIVE*
177	423	**3.4.9 Interpretation of results from conventional and real-time PCR**	**European Union** There is no section for the interpretation of results from LAMP tests, whereas they are cited as possible tests to be conducted. *Category : TECHNICAL*
178	423	**3.4.9 Interpretation of results from conventional and real-time PCR**	**EPPO** There is no section for the interpretation of results from LAMP tests, whereas they are cited as possible tests to be conducted. *Category : TECHNICAL*
179	423	**3.4.9 Interpretation of results from conventional and real-time PCR**	**Singapore** How about interpretation of results from LAMP which had not been covered under 3.4.9. Suggest to include for LAMP for consistency in DP. *Category : SUBSTANTIVE*
180	437	The minimum requirements for ~~identification~~ detection in plant extracts are positive results from two detection tests based on different biological principles or from two molecular tests that amplify different genetic loci. However, if the outcome is critical (e.g. post-entry quarantine samples, new host record, new country record), it is recommended that the bacterium is isolated and the requirements for Koch's postulates fulfilled.	**European Union** *Category : TECHNICAL*
181	437	The minimum requirements for identification are positive results from two tests based on different biological principles or from two molecular tests that amplify different genetic loci. However, if the outcome is critical (e.g. post-entry quarantine samples, new host record, new country record), it is recommended that the bacterium is isolated and the requirements for Koch's postulates fulfilled.	**European Union** In the EPPO Diagnostic protocol PM 7/24 (2) Sep. 2016: "For areas where the pest is known to be present or in buffer zones one positive test is sufficient to consider a sample as 'sample with suspected presence of X. Fastidiosa"

#	Para	Text	Comment
			This includes symptomatic but also asymptomatic plants. "Testing for asymptomatic plants in an outbreak area or a buffer zone around an outbreak often implies that a large number of tests need to be performed. In such a situation, and given that the concentration of the bacterium is expected to be higher than in an area thought to be pest free, a single test including serological tests (e.g. ELISA) may be performed." Could such an approach be considered in the IPPC Standard? *Category : TECHNICAL*
182	437	The minimum requirements for identification are positive results from two tests based on different biological principles or from two molecular tests that amplify different genetic loci. However, if the outcome is critical (e.g. post-entry quarantine samples, new host record, new country record), it is recommended that the bacterium is isolated and the requirements for Koch's postulates fulfilled.	**EPPO** ES In the EPPO Diagnostic protocol PM 7/24 (2) Sep. 2016. "For areas where the pest is known to be present or in buffer zones one positive test is sufficient to consider a sample as 'sample with suspected presence of X. Fastidiosa" This includes symptomatic but also asymptomatic plants "Testing for asymptomatic plants in an outbreak area or a buffer zone around an outbreak often implies that a large number of tests need to be performed. In such a situation, and given that the concentration of the bacterium is expected to be higher than in an area thought to be pest free, a single test including serological tests (e.g. ELISA) may be performed." Could such an approach be considered in the IPPC Standard? *Category : TECHNICAL*
183	437	The minimum requirements for ~~identification~~ detection in plant extracts are positive results from two detection tests based on different biological principles or from two molecular tests that amplify different genetic loci. However, if the outcome is critical (e.g. post-entry quarantine samples, new host record, new country record), it is recommended that the bacterium is isolated and the requirements for Koch's postulates fulfilled.	**EPPO** *Category : TECHNICAL*
184	442	It is very important to surface sterilize the sample in order to avoid contaminants, because *X. fastidiosa* grows very slowly (up to 30 days) and can be readily overgrown by other micro-organisms. Petiole or midrib samples are surface sterilized with 1% bleach for 2 min and then by immersion in 70% ethanol for 1 ~~min and then with 1% bleach for 2~~ min, followed by two rinses in sterile distilled water. Alternatively they can also be flamed. Surface-sterilized plant tissue segments are cut in the middle, squeezed with flame-sterilized needle-nose pliers, and the sap that exudes can be blotted directly onto media (Hopkins, 2001). Alternatively, tissue is ground in PBS at ratios of 1:10 and 1:100 with a mortar and pestle or a homogenizer (e.g. Homex)[1] and then plated onto two	**European Union** IPPC recommends first ethanol and then bleach, EPPO the other way around. The IPPC recommendation is simply not logical. Another alternative way to sterilize is to flame plant surface as cited in EPPO (2016). *Category : TECHNICAL*

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		different types of specific media (e.g. PD2, BCYE, PWG).	
185	442	It is very important to surface sterilize the sample in order to avoid contaminants, because *X. fastidiosa* grows very slowly (up to 30 days) and can be readily overgrown by other micro-organisms. Petiole or midrib samples are surface sterilized by immersion in 70% ethanol for 1 min and then with 1% bleach for 2 min, followed by two rinses in sterile distilled water. Surface-sterilized plant tissue segments are cut in the middle, squeezed with flame-sterilized needle-nose pliers, and the sap that exudes can be blotted directly onto media (Hopkins, 2001). Alternatively, tissue is ground in PBS at ratios of 1:10 and 1:100 with a mortar and pestle or a homogenizer (e.g. Homex)[1] and then plated onto two different types of specific media (e.g. PD2, BCYE, PWG).	**European Union** Could ultrasonication be considered for the protocol and a sentence added at the end of the paragraph Bergsma-Vlami et al. 2017 abstract: "Isolation of X. fastidiosa from leaf petioles and midribs of infected C. arabica plants was successfully performed only after the application of an additional ultrasonication step during the extraction procedure". Extracts of the main text: ...After homogenisation and addition of PBS (0,01 M pH 7,4), the crushed plant material was initially ultrasonicated for 30 sec, 45 sec and 60 sec respectively. Ultrasonication (Branson Ultrasonic Cleaner; Branson Ultrasonics, Danbury, CT) was performed at a frequency of 40 kHz for each time duration... ...The duration of the ultrasonication did not actually influence the number of isolates obtained. The application of ultrasonication in order to disrupt the biofilm-like colonies of X. fastidiosa inside the xylem vessels of C. arabica midribs and petioles followed by isolation seems to be a sensitive method for a succesful isolation from asymptomatic C. arabica plants. Bergsma-Vlami, M., van de Bilt, J. L. J., Tjou-Tam-Sin, N. N. A., Helderman, C. M., Gorkink-Smits, P. P. M. A., Landman, N. M., van Nieuwburg, J. G. W., van Veen, E. J. and Westenberg, M. (2017), Assessment of the genetic diversity of Xylella fastidiosa in imported ornamental Coffea arabica plants. Plant Pathol, 66: 1065–1074. doi:10.1111/ppa.12696, There is also a good experience with ultrasonication in Mallorca. *Category : TECHNICAL*
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			...The duration of the ultrasonication did not actually influence the number of isolates obtained. The application of ultrasonication in order to disrupt the biofilm-like colonies of X. fastidiosa inside the xylem vessels of C. arabica midribs and petioles followed by isolation seems to be a sensitive method for a succesful isolation from asymptomatic C. arabica plants. Bergsma-Vlami, M., van de Bilt, J. L. J., Tjou-Tam-Sin, N. N. A., Helderman, C. M., Gorkink-Smits, P. P. M. A., Landman, N. M., van Nieuwburg, J. G. W., van Veen, E. J. and Westenberg, M. (2017), Assessment of the genetic diversity of Xylella fastidiosa in imported ornamental Coffea arabica plants. Plant Pathol, 66: 1065–1074. doi:10.1111/ppa.12696, THere is also a good experience with ultrasonication in Mallorca. *Category : TECHNICAL*
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188	445	**4.1.1 Culture media**	**European Union** General comment on this section: there are several differences in media composition with EPPO protocol (EPPO, 2016). Furthermore, it has been shown through the EPPO members's experience that specific chemicals should be used to get reliable and reproducible results. EPPO (2016) provides specific references fort the relevant chemicals. *Category : SUBSTANTIVE*
189	445	**4.1.1 Culture media**	**European Union** The companies Difco and DB merged and one name 'BD Difco' should be used throughout the protocol. *Category : TECHNICAL*
190	445	**4.1.1 Culture media**	**EPPO** The companies Difco and DB merged and one name 'BD Difco' should be used throughout the protocol *Category : TECHNICAL*
191	445	**4.1.1 Culture media**	**EPPO** General comment: there are several differences in media composition with EPPO protocol (EPPO, 2016). Furthermore, it has been shown through the EPPO members's experience that specific chemicals should be used to get reliable and reproducible results. EPPO (2016)

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			provides specific references fort he relevant chemicals. *Category : SUBSTANTIVE*
192	468	Bacto agar (Difco1)	**European Union** As en example, in the EPPO protocol (EPPO, 2016), the bacto agar is mentioned as Microbiological grade agar (Oxoid, LP0028). In the revision of the EPPO protocol 'BD Difco' will be added as it is used in many labs. *Category : TECHNICAL*
193	468	Bacto agar (Difco1)	**EPPO** As en example, in the EPPO protocol (EPPO, 2016), the bacto agar is mentionned as Microbiological grade agar (Oxoid, LP0028). In the revision of the EPPO prtoocol BD Difco' will be added as it is used in many labs. *Category : TECHNICAL*
194	473	*PD2 medium* (Table 7). All constituents except BSA (bovine serum albumin) and hemin chloride stock solution are added to 980 ml of distilled water prior to autoclaving. The pH is adjusted to 7.0 after dissolving the agar. After autoclaving, the BSA (dissolved in distilled water) and hemin chloride stock solution are filter-sterilized (0.2 µm membrane) and added to the cooled (45–50 °C) sterile basal medium.	**European Union** The target pH is defined as 6,9 in the EPPO protocol (EPPO, 2016). *Category : TECHNICAL*
195	473	*PD2 medium* (Table 7). All constituents except BSA (bovine serum albumin) and hemin chloride stock solution are added to 980 ml of distilled water prior to autoclaving. The pH is adjusted to 7.0 after dissolving the agar. After autoclaving, the BSA (dissolved in distilled water) and hemin chloride stock solution are filter-sterilized (0.2 µm membrane) and added to the cooled (45–50 °C) sterile basal medium.	**EPPO** The target pH is defined as 6,9 in the EPPO protocol (EPPO, 2016). *Category : TECHNICAL*
196	473	*PD2 medium* (Table 7). All ~~constituents~~ components except BSA (bovine serum albumin) and hemin chloride stock solution are added to 980 ml of distilled water prior to autoclaving. The pH is adjusted to 7.0 after dissolving the agar. After autoclaving, the BSA (dissolved in distilled water) and hemin chloride stock solution are filter-sterilized (0.2 µm membrane) and added to the cooled (45–50 °C) sterile basal medium.	**Philippines** *Category : EDITORIAL*
197	487	Bacto agar (Difco1)	**European Union** In EPPO, it is recommended Agar N°1 (Oxoid/LP011). In the revision of the EPPO protocol 'BD Difco' will be added as it is used in many laboratories. *Category : TECHNICAL*
198	487	Bacto agar (Difco1)	**EPPO** In EPPO, it is recommended Agar N°1 (Oxoid/LP011). in the revision of the EPPO protocol BBD Difco will be added as it is used in many laboratories. *Category : TECHNICAL*
199	492	**Table 9.** Modified PWG medium (based on Hill and Purcell, 1995 and information provided in EPPO ~~(2016)~~(2016))	**European Union** Typo. *Category : EDITORIAL*
200	492	**Table 9.** Modified PWG medium (based on Hill and Purcell, 1995 and information provided in EPPO ~~(2016)~~(2016))	**EPPO** Typo *Category : EDITORIAL*

#	Para	Text	Comment
201	499	Bacto tryptone (Oxoid1)	**European Union** Another example is that Bacto Tryptone is refered as BD Difco 211705 in the EPPO protocol (EPPO, 2016). *Category : TECHNICAL*
202	499	Bacto tryptone (Oxoid1)	**EPPO** Another example is that Bacto Tryptone is refered as BD Difco 211705 in the EPPO protocol (EPPO, 2016) *Category : TECHNICAL*
203	515	Bacto agar (Difco1)	**European Union** It is probably an error, the fact that there is already Gelrite (gelrite replaces agar). *Category : TECHNICAL*
204	515	Bacto agar (Difco1)	**EPPO** It is probably an error, the fact that there is already Gelrite (gelrite replaces agar). *Category : TECHNICAL*
205	520	*Modified PWG medium* (Table 9). All ~~constituents~~ components except L-glutamine, hemin chloride stock solution and BSA are added prior to autoclaving. Bovine serum albumin (3 g) is dissolved in 15 ml distilled water, and 4 g L-glutamine is dissolved in 50 ml distilled water over a low heat (c. 50 °C). Hemin chloride stock is 0.1 % bovine hemin chloride dissolved in 0.05 N NaOH. These three solutions are filter sterilized (0.2 µm membrane) and added to the cooled sterile basal medium.	**Philippines** *Category : EDITORIAL*
206	541	The reference *X. fastidiosa* strains available from different collections are listed in Table 10. These strains are suggested for use as positive controls in biochemical and molecular tests.	**Philippines** This addresses the concern on difficulty of obtaining positive controls. Section for requesting positive may also be included. NPPO needs guidance on how requests will be conducted. *Category : SUBSTANTIVE*
207	545	~~Catalase~~Gram stain - Catalase	**Kenya** Gram stain - *Category : TECHNICAL*
208	566	Pathogenicity tests should use plants of the same host from which the suspect *X. fastidiosa* was isolated. Where possible, the most susceptible cultivars should be used. Some recommended examples include: for *Vitis vinifera*, the cultivars Chardonnay, Cabernet sauvignon, Chenin Blanc and Pinot Noir; for *Citrus sinensis*, Pera, Hamlin, Natal and Valencia; and for *Olea europaea*, Cellina di Nardo, Frantoio and Leccino (EPPO, 2016). ~~Madagascar periwinkle (~~*Catharanthus ~~roseus~~roseus* ~~) is a herbaceous-grown plant that is easily grown in a greenhouse and is susceptible to~~ (Madagascar periwinkle) is a herbaceous-grown plant that is easily grown in a greenhouse and is susceptible to *X. fastidiosa* (Monteiro *et al.*, 2001).	**European Union** Latin name to be put before common name (see for example paragraph 571). *Category : EDITORIAL*
209	566	Pathogenicity tests should use plants of the same host from which the suspect *X. fastidiosa* was isolated. Where possible, the most susceptible cultivars should be used. Some recommended examples include: for *Vitis vinifera*, the cultivars Chardonnay, Cabernet sauvignon, Chenin Blanc and Pinot Noir; for *Citrus sinensis*,	**EPPO** Latin name to be put before common name (see for example paragraph 571). *Category : EDITORIAL*

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		Pera, Hamlin, Natal and Valencia; and for *Olea europaea*, Cellina di Nardo, Frantoio and Leccino (EPPO, 2016). ~~Madagascar periwinkle (~~*Catharanthus* ~~*roseus*~~*roseus* (Madagascar periwinkle)~~)~~ is a herbaceous-grown plant that is easily grown in a greenhouse and is susceptible to *X. fastidiosa* (Monteiro *et al.*, 2001).	
210	577	An MLST approach has been described for the identification of *X. fastidiosa* subspecies and is recommended for the characterization of new strains (Scally *et al.*, 2005; Yuan *et al.*, 2010; Jacques *et al.*, 2016). This approach can be used on DNA extracted from either bacterial cultures or infected plants tested positive for *X. fastidiosa* (Loconsole *et al.*, 2016). For amplification of DNA direct from plant tissue, it has been observed that the quality of the target DNA may not always be suitable for obtaining all amplicons (EPPO, 2016). Primers and conditions for the sequencing and analysis of seven housekeeping genes (*cysG*, *gltT*, *holC*, *leuA*, *malF*, *nuoL* and *petC*) are described by Yuan *et al.* (2010) and further details regarding analysis can be found on the *X. fastidiosa* MLST website (http://pubmlst.org/xfastidiosa/). The annealing temperature described in Yuan *et al.* (2010) is 65 °C but other researchers have observed that an annealing temperature of 60 °C improves the reliability of the method (French National Institute for Agricultural Research–Research Institute of Horticulture and Seeds, personal communication, 2016).	**European Union** The strong recommendation to use the MLST analysis instead of the PCR (markers) for subspecies determination and the argumentation for this is missing and should be added from the EPPO protocol. *Category : TECHNICAL*
211	577	An MLST approach has been described for the identification of *X. fastidiosa* subspecies and is recommended for the characterization of new strains (Scally *et al.*, 2005; Yuan *et al.*, 2010; Jacques *et al.*, ~~2016)~~2016, Bergsma *et al.*, 2017). This approach can be used on DNA extracted from either bacterial cultures or infected plants tested positive for *X. fastidiosa* (Loconsole *et al.*, 2016). For amplification of DNA direct from plant tissue, it has been observed that the quality of the target DNA may not always be suitable for obtaining all amplicons (EPPO, 2016). Primers and conditions for the sequencing and analysis of seven housekeeping genes (*cysG*, *gltT*, *holC*, *leuA*, *malF*, *nuoL* and *petC*) are described by Yuan *et al.* (2010) and further details regarding analysis can be found on the *X. fastidiosa* MLST website (http://pubmlst.org/xfastidiosa/). The annealing temperature described in Yuan *et al.* (2010) is 65 °C but other researchers have observed that an annealing temperature of 60 °C improves the reliability of the method (French National Institute for Agricultural Research–Research Institute of Horticulture and Seeds, personal communication, 2016).	**European Union** *Category : TECHNICAL*
212	577	An MLST approach has been described for the identification of *X. fastidiosa* subspecies and is recommended for the characterization of new strains (Scally *et al.*, 2005; Yuan *et al.*, 2010; Jacques *et al.*, 2016). This approach can be used on DNA extracted from either bacterial cultures or infected plants tested positive for *X. fastidiosa* (Loconsole *et al.*, 2016). For amplification of DNA direct from plant tissue, it has been observed that the quality of the target DNA may not always be suitable for obtaining all	**European Union** MLST has mainly been developed on pure cultures. It can be used on DNA extract from plants, however, it is recognized that the quantity and quality of target DNA, or the occurrence of possible mixed infections may prevent obtaining all amplicons or clear assignment of sub-species. A warning about this is included in the IPPC protocol. Regarding the adaptation of the annealing temperature, it is noted in our region that reliability is only increased for some genes and not all, and it also depends on the host plant tested.

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		amplicons (EPPO, 2016). Primers and conditions for the sequencing and analysis of seven housekeeping genes (*cysG*, *gltT*, *holC*, *leuA*, *malF*, *nuoL* and *petC*) are described by Yuan *et al.* (2010) and further details regarding analysis can be found on the *X. fastidiosa* MLST website (http://pubmlst.org/xfastidiosa/). The annealing temperature described in Yuan *et al.* (2010) is 65 °C but other researchers have observed that an annealing temperature of 60 °C improves the reliability of the method (French National Institute for Agricultural Research–Research Institute of Horticulture and Seeds, personal communication, 2016).	A more general sentence could be considered such as: "If erratic amplification occurs, the following PCR parameters can be adjusted: dilution of the DNA extract (to limit inhibition) or increase of DNA input, use of a different Taq polymerase/Mastermix, decrease of annealing temperature from 65°C to 60°C or 58°C or increase of primer concentration from 0.3 to 0.5 µM." This sentence is currently proposed for addition to a revision the EPPO Protocol. *Category : SUBSTANTIVE*
213	577	An MLST approach has been described for the identification of *X. fastidiosa* subspecies and is recommended for the characterization of new strains (Scally *et al.*, 2005; Yuan *et al.*, 2010; Jacques *et al.*, 2016). This approach can be used on DNA extracted from either bacterial cultures or infected plants tested positive for *X. fastidiosa* (Loconsole *et al.*, 2016). For amplification of DNA direct from plant tissue, it has been observed that the quality of the target DNA may not always be suitable for obtaining all amplicons (EPPO, 2016). Primers and conditions for the sequencing and analysis of seven housekeeping genes (*cysG*, *gltT*, *holC*, *leuA*, *malF*, *nuoL* and *petC*) are described by Yuan *et al.* (2010) and further details regarding analysis can be found on the *X. fastidiosa* MLST website (http://pubmlst.org/xfastidiosa/). The annealing temperature described in Yuan *et al.* (2010) is 65 °C but other researchers have observed that an annealing temperature of 60 °C improves the reliability of the method (French National Institute for Agricultural Research–Research Institute of Horticulture and Seeds, personal communication, 2016).	**EPPO** MLST has mainly been developed on pure cultures. It can be used on DNA extract from plants, however, it is recognized that the quantity and quality of target DNA, or the occurrence of possible mixed infections may prevent obtaining all amplicons or clear assignment of sub-species. A warning about this is included in the IPPC protocol. Regarding the adaptation of the annealing temperature, it is noted in our region that reliability is only increased for some genes and not all, and it also depends on the host plant tested. A more general sentence could be considered such as If erratic amplification occurs, the following PCR parameters can be adjusted: dilution of the DNA extract (to limit inhibition) or increase of DNA input, use of a different Taq polymerase/Mastermix, decrease of annealing temperature from 65°C to 60°C or 58°C or increase of primer concentration from 0.3 to 0.5 µM. This sentence is currently proposed for addition to a revision the EPPO Protocol. *Category : SUBSTANTIVE*
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215	577	An MLST approach has been described for the identification of *X. fastidiosa* subspecies	**EPPO**

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216	582	There are a number of specific tests using PCR that enable *X. fastidiosa* subspecies determination (Pooler and Hartung 1995; Hernandez-Martinez *et al.*, 2006; Li *et al.*, 2013). The PCR described by Hernandez-Martinez *et al.*, 2006) can allow the identification of cultures of subspecies *fastidiosa*, *multiplex* and *sandyi*. Pooler and Hartung (1995) developed a conventional PCR that identifies subspecies *pauca*. The citrus variegated chlorosis strains of *X. fastidiosa* can be identified by using either a conventional PCR (Pooler and Hartung, 1995) or a real-time PCR (Li *et al.*, 2013). The oleander leaf scorch strains of *X. fastidiosa* can be specifically detected and differentiated from other strains by PCR (Huang, 2009) or real-time PCR (Guan *et al*., 2013). Recently, a PCR has been developed to allow specific detection and identification of American mulberry-infecting strains of *X. fastidiosa* and the newly discovered strains *X. fastidiosa* associated with Italian ~~olives~~ olive trees (Guan *et al*., 2015).	**European Union** Clearer. *Category : EDITORIAL*
217	582	There are a number of specific tests using PCR that enable *X. fastidiosa* subspecies determination (Pooler and Hartung 1995; Hernandez-Martinez *et al.*, 2006; Li *et al.*, 2013). The PCR described by Hernandez-Martinez *et al.*, 2006) can allow the identification of cultures of subspecies *fastidiosa*, *multiplex* and *sandyi*. Pooler and Hartung (1995) developed a conventional PCR that identifies subspecies *pauca*. The citrus variegated chlorosis strains of *X. fastidiosa* can be identified by using either a conventional PCR (Pooler and Hartung, 1995) or a real-time PCR (Li *et al.*, 2013). The oleander leaf scorch strains of *X. fastidiosa* can be specifically detected and differentiated from other strains by PCR (Huang, 2009) or real-time PCR (Guan *et al*., 2013). Recently, a PCR has been developed to allow specific detection and identification of American mulberry-infecting strains of *X. fastidiosa* and the newly discovered strains *X. fastidiosa* associated with Italian olives (Guan *et al*., 2015).	**European Union** We note that in the IPPC protocol the PCR Hernandez-Martinez et al., 2006 is recommended to be performed on cultures, but it should be noted that on plant extracts, there are some issues with unspecific amplification (in particular with the multiplex version of the PCR). *Category : SUBSTANTIVE*

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219	582	There are a number of specific tests using PCR that enable *X. fastidiosa* subspecies determination (Pooler and Hartung 1995; Hernandez-Martinez *et al.*, 2006; Li *et al.*, 2013). The PCR described by Hernandez-Martinez *et al.*, 2006) can allow the identification of cultures of subspecies *fastidiosa*, *multiplex* and *sandyi*. Pooler and Hartung (1995) developed a conventional PCR that identifies subspecies *pauca*. The citrus variegated chlorosis strains of *X. fastidiosa* can be identified by using either a conventional PCR (Pooler and Hartung, 1995) or a real-time PCR (Li *et al.*, 2013). The oleander leaf scorch strains of *X. fastidiosa* can be specifically detected and differentiated from other strains by PCR (Huang, 2009) or real-time PCR (Guan *et al*., 2013). Recently, a PCR has been developed to allow specific detection and identification of American mulberry-infecting strains of *X. fastidiosa* and the newly discovered strains *X. fastidiosa* associated with Italian ~~olives~~ olive trees (Guan *et al*., 2015).	**EPPO** Clearer. *Category : EDITORIAL*
220	586	~~**6. Contact Points for Further Information**~~	**Viet Nam** This Section move to Appendix 1 *Category : EDITORIAL*
221	587	~~Further information on this protocol can be obtained from (in alphabetical order):~~	**Viet Nam** This para move to Appendix 1 *Category : EDITORIAL*
222	588	~~Austrian Agency for Health and Food Safety (AGES), Plant Health Laboratory, Spargelfeldstraße 191, 1220 Vienna, the Republic of Austria (Helga Reisenzein; email: Helga.reisenzein@ages.at; tel: +43 50 555 33340).~~	**Viet Nam** This para move to Appendix 1 *Category : EDITORIAL*
223	589	~~Ministry for Primary Industries, Plant Health and Environment Laboratory, PO Pox 2095, Auckland 1140, New Zealand (Robert Taylor; email: Robert.taylor@mpi.govt.nz).~~	**Viet Nam** This para move to Appendix 1 *Category : EDITORIAL*
224	590	~~United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Molecular Plant Pathology Laboratory, Beltsville Agriculture Research Center West, 10300 Baltimore Avenue, Beltsville, MD 20705, the United States of America (John~~	**Viet Nam** This para move to Appendix 1 *Category : EDITORIAL*

#	Para	Text	Comment
		~~Hartung; email: John.hartung@ars.usda.gov).~~	
225	591	~~USDA Animal Plant Health and Inspection Service, (APHIS), Plant Protection and Quarantine (PPQ), Phytosanitary Issues Management, 4700 River Road, Riverdale, MD 20737, the United States of America (Wenbin Li; email: Wenbin.li@aphis.usda.gov).~~	**Viet Nam** This para move to Appendix 1 *Category : EDITORIAL*
226	591	USDA Animal Plant Health and Inspection ~~Service,~~ Service (APHIS), Plant Protection and Quarantine (PPQ), Phytosanitary Issues Management, 4700 River Road, Riverdale, MD 20737, the United States of America (Wenbin Li; email: Wenbin.li@aphis.usda.gov).	**European Union** Typo. *Category : EDITORIAL*
227	591	USDA Animal Plant Health and Inspection ~~Service,~~ Service (APHIS), Plant Protection and Quarantine (PPQ), Phytosanitary Issues Management, 4700 River Road, Riverdale, MD 20737, the United States of America (Wenbin Li; email: Wenbin.li@aphis.usda.gov).	**EPPO** Typo *Category : EDITORIAL*
228	592	~~A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will forward it to the Technical Panel on Diagnostic Protocols (TPDP).~~	**Viet Nam** This para move to Appendix 1 *Category : EDITORIAL*
229	593	~~7. Acknowledgements~~	**Viet Nam** This section move to Appendix 2 *Category : EDITORIAL*
230	594	~~This diagnostic protocol was drafted by Marta Francis (formerly USDA), Robert Taylor (Plant Health and Environment Laboratory, Ministry for Primary Industries, New Zealand (see preceding section)), Helga Reisenzein (Austrian Agency for Health and Food Safety, Plant Health Laboratory, the Republic of Austria (see preceding section)), John Hartung (USDA ARS, Molecular Plant Pathology Laboratory, Beltsville Agriculture Research Center-West, the United States of America (see preceding section)), Wenbin Li (USDA APHIS-PPQ, the United States of America (see preceding section)).~~	**Viet Nam** This para move to Appendix 2 *Category : EDITORIAL*
231	594	This diagnostic protocol was drafted by Marta Francis (formerly USDA), Robert Taylor (Plant Health and Environment Laboratory, Ministry for Primary Industries, New Zealand (see preceding section)), Helga Reisenzein (Austrian Agency for Health and Food Safety, Plant Health Laboratory, the Republic of Austria (see preceding section)), John Hartung (USDA ARS, Molecular Plant Pathology Laboratory, Beltsville Agriculture Research Center-West, the United States of America (see preceding section)), and Wenbin Li (USDA APHIS-PPQ, the United States of America (see preceding section)).	**European Union** Last author. *Category : EDITORIAL*
232	594	This diagnostic protocol was drafted by Marta Francis (formerly USDA), Robert Taylor (Plant Health and Environment Laboratory, Ministry for Primary Industries, New Zealand (see preceding section)), Helga Reisenzein (Austrian Agency for Health and	**EPPO** Last author. *Category : EDITORIAL*

#	Para	Text	Comment
		Food Safety, Plant Health Laboratory, the Republic of Austria (see preceding section)), John Hartung (USDA ARS, Molecular Plant Pathology Laboratory, Beltsville Agriculture Research Center-West, the United States of America (see preceding section)), and Wenbin Li (USDA APHIS-PPQ, the United States of America (see preceding section)).	
233	595	~~In addition, Ed Civerolo (formerly USDA) was involved in the development of this protocol. The diagnostic protocol developed for the detection of *X. fastidiosa* in the European and Mediterranean Plant Protection Organization (EPPO) region (EPPO, 2016) was used as an important contribution to the drafting of this protocol.~~	**Viet Nam** This para move to Appendix 2 *Category : EDITORIAL*
234	597	~~No figures are included in the protocol itself. Pictures of symptoms are accessible at https://gd.eppo.int/taxon/XYLEFA/photos.~~Pictures of symptoms:	**Viet Nam** Detail some pictures in this section. Do not use link in this section. *Category : EDITORIAL*
235	606	**Bergsma-Vlami, M., van de Bilt, J.L.J., Tjou-Tam-Sin, N.N.A., van de Vossenberg, B.T.L.H. & Westenberg, M.** 2015. *Xylella fastidiosa* in *Coffea arabica* ornamental plants imported from Costa Rica and Honduras in the Netherlands. *Journal of Plant Pathology*, 97, 395. **Bergsma et al.,** 2017 in Plant Pathology on MLST analysis (Bergsma- Vlami, M., van de Bilt, J. L. J., Tjou- Tam- Sin, N. N. A., Helderman, C. M., Gorkink- Smits, P. P. M. A., Landman, N. M., … & Westenberg, M. (2017). Assessment of the genetic diversity of Xylella fastidiosa in imported ornamental Coffea arabica plants. Plant Pathology DOI: 10.1111/ppa.12696.	**European Union** *Category : TECHNICAL*
236	606	**Bergsma-Vlami, M., van de Bilt, J.L.J., Tjou-Tam-Sin, N.N.A., van de Vossenberg, B.T.L.H. & Westenberg, M.** 2015. *Xylella fastidiosa* in *Coffea arabica* ornamental plants imported from Costa Rica and Honduras in the Netherlands. *Journal of Plant Pathology*, 97, 395. Bergsma et al., 2017 in Plant Pathology on MLST analysis (Bergsma- Vlami, M., van de Bilt, J. L. J., Tjou- Tam- Sin, N. N. A., Helderman, C. M., Gorkink- Smits, P. P. M. A., Landman, N. M., … & Westenberg, M. (2017). Assessment of the genetic diversity of Xylella fastidiosa in imported ornamental Coffea arabica plants. Plant Pathology DOI: 10.1111/ppa.12696	**EPPO** *Category : TECHNICAL*
237	613	~~Bull, C.T., De Boer, S.H., Denny, T.P., Firrao, G., Fischer-Le Saux, M., Saddler, G.S., Scortichini, M., Stead, D.E. & Takikawa, Y.~~ **Bull, C.T., De Boer, S.H., Denny, T.P., Firrao, G., Fischer-Le Saux, M., Saddler, G.S., Scortichini, M., Stead, D.E. & Takikawa, Y.** 2012. List of new names of plant pathogenic bacteria (2008–2010). *Journal of Plant Pathology*, 94: 21–27.	**European Union** Typo: authors to be in bold. *Category : EDITORIAL*
238	613	~~Bull, C.T., De Boer, S.H., Denny, T.P., Firrao, G., Fischer-Le Saux, M., Saddler, G.S., Scortichini, M., Stead, D.E. & Takikawa, Y.~~ **Bull, C.T., De Boer, S.H., Denny, T.P., Firrao, G., Fischer-Le Saux, M., Saddler, G.S., Scortichini, M., Stead, D.E. &**	**EPPO** Typo: authors to be put in bold. *Category : EDITORIAL*

#	Para	Text	Comment
		Takikawa, Y. 2012. List of new names of plant pathogenic bacteria (2008–2010). *Journal of Plant Pathology*, 94: 21–27.	
239	621	**Coletta-Filho, H.D., Francisco, C.S., Lopes, J.R.S., De Oliveira, A.F. & Da Silva, L.F.O.** 2016. First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. ~~pauca~~*pauca*. *Phytopathologia Mediterranea*, 55: 130–135.	**European Union** Typo: subsp. to be in italic. *Category : EDITORIAL*
240	621	**Coletta-Filho, H.D., Francisco, C.S., Lopes, J.R.S., De Oliveira, A.F. & Da Silva, L.F.O.** 2016. First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. ~~pauca~~*pauca*. *Phytopathologia Mediterranea*, 55: 130–135.	**EPPO** Typo: subspecies to be put in italics. *Category : EDITORIAL*
241	637	**Guan, W., Shao, J., Elbeaino, T., Davis, R.E., Zhao, T. & Huang, Q.** 2015~~.~~. Specific detection and identification of American mulberry-infecting and Italian olive-associated strains of *Xylella fastidiosa* by polymerase chain reaction. *PLoS ONE*, 10(6): e0129330. doi:10.1371/journal.pone.0129330.	**European Union** Typo: missing space. *Category : EDITORIAL*
242	637	**Guan, W., Shao, J., Elbeaino, T., Davis, R.E., Zhao, T. & Huang, Q.** 2015~~.~~. Specific detection and identification of American mulberry-infecting and Italian olive-associated strains of *Xylella fastidiosa* by polymerase chain reaction. *PLoS ONE*, 10(6): e0129330. doi:10.1371/journal.pone.0129330.	**EPPO** Typo: missing space. *Category : EDITORIAL*
243	676	**Rossetti, V., Garnier, M., Bové, J.M., Beretta, M.J.G., Teixeira, A.R.R., Quaggio, J.A. & de Negri, J.D.** 1990. Occurrence of xylem-restricted bacteria in sweet orange trees affected by chlorotic variegation, a new citrus disease in Brazil. *Comptes Rendus de ~~l'Academie~~ l'Académie des Sciences ~~Series~~ Série 3*, 310: 345–349.	**European Union** Typos: French spelling. *Category : EDITORIAL*
244	676	**Rossetti, V., Garnier, M., Bové, J.M., Beretta, M.J.G., Teixeira, A.R.R., Quaggio, J.A. & de Negri, J.D.** 1990. Occurrence of xylem-restricted bacteria in sweet orange trees affected by chlorotic variegation, a new citrus disease in Brazil. *Comptes Rendus de ~~l'Academie~~ l'Académie des Sciences ~~Series~~ Série 3*, 310: 345–349.	**EPPO** Typos (French spelling) *Category : EDITORIAL*