2023 FIRST CONSULTATION

1 July – 30 September 2023

Compiled comments for 2023 First Consultation: 2021-003_Revision_DP25_Xylella

Summary

Title	2023 First consultation: Draft annex to ISPM 27: Revision of DP 25 - Xylella fastidiosa (2021-003) (Id 1439)
Description	
End Date	30 Sep 2023 11:45 PM
Review Status	Completed (2 Oct 2023 10:08 AM)

Participants

Name	Summary
Australia	Comments provided
Azerbaijan	no comment
European Union Σ	The comments on the draft standard are submitted by the European Commission on behalf of the European Union and its 27 Member States.
Gabon	annexe validée
Malawi	We support the draft revision of DP25
Singapore	Singapore is supportive of this draft annex.

T (Type) - B = Bullet, C = Comment, P = Proposed Change, R = Rating
S (Status) - A = Accepted, C = Closed, O = Open, W = Withdrawn, M = Merged

Para	Text	T	Comment
G	(General Comment)	C	*Category : SUBSTANTIVE* **(322) Argentina (1 Oct 2023 4:16 AM)** Argentina supports the COSAVE comments
G	(General Comment)	C	*Category : TECHNICAL* **(321) Zambia (30 Sep 2023 10:07 PM)** The diagnostic protocol is endorsed.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(320) Barbados (30 Sep 2023 6:33 PM)** Barbados has not objections to the adoption of this protocol.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(319) Costa Rica (30 Sep 2023 1:44 AM)** We have no comments
G	(General Comment)	C	*Category : EDITORIAL* **(313) Paraguay (29 Sep 2023 8:51 PM)** Paraguay de acuerdo con los comentarios de

			COSAVE.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(297) Russian Federation (29 Sep 2023 4:37 PM)** General Comment: The Russian Federation would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(285) Belarus (29 Sep 2023 4:10 PM)** General comment: Republic of Belarus, would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System
G	(General Comment)	C	*Category : EDITORIAL* **(269) Switzerland (29 Sep 2023 3:16 PM)** Switzerland would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(258) European Union (29 Sep 2023 2:46 PM)** In addition to the following technical and substantive comments, we also support editorial comments submitted by EPPO.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(256) Philippines (29 Sep 2023 4:50 AM)** The PH has no further comments on the Draft annex to ISPM 27: Revision of DP 25 - Xylella fastidiosa
G	(General Comment)	C	*Category : TECHNICAL* **(242) Colombia (27 Sep 2023 5:28 PM)** For real-time PCR, it is suggested to include the expected result according to the primers.
G	(General Comment)	C	*Category : TECHNICAL* **(241) Colombia (27 Sep 2023 5:27 PM)** It is suggested to include an image of the gel with the amplifications.
G	(General Comment)	C	*Category : TECHNICAL* **(240) Colombia (27 Sep 2023 5:27 PM)** It is suggested to include images of the symptoms.

G	(General Comment)	C	*Category : SUBSTANTIVE* **(218) United Kingdom (26 Sep 2023 5:22 PM)** The UK supports the comments the EPPO secretariat have submitted on behalf of those EPPO member countries which are not part of the European Union.
G	(General Comment)	C	*Category : TECHNICAL* **(217) Mozambique (26 Sep 2023 11:01 AM)** Mozambique agrees with the proposed Diagnostic Protocol of Xylella fastidiosa
G	(General Comment)	C	*Category : SUBSTANTIVE* **(89) IPPC Regional Workshop Africa (23 Sep 2023 3:51 PM)** We support the draft Annex to ISPM 27:Revision of DP25
G	(General Comment)	C	*Category : TECHNICAL* **(88) IPPC Regional Workshop Africa (23 Sep 2023 3:51 PM)** Very elaborate.
G	(General Comment)	C	*Category : SUBSTANTIVE* **(87) Malawi (23 Sep 2023 2:43 PM)** We support the draft revision of DP25
G	(General Comment)	C	*Category : SUBSTANTIVE* Mexico **(74) Mexico (15 Sep 2023 7:14 PM)** Mexico has reviewed and supports the Draft annex to ISPM 27: Revision of DP 25 - Xylella fastidiosa (2021-003) in its current format.
G	(General Comment)	C	*Category : TECHNICAL* **(73) United States of America (15 Sep 2023 3:52 PM)** Adding pictures of some procedures would be very helpful explaining the text.
G	(General Comment)	C	*Category : TECHNICAL* **(60) COSAVE (13 Sep 2023 6:09 PM)** No comments. Cosave agrees with the document as it is
G	(General Comment)	C	*Category : SUBSTANTIVE* **(44) United States of America (7 Sep 2023 4:40 PM)** We consider that Revision of DP 25 - Xylella fastidiosa very well written.

G	(General Comment)	C	*Category : EDITORIAL* **(43) Guyana (4 Sep 2023 12:50 AM)** Guyana has no objection to the revision of the annex to ISPM 27: DP 25 - Xylella fastidiosa
G	(General Comment)	C	*Category : SUBSTANTIVE* **(16) Egypt (27 Aug 2023 8:36 PM)** The diagnostic protocol for Xylella fastidiosa is highly comprehensive, encompassing a series of laboratory tests that cover all identification profiles for this pathogen. Specifically, it includes a range of tests that utilize diverse biological methodologies. Supporting the adoption of this protocol will be helpful in safeguarding plant health and facilitate trade.
G	(General Comment)	C	*Category : TECHNICAL* **(15) Congo (23 Aug 2023 9:37 AM)** i agree with this annex of ISPM 27. Nothing to add
G	(General Comment)	C	*Category : SUBSTANTIVE* **(14) Thailand (22 Aug 2023 5:38 AM)** Thailand agreed with the proposed draft revision of DP◦25: Xylella fastidiosa
G	(General Comment)	C	*Category : SUBSTANTIVE* **(13) New Zealand (17 Aug 2023 2:53 AM)** New Zealand supports this DP.
G	(General Comment)	C	*Category : EDITORIAL* **(12) Papua New Guinea (16 Aug 2023 5:29 AM)** Very important plant pest because it will have significant impact on cultivation of many perennial crops in the tropics. Identification of the pest in the field will be very difficult and use of molecular techniques to detect it will be also difficult for laboratories in many developing countries.
G	(General Comment)	C	*Category : TECHNICAL* **(11) Uruguay (15 Aug 2023 3:58 PM)** No comments, we agree with the document as it is
G	(General Comment)	C	*Category : SUBSTANTIVE* **(9) Chile (14 Aug 2023 4:22 PM)**

			Teccnicas de serología y PCR convencionales no aceptados por la CE pese a estar en el IT EPPO.
1	**DRAFT REVISION OF DP 25: *Xylella fastidiosa* (2021-003)**	C	*Category : SUBSTANTIVE* **(246) China (28 Sep 2023 8:14 AM)** We recommend including information introducing how to select the suitable method. For example, add a flowchart. 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. It is critical to select the suitable method.
50	**1. Pest information**	C	*Category : SUBSTANTIVE* **(247) China (28 Sep 2023 8:15 AM)** Change the paragraph to "X. fastidiosa is genetically diverse and consists of several subspecies. Four subspecies are widely reported: X. fastidiosa subsp. fastidiosa causes Pierce's disease and infects a large host range including Acer spp., Citrus spp., Medicago sativa, P. dulcis, and V. vinifera. (EPPO, 2023b, Schuenzel et al., 2005)); X. fastidiosa subsp. multiplex is associated with scorch diseases of a range of trees that include Platanus occidentalis, P. dulcis, P. persica, and Quercus spp., and ornamentals (e.g. Helichrysum italicum, Polygala myrtifolia); and X. fastidiosa subsp. pauca (Schadd et al., 2004) infects most Citrus species (mainly C. sinensis), Coffea species, and O. europaea; X. fastidiosa subsp. sandyi causes oleander leaf scorch in Nerium oleander (Schuenzel et al., 2005). Two other subspecies are currently described: X. fastidiosa subsp. tashke causes leaf scorch in Chitalpa tashkentensis ; and X. fastidiosa subsp. morus (Nunney et al., 2014) infects Morus." Based on two referenced papers (Denance et al., 2017; Denance et al., 2019), X. fastidiosa comprises a total of six subspecies classifications, with four of them being relatively well-documented. Therefore, it is

			recommended to revise the content related to the subspecies classification.
51	*Xylella fastidiosa* Wells *et al.*, 1987 is a xylem-limited bacterium and is the causal agent of many economically important plant diseases of agronomic, horticultural or forestry 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 (EFSA, 2021; EFSA, 2023). *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, 2023a).	C	*Category : TECHNICAL* **(259) European Union (29 Sep 2023 2:48 PM)** Outbreaks in Europe are not that recent anymore, this sentence from the previous version of the protocol could be rephrased and updated as the refence was also changed from 2015 to 2023.
51	*Xylella fastidiosa* Wells *et al.*, 1987 is a xylem-limited bacterium and is the causal agent of many economically important plant diseases of agronomic, horticultural or forestry 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 (EFSA, 2021; EFSA, 2023). *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and Nunney, ~~2015), but there~~ 2015) and outbreaks have ~~now~~ been ~~reports of outbreaks~~ confirmed in Asia and Europe (EPPO, 2023a).	P	*Category : EDITORIAL* **(219) Australia (27 Sep 2023 8:10 AM)** Changing language to make it clear that outbreaks are confirmed, they are not only 'reports' of outbreaks.
51	*Xylella fastidiosa* Wells *et al.*, 1987 is a xylem-limited bacterium and is the causal agent of many economically important plant diseases of agronomic, horticultural or forestry 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 (EFSA, 2021; EFSA, 2023). *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, 2023a).	C	*Category : EDITORIAL* **(91) EPPO (25 Sep 2023 8:22 AM)** To be deleted? The EFSA 2023 update is maybe sufficient?
51	*Xylella fastidiosa* Wells *et al.*, 1987 is a xylem-limited bacterium and is the causal agent of many economically important plant diseases of agronomic, horticultural or forestry 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 (EFSA, 2021; EFSA, 2023). *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and	C	*Category : TECHNICAL* **(90) EPPO (25 Sep 2023 8:22 AM)** Outbreaks in Europe are not that recent anymore, this sentence from the previous version of the protocol could be rephrased and updated as the refence was also changed from 2015 to 2023.

	Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2023a).		
51	*Xylella fastidiosa* Wells *et al.*, 1987 is a xylem-limited bacterium ~~and is the causal agent of~~ that causes many economically important plant diseases of agronomic, horticultural or forestry 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 (EFSA, 2021; EFSA, 2023). *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~~ Asia, Europe and the Middle East (EPPO, 2023a).	P	*Category : TECHNICAL* **(45) United States of America (7 Sep 2023 4:43 PM)** is this verified?
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*, , . (EPPO, 2023b, Schuenzel *et al.*, 2005)); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.	C	*Category : TECHNICAL* **(260) European Union (29 Sep 2023 2:49 PM)** Citrus was reported as a host (see EPPO 2023b) but is not known to be a major host of X. fastidiosa subsp. fastidiosa. It is suggested to delete Citrus from the list of host range. More generally, it is suggested to list the major hosts or the most relevant for the different subspecies.
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*, , . (EPPO, 2023b, Schuenzel *et al.*, 2005)); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.	C	*Category : EDITORIAL* **(255) South Africa (28 Sep 2023 12:25 PM)** Suggestion for deletion commas in order to ensure grammatical correctness.

52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*~~, , .~~ (EPPO, 2023b, Schuenzel *et al.*, 2005)~~)~~; *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.	P	*Category : EDITORIAL* **(243) Colombia (27 Sep 2023 5:33 PM)** There are punctuation marks left over
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease in *V. vinifera* and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*~~,~~, and *P. dulcis*~~, and (V. vinifera, , .~~ (EPPO, 2023b, Schuenzel *et al.*, ~~2005)~~2005); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp.,~~ and ornamentals (e.g.~~ as well as ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* ~~(Schadd~~ (Schaad *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.	P	*Category : EDITORIAL* **(93) EPPO (25 Sep 2023 8:22 AM)** Typos Move the example of the host V. vinifera here as Pierce's disease is observed in this host. It is suggested to replace the second 'and' by 'as well as' to clarify.
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease in *V. vinifera* and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, and *P. dulcis* (EPPO, 2023b, Schuenzel *et al.*, 2005); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schaad *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes	C	*Category : TECHNICAL* **(92) EPPO (25 Sep 2023 8:22 AM)** Citrus was reported as a host (see EPPO 2023b) but is not known to be a major host of X. fastidiosa subsp. fastidiosa. It is suggested to delete Citrus from the list of host range. More generally, it is suggested to list the major hosts or the most relevant for the different subspecies.

	oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.		
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*, coffee, . (EPPO, 2023b, Schuenzel *et al.*, 2005)); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., *blueberries,* and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp. Recent work has shown that X. fastidiosa taxonomy needs reevaluation (Kahn and Almeida 2022)	P	*Category : TECHNICAL* **(64) United States of America (15 Sep 2023 3:17 PM)**
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*, , , . (EPPO, 2023b, Schuenzel *et al.*, 2005)); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.	P	*Category : EDITORIAL* **(59) Canada (12 Sep 2023 5:41 PM)**
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*~~, , .~~,(EPPO, 2023b, Schuenzel *et al.*, 2005)); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*),	P	*Category : EDITORIAL* **(58) Canada (12 Sep 2023 5:40 PM)** removing punctuation marks

	Coffea species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.		
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*~~, , .~~ (EPPO, 2023b, Schuenzel *et al.*, 2005)); *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.	P	*Category : EDITORIAL* **(46) United States of America (7 Sep 2023 4:44 PM)** Punctuation.
52	*X. fastidiosa* is genetically diverse and consists of several subspecies. Three subspecies are widely accepted: *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and ~~infects~~ has a large host range including *Acer* spp., *Citrus* spp., *Medicago sativa*, *P. dulcis*, and *V. vinifera*~~, , .~~ (EPPO, 2023b, Schuenzel *et al.*, 2005)~~);~~ *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *Platanus occidentalis*, *P. dulcis*, *P. persica*, and *Quercus* spp., and ornamentals (e.g. *Helichrysum italicum*, *Polygala myrtifolia*); and *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* species (mainly *C. sinensis*), *Coffea* species, and *O. europaea*. Two other subspecies are currently described: *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005); and *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp.	P	*Category : EDITORIAL* **(22) Ghana (30 Aug 2023 10:19 PM)**
53	*X. fastidiosa* is a Gram-negative 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,	C	*Category : TECHNICAL* **(261) European Union (29 Sep 2023 2:51 PM)** Delete non motile or rephrase to refer to twitching motility (see https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.2006/002311-0?crawler=true).

	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* show no symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant's shoot and root system systemically (Aldrich, Gould and Martin, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant.		
53	*X. fastidiosa* is a Gram-negative 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* show no symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant's shoot and root system systemically (Aldrich, Gould and Martin, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant.	C	*Category : TECHNICAL* **(94) EPPO (25 Sep 2023 8:22 AM)** Delete non motile or rephrase to refer to twitching motility (see https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.2006/002311-0?crawler=true)
53	*X. fastidiosa* is a Gram-negative 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. Plant responses to infection lead to tyloses formation that also blocks water transport. 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* show no symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant's shoot and root system systemically (Aldrich, Gould and Martin, 1992; He *et al.*, 2000; Li *et al.*, 2003).	P	*Category : TECHNICAL* **(65) United States of America (15 Sep 2023 3:23 PM)** What about twitching motility? Last sentence seems redundant.

	~~The pathogen overwinters in the xylem of the host plant.~~		
53	*X. fastidiosa* is a Gram-negative bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, ~~rod-shaped cells~~rod-shaped, 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* show no symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant's shoot and root system systemically (Aldrich, Gould and Martin, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of ~~the host plant~~its host.	P	*Category : EDITORIAL* **(23) Ghana (30 Aug 2023 10:22 PM)**
54	Insect transmission is considered the main factor for localized spread of *X. fastidiosa*. The vectors belong to the order Hemiptera, suborder Auchenorrhyncha, families Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee, Almeida and Lindow, 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 they then transmit the pathogen to other healthy plant hosts. While nymphs are able to acquire (and transmit) the bacterium, they lose it at each moult, so only continue to be infected if they reacquire the bacterium by feeding on infected plants after moulting (Almeida *et al.*, 2014). Once adults acquire the bacterium, they have it for life (as they do not moult). 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). There is no evidence of transovarial transmission (transmission from a female to her eggs) (Redak *et al.*, 2004). 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.	C	*Category : EDITORIAL* **(95) EPPO (25 Sep 2023 8:22 AM)** Suggestion: rephrase or delete this sentence and move the information that adults keep transmitting the bacterium because they do not moult into the next sentence?
54	Insect transmission is ~~considered the main factor~~ responsible for ~~localized~~ local spread of *X. fastidiosa*. The vectors belong to the order Hemiptera, suborder	P	*Category : TECHNICAL* **(47) United States of America (7 Sep 2023 4:45 PM)**

	Auchenorrhyncha, families Cicadellidae (sharpshooter leafhopper), Cercopidae and Aphrophoridae (spittlebugs) (Redak *et al*., 2004; Chatterjee, Almeida and Lindow, 2008), ~~Aphrophoridae~~ and potentially 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 they then transmit the pathogen to ~~other healthy~~ susceptible plant hosts. While nymphs are able to acquire (and ~~transmit)~~ inoculate) the bacterium, they lose ~~it~~ the infection at each moult, ~~so only continue to be infected if they~~ the insects must reacquire the bacterium by feeding on infected plants after moulting (Almeida *et al.*, 2014)~~.~~. Once ~~adults acquire the bacterium, they have it for life (as they do not moult). 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). There is no evidence of transovarial transmission (transmission from a female to her ~~eggs)~~ eggs Freitag 1956??) (Redak *et al.*, 2004). The movement of infected plants and planting material (e.g. ~~budwood,~~ budwood and seedlings) is assumed to be responsible for the long-distance spread of the disease and its ~~entry~~ introduction into new areas.		perhaps add both work suggesting transmission and work suggesting no transmission as references
54	Insect transmission is considered the main factor for localized spread of *X. fastidiosa*. The vectors belong to the order Hemiptera, suborder Auchenorrhyncha, families Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee, Almeida and Lindow, 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 they then transmit the pathogen to other healthy plant hosts. While nymphs are able to acquire (and transmit) the bacterium, they lose it at each moult, so only continue to be infected if they reacquire the bacterium by feeding on infected plants after moulting (Almeida *et al.*, 2014). Once adults acquire the bacterium, they have it for life (as they do not moult). Once infected, adults can transmit throughout their whole lifetime, as the bacterium multiplies and persists in the ~~vector~~ foregut of the vector (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). There is no evidence of transovarial transmission (transmission from a female to her eggs) (Redak *et al.*, 2004). 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.	P	*Category : EDITORIAL* **(24) Ghana (30 Aug 2023 10:24 PM)**

54	Insect transmission is considered the main factor for localized spread of *X. fastidiosa*. The vectors belong to the order Hemiptera, suborder Auchenorrhyncha, families Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee, Almeida and Lindow, 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 they then transmit the pathogen to other healthy plant hosts. While nymphs are able to acquire (and transmit) the bacterium, they lose it at each moult, so only continue to be infected if they reacquire the bacterium by feeding on infected plants after moulting (Almeida *et al.*, 2014). Once adults acquire the bacterium, they have it for life (as they do not moult). 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). There is no evidence of transovarial transmission (transmission from a female to her eggs) (Redak *et al.*, 2004). The movement of infected ~~plants~~ plant materials and ~~planting material~~ propagative (e.g. budwood, ~~seedlings)~~ grafts, seedlings, etc.) is assumed to be responsible for the long-distance spread of the disease and its entry into ~~new areas.~~newenvironments	P	*Category : TECHNICAL* **(18) Kenya (28 Aug 2023 3:53 PM)**
54	Insect transmission is considered the main factor for localized spread of *X. fastidiosa*. The vectors belong to the order Hemiptera, suborder Auchenorrhyncha, families Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee, Almeida and Lindow, 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 they then transmit the pathogen to other healthy plant hosts. While nymphs are able to acquire (and transmit) the bacterium, they lose it at each moult, so only continue to be infected if they reacquire the bacterium by feeding on infected plants after moulting (Almeida *et al.*, 2014). Once adults acquire the bacterium, they have it for life (as they do not moult). 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). There is no evidence of transovarial transmission (transmission from a female to her eggs) (Redak *et al.*, 2004). The movement of infected plants and planting material	C	*Category : TECHNICAL* **(17) Kenya (28 Aug 2023 3:46 PM)** Planting materials is general and may include both plants, media, containers and other agricultural inputs

	(e.g. budwood, seedlings) is assumed to be responsible for the long-distance spread of the disease and its entry into new areas.		
58	**Taxonomic position:** Bacteria, ~~Proteobacteria~~Pseudomonadota (ex-Proteobacteria), Gammaproteobacteria, Lysobacterales (ex-Xanthomonadales), Lysobacteraceae (ex-Xanthomonadaceae) (Tindall, 2014; Whitman, Lawson and Losey, 2015)	P	*Category : TECHNICAL* **(220) Australia (27 Sep 2023 8:11 AM)** Proteobacteria was recently renamed. https://doi.org/10.1099/ijsem.0.005056
60	Recent studies have split *X. fastidiosa* into several subspecies (Schaad *et al.*, 2004; Scally *et al.*, 2005; Schuenzel *et al.*, 2005; Randall *et al.*, 2009; Yuan *et al.*, 2010; Nunney *et al.*, 2014). Currently, only the subspecies *fastidiosa* and *multiplex* are considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (Bull *et al.*, 2012). Other additional *X. fastidiosa* subspecies proposed are "*pauca*" (Schaad *et al.*, 2004), "*sandyi*" (Schuenzel *et al.*, 2005) and "*morus*" (Nunney *et al.*, 2014). Recently, a revision of the *X. fastidiosa* subspecies has been proposed based on comparative genomic analysis (Marceletti and Scortichini, 2016; Denancé *et al*., 2019), in which the subspecies *morus* and *sandyi* are merged with subspecies *fastidiosa* in the subspecies *fastidiosa sensu lato*. The strains associated with olive quick decline syndrome in Argentina, Brazil and Italy, have been found to consistently belong to *X. fastidiosa* subsp. *pauca* but to different sequence types (Haelterman *et al.*, 2015; Giampetruzzi *et al.*, 2017; Safady *et al*., 2019). The *Xylella* species associated with pear leaf scorch in the Taiwan Province of China (Leu and Su, 1993) is a new species, *X. taiwanensis* (Su *et al*., 2016), which is phylogenetically related to *X. fastidiosa* subsp. *sandyi* (Weng *et al.*, 2021).	C	*Category : TECHNICAL* **(263) European Union (29 Sep 2023 2:56 PM)** Rephrase, not that recent anymore.
60	Recent studies have split *X. fastidiosa* into several subspecies (Schaad *et al.*, 2004; Scally *et al.*, 2005; Schuenzel *et al.*, 2005; Randall *et al.*, 2009; Yuan *et al.*, 2010; Nunney *et al.*, 2014). Currently, only the subspecies *fastidiosa* and *multiplex* are considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (Bull *et al.*, 2012). Other additional *X. fastidiosa* subspecies proposed are "*pauca*" (Schaad *et al.*, 2004), "*sandyi*" (Schuenzel *et al.*, 2005) and "*morus*" (Nunney *et al.*, 2014). Recently, a revision of the *X. fastidiosa* subspecies has been proposed based on comparative genomic analysis (Marceletti and Scortichini, 2016; Denancé *et al*., 2019), in which the subspecies *morus* and *sandyi* are merged with subspecies *fastidiosa* in the subspecies *fastidiosa sensu lato*. The strains associated with olive quick decline syndrome in Argentina, Brazil and Italy, have been found to consistently belong to	P	*Category : TECHNICAL* **(262) European Union (29 Sep 2023 2:55 PM)** It is suggested to deleted this information because it is not supported by all the data presented in Weng et al. 2021 and it is not relevant here for the protocol.

	X. fastidiosa subsp. *pauca* but to different sequence types (Haelterman *et al.*, 2015; Giampetruzzi *et al.*, 2017; Safady *et al*., 2019). The *Xylella* species associated with pear leaf scorch in the Taiwan Province of China (Leu and Su, 1993) is a new species, *X. taiwanensis* (Su *et al*., 2016)~~, which is phylogenetically related to *X. fastidiosa* subsp. *sandyi* (Weng *et al*., 2021).~~		
60	Recent studies have split *X. fastidiosa* into several subspecies (Schaad *et al.*, 2004; Scally *et al.*, 2005; Schuenzel *et al.*, 2005; Randall *et al.*, 2009; Yuan *et al.*, 2010; Nunney *et al.*, 2014). Currently, only the subspecies *fastidiosa* and *multiplex* are considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (Bull *et al.*, 2012). Other additional *X. fastidiosa* subspecies proposed are "*pauca*" (Schaad *et al.*, 2004), "*sandyi*" (Schuenzel *et al.*, 2005) and "*morus*" (Nunney *et al.*, 2014). Recently, a revision of the *X. fastidiosa* subspecies has been proposed based on comparative genomic analysis (Marceletti and Scortichini, 2016; Denancé *et al*., 2019), in which the subspecies *morus* and *sandyi* are merged with subspecies *fastidiosa* in the subspecies *fastidiosa sensu lato*. The strains associated with olive quick decline syndrome in Argentina, Brazil and Italy, have been found to consistently belong to *X. fastidiosa* subsp. *pauca* but to different sequence types (Haelterman *et al.*, 2015; Giampetruzzi *et al.*, 2017; Safady *et al*., 2019). The *Xylella* species associated with pear leaf scorch in the Taiwan Province of China (Leu and Su, 1993) is a new species, *X. taiwanensis* (Su *et al*., 2016)~~, which is phylogenetically related to *X. fastidiosa* subsp. *sandyi* (Weng *et al*., 2021).~~	P	*Category : TECHNICAL* **(97) EPPO (25 Sep 2023 8:22 AM)** It is suggested to deleted this information because it is not supported by all the data presented in Weng et al. 2021 and it is not relevant here for the protocol.
60	Recent studies have split *X. fastidiosa* into several subspecies (Schaad *et al.*, 2004; Scally *et al.*, 2005; Schuenzel *et al.*, 2005; Randall *et al*., 2009; Yuan *et al.*, 2010; Nunney *et al.*, 2014). Currently, only the subspecies *fastidiosa* and *multiplex* are considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (Bull *et al.*, 2012). Other additional *X. fastidiosa* subspecies proposed are "*pauca*" (Schaad *et al.*, 2004), "*sandyi*" (Schuenzel *et al.*, 2005) and "*morus*" (Nunney *et al.*, 2014). Recently, a revision of the *X. fastidiosa* subspecies has been proposed based on comparative genomic analysis (Marceletti and Scortichini, 2016; Denancé *et al*., 2019), in which the subspecies *morus* and *sandyi* are merged with subspecies *fastidiosa* in the subspecies *fastidiosa sensu lato*. The strains associated with olive quick decline syndrome in Argentina, Brazil and Italy, have been found to consistently belong to *X. fastidiosa* subsp. *pauca* but to different sequence	C	*Category : TECHNICAL* **(96) EPPO (25 Sep 2023 8:22 AM)** Rephrase, not that recent anymore

	types (Haelterman *et al.*, 2015; Giampetruzzi *et al.*, 2017; Safady *et al*., 2019). The *Xylella* species associated with pear leaf scorch in the Taiwan Province of China (Leu and Su, 1993) is a new species, *X. taiwanensis* (Su *et al*., 2016), which is phylogenetically related to *X. fastidiosa* subsp. *sandyi* (Weng *et al.*, 2021).		
60	Recent studies have split *X. fastidiosa* into several subspecies (Schaad *et al.*, 2004; Scally *et al.*, 2005; Schuenzel *et al.*, 2005; Randall *et al.*, 2009; Yuan *et al.*, 2010; Nunney *et al.*, 2014). Currently, only the subspecies *fastidiosa* and *multiplex* are considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (Bull *et al.*, 2012). Other additional *X. fastidiosa* subspecies proposed are "*pauca*" (Schaad *et al.*, 2004), "*sandyi*" (Schuenzel *et al.*, 2005) and "*morus*" (Nunney *et al.*, 2014). Recently, a revision of the *X. fastidiosa* subspecies has been proposed based on comparative genomic analysis (Marceletti and Scortichini, 2016; Denancé *et al*., 2019), in which the subspecies *morus* and *sandyi* are merged with subspecies *fastidiosa* in the subspecies *fastidiosa sensu lato*. The strains associated with olive quick decline syndrome in Argentina, Brazil and Italy, have been found to consistently belong to *X. fastidiosa* subsp. *pauca* but to different sequence types (Haelterman *et al.*, 2015; Giampetruzzi *et al.*, 2017; Safady *et al*., 2019). The *Xylella* species associated with pear leaf scorch in the Taiwan Province of China (Leu and Su, 1993) is a new species, *X. taiwanensis* (Su *et al*., 2016), which is phylogenetically related to *X. fastidiosa* subsp. *sandyi* (Weng *et al.*, 2021).	C	*Category : TECHNICAL* **(66) United States of America (15 Sep 2023 3:34 PM)** Perhaps you should add Kahn and Almeida 2022 as that analyses shows the importance of data sets in tree topology - and taxonomy
60	Recent studies ~~have~~ split *X. fastidiosa* into several subspecies (Schaad *et al.*, 2004; Scally *et al.*, 2005; Schuenzel *et al.*, 2005; Randall *et al.*, 2009; Yuan *et al.*, 2010; Nunney *et al.*, 2014). Currently, only the subspecies *fastidiosa* and *multiplex* are considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (Bull *et al.*, 2012). Other additional *X. fastidiosa* subspecies proposed are "*pauca*" (Schaad *et al.*, 2004), "*sandyi*" (Schuenzel *et al.*, 2005) and "*morus*" (Nunney *et al.*, 2014). Recently, a revision of the *X. fastidiosa* subspecies has been proposed based on comparative genomic analysis (Marceletti and Scortichini, 2016; Denancé *et al*., 2019), in which the subspecies *morus* and *sandyi* are merged with subspecies *fastidiosa* in the subspecies *fastidiosa sensu lato*. The strains associated with olive quick decline syndrome in Argentina, Brazil and Italy, have been found to consistently belong to *X. fastidiosa* subsp. *pauca* but to different sequence types (Haelterman *et al.*, 2015; Giampetruzzi *et al.*, 2017; Safady *et al*., 2019). The *Xylella* species associated with	P	*Category : EDITORIAL* **(25) Ghana (30 Aug 2023 10:26 PM)**

	pear leaf scorch in the Taiwan Province of China (Leu and Su, 1993) is a new species, *X. taiwanensis* (Su *et al.*, 2016), which is phylogenetically related to *X. fastidiosa* subsp. *sandyi* (Weng *et al.*, 2021).		
62	Plants infected with *X. fastidiosa* may be asymptomatic (Almeida and Purcell, 2003) or the symptoms may be similar to those associated with water stress or physiological disorders. Isolation methods are not recommended for detection because of the difficulty in isolating *X. fastidiosa* from plant tissue. Therefore, detection is based on inspection for symptoms and the use of specific serological and molecular tests on symptomatic plant material. There is limited information available on testing asymptomatic plants and the concentration of *X. fastidiosa* is likely to be lower than in symptomatic plants (Almeida and Nunney, 2015). Therefore, it is advisable to include molecular methods for testing asymptomatic plant material.	C	*Category : TECHNICAL* **(264) European Union (29 Sep 2023 2:57 PM)** It is confusing whether this statement is a general statement on the tests that can be used or if inspection of symptoms and serological tests and molecular tests are mandatory. Suggestion: Therefore, detection is generally based on inspection for symptoms and the use of specific serological and/or molecular tests on symptomatic plant material.
62	Plants infected with *X. fastidiosa* may be asymptomatic (Almeida and Purcell, 2003) or the symptoms may be similar to those associated with water stress or physiological disorders. Isolation methods are not recommended for detection because of the difficulty in isolating *X. fastidiosa* from plant tissue. Therefore, detection is based on inspection for symptoms and the use of specific serological and molecular tests on symptomatic plant material. There is limited information available on testing asymptomatic plants and the concentration of *X. fastidiosa* is likely to be lower than in symptomatic plants (Almeida and Nunney, 2015). Therefore, it is advisable to include molecular methods for testing asymptomatic plant material.	C	*Category : TECHNICAL* **(214) EPPO (25 Sep 2023 8:48 AM)** It is confusing whether this statement is a general statement on the tests that can be used or if inspection of symptoms and serological tests and molecular tests are mandatory. Suggestion: Therefore, detection is generally based on inspection for symptoms and the use of specific serological and/or molecular tests on symptomatic plant material.
62	Plants infected with *X. fastidiosa* may be asymptomatic (Almeida and Purcell, 2003) or the symptoms may be similar to those associated with water stress or physiological disorders. Isolation methods are not recommended for detection because of the difficulty in isolating *X. fastidiosa* from plant tissue. Therefore, detection is based on inspection for symptoms and the use of specific serological and molecular tests on symptomatic plant material. There is limited information available on testing asymptomatic plants and the concentration of *X. fastidiosa* is likely to be lower than in symptomatic plants (Almeida and Nunney, 2015). Therefore, it is advisable to include molecular methods such as qPCR for testing asymptomatic plant material.	P	*Category : SUBSTANTIVE* **(26) Ghana (30 Aug 2023 10:28 PM)**
62	Plants infected with *X. fastidiosa* may be asymptomatic (Almeida and Purcell,	P	*Category : TECHNICAL* **(19) Kenya (28 Aug 2023 3:54 PM)**

	2003) or the symptoms may be similar to those associated with water ~~stress~~ stress, salinity or physiological disorders. Isolation methods are not recommended for detection because of the difficulty in isolating *X. fastidiosa* from plant tissue. Therefore, detection is based on inspection for symptoms and the use of specific serological and molecular tests on symptomatic plant material. There is limited information available on testing asymptomatic plants and the concentration of *X. fastidiosa* is likely to be lower than in symptomatic plants (Almeida and Nunney, 2015). Therefore, it is advisable to include molecular methods for testing asymptomatic plant material.		
62	Plants infected with *X. fastidiosa* may be asymptomatic (Almeida and Purcell, 2003) or the symptoms may be similar to those associated with water stress or physiological disorders. Isolation methods are not recommended for detection because of the difficulty in isolating *X. fastidiosa* from plant tissue. Therefore, detection is based on inspection for symptoms and the use ~~of specific serological~~ and molecular tests on symptomatic plant material. There is limited information available on testing asymptomatic plants and the concentration of *X. fastidiosa* is likely to be lower than in symptomatic plants (Almeida and Nunney, 2015). Therefore, it is advisable to include molecular methods for testing asymptomatic plant material.	P	*Category : TECHNICAL* **(1) Chile (14 Aug 2023 4:13 PM)** serología y PCR convencionales no aceptados por la CE pese a estar en el IT EPPO.
65	The presence of *X. fastidiosa* can have a broad impact on its host: from symptomless to plant death. Most host plants infected with *X. fastidiosa* do not 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 temperature or by drought) and they can vary according to the plant species or ~~cultivar~~ cultivar, *X.fastidiosa* strain and environmental conditions (Janse and Obradovic, 2010; CABI, 2023).	P	*Category : TECHNICAL* **(265) European Union (29 Sep 2023 2:59 PM)** Appropriate addition.
65	The presence of *X. fastidiosa* can have a broad impact on its host: from symptomless to plant death. Most host plants infected with *X. fastidiosa* do not 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 temperature or by drought) and they can vary according to the plant species or ~~cultivar~~ cultivar, *X. fastidiosa* strain, and environmental conditions (Janse and Obradovic, 2010; CABI, 2023).	P	*Category : TECHNICAL* **(98) EPPO (25 Sep 2023 8:22 AM)**

65	The presence of *X. fastidiosa* can have a broad impact on its host: from symptomless to plant death. Most host plants infected with *X. fastidiosa* do not 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 temperature or ~~by~~ drought) and they can vary according to the plant species or cultivar and environmental conditions (Janse and Obradovic, 2010; CABI, 2023).	P	*Category : EDITORIAL* **(48) United States of America (7 Sep 2023 4:47 PM)**
66	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.). Pictures of symptoms on various hosts can be found at and . ~~Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination.~~ The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.	P	*Category : TECHNICAL* **(268) European Union (29 Sep 2023 3:11 PM)** Repetition from the previous paragraph.
66	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.). Pictures of symptoms on various hosts can be found at and . Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.	C	*Category : SUBSTANTIVE* **(267) European Union (29 Sep 2023 3:08 PM)** Move this text in the introduction, in the paragraph describing host range.
66	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.). Pictures of symptoms on various hosts can be found	C	*Category : TECHNICAL* **(266) European Union (29 Sep 2023 3:05 PM)** A reference with pictures could be added

	at and . Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.		e.g. the following document from the French ministry of agriculture which describes/shows potential confusions. https://agriculture.gouv.fr/telecharger/85855.
66	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.). Pictures of symptoms on various hosts can be found at and . The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.	C	*Category : TECHNICAL* **(101) EPPO (25 Sep 2023 8:22 AM)** A reference with pictures could be added e.g. the following document from the French ministry of agriculture which describes/shows potential confusions. https://agriculture.gouv.fr/telecharger/85855
66	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.). Pictures of symptoms on various hosts can be found at and . Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.	C	*Category : SUBSTANTIVE* **(100) EPPO (25 Sep 2023 8:22 AM)** Move this text in the introduction, in the paragraph describing host range.
66	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.). Pictures of symptoms on various hosts can be found at	P	*Category : TECHNICAL* **(99) EPPO (25 Sep 2023 8:22 AM)** Repetition from the previous paragraph.

	and ~~. Symptoms may vary depending on the host and~~ .*X. fastidiosa* ~~subspecies combination.~~ The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.		
66	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.). Pictures of symptoms on various hosts can be found at and . Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.	P	*Category : EDITORIAL* **(49) United States of America (7 Sep 2023 4:48 PM)**
66	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.). Pictures of symptoms on various hosts can be found at and . Symptoms may vary depending on the genetic make-up of the host and *X. fastidiosa* subspecies combination. The host range can be markedly different between subspecies; however, there is some uncertainty with regard to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subsp. *fastidiosa* not only infects grapes but also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.	P	*Category : SUBSTANTIVE* **(27) Ghana (30 Aug 2023 10:30 PM)**
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and	C	*Category : TECHNICAL* **(271) European Union (29 Sep 2023**

	local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis rotundifolia* (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2023c).		**3:18 PM)** Add this recent paper? Alexandra Katz Kahn, Anne Sicard, Monica Cooper, Matt Daugherty, Monica Ann Donegan and Rodrigo Almeida (2023) Progression of Xylella fastidiosa infection in grapevines under field conditions https://doi.org/10.1094/PHYTO-01-23-0008-R.
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis rotundifolia* (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years,	C	*Category : TECHNICAL* **(270) European Union (29 Sep 2023 3:17 PM)** Abdelrazek et al., 2023 (DOI: 10.1094/PHYTO-06-23-0212-R) reports results illustrating the presence of Xylella fastidiosa subsp. multiplex and not only Xylella fastidiosa subsp. fastidiosa in symptomatic grapevines. It is suggested to add this information at the end of this paragraph as this is relevant for grapevine.

	although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2023c).		
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis rotundifolia* (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as angular leaf scorch ~~rotbrenner~~ and ~~esca~~ escald (EPPO, 2023c).	P	*Category : TECHNICAL* **(245) Colombia (27 Sep 2023 5:39 PM)** It is considered that the correct symptom is "angular leaf scorch and escald".
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis rotundifolia* (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf ~~lamina~~blade, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid	P	*Category : EDITORIAL* **(244) Colombia (27 Sep 2023 5:37 PM)** It is suggested to change "lamina" to "blade"

	and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2023c).		
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis rotundifolia* (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, ~~in particular~~ particularly along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2023c).	P	*Category : EDITORIAL* **(221) Australia (27 Sep 2023 8:12 AM)** Simplification of language
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis*	C	*Category : TECHNICAL* **(103) EPPO (25 Sep 2023 8:22 AM)** Abdelrazek et al., 2023 (DOI: 10.1094/PHYTO-06-23-0212-R) reports

	rotundifolia (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2023c).		results illustrating the presence of Xylella fastidiosa subsp. multiplex and not only Xylella fastidiosa subsp. fastidiosa in symptomatic grapevines. It is suggested to add this information at the end of this paragraph as this is relevant for grapevine.
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis rotundifolia* (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years,	C	*Category : TECHNICAL* **(102) EPPO (25 Sep 2023 8:22 AM)** Add this recent paper? Alexandra Katz Kahn, Anne Sicard, Monica Cooper, Matt Daugherty, Monica Ann Donegan and Rodrigo Almeida (2023) Progression of Xylella fastidiosa infection in grapevines under field conditions https://doi.org/10.1094/PHYTO-01-23-0008-R

	although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2023c).		
68	Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). *Vitis rotundifolia* (Muscadinia) and indigenous American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly, showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2023c).	C	*Category : TECHNICAL* **(67) United States of America (15 Sep 2023 3:38 PM)** A paper showing multiplex infecting grapevines just accepted in Phytopathology, and perhaps should be added here. Please incorporate the results of Kahn et al. 2023 Phytopathology here. Symptom development was studied in detail and does not match the paradigm very well. Suggest deletion, this is not correct. In hot conditions you can see good symptoms in year one.
72	Symptoms of coffee leaf scorch appear on young flushes of field plants as large marginal and apical scorched zones on recently matured leaves (EPPO, 2023c). Affected leaves drop prematurely, shoot growth is stunted, and apical leaves are small and chlorotic. Symptoms may progress to shoot dieback and overall plant stunting. Fruit size and yield are generally reduced (De Lima *et al.*, 1998). Side branches have no leaves and fruit, the exception being a tuft of leaves at the branch tip. Infection of coffee plants by *X. fastidiosa* can also lead to the "crespera" disease, which has been reported in Costa Rica (Montero-Astúa *et al.*, 2008). Symptoms range from mild to severe curling of leaf margins, chlorosis and deformation of leaves, asymmetry (Bergsma-Vlami *et al*., 2015), stunting of plants,	P	*Category : EDITORIAL* **(28) Ghana (30 Aug 2023 10:31 PM)** for consistency

	shortening of internodes and dieback of branches (Montero-Astúa *et al.*, 2008). Infected ~~*Coffea*~~Coffea plants may remain asymptomatic (De Lima *et al.*, 1998; Montero-Astúa *et al.*, 2008).		
74	In ~~two,~~ two different, distant regions around the world (southern Europe and South America), leaf scorching symptoms on *O. europaea* trees have been associated with *X. fastidiosa* (Saponari *et al.*, 2013; Haelterman *et al.*, 2015; Coletta-Filho *et al.*, 2016). 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 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 the death of the entire tree. *X. fastidiosa* has also been detected in young olive trees with leaf scorching and quick decline (EPPO, 2023c).	P	*Category : EDITORIAL* **(50) United States of America (7 Sep 2023 4:51 PM)**
76	The most characteristic symptoms of almond leaf scorch disease are leaf scorching followed by decreased productivity and general decline. Strains of *X. fastidiosa* subsp. *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. The disease is sometimes described as golden death, due to the golden yellow color of the canopy of infected trees (Marco-Noales et al., 2021).	P	*Category : SUBSTANTIVE* **(272) European Union (29 Sep 2023 3:22 PM)** This disease is often refered to the golden death of almond (Marco-Noales et al., 2021 https://ipm.ucanr.edu/agriculture/almond/almond-leaf-scorch/, …).
76	The most characteristic symptoms of almond leaf scorch disease are leaf scorching followed by decreased productivity and general decline. Strains of *X. fastidiosa* subsp. *fastidiosa* and subsp. *multiplex* have been reported to cause almond leaf scorch disease (Yuan *et al.*, 2010). In early summer, leaves appear with marginal	P	*Category : SUBSTANTIVE* **(104) EPPO (25 Sep 2023 8:22 AM)** This disease is often refered to the golden death of almond (Marco-Noales et al., 2021 https://ipm.ucanr.edu/agriculture/almond/almond-leaf-scorch/, …)

	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. The disease is sometimes described as golden death, due to the golden yellow color of the canopy of infected trees (Marco-Noales et al., 2021).		
76	The most characteristic symptoms of almond leaf scorch disease are leaf scorching followed by decreased productivity and general decline. Strains of *X. fastidiosa* subsp. *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.	C	*Category : TECHNICAL* **(68) United States of America (15 Sep 2023 3:39 PM)** Is there data supporting this statement?
80	The first symptom of bacterial leaf scorch of blueberry is ~~a~~ marginal leaf scorching, and the scorched leaf zone may be bordered by a darker band (Brannen *et al.*, 2022; EPPO, 2023c). In the early stages of disease progression, symptoms may be localized, but over time, symptoms can become uniformly distributed throughout the foliage. Newly developed shoots can be abnormally thin with a reduced number of flower buds. Leaf drop occurs, and twigs and stems have a distinct “skeletal” yellow appearance. Following leaf drop, the plant dies, typically during the second year after symptoms are observed (Chang *et al.*, 2009).	P	*Category : EDITORIAL* **(222) Australia (27 Sep 2023 8:13 AM)** Removal of unnecessary word.
84	The main symptom of alfalfa dwarf is stunted regrowth after cutting. This stunting may not be apparent until many months after initial infection. Leaflets on affected plants are smaller and often slightly darker in colour than those on uninfected plants, but not distorted, cupped, mottled or yellow. The tap-root is of a normal size, but the lignified tissue has an abnormally yellowish colour, with fine dark streaks of dead tissue scattered throughout. In newly infected plants, the yellowing is mostly in a ring beginning under the bark, with a normal white-coloured cylinder	P	*Category : TECHNICAL* **(273) European Union (29 Sep 2023 3:28 PM)** Current preferred name (please see the EPPO Global Database: https://gd.eppo.int/taxon/CORBIN)

	of tissue inside the yellowed outer layer of wood (EPPO, 2023c). The inner bark is not discoloured, nor do large brown or yellow patches appear as in bacterial wilt (caused by *Clavibacter ~~michiganensis subsp.~~ insidiosus*). Alfalfa dwarf progressively worsens over the first one to two years after the symptoms appear, and eventually kills infected plants.		
84	The main symptom of alfalfa dwarf is stunted regrowth after cutting. This stunting may not be apparent until many months after initial infection. Leaflets on affected plants are smaller and often slightly darker in colour than those on uninfected plants, but not distorted, cupped, mottled or yellow. The tap-root is of a normal size, but the lignified tissue has an abnormally yellowish colour, with fine dark streaks of dead tissue scattered throughout. In newly infected plants, the yellowing is mostly in a ring beginning under the bark, with a normal white-coloured cylinder of tissue inside the yellowed outer layer of wood (EPPO, 2023c). The inner bark is not discoloured, nor do large brown or yellow patches appear as in bacterial wilt (caused by *Clavibacter ~~michiganensis subsp.~~ insidiosus*). Alfalfa dwarf progressively worsens over the first one to two years after the symptoms appear, and eventually kills infected plants.	P	*Category : TECHNICAL* **(105) EPPO (25 Sep 2023 8:22 AM)** Current preferred name (please see the EPPO Global Database: https://gd.eppo.int/taxon/CORBIN)
84	The main symptom of alfalfa dwarf is stunted regrowth after cutting. This stunting may not be apparent until many months after initial infection. Leaflets on affected plants are smaller and often slightly darker in colour than those on uninfected plants, but not distorted, cupped, mottled or yellow. The ~~tap-root~~ taproot is of a normal size, but the lignified tissue has an abnormally yellowish colour, with fine dark streaks of dead tissue scattered throughout. In newly infected plants, the yellowing is mostly in a ring beginning under the bark, with a normal white-coloured cylinder of tissue inside the yellowed outer layer of wood (EPPO, 2023c). The inner bark is not discoloured, nor do large brown or yellow patches appear as in bacterial wilt (caused by *Clavibacter michiganensis* subsp. *insidiosus*). Alfalfa dwarf progressively worsens over the first one to two years after the symptoms appear, and eventually kills infected plants.	P	*Category : EDITORIAL* **(51) United States of America (7 Sep 2023 4:53 PM)**
86	*X. fastidiosa* has been detected on a number of different hosts in the recent European outbreaks. Most symptomatic plants display ~~typical~~ leaf scorching symptoms. On *N. oleander*, necrosis develops on the leaf margin and infection may lead to death of entire plants (EPPO, 2023c). *P. myrtifolia* and *Spartium junceum* have been found to be two of the most susceptible hosts in the recent European outbreaks. Infected plants show scorched leaves, with desiccation starting from the	P	*Category : TECHNICAL* **(274) European Union (29 Sep 2023 3:29 PM)** It is not clear what is meant by 'typical' here so it is suggested to delete.

	tip and progressing to the entire blade (EPPO, 2023c). Symptoms can be seen at		
86	*X. fastidiosa* has been detected on a number of different hosts in the recent European outbreaks. Most symptomatic plants display typical leaf scorching symptoms. On *N. oleander*, necrosis develops on the leaf margin and infection may lead to death of entire plants (EPPO, 2023c). *P. myrtifolia* and *Spartium junceum* have been found to be two of the most susceptible hosts in the recent European outbreaks. Infected plants show scorched leaves, with desiccation starting from the tip and progressing to the entire blade (EPPO, 2023c). Symptoms can be seen at	C	*Category : TECHNICAL* **(106) EPPO (25 Sep 2023 8:22 AM)** It is not clear what is meant by 'typical' here so it is suggested to delete.
88	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. 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 may also be sampled. Guidance on the number of leaves (including their petioles), twigs or stems and approximate weights to be used in the laboratory sample is presented in Table 1 for the testing of individual plants and in Table 2 and Table 3 for the testing of ~~many plants~~ composite samples (EPPO, 2023c). For detailed guidance on the sampling of composite samples, see also Loconsole *et al*. (2021).	P	*Category : EDITORIAL* **(107) EPPO (25 Sep 2023 8:22 AM)** Delete from (typo). Replace 'many plants' by 'composite samples': this corresponds better to the title of Tables 2 and 3 and to the fact that Table 2 correspond to a "small" number of samples.
89	**Table 1.** Number of leaves (including their petioles) or other plant material to be used, and approximate weight of the laboratory sample, when testing individual plants	C	*Category : SUBSTANTIVE* **(248) China (28 Sep 2023 8:15 AM)** It is recommended to add a column to indicate the sampling size of leaves or other parts for different plant species, such as 1cm*1cm, etc. This will help to ensure a more accurate sampling process. Listing the specific dimensions of each leaf/part during sampling will help achieve a more accurate sampling process.
115	*Notes:* † The superficial bark should be removed, and ~~scraping~~ scrapings taken from the active tissues (youngest external ring).	P	*Category : TECHNICAL* **(52) United States of America (7 Sep 2023 4:55 PM)** More than one
117	*Source:* EPPO (European and Mediterranean Plant Protection ~~organization)~~Organization). 2023c.	P	*Category : EDITORIAL* **(29) Ghana (30 Aug 2023 10:32 PM)**
119	The distribution and concentration of *X. fastidiosa* within the plant can be variable	P	*Category : EDITORIAL* **(223) Australia (27 Sep 2023 8:19 AM)**

	and is dependent upon the plant species and seasonal and environmental factors. ~~To maximize the likelihood of detection~~As a result, sampling ~~should preferably be performed~~ during the period of active growth of the plants (Hopkins, ~~1981)~~1981) maximizes the likelihood of detection. For tropical plant species grown indoors, such as coffee plants, sampling may be performed all year round when plants are exhibiting periods of active growth (EPPO, 2023c).		Changing the sentence to remove the phrase "should preferably", an ambiguous phrase in ISPMs.
119	The distribution and concentration of *X. fastidiosa* within the plant can be variable and is dependent upon the plant species and seasonal and environmental factors. To maximize the likelihood of detection, sampling should preferably be performed during the period of active growth of the plants (Hopkins, 1981). For tropical plant species grown indoors, such as coffee plants, sampling may be performed ~~all year round~~ year-round when plants are exhibiting periods of active growth (EPPO, 2023c).	P	*Category : EDITORIAL* **(53) United States of America (7 Sep 2023 4:58 PM)**
121	*O. europaea* and *N. oleander* sampling can be performed ~~all year around~~ year-around with no decrease in the diagnostic sensitivity throughout the year, excluding the warmest and coldest periods (D'Onghia *et al.*, 2022). These observations are considered valid for areas with a Mediterranean climate.	P	*Category : EDITORIAL* **(54) United States of America (7 Sep 2023 4:59 PM)**
125	In temperate zones of the world where *V. vinifera* or deciduous trees (e.g. *Prunus cerasus*, *P. dulcis*) have been infected for some time, the bacteria do not move into the new season's growth until the middle of summer, when symptoms may also become visible. For example, the most suitable time for searching for symptoms in grapevine is late summer to early autumn when weather conditions are predominately hot and dry or when grape plants are exposed to drought stress (Galvez *et al.*, 2010).	C	*Category : TECHNICAL* **(69) United States of America (15 Sep 2023 3:41 PM)** Is there are data supporting this statement?
126	In a tropical climate, such as Brazil, *X.* ~~*fastidiosa*~~*fastidiosa*~~- associated~~ ssociated symptoms and detection occurs throughout the year for olive quick decline syndrome (Safady *et al*., 2019) and mainly from February to June for CVC in the southern hemisphere (Bassanezi and Primiano, 2021). The vigorous sprout growth can make it difficult to identify symptoms during periods of rain and high temperatures.	P	*Category : EDITORIAL* **(30) Ghana (30 Aug 2023 10:34 PM)**
127	**3.2.2 Plant sample collection**	C	*Category : TECHNICAL* **(275) European Union (29 Sep 2023 3:37 PM)** This section presents general aspects that are also presented in 3.2. The two paragraphs could be combined.

127	**3.2.2 Plant sample collection**	C	*Category : TECHNICAL* **(108) EPPO (25 Sep 2023 8:22 AM)** This section presents general aspects that are also presented in 3.2. The two paragraphs could be combined.
129	**3.2.3 Sampling of symptomatic plants**	C	*Category : EDITORIAL* **(109) EPPO (25 Sep 2023 8:22 AM)** Based on the content and title, this section could be a subsection of 3.2.2. (i.e. 3.2.2.1). This comment can be ignored if 3.2.2 is combined with the introductory text 3.2.
130	The sample should consist of branches or cuttings representative of the symptoms seen on the plant or plants and containing at least ~~10~~ 5 to 25 leaves or twigs (or a combination of leaves and twigs) depending on leaf size. The approximate weight needed for laboratory samples is between 0.5 g and 1 g leaf petioles, midribs or other plant material from each individual plant (EPPO, 2023c) (Table 1). Symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected. It is recommended that, when testing pooled samples, the limit of detection for each detection test be confirmed.	P	*Category : TECHNICAL* **(276) European Union (29 Sep 2023 3:38 PM)** According to Table 1, the minimum is 5 for leaves of large size.
130	The sample should consist of branches or cuttings representative of the symptoms seen on the plant or plants and containing at least ~~10~~ 5 to 25 leaves or twigs (or a combination of leaves and twigs) depending on leaf size. The approximate weight needed for laboratory samples is between 0.5 g and 1 g leaf petioles, midribs or other plant material from each individual plant (EPPO, 2023c) (Table 1). Symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected. It is recommended that, when testing pooled samples, the limit of detection for each detection test be confirmed.	P	*Category : TECHNICAL* **(215) EPPO (25 Sep 2023 8:56 AM)** According to Table 1, the minimum is 5 for leaves of large size.
130	The sample should consist of branches or cuttings representative of the symptoms seen on the plant or plants and containing at least 10 to 25 leaves or twigs (or a combination of leaves and twigs) depending on leaf size. The approximate weight needed for laboratory samples is between 0.5 g and 1 g leaf petioles, ~~midribs~~ midribs, or other plant material from each individual plant (EPPO, 2023c) (Table 1). Symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected. It is recommended that, when testing pooled samples, the limit of detection for each detection test be confirmed.	P	*Category : EDITORIAL* **(55) United States of America (7 Sep 2023 5:07 PM)**

131	**3.2.4 Sampling of asymptomatic plants**	C	*Category : EDITORIAL* **(111) EPPO (25 Sep 2023 8:22 AM)** Based on the content and title, this section could be a subsection of 3.2.2. (i.e. 3.2.2.2). This comment can be ignored if 3.2.2 is combined with the introductory text 3.2.
132	For asymptomatic plants, the sample should be representative of the entire aerial part of the plant. Recent experimental data on detection of *X. fastidiosa* in ~~monumental~~ ornamental and ancient *O. europaea* trees showed that detection was more reliable when sampling the medium–upper part of the canopy (Valentini and Porcelli, 2016). As mentioned in section 3.2.2, olive tree twigs were a better matrix than leaves for the detection of the bacterium. For testing individual asymptomatic plants, at least four to ten branches should be collected, depending on the host and plant size. Detailed guidance on collecting the minimum amount of tissue from a plant to achieve consistent and reliable detection can be found in Loconsole *et al*. (2021).	P	*Category : EDITORIAL* **(224) Australia (27 Sep 2023 8:19 AM)** correcting typo
133	Further information on the number of samples to be collected per lot can be found in ISPM 31 (*Methodologies for sampling of consignments*). Sampling details for tests of composite samples composed of a small amount of ~~samples~~ tissue are presented in Table 2. Sampling details for tests of samples composed of a large amount of tissue are presented in Table 3. Validation data are available in the European and Mediterranean Plant Protection Organization (EPPO) diagnostic database on diagnostic expertise (EPPO, 2023d).	P	*Category : TECHNICAL* **(277) European Union (29 Sep 2023 3:43 PM)** It is suggested to use 'tissue' as in EPPO,2023c.
133	Further information on the number of samples to be collected per lot can be found in ISPM 31 (*Methodologies for sampling of consignments*). Sampling details for tests of composite samples composed of a small amount of ~~samples~~ tissue are presented in Table 2. Sampling details for tests of samples composed of a large amount of tissue are presented in Table 3. Validation data are available in the European and Mediterranean Plant Protection Organization (EPPO) diagnostic database on diagnostic expertise (EPPO, 2023d).	P	*Category : TECHNICAL* **(112) EPPO (25 Sep 2023 8:22 AM)** It is suggested to use 'tissue' as in EPPO,2023c
133	Further information on the number of samples to be collected per lot can be found in ISPM 31 (*Methodologies for sampling of consignments*). Sampling details for tests of composite samples composed of a small ~~amount~~ number of samples are presented in Table 2. Sampling details for tests of samples composed of a large amount of tissue are presented in Table 3. Validation data are available in the European and Mediterranean Plant Protection Organization (EPPO) diagnostic	P	*Category : EDITORIAL* **(56) United States of America (7 Sep 2023 5:09 PM)**

	database on diagnostic expertise (EPPO, 2023d).		
134	**Table 2.** Guidance for both sampling and laboratory testing of composite samples composed of a small ~~number~~ amount of ~~samples~~tissue	P	*Category : TECHNICAL* **(278) European Union (29 Sep 2023 3:44 PM)** It is suggested to use 'amount of tissue' as in EPPO,2023c and as also suggested above.
134	**Table 2.** Guidance for both sampling and laboratory testing of composite samples composed of a small ~~number~~ amount of ~~samples~~tissue	P	*Category : TECHNICAL* **(113) EPPO (25 Sep 2023 8:22 AM)** It is suggested to use 'amount of tissue' as in EPPO,2023c and as also suggested above.
136	**Minimum no. of ~~petioles or twigs~~leaves/twigs/stems**	P	*Category : TECHNICAL* **(279) European Union (29 Sep 2023 3:47 PM)** It is suggested to align the text with EPPO 2023c i.e. Minimum no. of leaves/twigs/stems per plant to be collected.
136	**Minimum no. of ~~petioles or twigs~~leaves/twigs/stems per plant to be collected**	P	*Category : TECHNICAL* **(114) EPPO (25 Sep 2023 8:22 AM)** It is suggested to align the text with EPPO 2023c i.e. Minimum no. of leaves/twigs/stems per plant to be collected
140	4 ~~(petioles~~ (leaf midribs or petioles or leaf ~~midribs)~~basal part for each leaf)	P	*Category : TECHNICAL* **(280) European Union (29 Sep 2023 3:50 PM)** It is suggested to align the text with EPPO 2023c.
140	4 ~~(petioles~~ (leaf midribs or petioles or leaf ~~midribs)~~basal part for each leaf)	P	*Category : TECHNICAL* **(115) EPPO (25 Sep 2023 8:22 AM)** It is suggested to align the text with EPPO 2023c.
158	**Table 3.** Guidance on sampling for lots of large composite ~~plant~~ samples (e.g. composite samples from consignments or places of production of plants for planting)	P	*Category : EDITORIAL* **(116) EPPO (25 Sep 2023 8:22 AM)** For consistency with the title of Table 2 (otherwise add "plant" in the title of Table 2).
177	250 shoot pieces of 1.5–2 cm (up to ~~2 0~~ 20 g)	P	*Category : EDITORIAL* **(117) EPPO (25 Sep 2023 8:22 AM)** Typo
195	*Notes:* The extraction procedure performed includes a bacterial concentration step: the volume of extraction buffer is 1:3 w:v for all samples except for herbaceous plantlets (1:1 or 1:1.5) and *Coffea* (1:4) ~~EPPO 2023c~~(EPPO 2023c).	P	*Category : EDITORIAL* **(118) EPPO (25 Sep 2023 8:22 AM)** Typos
203	Once samples are collected, they should be kept cool (e.g. 4–15 °C) and transported to the laboratory as quickly as possible (within no more than two days)	C	*Category : TECHNICAL* **(314) United States of America (29 Sep**

	in clean, transparent, plastic sample bags. 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 them. Samples should be processed as soon as possible after arrival at the laboratory. If the plant samples originate from areas where infected vectors may occur, it is recommended that the samples are checked for the presence of insects before opening the sample bags. If any insects are present, samples should be stored in the refrigerator for approximately 12 h.		**2023 9:16 PM)** Wrapping plant tissue in paper towels may also help to preserve integrity.
203	Once samples are collected, they should be kept cool (e.g. 4–15 °C) and transported to the laboratory as quickly as possible (within no more than two days) in clean, transparent, plastic sample bags. 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 them. Samples~~ should be processed as soon as possible after arrival at the laboratory. If the plant samples originate from areas where infected vectors may occur, it is recommended that the samples are checked for the presence of insects before opening the sample bags. If any insects are present, samples should be stored in the refrigerator for approximately 12 h.	P	*Category : TECHNICAL* **(281) European Union (29 Sep 2023 3:52 PM)** It seems contradictory to say that 'for culture isolation work samples should be processed immediately' and 'Samples for isolation (see section 4.1) may be kept refrigerated (e.g. 4 °C) for up to three days'. Proposal: delete 'for culture isolation work samples should be processed immediately' here and include the information below.
203	Once samples are collected, they should be kept cool (e.g. 4–15 °C) and transported to the laboratory as quickly as possible (within no more than two days) in clean, transparent, plastic sample bags. 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 them. Samples~~ should be processed as soon as possible after arrival at the laboratory. If the plant samples originate from areas where infected vectors may occur, it is recommended that the samples are checked for the presence of insects before opening the sample bags. If any insects are present, samples should be stored in the refrigerator for approximately 12 h.	P	*Category : TECHNICAL* **(119) EPPO (25 Sep 2023 8:22 AM)** It seems contradictory to say that 'for culture isolation work samples should be processed immediately' and 'Samples for isolation (see section 4.1) may be kept refrigerated (e.g. 4 °C) for up to three days'. Proposal: delete 'for culture isolation work samples should be processed immediately' here and include the information below.
203	Once samples are collected, they should be kept cool (e.g. 4–15 °C) and transported to the laboratory as quickly as possible (within no more than two days) in clean, transparent, plastic sample bags. 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 them. Samples should be processed as soon as possible after arrival at the laboratory. If the plant samples originate from areas where infected vectors	C	*Category : TECHNICAL* **(21) Kenya (28 Aug 2023 3:57 PM)** To specify the required storage temperatures

	may occur, it is recommended that the samples are checked for the presence of insects before opening the sample bags. If any insects are present, samples should be stored in the refrigerator for approximately 12 h.		
203	Once samples are collected, they should be kept ~~cool~~ in cooled containers (e.g. 4–15 °C) and transported to the laboratory as quickly as possible (within no more than two days) in clean, transparent, plastic sample bags. 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 them. Samples should be processed as soon as possible after arrival at the laboratory. If the plant samples originate from areas where infected vectors may occur, it is recommended that the samples are checked for the presence of insects before opening the sample bags. If any insects are present, samples should be stored in the refrigerator for approximately 12 h.	P	*Category : EDITORIAL* **(20) Kenya (28 Aug 2023 3:56 PM)**
204	Samples for isolation (see section 4.1) ~~may~~ should be ~~kept~~ processed immediately or stored refrigerated (e.g. 4 °C) for up to three days. For isolation, samples should be surface disinfected. For other tests, samples may be refrigerated for up to one week. For longer term storage, plant samples may be stored at −20 °C or −80 °C for up to one year for molecular or serological detection. Samples should be inspected for symptoms and, if present, symptomatic leaves (including their petioles) and twigs should be selected and processed (removing the necrotic and dead tissue). If no symptoms are noted, leaves should be representative of the entire sample received in the laboratory.	P	*Category : TECHNICAL* **(283) European Union (29 Sep 2023 3:59 PM)** See comment in previous paragraph.
204	Samples for isolation (see section 4.1) may be kept refrigerated (e.g. 4 °C) for up to three days. For isolation, samples should be surface disinfected. For other tests, samples may be refrigerated for up to one week. For longer term storage, plant samples may be stored at −20 °C or −80 °C for up to one year for molecular or serological detection. Samples should be inspected for symptoms and, if present, symptomatic leaves (including their petioles) and twigs should be selected and processed (removing the necrotic and dead tissue). If no symptoms are noted, leaves should be representative of the entire sample received in the laboratory.	C	*Category : TECHNICAL* **(282) European Union (29 Sep 2023 3:54 PM)** Note that this refers to subsampling in the laboratory (first step of the sample processing in the laboratory) and not to sample transport and storage. It is suggested to move the information in an appropriate section or to change the heading of 3.2.5.
204	Samples for isolation (see section 4.1) may be kept refrigerated (e.g. 4 °C) for up to three days. For isolation, samples should be surface disinfected. For other tests, samples may be refrigerated for up to one week. For longer term storage, plant samples may be stored at −20 °C or −80 °C for up to one year for molecular or serological detection. Samples should be inspected for symptoms and, if present,	P	*Category : EDITORIAL* **(225) Australia (27 Sep 2023 8:23 AM)** For clarity. It was noted above that twig material is better for resistant olive varieties.

	symptomatic leaves (including their petioles) and twigs should be selected and processed (removing the necrotic and dead tissue). If no symptoms are noted, ~~leaves~~ plant samples (e.g. leaves, twigs) should be representative of the entire sample received in the laboratory.		
204	Samples for isolation (see section 4.1) may be kept refrigerated (e.g. 4 °C) for up to three days. For isolation, samples should be surface disinfected. For other tests, samples may be refrigerated for up to one week. For longer term storage, plant samples may be stored at −20 °C or −80 °C for up to one year for molecular or serological detection. Samples should be inspected for symptoms and, if present, symptomatic leaves (including their petioles) and twigs should be selected and processed (removing the necrotic and dead tissue). If no symptoms are noted, leaves should be representative of the entire sample received in the laboratory.	C	*Category : TECHNICAL* **(121) EPPO (25 Sep 2023 8:22 AM)** Note that this refers to subsampling in the laboratory (first step of the sample processing in the laboratory) and not to sample transport and storage. It is suggested to move the information in an appropriate section or to change the heading of 3.2.5.
204	Samples for isolation (see section 4.1) ~~may~~ should be ~~kept~~ processed immediately or stored refrigerated (e.g. 4 °C) for up to three days. For isolation, samples should be surface disinfected. For other tests, samples may be refrigerated for up to one week. For longer term storage, plant samples may be stored at −20 °C or −80 °C for up to one year for molecular or serological detection. Samples should be inspected for symptoms and, if present, symptomatic leaves (including their petioles) and twigs should be selected and processed (removing the necrotic and dead tissue). If no symptoms are noted, leaves should be representative of the entire sample received in the laboratory.	P	*Category : TECHNICAL* **(120) EPPO (25 Sep 2023 8:22 AM)** See comment in previous paragraph
206	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 may 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 carried out from late spring until autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol at −20 °C or at −80 °C with or without ethanol. Sticky traps with captured insects can also be stored at −20 °C. A video on insect collection has been published by the European Food Safety Authority.[1] Identification keys with pictures to distinguish the suborder Cercopoidea from Delphacidae and Cicadellidae are available online (Purcell *et al.*, 2014).	C	*Category : TECHNICAL* **(318) United States of America (29 Sep 2023 9:20 PM)** This citation is regarding Xylella vectors in Europe only and is quite sparse as an ID key. Unclear why users need to specifically distinguish Cercopoidea from Delphacidae and Cicadellidae. Recommend either additional citations for other world regions, or citations of ID guides for the four families of vectors listed above or all of Auchenorrhyncha. Consider the following alternatives: https://idtools.dpi.nsw.gov.au/keys/auch/index.html; https://trinidad.tamu.edu/wp-content/uploads/sites/11/2017/09/Shirley_Xanthe_2012.pdf; C. H. Dietrich "KEYS TO THE FAMILIES OF CICADOMORPHA AND SUBFAMILIES AND TRIBES OF

			CICADELLIDAE (HEMIPTERA: AUCHENORRHYNCHA)," Florida Entomologist, 88(4), 502-517, (1 December 2005)
206	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 may 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 carried out from late spring until autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol at −20 °C or at −80 °C with or without ethanol. Sticky traps with captured insects can also be stored at −20 °C. A video on insect collection has been published by the European Food Safety Authority.[1] Identification keys with pictures to distinguish the suborder Cercopoidea from Delphacidae and Cicadellidae are available online (Purcell *et al*., 2014).	C	*Category : TECHNICAL* **(317) United States of America (29 Sep 2023 9:19 PM)** This video is very specific to collecting spittlebugs in olive in Europe. Add clarifying language about the specificity of the video, or add additional resources for sampling other vectors like leafhoppers and other cropping systems.
206	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 may 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 carried out from late spring until autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol at −20 °C or at −80 °C with or without ethanol. Sticky traps with captured insects can also be stored at −20 °C. A video on insect collection has been published by the European Food Safety Authority.[1] Identification keys with pictures to distinguish the suborder Cercopoidea from Delphacidae and Cicadellidae are available online (Purcell *et al*., 2014).	C	*Category : TECHNICAL* **(316) United States of America (29 Sep 2023 9:18 PM)** Although Dr. Purcell is an expert on Xf/vectoring, he has never done trap efficacy studies so should not be used to cite effectiveness or not. There are actual studies done on various leafhoppers that show yellow panel traps are visually effective as lures to the color. This citation listed is very misleading and generalized without being accurate or from a scientist who has done trap studies. -I would suggest citing researcher(s) who have actually done trap/lure studies for GWSS (other vectors) on the effectiveness of the trap vs. citing S. Purcell who has not done such studies. The citation appears to be info from a workshop manual put together for a workshop in Italy. Either info from him was second hand, or opinion, neither of which I think should be in an ISPM document with high integrity. All citations in an ISPM document I would hope have been checked for opinion/2nd

			hand information vs. actual work done on the topic, or at least clarify which xylem feeding vectors they are being included in the sweeping generalizations without actual citing of trap studies for each or assumptions may be made about specific pests which are incorrect and could cause a view of lack of integrity for a country's pest programs. Raymond Hix - ARS – Gary Puterka Development of Trapping Systems to Trap the Glassy-Winged Sharpshooter Homalodisca Coagulata Adults and Nymphs in Grape – American Vineyard Foundation (avf.org) 2001_55-57.pdf (ca.gov) CDFA - Pierce's Disease Research - Paper #86 - DEVELOPMENT OF TRAPPING SYSTEMS TO TRAP GLASSY-WINGED SHARPSHOOTER (HOMALODISCA COAGULATA) ADULTS AND NYMPHS IN GRAPE UC GWSS monitoring handout.pdf (ucr.edu) Blue-green most important vector of Xf in coastal California grape growing areas (no GWSS present). (Green, willow and red-headed also present) Blue-green and GWSS ARE attracted to yellow panel traps. Green and red headed sharpshooter NOT attracted to yellow panel traps so much use sweep net Sharpshooters / Grape / Agriculture: Pest Management Guidelines / UC Statewide IPM Program (UC IPM) (ucanr.edu)
206	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	C	*Category : TECHNICAL* **(315) United States of America (29 Sep 2023 9:17 PM)**

	insects may be trapped accidentally and specimens collected from sticky traps may 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 carried out from late spring until autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol at −20 °C or at −80 °C with or without ethanol. Sticky traps with captured insects can also be stored at −20 °C. A video on insect collection has been published by the European Food Safety Authority.[1] Identification keys with pictures to distinguish the suborder Cercopoidea from Delphacidae and Cicadellidae are available online (Purcell *et al*., 2014).		This is not always true. Manual cited says "...sticky traps are sometimes one of the most useful methods of relative sampling for vector leafhoppers." Other literature supports the use of yellow sticky traps for some important Xylella vectors like sharpshooters. Trapped insects can be used for testing but it should be pointed out that old or degraded samples may give false negative results.
206	Vectors can be collected with sweeping nets (adults), light trapping or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell ~~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 may 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~~ can be carried out from late spring until autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol at −20 °C or at −80 °C with or without ethanol. Sticky traps with captured insects can also be stored at −20 °C. A video on insect collection has been published by the European Food Safety Authority.[1] Identification keys with pictures to distinguish the suborder Cercopoidea from Delphacidae and Cicadellidae are available online (Purcell *et al*., 2014).	P	*Category : SUBSTANTIVE* **(226) Australia (27 Sep 2023 8:25 AM)** Use of "can" instead of "should preferably", an ambiguous phrase in ISPMs. Include light trapping as a method to collect vectors such as Cercopoidea (spittlebugs) and Cicadellini (sharpshooters) Sticky traps rarely capture either, and sweep netting will depend on how easy it is to get near vegetation – sweep nets get shredded by the sharp vegetation. Light trapping works well on Cercopoidea, some cicadas and Cicadellini without being dependant on the ability to traverse the countryside.
206	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 may 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 carried out from late spring until autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol at −20 °C or at −80 °C with or without ethanol. Sticky traps with	C	*Category : TECHNICAL* **(70) United States of America (15 Sep 2023 3:43 PM)** Traps are rather effective for sharpshooters, certainly not for spittlebugs. I think the statement should be modified or at least toned down. Why Delphacidae are included here?

	captured insects can also be stored at −20 °C. A video on insect collection has been published by the European Food Safety Authority.[1] Identification keys with pictures to distinguish the suborder Cercopoidea from Delphacidae and Cicadellidae are available online (Purcell *et al*., 2014).		
208	Insects collected may 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, despite its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). According to Cornara *et al* (2016), there is a saturation point for the number of cells detected in the mouthparts of spittlebug *Philaenus spumarius* and the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells. In France, the bacterial load of *P. spumarius* collected in the Corsica region was estimated by droplet digital PCR and ranged from 10^1 to 10^6 cells per insect (Cunty *et al*, 2020). In Italy, the bacterial load can be about 10^4 cells per insect (Cavalieri *et al*., 2019). In the United States of America, the number of cells can be higher than 10^4 cells per insect in sharpshooter vectors (Retchless *et al*., 2014).	C	*Category : SUBSTANTIVE* **(286) European Union (29 Sep 2023 4:10 PM)** This paragraph is not about sampling of insects. Information should be moved in another section.
208	Insects collected may 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, despite its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). According to Cornara *et al* (2016), there is a saturation point for the number of cells detected in the mouthparts of spittlebug *Philaenus spumarius* and the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells. In France, the bacterial load of *P. spumarius* collected in the Corsica region was estimated by droplet digital PCR and ranged from 10^1 to 10^6 cells per insect (Cunty *et al*, 2020). In Italy, the bacterial load can be about 10^4 cells per insect (Cavalieri *et al*., 2019). In the United States of America, the number of cells can be higher than 10^4 cells per insect in sharpshooter vectors (Retchless *et al*., 2014).	C	*Category : TECHNICAL* **(284) European Union (29 Sep 2023 4:09 PM)** Recent paper on detection by droplet digital PCR on vector in Italy: Detection of Xylella fastidiosa in Host Plants and Insect Vectors by Droplet Digital PCR (2023) Serafina Serena Amoia, Angelantonio Minafra, Angela Ligorio, Vincenzo Cavalieri, Donato Boscia, Maria Saponari and Giuliana Loconsole. Agriculture 2023, 13(3), 716; https://doi.org/10.3390/agriculture13030716 Information on the bacterial load found per insect in this paper could also added.
208	Insects collected may 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, despite its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). According to Cornara *et al* (2016), there is a saturation point	C	*Category : TECHNICAL* **(124) EPPO (25 Sep 2023 8:22 AM)** Recent paper on detection by droplet digital PCR on vector in Italy: Detection of Xylella fastidiosa in Host Plants and Insect Vectors by Droplet Digital PCR (2023) Serafina Serena Amoia, Angelantonio

	for the number of cells detected in the mouthparts of spittlebug *Philaenus spumarius* and the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells. In France, the bacterial load of *P. spumarius* collected in the Corsica region was estimated by droplet digital PCR and ranged from 10^1 to 10^6 cells per insect (Cunty *et al.*, 2020). In Italy, the bacterial load can be about 10^4 cells per insect (Cavalieri *et al.*, 2019). In the United States of America, the number of cells can be higher than 10^4 cells per insect in sharpshooter vectors (Retchless *et al.*, 2014).		Minafra, Angela Ligorio, Vincenzo Cavalieri, Donato Boscia, Maria Saponari and Giuliana Loconsole. Agriculture 2023, 13(3), 716; https://doi.org/10.3390/agriculture13030716 Information on the bacterial load found per insect in this paper could also added.
208	Insects collected may 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, despite its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). According to Cornara *et al* (2016), there is a saturation point for the number of cells detected in the mouthparts of spittlebug *Philaenus spumarius* and the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells. In France, the bacterial load of *P. spumarius* collected in the Corsica region was estimated by droplet digital PCR and ranged from 10^1 to 10^6 cells per insect (Cunty *et al*, 2020). In Italy, the bacterial load can be about 10^4 cells per insect (Cavalieri *et al.*, 2019). In the United States of America, the number of cells can be higher than 10^4 cells per insect in sharpshooter vectors (Retchless *et al.*, 2014).	C	*Category : SUBSTANTIVE* **(123) EPPO (25 Sep 2023 8:22 AM)** This paragraph is not about sampling of insects. Information should be moved in another section.
208	Insects collected may 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, despite its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). According to Cornara *et al* (2016), there is a saturation point for the number of cells detected in the mouthparts of spittlebug *Philaenus spumarius* and the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells. In France, the bacterial load of *P. spumarius* collected in the Corsica region was estimated by droplet digital PCR and ranged from 10^1 to 10^6 cells per insect (Cunty *et al.*, 2020). In Italy, the bacterial load can be about 10^4 cells per insect (Cavalieri *et al.*, 2019). In the United States of America, the number of cells can be higher than 10^4 cells per insect in sharpshooter vectors (Retchless *et al.*, 2014).	P	*Category : EDITORIAL* **(122) EPPO (25 Sep 2023 8:22 AM)**
208	Insects collected may be analysed by polymerase chain reaction (PCR) to detect *X. fastidiosa*. Enzyme-linked immunosorbent assay (ELISA: see section 3.3)	C	*Category : TECHNICAL* **(71) United States of America (15 Sep 2023 3:45 PM)**

	is not sensitive enough, as the bacterium only colonizes the insect foregut where, despite its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). According to Cornara *et al* (2016), there is a saturation point for the number of cells detected in the mouthparts of spittlebug *Philaenus spumarius* and the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells. In France, the bacterial load of *P. spumarius* collected in the Corsica region was estimated by droplet digital PCR and ranged from 10^1 to 10^6 cells per insect (Cunty *et al*, 2020). In Italy, the bacterial load can be about 10^4 cells per insect (Cavalieri *et al*., 2019). In the United States of America, the number of cells can be higher than 10^4 cells per insect in sharpshooter vectors (Retchless *et al*., 2014).		Killiny 2009 AEM might be a good reference here, better than Retchless. Also, Ranieri et al. 2020 should be cited as it also considers potential size of populations.
208	Insects collected may 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, despite its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). According to Cornara *et al* (2016), there is a saturation point for the number of cells detected in the mouthparts of spittlebug (*Philaenus ~~spumarius~~spumarius)* and the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells. In France, the bacterial load of *P. spumarius* collected in the Corsica region was estimated by droplet digital PCR and ranged from 10^1 to 10^6 cells per insect (Cunty *et al*, 2020). In Italy, the bacterial load can be about 10^4 cells per insect (Cavalieri *et al*., 2019). In the United States of America, the number of cells can be higher than 10^4 cells per insect in sharpshooter vectors (Retchless *et al*., 2014).	P	*Category : EDITORIAL* **(32) Ghana (30 Aug 2023 10:43 PM)**
209	~~**3.3 Serological detection**~~	P	*Category : SUBSTANTIVE* **(2) Chile (14 Aug 2023 4:16 PM)** serología y PCR convencionales no aceptados por la CE pese a estar en el IT EPPO.
210	A number of serological methods have been developed for the detection of *X. fastidiosa*, ~~including methods using~~ including ELISA (Sherald and Lei, 1991), membrane entrapment immunofluorescence (Hartung *et al.*, 1994), dot immunobinding assay (Lee *et al.*, 1992), western blotting (Chang *et al.*, 1993) and immunofluorescence (Carbajal, Morano and Morano, 2004). More recently, direct tissue blot immunoassay has been reported as an alternative means of rapidly screening *O. europaea* samples for *X. fastidiosa* (Djelouah *et al*., 2014). Instructions for performing an ELISA (including tissue print, squash or dot ELISA)	P	*Category : EDITORIAL* **(125) EPPO (25 Sep 2023 8:22 AM)**

	or an immunofluorescence test can be found in EPPO (2009, 2010). Serological methods are not sensitive enough for use early in the growing season, when no symptoms of the disease are observed, because of the low concentration of bacteria likely to be present in young asymptomatic tissue.		
211	**3.3.1 Preparation of material**	C	*Category : EDITORIAL* **(126) EPPO (25 Sep 2023 8:22 AM)** It is suggested to merge this subsection with the text under 3.3 as it is not really a description of how to prepare the material.
218	The specificity and sensitivity of DAS-ELISA to detect *X. fastidiosa* on *O. europaea*, using a kit from Loewe, were evaluated by Loconsole *et al.* (2014).[2] Additionally, a test performance study ~~performed~~ at the Institute for Sustainable Plant Protection (Bari, Italy) was conducted on serological kits from Agritest, Agdia and Loewe.[2] 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 in the EPPO database on diagnostic expertise (EPPO, 2023d).	P	*Category : EDITORIAL* **(33) Ghana (30 Aug 2023 10:45 PM)**
222	The ELISA is negative if the average absorbance reading of duplicate wells containing tissue macerate is <2× the average absorbance reading of the negative control wells containing healthy host tissue macerate.	P	*Category : EDITORIAL* **(127) EPPO (25 Sep 2023 8:22 AM)**
223	The ELISA is positive if the average absorbance reading of duplicate ~~sample~~ wells is ≥2× the average absorbance reading of the negative control wells containing healthy host tissue macerate.	P	*Category : EDITORIAL* **(128) EPPO (25 Sep 2023 8:22 AM)**
226	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; Oliveira *et al*. 2002; Schaad, Opgenorth and Gaush, 2002; Rodrigues *et al.*, 2003; Francis *et al.*, 2006; Harper, Ward and Clover, 2010; Li *et al.*, 2013; Ouyang *et al.*, 2013, Bonants *et al*., 2019, Dupas *et al*., 2019b, Hodgetts *et al*., 2021). The latter two tests allow subspecies assignment. A digital PCR (Dupas *et al*., 2019a) and isothermal amplification for use in the field without prior extraction steps (loop-mediated isothermal amplification (LAMP) (Yaseen *et al*., 2015) or recombinase polymerase amplification (Cesbron, Dupas and Jacques, 2022)) have also been developed or evaluated.[3] The conventional PCRs developed by Minsavage *et al.* (1994), Pooler and Hartung (1995) and Rodrigues *et al*. (2003), two real-time PCRs (Harper, Ward and Clover, 2010; Li *et al.*, 2013), and the two	C	*Category : TECHNICAL* **(287) European Union (29 Sep 2023 4:16 PM)** In this section on molecular detection (section 3.4), tests are also refered as being used for identification which is somehow confusing as identification should be covered in 4.5. In addition, in this sentence it is indicated that all the tests described from 3.4.3 to 3.4.10 can be used for the detection and identification of Xylella fastidiosa. However, in the specific subsections, the tests are either presented as being used for detection only (e.g. 3.4.3 and 3.4.6), or for both detection and identification (e.g. 3.4.8,

	real-time PCRs allowing subspecies assignment (Dupas *et al.*, 2019b; Hodgetts *et al.,* 2021) are described in this protocol for the detection and identification of *X. fastidiosa*. The real-time methods using isothermal amplification such as LAMP are also described in this protocol.[3] The PCR methods described hereafter are as described in the original publications.		3.4.9). For some tests, nothing is indicated. (e.g. 3.4.4 and 3.4.5). The matrices (including bacterial colonies) are sometimes indicated but sometimes not. In section 4.5, there is only a general sentence to mention that tests described in section 3.4 can be used for the identification of suspect X. fastidiosa isolates. So overall, the tests that can be used for detection and/or identification and the matrices that can be used is confusing.
226	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; Oliveira *et al*. 2002; Schaad, Opgenorth and Gaush, 2002; Rodrigues *et al.*, 2003; Francis *et al.*, 2006; Harper, Ward and Clover, 2010; Li *et al.*, 2013; Ouyang *et al.*, 2013, Bonants *et al*., 2019, Dupas *et al*., 2019b, Hodgetts *et al*., 2021). The latter two tests allow subspecies assignment. A digital PCR (Dupas *et al*., 2019a) and isothermal amplification for use in the field without prior extraction steps (loop-mediated isothermal amplification (LAMP) (Yaseen *et al*., 2015) or recombinase polymerase amplification (Cesbron, Dupas and Jacques, 2022)) have also been developed or evaluated.[3] The conventional PCRs developed by Minsavage *et al.* (1994), Pooler and Hartung (1995) and Rodrigues *et al*. (2003), two real-time PCRs (Harper, Ward and Clover, 2010; Li *et al.*, 2013), and the two real-time PCRs allowing subspecies assignment (Dupas *et al.*, 2019b; Hodgetts *et al.,* 2021) are described in this protocol for the detection and identification of *X. fastidiosa*. The real-time methods using isothermal amplification such as LAMP are also described in this protocol.[3] The PCR methods described hereafter are as described in the original publications.	C	*Category : TECHNICAL* **(129) EPPO (25 Sep 2023 8:22 AM)** In this section on molecular detection (section 3.4), tests are also refered as being used for identification which is somehow confusing as identification should be covered in 4.5. In addition, in this sentence it is indicated that all the tests described from 3.4.3 to 3.4.10 can be used for the detection and identification of Xylella fastidiosa. However, in the specific subsections, the tests are either presented as being used for detection only (e.g. 3.4.3 and 3.4.6), or for both detection and identification (e.g. 3.4.8, 3.4.9). For some tests, nothing is indicated. (e.g. 3.4.4 and 3.4.5). The matrices (including bacterial colonies) are sometimes indicated but sometimes not. In section 4.5, there is only a general sentence to mention that tests described in section 3.4 can be used for the identification of suspect X. fastidiosa isolates. So overall, the tests that can be used for detection and/or identification and the matrices that can be used is confusing.
228	**3.4.1 DNA extraction from plant material**	C	*Category : SUBSTANTIVE* **(249) China (28 Sep 2023 8:17 AM)** Explain advantages for each DNA extraction methods from plant materials, for example,

			CTAB is suitable for wide range of plant species; Dneasy Plant Mini Kit is easy-to-use protol, requiring minimal hands-on time; DNeasy mericon Food kit is designed for food sample, providing efficient DNA extraction from a variety of food matrices; QuickPick SML Plant DNA Kit is rapid and straighforwad, ideal for high-thought applications. InviMAG Plant DNA Mini Kit is automated magnetic bead-based system, suitable for medium to high-throughput DNA extraction. Listing the advantages of each DNA extraction method aims to help practitioners choose suitable reagents and methods。
229	A number of methods have been described for the extraction of the DNA of *X. fastidiosa* from bacterial colonies and from plant material (Minsavage *et al.*, 1994; Pooler and Hartung, 1995; Francis *et al.*, 2006; Huang, Bentz and Sherald, 2006; Harper, Ward and Clover, 2010; Li *et al.*, 2013). Extraction can be achieved using a number of standard commercial kits (e.g. Bextine and Child, 2007; Huang, Bentz and Sherald, 2006). The following methods are a selection of those widely used in laboratories. Many other similar DNA extraction kits will also readily extract *Xylella* DNA from plant material. Validation data on the sensitivities associated with the different nucleic acid extraction methods can be found in the EPPO database on diagnostic expertise (EPPO, 2023d). A PCR can be readily conducted on boiled or heated preparations (e.g. suspensions of 10^8 c.f.u. /mL heated at 95 °C for 15 min or 100 °C for 5 min) of bacterial colonies, or on DNA extracts purified using the methods below.	C	*Category : TECHNICAL* **(289) European Union (29 Sep 2023 4:18 PM)** Section 3 is about detection whereas identification of colonies is covered in section 4. So it is suggested to delete the reference to bacterial colonies here.
229	A number of methods have been described for the extraction of the DNA of *X. fastidiosa* from bacterial colonies and from plant material (Minsavage *et al.*, 1994; Pooler and Hartung, 1995; Francis *et al.*, 2006; Huang, Bentz and Sherald, 2006; Harper, Ward and Clover, 2010; Li *et al.*, 2013). Extraction can be achieved using a number of standard commercial kits (e.g. Bextine and Child, 2007; Huang, Bentz and Sherald, 2006). The following methods are a selection of those widely used in laboratories. Many other similar DNA extraction kits will also readily extract *Xylella* DNA from plant material. Validation data on the sensitivities associated with the different nucleic acid extraction methods can be found in the EPPO database on diagnostic expertise (EPPO, 2023d). A PCR can be	C	*Category : TECHNICAL* **(288) European Union (29 Sep 2023 4:17 PM)** Section 3 is about detection whereas identification of colonies is covered in section 4. So the EWG suggests to move the information in section 4.5.

	readily conducted on boiled or heated preparations (e.g. suspensions of 10^8 c.f.u. /mL heated at 95 °C for 15 min or 100 °C for 5 min) of bacterial colonies, or on DNA extracts purified using the methods below.		
229	A number of methods have been described for the extraction of the DNA of *X. fastidiosa* from bacterial colonies and from plant material (Minsavage *et al.*, 1994; Pooler and Hartung, 1995; Francis *et al.*, 2006; Huang, Bentz and Sherald, 2006; Harper, Ward and Clover, 2010; Li *et al.*, 2013). Extraction can be achieved using a number of standard commercial kits (e.g. Bextine and Child, 2007; Huang, Bentz and Sherald, 2006). The following methods are a selection of those widely used in laboratories. Many other similar DNA extraction kits will also readily extract *Xylella* DNA from plant material. Validation data on the sensitivities associated with the different nucleic acid extraction methods can be found in the EPPO database on diagnostic expertise (EPPO, 2023d). A PCR can be readily conducted on boiled or heated preparations (e.g. suspensions of 10^8 c.f.u. /mL heated at 95 °C for 15 min or 100 °C for 5 min) of bacterial colonies, or on DNA extracts purified using the methods below.	C	*Category : TECHNICAL* **(131) EPPO (25 Sep 2023 8:22 AM)** Section 3 is about detection whereas identification of colonies is covered in section 4. So the EWG suggests to move the information in section 4.5.
229	A number of methods have been described for the extraction of the DNA of *X. fastidiosa* from bacterial colonies and from plant material (Minsavage *et al.*, 1994; Pooler and Hartung, 1995; Francis *et al.*, 2006; Huang, Bentz and Sherald, 2006; Harper, Ward and Clover, 2010; Li *et al.*, 2013). Extraction can be achieved using a number of standard commercial kits (e.g. Bextine and Child, 2007; Huang, Bentz and Sherald, 2006). The following methods are a selection of those widely used in laboratories. Many other similar DNA extraction kits will also readily extract *Xylella* DNA from plant material. Validation data on the sensitivities associated with the different nucleic acid extraction methods can be found in the EPPO database on diagnostic expertise (EPPO, 2023d). A PCR can be readily conducted on boiled or heated preparations (e.g. suspensions of 10^8 c.f.u. /mL heated at 95 °C for 15 min or 100 °C for 5 min) of bacterial colonies, or on DNA extracts purified using the methods below.	C	*Category : TECHNICAL* **(130) EPPO (25 Sep 2023 8:22 AM)** Section 3 is about detection whereas identification of colonies is covered in section 4. So it is suggested to delete the reference to bacterial colonies here.
230	The analytical sensitivity of PCR tests can be improved when an additional ultrasonication (1 min at 35–40 kHz) is performed on the plant extract before DNA extraction (Bergsma-Vlami *et al*. 2017; Dupas *et al*., 2019b). This has improved the release of bacteria from biofilms, in particular with difficult matrices such as *O. europaea* and *Quercus* spp. Validation data from the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) are available ~~on~~ in the	P	*Category : EDITORIAL* **(132) EPPO (25 Sep 2023 8:22 AM)** Better English?

	EPPO database on diagnostic expertise ().		
231	**CTAB-based extraction (EPPO, 2023c)**. In brief, 0.5–1 g midrib, petiole or twig tissue is placed into an extraction bag with 5 mL extraction buffer (hexadecyltrimethylammonium bromide (CTAB) buffer: 100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 10 mM ethylenediaminetetraacetic acid (EDTA); 2% CTAB; 3% PVP-40) and homogenized using a homogenizer (e.g. Homex, Polytron).[2] 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~~ 16000 *g* for ~~15~~ 10 min. The aqueous layer (the upper layer – approximately 700 µL) is carefully transferred to a new tube and mixed with 490 µL ice-cold isopropanol. The suspension is mixed gently and incubated for at least ~~30~~ 20 min at −20 °C. After this DNA precipitation step, the suspension is centrifuged at 16 000 *g* for ~~15~~ 20 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 last centrifugation step. After washing and decanting the supernatant, the pellet is air-dried and suspended in 100 µL of TE buffer or deoxyribonuclease-free water.	P	*Category : TECHNICAL* **(290) European Union (29 Sep 2023 4:28 PM)** The protocol described should be the same as in EPPO 2023c. See the changes in the text.
231	**CTAB-based extraction (EPPO, 2023c)**. In brief, 0.5–1 g midrib, petiole or twig tissue is placed into an extraction bag with 5 mL extraction buffer (hexadecyltrimethylammonium bromide (CTAB) buffer: 100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 10 mM ethylenediaminetetraacetic acid (EDTA); 2% CTAB; 3% PVP-40) and homogenized using a homogenizer (e.g. Homex, Polytron).[2] 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~~ 16 000 *g* for ~~15~~ 10 min. The aqueous layer (the upper layer – approximately 700 µL) is carefully transferred to a new tube and mixed with 490 µL ice-cold isopropanol. The suspension is mixed gently and incubated for at least ~~30~~ 20 min at −20 °C. After this DNA precipitation step, the suspension is centrifuged at 16 000 *g* for ~~15~~ 20 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 last centrifugation step. After washing and decanting the supernatant, the pellet is air-dried and suspended in 100 µL of TE buffer or deoxyribonuclease-free water.	P	*Category : TECHNICAL* **(133) EPPO (25 Sep 2023 8:22 AM)** The protocol described should be the same as in EPPO 2023c. See the changes in the text.

232	**DNeasy® Plant Mini Kit** (QIAGEN).[2] DNA is extracted from 0.5–1.0 g plant tissue (leaf midrib, petiole or twig tissue) and macerated in lysis buffer using homogenizing equipment (e.g. Homex, Polytron).[2] Alternatively, plant tissue can be ground to a fine powder in liquid nitrogen before extraction. These extracts are then treated according to the manufacturer's instructions.	C	*Category : TECHNICAL* **(291) European Union (29 Sep 2023 4:31 PM)** Note that the manufacturer instructions recommend the use of 100 mg of starting material and that EPPO recommends 200 mg (see EPPO 2023c).
232	**DNeasy® Plant Mini Kit** (QIAGEN).[2] DNA is extracted from 0.5–1.0 g plant tissue (leaf midrib, petiole or twig tissue) and macerated in lysis buffer using homogenizing equipment (e.g. Homex, Polytron).[2] Alternatively, plant tissue can be ground to a fine powder in liquid nitrogen before extraction. These extracts are then treated according to the manufacturer's instructions.	C	*Category : TECHNICAL* **(134) EPPO (25 Sep 2023 8:22 AM)** Note that the manufacturer instructions recommend the use of 100 mg of starting material and that EPPO recommends 200 mg (see EPPO 2023c).
233	**DNeasy® *mericon*® Food Kit** (Standard Protocol) (QIAGEN) and **Maxwell® RSC PureFood GMO and Authentication Kit** (Promega).[2] The first of these kits was adapted to recover high-quality DNA from a wide range of plant species. An evaluation by (EPPO, 2023c) showed that both kits performed well with DNA extracts from large amount of tissue.	C	*Category : TECHNICAL* **(293) European Union (29 Sep 2023 4:34 PM)** Maxwell® HT Environmental TNA kit (Promega) proposed into the French official method MA039v6 (February 2023) allows a very good sensitivity on Olea europaea and Quercus spp.. Validation data from the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) will be soon available on the EPPO database on diagnostic expertise (http://dc.eppo.int/validationlist.php).
233	**DNeasy® *mericon*® Food Kit** (Standard Protocol) (QIAGEN) and **Maxwell® RSC PureFood GMO and Authentication Kit** (Promega).[2] The first of these kits was adapted to recover high-quality DNA from a wide range of plant ~~species. An evaluation by (EPPO, 2023c) showed that~~ species and both kits performed well with DNA extracts from large amount of ~~tissue~~tissue (EPPO, 2023c).	P	*Category : TECHNICAL* **(292) European Union (29 Sep 2023 4:33 PM)** It was rephrased as EPPO is not performing evaluations.
233	**DNeasy® *mericon*® Food Kit** (Standard Protocol) (QIAGEN) and **Maxwell® RSC PureFood GMO and Authentication Kit** (Promega).[2] The first of these kits was adapted to recover high-quality DNA from a wide range of plant ~~species. An evaluation by (EPPO, 2023c) showed that~~ species and both kits performed well with DNA extracts from large amount of ~~tissue.~~tissue (EPPO, 2023c).	P	*Category : TECHNICAL* **(136) EPPO (25 Sep 2023 8:22 AM)** It was rephrased as EPPO is not performing evaluations.
233	**DNeasy® *mericon*® Food Kit** (Standard Protocol) (QIAGEN) and **Maxwell® RSC PureFood GMO and Authentication Kit** (Promega).[2] The first of these kits was adapted to recover high-quality DNA from a wide range of plant species. An evaluation by (EPPO, 2023c) showed that both kits performed well with DNA extracts from large amount of tissue.	C	*Category : TECHNICAL* **(135) EPPO (25 Sep 2023 8:22 AM)** Maxwell® HT Environmental TNA kit (Promega) proposed into the French official method MA039v6 (February 2023) allows a very good sensitivity on Olea europaea and

			Quercus spp.. Validation data from the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) will be soon available on the EPPO database on diagnostic expertise (http://dc.eppo.int/validationlist.php).
233	**DNeasy® *mericon*® Food Kit** (Standard Protocol) (QIAGEN) and **Maxwell® RSC PureFood GMO and Authentication Kit** (Promega).[2] The first of these kits was adapted to recover high-quality DNA from a wide range of plant species. An evaluation by ~~(EPPO~~EPPO, ~~2023c)~~ (2023c) showed that both kits performed well with DNA extracts from large amount of tissue.	P	*Category : EDITORIAL* **(34) Ghana (30 Aug 2023 10:47 PM)**
234	**QuickPick™ SML Plant DNA Kit** (Bio-Nobile).[2] 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 tissue 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 parts 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™ mL (15 samples) or KingFisher™ Flex (96 samples) Purification System (Thermo Fisher Scientific).[2] Validation data are available in the EPPO database on diagnostic expertise (EPPO, 2023d). Caution is needed for users who are not familiar with this method, if performing manually, because the risk of cross-contamination between samples is high.	C	*Category : TECHNICAL* **(294) European Union (29 Sep 2023 4:35 PM)** "Bio-Nobile" to be replaced by "QRET Technologies" because the manufacturer is no longer Bio-Nobile but now QRET Technologies.
234	**QuickPick™ SML Plant DNA Kit** (Bio-Nobile).[2] 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 tissue 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–	C	*Category : TECHNICAL* **(137) EPPO (25 Sep 2023 8:22 AM)** "Bio-Nobile" to be replaced by "QRET Technologies" because the manufacturer is no longer Bio-Nobile but now QRET Technologies

	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 parts 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™ mL (15 samples) or KingFisher™ Flex (96 samples) Purification System (Thermo Fisher Scientific).[2] Validation data are available in the EPPO database on diagnostic expertise (EPPO, 2023d). Caution is needed for users who are not familiar with this method, if performing manually, because the risk of cross-contamination between samples is high.		
237	DNA may be extracted from a single insect head or a pool of up to ten heads (EPPO, 2023c). Only the heads of insects are used, because they contain the foregut and mouthparts where *X. fastidiosa* resides (Bextine *et al.*, 2004). For DNA extraction from insects with ~~big~~ larger heads (e.g. *Cicadella viridis*, *Cicada orni*), only a single head should be used. The removal of the eye tissue, a potential source of PCR inhibitors, is recommended as it has been reported that this increases sensitivity (Bextine *et al.*, 2004; Purcell *et al*., 2014). Insect tissue can be ground in lysis buffer, or homogenized using a bead-beater system such as MagNA Lyser (Roche) or by vacuum application and release (Bextine *et al.*, 2004, 2005; Huang Bentz and Sherald, 2006).[2] A number of DNA extraction methods have been evaluated for the detection of *X. fastidiosa* in insect vectors. The following methods are a selection of those widely used in laboratories.	P	*Category : EDITORIAL* **(138) EPPO (25 Sep 2023 8:22 AM)** It should be 'a pool of up to ten heads' according to EPPO,2023
240	**QuickPick™ SML Plant DNA Kit** (Bio-Nobile).[2] The homogenization of individual insect heads or groups of up to ten heads can be performed in 200 µL sterile distilled water using a bead-beater system such as the Mixer Mill MM400 (Retsch).[2] Samples are homogenized for 2 min at 30 Hertz using ten stainless steel beads (diameter 3 mm) per 2 mL microcentrifuge tube. The microcentrifuge tube is placed on a magnet and the supernatant is transferred to a new microcentrifuge tube. The extract is centrifuged for 20 min at 20 000 *g*. The pellet is then suspended in 37.5 µL lysis buffer with 2.5 µL proteinase K, and the manufacturer's instructions followed. This kit can be used either manually or with the KingFisher™ mL (15 samples) or KingFisher™ Flex (96 samples) Purification System (Thermo Scientific) (Cunty *et al*., 2020).[2]	C	*Category : TECHNICAL* **(295) European Union (29 Sep 2023 4:36 PM)** "Bio-Nobile" to be replaced by "QRET Technologies" because the manufacturer is no longer Bio-Nobile but now QRET Technologies.

240	**QuickPick™ SML Plant DNA Kit** (Bio-Nobile).[2] The homogenization of individual insect heads or groups of up to ten heads can be performed in 200 µL sterile distilled water using a bead-beater system such as the Mixer Mill MM400 (Retsch).[2] Samples are homogenized for 2 min at 30 Hertz using ten stainless steel beads (diameter 3 mm) per 2 mL microcentrifuge tube. The microcentrifuge tube is placed on a magnet and the supernatant is transferred to a new microcentrifuge tube. The extract is centrifuged for 20 min at 20 000 *g*. The pellet is then suspended in 37.5 µL lysis buffer with 2.5 µL proteinase K, and the manufacturer's instructions followed. This kit can be used either manually or with the KingFisher™ mL (15 samples) or KingFisher™ Flex (96 samples) Purification System (Thermo Scientific) (Cunty *et al*., 2020).[2]	C	*Category : TECHNICAL* **(139) EPPO (25 Sep 2023 8:22 AM)** "Bio-Nobile" to be replaced by "QRET Technologies" because the manufacturer is no longer Bio-Nobile but now QRET Technologies
242	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 laboratories for the detection of *X. fastidiosa* in different host plants and vectors. Analytical specificity was validated later by Harper, Ward and Clover (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 *Quercus rubra* and *Quercus laevis* and several strains from grapevines were not detected with this PCR. The analytical sensitivity of the method as stated by Minsavage *et al.* (1994) is 1×10^2 c.f.u. /mL on *V. vinifera* and *P. persica*. Further validation data on other hosts are available in the EPPO database on diagnostic expertise (EPPO, 2023d).	C	*Category : TECHNICAL* **(296) European Union (29 Sep 2023 4:37 PM)** It should be noted that the test has been adapted and validated in laboratories (EPPO 2023c) and may not be used anymore as described in Table 4.
242	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 laboratories for the detection of *X. fastidiosa* in different host plants and vectors. Analytical specificity was validated later by Harper, Ward and Clover (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 *Quercus rubra* and *Quercus laevis* and several strains from grapevines were not detected with this PCR. The analytical sensitivity of the method as stated by Minsavage *et al.* (1994) is 1×10^2 c.f.u. /mL on *V. vinifera* and *P. persica*. Further validation data on other hosts are available in the EPPO database on diagnostic expertise (EPPO, 2023d).	C	*Category : SUBSTANTIVE* **(250) China (28 Sep 2023 8:18 AM)** Suggestion to consolidate all traditional PCR-Related content into one section, including subheadings. The paragraph divisions in this section are unclear and should be consolidated into one comprehensive section, contrasting with the subsequent section on RT-PCR.
242	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 laboratories for	C	*Category : TECHNICAL* **(140) EPPO (25 Sep 2023 8:22 AM)** It should be noted that the test has been

	the detection of *X. fastidiosa* in different host plants and vectors. Analytical specificity was validated later by Harper, Ward and Clover (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 *Quercus rubra* and *Quercus laevis* and several strains from grapevines were not detected with this PCR. The analytical sensitivity of the method as stated by Minsavage *et al.* (1994) is 1×10^2 c.f.u. /mL on *V. vinifera* and *P. persica*. Further validation data on other hosts are available in the EPPO database on diagnostic expertise (EPPO, 2023d).		adapted and validated in laboratories (EPPO 2023c) and may not be used anymore as described in Table 4.
285	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(141) EPPO (25 Sep 2023 8:22 AM)** No page footnote 2.
288	~~**3.4.4 Conventional polymerase chain reaction (PCR) using the primers of Pooler and Hartung (1995)**~~	P	*Category : SUBSTANTIVE* **(3) Chile (14 Aug 2023 4:17 PM)** serología y PCR convencionales no aceptados por la CE pese a estar en el IT EPPO.
289	This PCR was designed by Pooler and Hartung (1995) with primers that target a specific, randomly amplified, polymorphic DNA (RAPD) fragment present in *X. fastidiosa*. The primers 272-1-int and 272-2-int are known to detect all known strains of *X. fastidiosa*. Analytical specificity has been validated with 57 different *X. fastidiosa* strains collected from different regions of Brazil and the United States of America (Huang, 2009; Reisenzein, 2017).	P	*Category : SUBSTANTIVE* **(35) Ghana (30 Aug 2023 10:48 PM)**
332	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(142) EPPO (25 Sep 2023 8:22 AM)**
335	~~**3.4.5 Conventional PCR using the primers of Rodrigues *et al.* (2003)**~~	P	*Category : SUBSTANTIVE* **(4) Chile (14 Aug 2023 4:18 PM)** serología y PCR convencionales no aceptados por la CE pese a estar en el IT EPPO.
336	This PCR is based on primers for the 16S ribosomal (r)RNA and *gyrB* genes and 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 (16S rRNA and *gyrB* primers combined) were evaluated using 30 *X. fastidiosa* strains from different plant hosts and 36 closely related or ~~host related~~ host-related non-target bacterial strains. The specific sets of primers for the 16S rRNA or *gyrB* genes can be used in either simplex or multiplex PCR. The analytical sensitivity for the multiplex PCR is similar to the simplex reactions,	P	*Category : EDITORIAL* **(57) United States of America (7 Sep 2023 5:18 PM)**

	which is approximately 10^2 c.f.u. /mL.		
339	S-S-X.fas-0067-a-S-19 (forward): 5′-CGG CAG CAC ATT GGT AGT A-3′	C	*Category : TECHNICAL* **(61) United States of America (15 Sep 2023 2:39 PM)** This primer sequence contains the SNPs as exemplified and highlighted in the below for several Xf isolates/strains, the effects of one SNP-G or -T in the middle of the primer sequence may be minor, but the SNPs at the 3'-end as for Xf taiwanensis Pear Leaf Scorch strain may lead to failure in PCR amplification of the target from Xf taiwanensis. CGGCAGCACGTTGGTAGTA for Xf CVC 9a5c found in Brazil and Xf pauca found in Italy, etc.; CGGCAGCATATTGGTAGTA for Xf from Coffee; CGGCAGCACAGTGGTAGCG for Xf taiwanensis Pear Leaf Scorch strain.
391	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(143) EPPO (25 Sep 2023 8:22 AM)**
437	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(144) EPPO (25 Sep 2023 8:22 AM)**
441	**3.4.6 Real-time PCR using the primers and probes of Harper, Ward and Clover (2010)**	C	*Category : SUBSTANTIVE* **(251) China (28 Sep 2023 8:18 AM)** Suggestion to consolidate all RT-PCR related content into One comprehensive section, including subheadings. As mentioned before, it is recommended to merge this section into one comprehensive unit and contrast it with the previous section on traditional PCR.
442	This PCR, developed by Harper, Ward and Clover (2010), is designed to amplify part of the 16S rRNA processing protein *rimM* gene. DNA can be amplified from bacterial cultures, infected leaves, cane tissue or insect vectors. This PCR is widely used in European laboratories for the detection of *X. fastidiosa*.	C	*Category : TECHNICAL* **(298) European Union (29 Sep 2023 4:38 PM)** It is a slightly adapted version of the test described in Table 8 that is used in EU countries (see EPPO 2023c).
442	This PCR, developed by Harper, Ward and Clover (2010), is designed to amplify part of the 16S rRNA processing protein *rimM* gene. DNA can be amplified from bacterial cultures, infected leaves, cane tissue or insect vectors. This PCR is widely used in European laboratories for the detection of *X. fastidiosa*.	C	*Category : TECHNICAL* **(145) EPPO (25 Sep 2023 8:22 AM)** It is a slightely adapted version of the test described in Table 8 that is used in EU countries (see EPPO 2023c).

443	Harper, Ward and Clover (2010) evaluated analytical specificity with 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. Only *X. fastidiosa* was detected. *X. taiwanensis* from the Taiwan Province of China was not detected. The PCR was further validated by Li *et al.* (2013). The diagnostic specificity and sensitivity, as determined using citrus and grape hosts, are both 100% (EPPO, 2023c). For *O. europaea* hosts when using CTAB extraction methods, the diagnostic specificity is 100% and the diagnostic sensitivity is 91% (EPPO, 2023c). Further validation data are available in the EPPO database on diagnostic expertise (EPPO, 2023d). The analytical sensitivity ~~(detection limit) is between 10~~varies depending on the host plant and the DNA extraction protocol (see EPPO 2023c)~~[3] c.f.u./mL for . *Citrus* spp. and *V. vinifera* and 10[4] c.f.u./mL for *O. europaea* (EPPO, 2023b).~~	P	*Category : TECHNICAL* **(299) European Union (29 Sep 2023 4:42 PM)** 2023c instead of 2023b (typo) It is suggested to replace with the following sentence: 'The analytical sensitivities varies depending on the host plant and the DNA extraction protocol (see EPPO 2023c).' because the analytical sensitivity depends on the extraction procedures which are not reported here. A general sentence seems more appropriate. Alternatively, the extraction methods should be reported and it should be noted that in EPPO 2023c the analytical sensitivity for Vitis vinifera is 10^3 cells/mL and the reference should be EPPO, 2023c.
443	Harper, Ward and Clover (2010) evaluated analytical specificity with 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. Only *X. fastidiosa* was detected. *X. taiwanensis* from the Taiwan Province of China was not detected. The PCR was further validated by Li *et al.* (2013). The diagnostic specificity and sensitivity, as determined using citrus and grape hosts, are both 100% (EPPO, 2023c). For *O. europaea* hosts when using CTAB extraction methods, the diagnostic specificity is 100% and the diagnostic sensitivity is 91% (EPPO, 2023c). Further validation data are available in the EPPO database on diagnostic expertise (EPPO, 2023d). The analytical ~~sensitivity (detection limit) is between 10~~sensitivities varies depending on the host plant and the DNA extraction protocol (see EPPO 2023c).~~[3] c.f.u./mL for *Citrus* spp. and *V. vinifera* and 10[4] c.f.u./mL for *O. europaea* (EPPO, 2023b).~~	P	*Category : TECHNICAL* **(146) EPPO (25 Sep 2023 8:22 AM)** 2023c instead of 2023b (typo) It is suggested to replace with the following sentence: 'The analytical sensitivities varies depending on the host plant and the DNA extraction protocol (see EPPO 2023c).' because the analytical sensitivity depends on the extraction procedures which are not reported here. A general sentence seems more appropriate. Alternatively, the extraction methods should be reported and it should be noted that in EPPO 2023c the analytical sensitivity for Vitis vinifera is 10^3 cells/mL and the reference should be EPPO, 2023c.
449	**Table 8.** Master mix composition and cycling parameters for real-time PCR using the primers and probes of Harper, Ward and Clover (2010)	C	*Category : EDITORIAL* **(147) EPPO (25 Sep 2023 8:22 AM)** Table 8 should be presented as Table 7 for Cycling parameters, without lines separating the steps that have to be repeated, such as denaturation and Annealing and elongation.
456	$MgCl_2$ ~~(to a final concentration of)~~	P	*Category : EDITORIAL* **(148) EPPO (25 Sep 2023 8:22 AM)** This is not said for Tables 4 to 7 and Table

			9. To be deleted.
476	Heating ramp speed	C	*Category : TECHNICAL* **(300) European Union (29 Sep 2023 4:42 PM)** Not found in the initial publication.
476	Heating ramp speed	C	*Category : TECHNICAL* **(149) EPPO (25 Sep 2023 8:22 AM)** Not found in the initial publication.
483	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(150) EPPO (25 Sep 2023 8:22 AM)**
486	~~**3.4.7 Real-time PCR using the primers and probes of Li *et al.* (2013)**~~	P	*Category : SUBSTANTIVE* **(5) Chile (14 Aug 2023 4:18 PM)** serología y PCR convencionales no aceptados por la CE pese a estar en el IT EPPO.
490	XF16Sf (forward primer): 5′-CGG CAG CAC GTT GGT AGT AA-3′	C	*Category : TECHNICAL* **(62) United States of America (15 Sep 2023 2:40 PM)** This primer sequence was studied with Xf from citrus. For other Xf isolates, it may contain the SNPs as highlighted in the below for several examples, the effects of one SNP-A in the middle of the primer sequence may be minor, but the SNPs at the 3'-end as for Xf taiwanensis Pear Leaf Scorch strain may lead to failure in real-time PCR amplification of the target from Xf taiwanensis. CGGCAGCACATTGGTAGTAA for Xf from grapevine, morus, oak, etc.; CGGCAGCATATTGGTAGTAA for Xf from Coffee; CGGCAGCACAGTGGTAGCGA for Xf taiwanensis Pear Leaf Scorch strain.
491	XF16Sr (reverse primer): 5′-CCG ATG TAT TCC TCA CCC GT-3′	C	*Category : TECHNICAL* **(227) Australia (27 Sep 2023 8:30 AM)** Request that this primer sequence is checked/confirmed.
491	XF16Sr (reverse primer): 5′-CCG ATG TAT TCC TCA CCC ~~GT-3~~GTC-3′	P	*Category : TECHNICAL* **(75) Japan (20 Sep 2023 7:58 AM)** Primer sequence should be consistent with the reference (Li et al. 2013).
494	**Table 9.** Master mix composition and cycling parameters for real-time PCR using the primers and probes of Li *et al.* (2013)	C	*Category : EDITORIAL* **(151) EPPO (25 Sep 2023 8:22 AM)** Table 9 should be presented as Table 7 for Cycling parameters, without lines separating

			the steps that have to be repeated, such as denaturation and Annealing and elongation.
528	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(152) EPPO (25 Sep 2023 8:22 AM)**
531	~~**Real-time PCR using the primers and probes of Dupas *et al.* (2019b)**~~	P	*Category : SUBSTANTIVE* **(6) Chile (14 Aug 2023 4:18 PM)** serología y PCR convencionales no aceptados por la CE pese a estar en el IT EPPO.
539	Dupas *et al.* (2019b) evaluated analytical specificity with 39 strains of *X. fastidiosa* from different subspecies and 30 non-target bacterial strains. Only *X. fastidiosa* was detected. The diagnostic specificity and sensitivity were 100% and 92%, respectively, on pure DNA extract and 100% on 10-fold diluted DNA. The analytical sensitivity (detection limit) was 10^5 cells/mL.	C	*Category : TECHNICAL* **(301) European Union (29 Sep 2023 4:43 PM)** The text needs to be clarified as it is unclear if the analytical sensitivity reported was evaluated on strains or plant material. Also the analytical sensitivity varies depending on the primers and the host matrix.
539	Dupas *et al.* (2019b) evaluated analytical specificity with 39 strains of *X. fastidiosa* from different subspecies and 30 non-target bacterial strains. Only *X. fastidiosa* was detected. The diagnostic specificity and sensitivity were 100% and 92%, respectively, on pure DNA extract and 100% on 10-fold diluted DNA. The analytical sensitivity (detection limit) was 10^5 cells/mL.	C	*Category : TECHNICAL* **(153) EPPO (25 Sep 2023 8:22 AM)** The text needs to be clarified as it is unclear if the analytical sensitivity reported was evaluated on strains or plant material. Also the analytical sensitivity varies depending on the primers and the host matrix.
543	Set 3: ~~XF–XFF–XFM–XMO~~XF–XFF–XFM–XFMO	P	*Category : TECHNICAL* **(76) Japan (20 Sep 2023 8:00 AM)**
552	**Sequence ~~(5′-3′)~~(5′→3′)**	P	*Category : EDITORIAL* **(36) Ghana (30 Aug 2023 10:52 PM)** Forward arrow to be inserted between 5 prime and 3 prime
600	~~HEX-CGCGTACCCACTCACGCCGC-BHQ1~~HEX-CGC GTA CCC ACT CAC GCC GC-BHQ1	P	*Category : TECHNICAL* **(77) Japan (20 Sep 2023 8:02 AM)**
648	~~FAM-ACGGAAGGGCACCACCAGGAGT-BHQ1~~FAM-ACG GAA GGG CAC CAC CAG GAG T-BHQ1	P	*Category : TECHNICAL* **(78) Japan (20 Sep 2023 8:05 AM)**
650	*Sources:* Dupas, E., Briand, M., Jacques, M.-A. & Cesbron, S. ~~2019~~2019b. Novel tetraplex quantitative PCR assays for simultaneous detection and identification of *Xylella fastidiosa* subspecies in plant tissues. *Frontiers in Plant Science*, 10: 1732.	P	*Category : EDITORIAL* **(154) EPPO (25 Sep 2023 8:22 AM)** For consistency with the title and last column of Table 10 and with Section 9 (References).
652	**Table 11.** Master mix composition and cycling parameters for tetraplex real-time PCR using the primers and probes of Dupas *et al.* ~~(2019)~~(2019b)	P	*Category : EDITORIAL* **(155) EPPO (25 Sep 2023 8:22 AM)**

			For consistency with paragraph [547] (i.e. the paragraph introducing Tables 10 and 11) and with Section 9 (References).
680	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(156) EPPO (25 Sep 2023 8:22 AM)**
682	*Source:* Dupas, E., Briand, M., Jacques, M.-A. & Cesbron, S. ~~2019~~2019b. Novel tetraplex quantitative PCR assays for simultaneous detection and identification of *Xylella fastidiosa* subspecies in plant tissues. *Frontiers in Plant Science*, 10: 1732.	P	*Category : EDITORIAL* **(157) EPPO (25 Sep 2023 8:22 AM)** For consistency with paragraph [547] (i.e. the paragraph introducing Tables 10 and 11) and with Section 9 (References).
684	This simplex real-time PCR is suitable for the detection and the identification of *X. fastidiosa* and assignment of subspecies on plant samples and cell cultures. Hodgetts *et al.* (2021) evaluated analytical specificity with eight strains of *X. fastidiosa* from different subspecies (*X. fastidiosa* subsp. *fastidiosa*, *X. fastidiosa* subsp. *morus*, *X. fastidiosa* subsp. *multiplex*, *X. fastidiosa* subsp. *pauca*, *X. fastidiosa* subsp. *sandyi*) and 50 non-target bacterial strains. Only *X. fastidiosa* was detected. The diagnostic specificity and sensitivity were both 100%. The analytical sensitivity (detection limit) was 124 fg DNA of *X. fastidiosa* subsp. *fastidiosa*, 59.2 fg DNA of *X. fastidiosa* subsp. *morus*, 182 fg DNA of *X. fastidiosa* subsp. *multiplex*, 84.2 fg DNA of *X. fastidiosa* subsp. *pauca*, and 908 fg DNA of *X. fastidiosa* subsp. *sandyi*. The sequences for the Hodgetts *et al.* (2021) primers and probes are presented in Table 12 and the corresponding master mix is described in Table 13.	P	*Category : EDITORIAL* **(158) EPPO (25 Sep 2023 8:22 AM)** For consistency with Section 3.4.8 (please see paragraph [547]).
697	~~FAM-ACC TGA GAA~~ FAM-TCG AAA ACA CCG GAC TTG ~~CCC TTA ATC G-BHQ1~~CCA ACA-BHQ1	P	*Category : TECHNICAL* **(79) Japan (20 Sep 2023 8:10 AM)** Probe sequence should be consistent with the reference (Hodgetts et al. 2021).
697	FAM-ACC TGA GAA TTG CCC TTA ATC G-BHQ1	C	*Category : TECHNICAL* **(233) Australia (27 Sep 2023 8:36 AM)** Technical: Request that this primer sequence is checked/confirmed.
712	CAA TCG CTT TTG AGG TCA TCC	C	*Category : TECHNICAL* **(234) Australia (27 Sep 2023 8:36 AM)** Technical: Request that this primer sequence is checked/confirmed.
712	~~CAA TCG CTT TTG AGG TCA TCC~~GCG ATT GTT TCT TCT CTA CAC CAA G	P	*Category : TECHNICAL* **(82) Japan (20 Sep 2023 8:18 AM)** Primer sequence should be consistent with

			the reference (Hodgetts et al. 2021).
721	GCA TCC TCA CCA CCG AAG G	C	*Category : TECHNICAL* **(235) Australia (27 Sep 2023 8:36 AM)** Technical: Request that this primer sequence is checked/confirmed.
721	~~GCA TCC TCA CCA CCG AAG G~~TCC ACA TCC AGC AAG GTG AC	P	*Category : TECHNICAL* **(83) Japan (20 Sep 2023 8:26 AM)** Primer sequence should be consistent with the reference (Hodgetts et al. 2021).
724	~~FAM-CCTTGGACGCGGATACCCGCA-BHQ1~~FAM-CCT TGG ACG CGG ATA CCC GCA-BHQ1	P	*Category : TECHNICAL* **(84) Japan (20 Sep 2023 8:27 AM)**
729	~~Xfs_4_Fwb_112076~~Xfs_4_Rv_112076	P	*Category : TECHNICAL* **(85) Japan (20 Sep 2023 8:29 AM)** Primer name should be consistent with the reference (Hodgetts et al. 2021).
732	~~Xfs_4_Fwb_112076~~Xfs_4_Pr_112076	P	*Category : TECHNICAL* **(86) Japan (20 Sep 2023 8:30 AM)** Probe name should be consistent with the reference (Hodgetts et al. 2021).
732	Xfs_4_Fwb_112076	C	*Category : TECHNICAL* **(236) Australia (27 Sep 2023 8:37 AM)** Technical: Request that this primer sequence is checked/confirmed.
763	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(159) EPPO (25 Sep 2023 8:22 AM)**
767	***3.4.10.1 LAMP of Harper, Ward and Clover (2010)***[3]	P	*Category : EDITORIAL* **(160) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
768	This LAMP method was developed by Harper, Ward and Clover (2010) and can be used on crude plant tissue and insect extracts or with the DNA extraction methods described in ~~section~~ sections 3.4.1. and 3.4.2[3] Hydroxynaphthol blue can be used as a means of detecting the end-point (Harper, Ward and Clover, 2010). Hydroxynaphthol blue or other dyes that can be added before amplification are recommended as they allow the LAMP to be performed as a closed-tube system.[3] This avoids the risk of opening tubes post amplification, which could lead to aerosol contamination because of the high titre of the LAMP amplicon.[3] The LAMP can also be performed in a real-time PCR thermocycler.[3]	P	*Category : EDITORIAL* **(161) EPPO (25 Sep 2023 8:22 AM)** Missing? (Section 3.4.2 is about DNA extraction from insect vectors) It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.

769	The LAMP, which targets the rimM gene, can detect 250 copies of the gene. In validation, only *X. fastidiosa* was detected among 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains.[3] All strains of *X. fastidiosa* were detected.	P	*Category : EDITORIAL* **(162) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
777	The master mix for the Harper, Ward and Clover (2010) LAMP is described in Table 14.[3]	P	*Category : EDITORIAL* **(163) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
778	**Table 14.** Master mix composition and test conditions for LAMP, according to Harper, Ward and Clover (2010)[3]	P	*Category : EDITORIAL* **(164) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
816	‡ See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(165) EPPO (25 Sep 2023 8:22 AM)**
821	**Figure 1.** Successful *rimM* loop-mediated isothermal amplification, visualized using hydroxynapthal blue dye. Samples that are positive for *Xylella fastidiosa* change to a blue colour (tubes 1 to 4); negative samples in which no amplification occurs remain violet (tubes 5 to 8).	C	*Category : EDITORIAL* **(166) EPPO (25 Sep 2023 8:22 AM)** Should not be highlighted in yellow.
823	***3.4.10.2 LAMP of Harper, Ward and Clover (2010) modified by Yaseen* et al. *(2015)***[3]	P	*Category : EDITORIAL* **(167) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
824	This method is based on the above LAMP primers developed by Harper, Ward and Clover (2010), and was modified by Yaseen *et al*. (2015).[3] The modifications consist of a simplified extraction method and reduced incubation times. Ready-to-use kits for the method are commercially available and they are performed in real time on a specific device or by using a standard real-time thermocycler (e.g. Enbiotech, Qualiplante, OptiGene).[2] The kits should be used as per the manufacturer's instructions. Diagnostic sensitivity and specificity using the Enbiotech and Qualiplante kits have been determined as being between 83% and 92%, respectively.[2] The analytical sensitivity (detection limit) of these kits is between 10^2 and 10^3 c.f.u./mL for *Citrus* spp., *V. vinifera* and *O. europaea*. Validation data are available in the EPPO database on diagnostic expertise (EPPO,	P	*Category : EDITORIAL* **(168) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.

	2023d).		
828	For LAMP, a positive nucleic acid (*X. fastidiosa*) control and a negative amplification control (no template control) are the minimum controls that should be used.[3]	P	*Category : EDITORIAL* **(169) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
829	Additional controls may be used for both LAMP and PCR as described below.[3]	P	*Category : EDITORIAL* **(170) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
834	For PCR, care needs to be taken to avoid cross-contamination resulting from aerosols from the positive control or from positive samples. If required, the positive control used in the laboratory ~~should~~ can be sequenced so that this sequence can be readily compared with sequences obtained from PCR amplicons of the correct size. Alternatively, synthetic positive controls can be made with a known sequence that, again, can be compared with PCR amplicons of the correct size.	P	*Category : EDITORIAL* **(237) Australia (27 Sep 2023 8:37 AM)** Removal of 'should' if the action is only if required and not mandatory.
840	the positive control produces the correct size amplicon for the bacterium; and	C	*Category : SUBSTANTIVE* **(252) China (28 Sep 2023 8:20 AM)** Suggested List of ladder products and their respective companies used for determining PCR amplification fragment sizes. Recommended ladder products and their respective companies used for determining PCR amplification fragment sizes
841	no ~~amplicons~~ amplicon of the correct size for the bacterium ~~are~~ is produced in the negative extraction control and the negative amplification control.	P	*Category : EDITORIAL* **(171) EPPO (25 Sep 2023 8:22 AM)** Make "amplicon" singular.
845	A pathogen-specific real-time PCR will be considered valid only if both these criteria are met:	C	*Category : SUBSTANTIVE* **(253) China (28 Sep 2023 8:21 AM)** Suggested Explanation of RT-PCR Curves. The provided RT-PCR curve results have not been specified. Please list them for practitioners' reference.
845	A pathogen-specific real-time PCR will be considered valid only if both of these criteria are met:	P	*Category : EDITORIAL* **(37) Ghana (30 Aug 2023 10:54 PM)**
850	***3.4.12.3 LAMP***[3]	P	*Category : EDITORIAL* **(172) EPPO (25 Sep 2023 8:22 AM)**

			It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
851	A LAMP will be considered valid only if both these criteria are met:[3]	P	*Category : EDITORIAL* **(173) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
851	A LAMP will be considered valid only if both of these criteria are met:[3]	P	*Category : EDITORIAL* **(38) Ghana (30 Aug 2023 10:54 PM)**
852	the positive nucleic acid control produces a specific reaction (the type of reaction varies with the technology used in the LAMP method (e.g. fluorescence, coloration, amplification curve); the specific reaction is described in the instructions of the kit providers or in the specific section of the protocol describing the LAMP method);[3] and	P	*Category : EDITORIAL* **(174) EPPO (25 Sep 2023 8:22 AM)** It seems sufficient to include this footnote only in the title of section 3.4.10 on the LAMP method and not to repeat it every time the LAMP method is mentioned.
857	Further tests may be done in instances where the national plant protection organization (NPPO) requires additional confidence in the identification of the *X. fastidiosa* subspecies or ~~strain~~ sequence type. Sequencing of the complete genome (Simpson *et al.*, 2000; Van Sluys *et al.*, 2003),or multilocus sequencing typing (MLST) (Scally *et al.*, 2005; Yuan *et al.*, 2010), is recommended for subspecies or sequence type identification or when atypical or undescribed strains are suspected (section 4.5.1).	P	*Category : TECHNICAL* **(302) European Union (29 Sep 2023 4:47 PM)** Refer to 'sequence type' + typo: missing space
857	Further tests may be done in instances where the national plant protection organization (NPPO) requires additional confidence in the identification of the *X. fastidiosa* subspecies or ~~strain~~ sequence type. Sequencing of the complete genome (Simpson *et al.*, 2000; Van Sluys *et al.*, 2003)~~,~~, or multilocus sequencing typing (MLST) (Scally *et al.*, 2005; Yuan *et al.*, 2010), is recommended for subspecies or sequence type identification or when atypical or undescribed strains are suspected (section 4.5.1).	P	*Category : TECHNICAL* **(175) EPPO (25 Sep 2023 8:22 AM)** Refer to 'sequence type' + typo: missing space
857	Further tests may be done in instances where the ~~national plant protection organization~~ National Plant Protection Organization (NPPO) requires additional confidence in the identification of the *X. fastidiosa* subspecies or strain type. Sequencing of the complete genome (Simpson *et al.*, 2000; Van Sluys *et al.*, 2003),or multilocus sequencing typing (MLST) (Scally *et al.*, 2005; Yuan *et al.*, 2010), is recommended for subspecies identification or when atypical or	P	*Category : EDITORIAL* **(40) Ghana (30 Aug 2023 10:56 PM)**

	undescribed strains are suspected (section 4.5.1).		
860	Midrib, petiole, ~~or~~ twig or stem tissue from symptomatic samples are considered the best sources for reliable isolation of *X. fastidiosa*. However, other sources of infected plant tissue from which the bacterium can be isolated include root sections (Hopkins, 2001). Also, it is technically possible to isolate *X. fastidiosa* from insect vectors (Hill and Purcell, 1995), but very few data are available on the performance of this method.	P	*Category : EDITORIAL* **(41) Ghana (30 Aug 2023 10:56 PM)**
861	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 microorganisms. Midrib, petiole, or twig or stem samples are surface sterilized by immersion in 70% ethanol for 1 min, then a one centimeter long piece of tissue is cut and transferred quickly into 96% ethanol, flamed rapidly, and placed in sterile demineralized water~~, and~~. The one centimeter long piece of tissue is then cut into very small pieces and subjected to gentle agitation for 15 min before plating. Alternatively, samples can be placed in 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); or tissue is cut in small pieces in PBS at ratios of 1:10 and 1:100, or ground with a mortar and pestle or a homogenizer (e.g. Homex), and then plated onto two different types of specific media (e.g. PD2, BCYE, PWG).	P	*Category : EDITORIAL* **(176) EPPO (25 Sep 2023 8:22 AM)** New paragraph. Lack of precision. Informations have been added
861	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 microorganisms. Midrib, petiole, ~~or~~ twig or stem samples are surface sterilized by immersion in 70% ethanol for 1 min, then transferred quickly into 96% ethanol, flamed rapidly, placed in sterile demineralized water, and then subjected to gentle agitation for 15 min before plating. Alternatively, samples can be placed in 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); or tissue is cut in small pieces in PBS at ratios of 1:10 and 1:100, or ground with a mortar and pestle or a homogenizer (e.g. Homex), and then plated onto two different types of specific media (e.g. PD2, BCYE, PWG).	P	*Category : EDITORIAL* **(42) Ghana (30 Aug 2023 10:57 PM)**
864	The plates should be incubated at 28 °C for 8–30 days, in plastic bags or sealed	C	*Category : TECHNICAL*

	with Parafilm® to prevent desiccation.[2] Plates are observed regularly for colony development using a binocular microscope. Colonies visible to the unaided eye within the first two days should be regarded as contaminants and can be carefully excised from the plate under aseptic conditions.		**(303) European Union (29 Sep 2023 4:47 PM)** if necessary
864	The plates should be incubated at 28 °C for 8–30 days, in plastic bags or sealed with Parafilm® or equivalent to prevent desiccation.[2] Plates are observed regularly for colony development using a binocular microscope. Colonies visible to the unaided eye within the first two days should be regarded as contaminants and can be carefully excised from the plate under aseptic conditions.	P	*Category : TECHNICAL* **(238) Australia (27 Sep 2023 8:38 AM)** For those NPPOs that do not have access to the specific branded Paraflim®
864	The plates should be incubated at 28 °C for 8–30 days, in plastic bags or sealed with Parafilm® to prevent desiccation.[2] Plates are observed regularly for colony development using a binocular microscope. Colonies visible to the unaided eye within the first two days should be regarded as contaminants and can be carefully excised from the plate under aseptic conditions.	C	*Category : TECHNICAL* **(177) EPPO (25 Sep 2023 8:22 AM)** if necessary
871	Phytone peptone ~~(BD~~ (e.g. BD BBL)†	P	*Category : EDITORIAL* **(178) EPPO (25 Sep 2023 8:22 AM)** To harmonize
873	Bacto tryptone ~~(Oxoid)~~(e.g. Oxoid)†	P	*Category : EDITORIAL* **(179) EPPO (25 Sep 2023 8:22 AM)** To harmonize
881	Bovine serum albumin (20% w/v) ~~(Sigma)~~(e.g. Sigma)†	P	*Category : EDITORIAL* **(180) EPPO (25 Sep 2023 8:22 AM)** To harmonize
893	*Notes:* † See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(181) EPPO (25 Sep 2023 8:22 AM)**
899	ACES buffer ~~(Sigma)~~(e.g. Sigma)†	P	*Category : EDITORIAL* **(182) EPPO (25 Sep 2023 8:22 AM)** To harmonize
903	Activated charcoal ~~(Norit)~~(e. g. Norit)†	P	*Category : EDITORIAL* **(183) EPPO (25 Sep 2023 8:22 AM)** To harmonize
905	L-cysteine hydrochloride-1-hydrate ~~(Sigma)~~(e.g. Sigma)†	P	*Category : EDITORIAL* **(184) EPPO (25 Sep 2023 8:22 AM)** To harmonize
907	Ferric pyrophosphate ~~(Sigma)~~(e.g. Sigma)†	P	*Category : EDITORIAL* **(185) EPPO (25 Sep 2023 8:22 AM)** To harmonize
913	*Notes:* † See ~~page~~ footnote 2. ACES, 2-[(2-amino-2-oxoethyl)amino]ethanesulfonic acid	P	*Category : EDITORIAL* **(186) EPPO (25 Sep 2023 8:22 AM)** To be moved from paragraph [915]

			(because not a source).
915	~~ACES, 2-[(2-amino-2-oxoethyl)amino]ethanesulfonic acid~~	P	*Category : EDITORIAL* **(187) EPPO (25 Sep 2023 8:22 AM)** To be moved after paragraph [913] (because not a source).
921	Gelrite gellan gum ~~(Sigma)~~(e.g. Sigma)†	P	*Category : EDITORIAL* **(188) EPPO (25 Sep 2023 8:22 AM)** To harmonize
929	L-glutamine ~~(Sigma)~~(e.g. Sigma)†	P	*Category : EDITORIAL* **(189) EPPO (25 Sep 2023 8:22 AM)** To harmonize
933	Bovine serum albumin ~~(Sigma)~~(e.g. Sigma)†	P	*Category : EDITORIAL* **(190) EPPO (25 Sep 2023 8:22 AM)** To harmonize
943	*Notes:* † See ~~page~~ footnote 2.	P	*Category : EDITORIAL* **(191) EPPO (25 Sep 2023 8:22 AM)**
949	**Table 18.** Reference *Xylella fastidiosa* strains	C	*Category : TECHNICAL* **(304) European Union (29 Sep 2023 4:49 PM)** Precise the subspecies and sequence type for each strain number CFBP 7969, 7970, = Xff ST2 LMG 17159 = Xff ST2 ICMP 11140, 15197 = Xff ST? NCPPB 4432 = Xff ST2 DSM 10026 = Xff ST2 CFBP 8073 = Xff ST75 CFBP 8173 = Xfm ST41
949	**Table 18.** Reference *Xylella fastidiosa* strains	P	*Category : TECHNICAL* **(192) EPPO (25 Sep 2023 8:22 AM)** Precise the subspecies and sequence type for each strain number CFBP 7969, 7970, = Xff ST2 LMG 17159 = Xff ST2 ICMP 11140, 15197 = Xff ST? NCPPB 4432 = Xff ST2 DSM 10026 = Xff ST2 CFBP 8073 = Xff ST75 CFBP 8173 = Xfm ST41
963	**4.1.3 Interpretation of isolation results**	C	*Category : SUBSTANTIVE* **(254) China (28 Sep 2023 8:22 AM)** It is recommended to add images of the colonies.

			It is recommended to add images of the colonies for reference by practitioners.
964	The isolation is negative if no bacterial colonies with growth characteristics and morphology similar to *X. fastidiosa* are observed after 14–30 days on any medium and typical *X. fastidiosa* colonies are found in the positive controls.	C	*Category : TECHNICAL* **(306) European Union (29 Sep 2023 4:51 PM)** In EPPO (2023), it is indicated to incubate plates for up to 6 weeks. Could the '14-30 days' be changed for '14-45' days'? If yes, should be changed also for positive results (next paragraph) and in section 4.1.
964	The isolation is negative if no bacterial colonies with growth characteristics and morphology similar to *X. fastidiosa* are observed after 14–30 days on any medium and typical *X. fastidiosa* colonies are found in the positive ~~controls~~controls if included.	P	*Category : TECHNICAL* **(305) European Union (29 Sep 2023 4:50 PM)** A positive control for isolation is not always used in laboratories. It is not always clear which strain(s) should be used as positive control. Suggestion: addition of 'if included'. A warning could be added that the media should be validated before use. Other tests should be used in addition to isolation.
964	The isolation is negative if no bacterial colonies with growth characteristics and morphology similar to *X. fastidiosa* are observed after 14–30 days on any medium and typical *X. fastidiosa* colonies are found in the positive ~~controls~~controls if included.	P	*Category : TECHNICAL* **(194) EPPO (25 Sep 2023 8:22 AM)** A positive control for isolation is not always used in laboratories. It is not always clear which strain(s) should be used as positive control. Suggestion: addition of 'if included'. A warning could be added that the media should be validated before use. Other tests should be used in addition to isolation.
964	The isolation is negative if no bacterial colonies with growth characteristics and morphology similar to *X. fastidiosa* are observed after 14–30 days on any medium and typical *X. fastidiosa* colonies are found in the positive controls.	C	*Category : TECHNICAL* **(193) EPPO (25 Sep 2023 8:22 AM)** In EPPO (2023), it is indicated to incubate plates for up to 6 weeks. Could the '14-30 days' be changed for '14-45' days'? If yes, should be changed also for positive results (next paragraph) and in section 4.1.
997	Pathogenicity assessment 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 *V. vinifera*, the cultivars	C	*Category : TECHNICAL* **(307) European Union (29 Sep 2023 4:53 PM)** Leccino is one of the most resistant olive

	'Cabernet sauvignon', 'Chardonnay', 'Chenin Blanc' and 'Pinot Noir'; for *C. sinensis*, 'Hamlin', 'Natal', 'Pera', and 'Valencia'; and for *O. europaea*, susceptible cultivars such as 'Cellina di Nardò', 'Coratina' 'Frantoio', and 'Leccino' (EPPO, 2023c). *Catharanthus roseus* (Madagascar periwinkle) is a herbaceous plant that is easily grown in a greenhouse and is susceptible to *X. fastidiosa* (Monteiro *et al.*, 2001).		tree cultivar. It is not really adapted for pathogenicty test. We propose to delete this reference to Leccino.
997	Pathogenicity assessment should use plants of the same host from which the suspect *X. fastidiosa* was isolated. ~~Where~~ When possible, the most susceptible cultivars should be used. Some recommended examples include: for *V. vinifera*, the cultivars 'Cabernet sauvignon', 'Chardonnay', 'Chenin Blanc' and 'Pinot Noir'; for *C. sinensis*, 'Hamlin', 'Natal', ~~'Pera',~~ 'Pera' and 'Valencia'; and for *O. europaea*, susceptible cultivars such as 'Cellina di Nardò', 'Coratina' ~~'Frantoio',~~ 'Frantoio' and 'Leccino' (EPPO, 2023c). *Catharanthus roseus* (Madagascar periwinkle) is a herbaceous plant that is easily grown in a greenhouse and is susceptible to *X. fastidiosa* (Monteiro *et al.*, 2001).	P	*Category : EDITORIAL* **(196) EPPO (25 Sep 2023 8:22 AM)** Typo + two unnecessary commas.
997	Pathogenicity assessment 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 *V. vinifera*, the cultivars 'Cabernet sauvignon', 'Chardonnay', 'Chenin Blanc' and 'Pinot Noir'; for *C. sinensis*, 'Hamlin', 'Natal', 'Pera', and 'Valencia'; and for *O. europaea*, susceptible cultivars such as 'Cellina di Nardò', 'Coratina' 'Frantoio', and 'Leccino' (EPPO, 2023c). *Catharanthus roseus* (Madagascar periwinkle) is a herbaceous plant that is easily grown in a greenhouse and is susceptible to *X. fastidiosa* (Monteiro *et al.*, 2001).	C	*Category : TECHNICAL* **(195) EPPO (25 Sep 2023 8:22 AM)** Leccino is one of the most resistant olive tree cultivar. It is not really adapted for pathogenicty test. We propose to delete this reference to Leccino.
998	To facilitate the rapid uptake of the inoculum by the transpiration system, inoculated plants should be young and should be grown in pots with dry soil. Cultures of bacteria grown for 8–10 days on suitable media should be used for pathogenicity tests. Bacteria are removed from solid media and suspended in PBS to produce a turbid suspension of approximately 10^8 c.f.u./mL (Abs_{600nm} = 0.2). A drop (20–50 µL) of inoculum is placed in a leaf axil and punctured through several times with a fine needle until the liquid is completely absorbed. Control plants are treated in the same way except that the suspending medium (PBS) is used instead of bacterial suspension. Plants must be maintained in the greenhouse or growing chambers at 26–28 °C.	C	*Category : TECHNICAL* **(308) European Union (29 Sep 2023 4:54 PM)** This may vary according to the plant species (e.g. for grape lower night temperature should be applied). The EPPO protocol indicates 25-28°C. Why is it different?
998	To facilitate the rapid uptake of the inoculum by the transpiration system,	C	*Category : TECHNICAL* **(197) EPPO (25 Sep 2023 8:22 AM)**

	inoculated plants should be young and should be grown in pots with dry soil. Cultures of bacteria grown for 8–10 days on suitable media should be used for pathogenicity tests. Bacteria are removed from solid media and suspended in PBS to produce a turbid suspension of approximately 10^8 c.f.u./mL (Abs_{600nm} = 0.2). A drop (20–50 µL) of inoculum is placed in a leaf axil and punctured through several times with a fine needle until the liquid is completely absorbed. Control plants are treated in the same way except that the suspending medium (PBS) is used instead of bacterial suspension. Plants must be maintained in the greenhouse or growing chambers at 26–28 °C.		This may vary according to the plant species (e.g. for grape lower night temperature should be applied). The EPPO protocol indicates 25-28°C. Why is it different?
1002	In addition, a bioassay can be performed on *Nicotiana tabacum* (tobacco) plants by inoculating the petioles with suspensions of *X. fastidiosa* (Francis, Civerolo and Bruening, 2008). Leaf scorch symptoms develop 10–14 days after inoculation.	C	*Category : TECHNICAL* **(309) European Union (29 Sep 2023 4:55 PM)** cv. Petit Havana (see EPPO, 2023c)
1002	In addition, a bioassay can be performed on *Nicotiana tabacum* (tobacco) plants by inoculating the petioles with suspensions of *X. fastidiosa* (Francis, Civerolo and Bruening, 2008). Leaf scorch symptoms develop 10–14 days after inoculation.	C	*Category : TECHNICAL* **(198) EPPO (25 Sep 2023 8:22 AM)** cv. Petit Havana (see EPPO, 2023c)
1010	The methods described in this section (section 4) have mainly been developed on pure cultures but can be used on DNA extracts from plants, except for the PCR by Hernandez-Martinez *et al.* (2006). However, it is recognized that the quantity and quality of target DNA, or the occurrence of possible mixed infections, may mean that not all amplicons are obtained or may prevent clear assignment of subspecies.	C	*Category : EDITORIAL* **(199) EPPO (25 Sep 2023 8:22 AM)** Rather "section 4.5"?
1012	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; Bergsma-Vlami *et al.*, 2017). This approach can be used on DNA extracted from either bacterial cultures or infected plants that have 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, ~~2023b)~~2023c). 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 () and in Appendix 16 of EPPO ~~(2023b)~~(2023c).	P	*Category : EDITORIAL* **(200) EPPO (25 Sep 2023 8:22 AM)** Correct reference to be quoted (twice).
1013	Expected amplicon sizes for the different housekeeping genes are: 600 bp for *cysG*, 654 bp for *gltT*, 379 bp for *holC*, 708 bp for *leuA*, 730 bp for *malF*, 557 bp for *nuoL* and 533 bp for *petC*.	C	*Category : TECHNICAL* **(310) European Union (29 Sep 2023 4:56 PM)** Indicate the amplicon size including primers

			or it should be clearly mentioned here that the size reported are without the primers.
1013	Expected amplicon sizes for the different housekeeping genes are: 600 bp for *cysG*, 654 bp for *gltT*, 379 bp for *holC*, 708 bp for *leuA*, 730 bp for *malF*, 557 bp for *nuoL* and 533 bp for *petC*.	C	*Category : TECHNICAL* **(201) EPPO (25 Sep 2023 8:22 AM)** Indicate the amplicon size including primers or it should be clearly mentioned here that the size reported are without the primers.
1041	A protocol for nested MLST, which has the same targets as in Yuan *et al.* (2010) but is more sensitive than MLST, has been described (Cesbron *et al*., 2020) and is appropriate when the MLST analysis (Yuan *et al*. 2010) is not successful. For Sanger sequencing (Scally *et al*., 2005; Yuan *et al*., 2010), the PCR product of at least two housekeeping genes, ~~such as~~ either *rpoD* (Minsavage *et al*., 1994) and *malF* (MLST analysis) or *cysG* and *malF* (MLST analysis), should be sequenced in both directions. The combination of ~~at least two~~ these genes ~~is equivalent to MLST for~~ allows the determination of the subspecies. Sequencing a combination of at least two genes may also allow possible recombinant strains to be detected. To determine the sequence type, however, the PCR products of all the seven housekeeping genes listed above for MLST are needed. Sequence data for *rpoD* and *malF* can be analysed using BLASTN, available at the National Center for Biotechnology Information (). *CysG* and *malF* sequences can be compared with data available in the pubMLST database for MLST genes (). The nested MLST protocol described by Cesbron *et al*. (2020) can be used with *cysG* and *malF*. In the case of inconsistent results for the two sequenced genes or atypical/new patterns, complete MLST analysis of the seven genes should be performed and sequences compared with data available in the pubMLST database as indicated.	P	*Category : TECHNICAL* **(311) European Union (29 Sep 2023 4:59 PM)** New paragraph. + The text was clarified according to EPPO 2023c + Suggestion to add a sentence regarding 'inconsistent results' from EPPO, 2023c
1041	A protocol for nested MLST, which has the same targets as in Yuan *et al.* (2010) but is more sensitive than MLST, has been described (Cesbron *et al*., 2020) and is appropriate when the MLST analysis (Yuan *et al*. 2010) is not successful. For Sanger sequencing (Scally *et al*., 2005; Yuan *et al*., 2010), the PCR product of at least two housekeeping genes, ~~such as~~ either *rpoD* (Minsavage *et al*., 1994) and *malF* (MLST analysis) or *cysG* and *malF* (MLST analysis), should be sequenced in both directions. The combination of ~~at least two~~ these genes ~~is equivalent to MLST for~~ allows the determination of the subspecies. Sequencing a combination of at least two genes may also allow possible recombinant strains to be detected. To determine the sequence type, however, the PCR products of all the seven housekeeping genes listed above for MLST are needed. Sequence data for *rpoD*	P	*Category : TECHNICAL* **(202) EPPO (25 Sep 2023 8:22 AM)** New paragraph. + The text was clarified according to EPPO 2023c + Suggestion to add a sentence regarding 'inconsistent results' from EPPO, 2023c

	and *malF* can be analysed using BLASTN, available at the National Center for Biotechnology Information (). *CysG* and *malF* sequences can be compared with data available in the pubMLST database for MLST genes (). The nested MLST protocol described by Cesbron *et al*. (2020) can be used with *cysG* and *malF*. In the case of inconsistent results for the two sequenced genes or atypical/new patterns, complete MLST analysis of the seven genes should be performed and sequences compared with data available in the pubMLST database as indicated.		
1041	A protocol for nested MLST, which has the same targets as in Yuan *et al.* (2010) but is more sensitive than MLST, has been described (Cesbron *et al*., 2020) and is appropriate when the MLST analysis (Yuan *et al*. 2010) is not successful. For Sanger sequencing (Scally *et al*., 2005; Yuan *et al*., 2010), the PCR product of at least two housekeeping genes, such as *rpoD* (Minsavage *et al*., 1994) and *malF* (MLST analysis) or *cysG* and *malF* (MLST analysis), should be sequenced in both directions. The combination of at least two genes is equivalent to MLST for the determination of the subspecies. Sequencing a combination of at least two genes may also allow possible recombinant strains to be detected. To determine the sequence type, however, the PCR products of all the seven housekeeping genes listed above for MLST are needed. Sequence data for *rpoD* and *malF* can be analysed using BLASTN, available at the National Center for Biotechnology Information (). *CysG* and *malF* sequences can be compared with data available in the pubMLST database for MLST genes (). The nested MLST protocol described by Cesbron *et al*. (2020) can be used with *cysG* and *malF*.	C	*Category : TECHNICAL* **(72) United States of America (15 Sep 2023 3:50 PM)** A statement should be included as to the limitations of this approach. MLST alone is known to lead to taxonomic errors, lack of monophyly, and phylogenetic resolution - with biological implications. While two loci may frequently work fine, that is different from stating that it is equivalent to seven loci - which we already know is not sufficient for certain types of questions.
1048	If relevant, DNA extracts should be kept at −80 °C and PCR amplification products at −20 °C.	C	*Category : SUBSTANTIVE* **(239) Australia (27 Sep 2023 8:39 AM)** Is there criteria for when DNA extracts should be kept? Suggest outlining the criteria or providing a relevant example as to when DNA extract should be kept.
1052	French Agency for Food, Environmental and Occupational Health & ~~Safety~~Safety (ANSES), Plant Health Laboratory, Bacteriology, Virology and GMO Unit, Angers, France (Bruno Legendre; email: tel.: (+33) 2 4120 7440).	P	*Category : EDITORIAL* **(203) EPPO (25 Sep 2023 8:22 AM)** Acronym.
1057	This protocol was revised by Helvecio Coletta (CCSM, Brazil (see preceding section)), Bruno Legendre (French Agency for Food, Environmental and Occupational Health & ~~Safety~~Safety (ANSES), France (see preceding section)), Toni Chapman (NSW Department of Primary Industries, Australia (see preceding section)) and Sophie Cesbron (University of Angers, France (see preceding	P	*Category : EDITORIAL* **(204) EPPO (25 Sep 2023 8:22 AM)** Acronym

	section)). The first version of this protocol, adopted in 2018, was written by Marta Francis (University of California Davis, United States of America), Robert Taylor (Biosecurity New Zealand, New Zealand), Helga Reisenzein (Austrian Agency for Health and Food Safety, Austria), John Hartung (United States Department of Agriculture (USDA), United States of America) and Wenbin Li (USDA Animal Plant Health and Inspection Service, United States of America). Ed Civerolo (formerly USDA) was also involved in the development of the originally adopted protocol.		
1103	**EFSA (European Food Safety Authority)**. 2021. Scientific report on the update of the *Xylella* spp. host plant database: systematic literature search up to 31 December 2020. *EFSA Journal* 19(6): 6674. 70 pp.	C	*Category : TECHNICAL* **(312) European Union (29 Sep 2023 5:00 PM)** Technical update: EFSA (European Food Safety Authority), Gibin D, Pasinato L and Delbianco A, 2023. Scientific Report on the update of the Xylella spp. host plant database – systematic literature search up to 31 December 2022. EFSA Journal 2023;21(6):8061, 73 pp. https://doi.org/10.2903/j.efsa.2023.8061
1103	**EFSA (European Food Safety Authority)**. 2021. Scientific report on the update of the *Xylella* spp. host plant database: systematic literature search up to 31 December 2020. *EFSA Journal* 19(6): 6674. 70 pp. **EFSA (European Food Safety Authority)**. 2023. Database of host plants found to be susceptible to *Xylella fastidiosa* in the Union territory. In: *Food safety*. European Commission. [Cited 15 April 2023]. https://ec.europa.eu/food/plants/plant-health-and-biosecurity/legislation/control-measures/xylella-fastidiosa/database-susceptible-host-plants_fr	P	*Category : EDITORIAL* **(206) EPPO (25 Sep 2023 8:22 AM)** To be moved from paragraph [1111] (alphabetical order).
1103	**EFSA (European Food Safety Authority)**. 2021. Scientific report on the update of the *Xylella* spp. host plant database: systematic literature search up to 31 December 2020. *EFSA Journal* 19(6): 6674. 70 pp.	C	*Category : TECHNICAL* **(205) EPPO (25 Sep 2023 8:22 AM)** Technical update: EFSA (European Food Safety Authority), Gibin D, Pasinato L and Delbianco A, 2023. Scientific Report on the update of the Xylella spp. host plant database – systematic literature search up to 31 December 2022. EFSA Journal 2023;21(6):8061, 73 pp. https://doi.org/10.2903/j.efsa.2023.8061
1106	**EPPO**. 2023a. *Xylella fastidiosa* (XYLEFA). In: *EPPO global database*. Paris.	P	*Category : EDITORIAL* **(207) EPPO (25 Sep 2023 8:22 AM)**

	[Cited 7 May ~~2023]~~2023].		Typo
1108	**EPPO**. 2023c. Diagnostic protocol for *Xylella fastidiosa*. PM 7/24 (5). *EPPO ~~Bulletin~~ Bulletin*~~[details to be added].~~, 53, 205–276. https://doi.or g /10.1111/e p p.12923	P	*Category : EDITORIAL* **(208) EPPO (25 Sep 2023 8:22 AM)** Precisions given.
1111	~~**EFSA (European Food Safety Authority)**. 2023. Database of host plants found to be susceptible to *Xylella fastidiosa* in the Union territory. In: *Food safety*. European Commission. [Cited 15 April 2023].~~	P	*Category : EDITORIAL* **(209) EPPO (25 Sep 2023 8:22 AM)** To be moved after paragraph [1103] (alphabetical order).
1115	**Galvez, L.C., Korus, K., Fernandez, J., Behn, J.L. & Banjara, N.** 2010. The threat of Pierce's disease to midwest wine and table grapes. *APSnet Features*. doi:10.1094/APSnetFeature-2010-1015. **Giampetruzzi, A., Saponari, M., Loconsole, G., Boscia, D., Savino, V. N., Almeida, R.P.P., Zicca, S., Landa, B.B., Chaćon-Diaz, C. & Saldarelli, P.** 2017. Genome-wide analysis provides evidence on the genetic relatedness of the emergent *Xylella fastidiosa* genotype in Italy to isolates from Central America. *Phytopathology*, 107(7): 816–827.	P	*Category : EDITORIAL* **(210) EPPO (25 Sep 2023 8:22 AM)** To be moved from paragraph [1117] (alphabetical order).
1117	~~**Giampetruzzi, A., Saponari, M., Loconsole, G., Boscia, D., Savino, V. N., Almeida, R.P.P., Zicca, S., Landa, B.B., Chaćon-Diaz, C. & Saldarelli, P.** 2017. Genome-wide analysis provides evidence on the genetic relatedness of the emergent *Xylella fastidiosa* genotype in Italy to isolates from Central America. *Phytopathology*, 107(7): 816–827.~~	P	*Category : EDITORIAL* **(211) EPPO (25 Sep 2023 8:22 AM)** To be moved after paragraph [1115] (alphabetical order).
1169	**Schaad, N.W., Postnikova, E., Lacy, G., Fatmi, M.B. & Chang, C.-J.** 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* ~~subsp~~ subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. *Systematic and Applied Microbiology*, 27: 290–300.	P	*Category : EDITORIAL* **(212) EPPO (25 Sep 2023 8:22 AM)** Typo
1174	**Tindall, B.J.** 2014. The family name Solimonadaceae Losey *et al.* 2013 is illegitimate, proposals to create the names 'Sinobacter soli' comb. nov. and 'Sinobacter variicoloris' contravene the Code, the family name Xanthomonadaceae Saddler and Bradbury 2005 and the order name Xanthomonadales Saddler and Bradbury 2005 are illegitimate and notes on the application of the family names Solibacteraceae Zhou *et al.* 2008, Nevskiaceae Henrici and Johnson 1935 (Approved Lists 1980) and Lysobacteraceae Christensen and Cook 1978 (Approved Lists 1980) and order name Lysobacteriales Christensen and Cook 1978 (Approved Lists 1980) with respect to the classification of the corresponding	C	*Category : EDITORIAL* **(213) EPPO (25 Sep 2023 8:22 AM)** Is it normal to have all these details in the reference?

	type genera *Solibacter* Zhou *et al.* 2008, *Nevskia* Famintzin 1892 (Approved Lists 1980) and *Lysobacter* Christensen and Cook 1978 (Approved Lists 1980) and importance of accurately expressing the link between a taxonomic name, its authors and the corresponding description/circumscription/emendation. *International Journal of Systematic and Evolutionary Microbiology*, 64(1): 293–297.		