2021 FIRST CONSULTATION

1 July – 30 September 2021

Compiled comments for Draft DP for *Candidatus Liberibacter* spp. on *Citrus* spp. (2004-010)

Summary

Name	Summary
European Union	The comments on this draft standard have been entered into the OCS by the European Commission on behalf of the EU and its member States.
Singapore	Singapore is supportive of this draft.
South Africa	The NPPOZA is in agreement with this draft and has no further comments
Venezuela	La parte técnica del Organismo Fitosanitario de Venezuela, al analizar el proyecto de NIMF:concluyo estar de acuerdo con lo planteado por el Grupo de debate sobre normas

T (Type) - B = Bullet, C = Comment, P = Proposed Change, R = Rating

FAO sequential number	Para	Text	T	Comment
1	G	(General Comment)	C	**Argentina** We support the comments submitted by COSAVE *Category : SUBSTANTIVE*
2	G	(General Comment)	C	**Guyana** Guyana has no objection to the proposed document at this time. *Category : SUBSTANTIVE*
3	G	(General Comment)	C	**Nepal** Nepal has no comments on Draft new annex to ISPM◦27 (Diagnostic protocols for regulated pests)- Candidatus Liberibacter on Citrus *Category : EDITORIAL*
4	G	(General Comment)	C	**Mexico** I support the document as it is and I have no comments *Category : SUBSTANTIVE*
5	G	(General Comment)	C	**Russian Federation** The Russian Federation would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System *Category : SUBSTANTIVE*
6	G	(General Comment)	C	**Canada** Canada supports the draft DP. *Category : SUBSTANTIVE*
7	G	(General Comment)	C	**European Union** The EU and its Member States support the EPPO comments on this draft DP. *Category : SUBSTANTIVE*

8	G	(General Comment)	C	**Switzerland** Switzerland would like to formally endorse the EPPO comments submitted via the IPPC Online Comment System *Category : TECHNICAL*
9	G	(General Comment)	C	**Barbados** Barbados agree with the proposed draft annex. *Category : SUBSTANTIVE*
10	G	(General Comment)	C	**COSAVE** We highlight the importance of having better traslation into Spanish in order to be consistent with the English version. Se destaca la importancia de contar con traducciones al español que reflejen mejor el contenido de la versión en inglés. *Category : TRANSLATION*
11	G	(General Comment)	C	**EPPO** In order to streamline the reading of the document, denomination of 'Candidatus Liberibacter asiaticus' should be shorten to CLas; africanus to CLaf; and americanus to CLam. These denominations are official and found in all recent literature. *Category : EDITORIAL*
12	G	(General Comment)	C	**Malawi** No substantive comment for draft annex to ISPM 27. We support it *Category : SUBSTANTIVE*
13	G	(General Comment)	C	**United States of America** In this draft protocol, versatile information on HLB (aka citrus greening) was clearly and concisely described, the focus was on molecular detection of three HLB associated bacterium, known as 'Candidatus Liberibacter asiaticus' (CLas), 'Candidatus Liberibacter africanus' (CLaf) and 'Candidatus Liberibacter americanus' (CLam). It briefly depicted individual conventional PCR methods and the well-known real-time PCRs published in 1990s and 2000s, such methods established the molecular diagnostics for HLB worldwide and in the United States. Several real-time PCRs published no later than 2014 were also presented. As only CLas has been found in the United States since the first detection of HLB in 2005 (in Florida), our practice-based comments to the draft protocol were limited to methods for detection of CLas. Comments for the contents in [86]3.4 were in accordance with the updated knowledge/sequences in the literature/GenBank and our experiences in testing thousands of samples collected from the US territories for HLB diagnostics in plants and ACPs. *Category : TECHNICAL*
14	G	(General Comment)	C	**Kenya** Clause 62 (3. detection) should come after clause 67 (3.1 symptoms). The document flow should first describe the disease symptoms before giving information about detection and also so

				that information about detection of this specific pathogen can flow in a systematic manner. That is, (clause 62 on detection to be followed by other types of disease detection mentioned in this draft as in clause 82 (3.3 biological detection), 86 (3.4 molecular detection). *Category : SUBSTANTIVE*
15	G	(General Comment)	C	**Thailand** Thailand has no objection on the Draft DP: Candidatus Liberibacter spp. on Citrus spp. *Category : SUBSTANTIVE*
16	G	(General Comment)	C	**Cuba** Con respecto a las técnicas de diagnóstico que en relacionan, las de PCR convencional, son las que comúnmente se han usado con éxito en el mundo, al igual que las de PCR en tiempo real. Sin embargo, consideramos que en este proyecto de protocolo de diagnóstico se debe mencionar y describir la PCR anidada convencional. Esta técnica es muy robusta y ha dado muy buenos resultados en el mundo, es además la alternativa en los países o laboratorios que no poseen el equipamiento para realizar PCR en tiempo real cuando se quiere ganar en sensibilidad analítica. *Category : SUBSTANTIVE*
17	G	(General Comment)	C	**New Zealand** list of references need to be checked and amended where needed to keep consistency through out. *Category : EDITORIAL*
18	1	**DRAFT ANNEX to ISPM 27: '*Candidatus* Liberibacter' spp. on *Citrus* spp. (2004-010)**	C	**Viet Nam** VN agrees with this draft annex to ISPM 27 *Category : SUBSTANTIVE*
19	24	Takayuki MATSUURA (Ministry of Agriculture, Forestry and Fisheries, Yokohama Plant Protection Station, Yokohama, JP)-	P	**EPPO** Typo (dot deleted) *Category : EDITORIAL*
20	43	Huanglongbing (HLB), caused by '*Candidatus* Liberibacter' species and also known as citrus greening, is one of the most destructive and widespread diseases of citrus in Asia, Africa and the Americas, affecting mainly *Citrus* species, cultivars and hybrids[1] and, to a lesser extent, some other hosts within the Rutaceae (EPPO, 2014; CABI, 2021). The '*Ca.* Liberibacter' species associated with the disease are transmitted by the psyllids *Diaphorina citri* (EPPO, 2005), *Trioza erytreae* and *Cacopsylla citrisuga* (Cen *et al*., 2012); '*Candidatus* Liberibacter asiaticus' was also detected in *Diaphorina communis* identified in Bhutan (Donovan *et al.*, 2012).	C	**EPPO** The reference EPPO, 2014 will shortly no longer be correct. The Protocol was revised and will be published in the EPPO Bulletin soon. (2021), PM 7/121 (2) 'Candidatus Liberibacter africanus', 'Candidatus Liberibacter americanus' and 'Candidatus Liberibacter asiaticus'. EPPO Bull. pp (will be available when published on paper) https://doi.org/10.1111/epp.12757 *Category : TECHNICAL*
21	43	Huanglongbing (HLB), caused by '*Candidatus* Liberibacter' species and also known as citrus greening, is one of the most destructive and widespread diseases of citrus in Asia, Africa and the Americas, affecting mainly *Citrus* species, cultivars and hybrids[1] and, to a lesser extent, some other hosts within the Rutaceae (EPPO,	P	**EPPO** Typo (a space deleted) *Category : EDITORIAL*

		2014; CABI, 2021). The '*Ca.* Liberibacter' species associated with the disease are transmitted by the psyllids *Diaphorina citri* (EPPO, 2005), *Trioza erytreae* and *Cacopsylla citrisuga* (Cen *et al*., 2012); '*Candidatus* Liberibacter asiaticus' was also detected in *Diaphorina communis* identified in Bhutan (Donovan *et al.*, 2012).		
1. Pest information				
22	46	The causal agents of HLB are fastidious Gram-negative bacteria in the '*Ca.* Liberibacter' genus (Garnier, Danel and Bové, 1984). '*Ca.* Liberibacter' species are restricted to the sieve tubes within the phloem tissues, occur at very low concentrations and are unevenly distributed within the host plant (Jagoueix, Bové and Garnier, 1994). The pathogenic '*Ca.* Liberibacter' species were discovered by electron microscopy in citrus trees with HLB symptoms. Three species of '*Ca.* Liberibacter' have been associated with HLB and are differentiated based on the nucleotide sequence in the 16S ribosomal gene operon (Jagoueix, Bové and Garnier, 1994). Ca. Liberibacter spp. can be transported both upward and downward throughout the tree, but their distribution is highly patchy (Li et al., 2009). The highest concentrations can be found in stem and midribs of flush. Flush is a newly developing cluster of very young leaves on the expanding terminal end of a shoot (Chiyaka et al, 2012).The three species are as follows:	P	**Colombia** Explain the distribution of the bacteria in a citrus plant to detect it. This way of distribution of the bacteria in the plant must be taken into account for the detection and diagnosis of the bacteria, which increases the probability of detecting and identifying it in the process of monitoring HLB disease in small or large citrus plantations. *Category : TECHNICAL*
23	46	The causal agents of HLB are fastidious Gram-negative bacteria in the '*Ca.* Liberibacter' genus (Garnier, Danel and Bové, 1984). '*Ca.* Liberibacter' species are restricted to the sieve tubes within the phloem ~~tissues, occur at very low concentrations~~ tissues and are unevenly distributed within the host plant (Jagoueix, Bové and Garnier, 1994). ~~The pathogenic~~ Bacterium titers in the phloem vary depending on 'Ca. Liberibacter' or plant species, plant organ, and the climatic or environmental conditions that plants are exposed to (Tatineneni et al., 2008; Lopes et al 2009; Lopes et al., 2017; Cifuentes Arenas et al., 2019) '*Ca.* Liberibacter' species were discovered by electron microscopy in citrus trees with HLB symptoms. Three species of '*Ca.* Liberibacter' have been associated with HLB and are differentiated based on the nucleotide sequence in the 16S ribosomal gene operon (Jagoueix, Bové and Garnier, 1994). The three species are as follows:	P	**COSAVE** Not always the bacteria occurs on very low concentrations, it depends upon many conditions. *Category : TECHNICAL*
24	46	The causal agents of HLB are fastidious Gram-negative bacteria in the '*Ca.* Liberibacter' genus (Garnier, Danel and Bové, 1984). '*Ca.* Liberibacter' species are restricted to the sieve tubes within the phloem ~~tissues, occur at very low concentrations~~ tissues and are unevenly distributed within the host plant (Jagoueix, Bové and Garnier, 1994). ~~The pathogenic~~ Bacterium titers in the phloem vary depending on '*Ca.* Liberibacter' ~~species~~ or plant species, plant organ, and the climatic or environmental conditions that plants are exposed to (Tatineneni et al., 2008; Lopes et al 2009; Lopes et al., 2017; Cifuentes Arenas et al., 2019).	P	**Uruguay** Not always the bacteria occurs on very low concentrations, it depends upon may conditions *Category : TECHNICAL*

		'Ca. Liberibacter' sprcies were discovered by electron microscopy in citrus trees with HLB symptoms. Three species of '*Ca.* Liberibacter' have been associated with HLB and are differentiated based on the nucleotide sequence in the 16S ribosomal gene operon (Jagoueix, Bové and Garnier, 1994). The three species are as follows:		
25	46	The causal agents of HLB are fastidious Gram-negative bacteria in the '*Ca.* Liberibacter' genus (Garnier, Danel and Bové, 1984). '*Ca.* Liberibacter' species are restricted to the sieve tubes within the phloem tissues, occur at very low concentrations and are unevenly distributed within the host plant (Jagoueix, Bové and Garnier, 1994). ~~The pathogenic~~ Bacterium titers in the phloem vary depending on '*Ca.* Liberibacter' or plant species, plant organ, and the climatic or environmental conditions that plants are exposed to (Tatineneni et al., 2008; Lopes et al., 2009; Lopes et al., 2017; Cifuentes Arenas et al., 2019). '*Ca.* Liberibacter' species were discovered by electron microscopy in citrus trees with HLB symptoms. Three species of '*Ca.* Liberibacter' have been associated with HLB and are differentiated based on the nucleotide sequence in the 16S ribosomal gene operon (Jagoueix, Bové and Garnier, 1994). The three species are as follows:	P	**Brazil** Explains the situations where concentration varies. *Category : TECHNICAL*
26	46	The causal agents of HLB are fastidious Gram-negative bacteria in the '*Ca.* Liberibacter' genus (Garnier, Danel and Bové, 1984). '*Ca.* Liberibacter' species are restricted to the sieve tubes within the phloem ~~tissues, occur at very low concentrations~~ tissues and are unevenly distributed within the host plant (Jagoueix, Bové and Garnier, 1994). The pathogenic '*Ca.* Liberibacter' species were discovered by electron microscopy in citrus trees with HLB symptoms. Three species of '*Ca.* Liberibacter' have been associated with HLB and are differentiated based on the nucleotide sequence in the 16S ribosomal gene operon (Jagoueix, Bové and Garnier, 1994). The three species are as follows:	P	**Brazil** Sometimes it happens but not generally. *Category : SUBSTANTIVE*
27	47	**'*Candidatus* Liberibacter asiaticus'**, transmitted by *Diaphorina citri*, is heat tolerant and induces symptoms in warm climates at optimal temperatures in the range 27–32 °C (Jagoueix *et al.*, 1996). Cell multiplication in plant tissues is partially limited at 38°C (Lopes *et al.*, 2009a). It is present in Asia, Africa, Oceania and North and South America (Bové, 2006; da Graça, 2010; CABI, 2021).	P	**COSAVE** Important information *Category : TECHNICAL*
28	47	**'*Candidatus* Liberibacter asiaticus'**, transmitted by *Diaphorina citri*, is heat tolerant and induces symptoms in warm climates at optimal temperatures in the range ~~27–32 °C~~ 27–32°C (Jagoueix *et al.*, 1996). It is present in Asia, Africa, Oceania and North and South America (Bové, 2006; da Graça, 2010; CABI, 2021).	P	**EPPO** Typo (space deleted). *Category : EDITORIAL*
29	47	**'*Candidatus* Liberibacter asiaticus'**, transmitted by *Diaphorina citri*, is heat tolerant and induces symptoms in warm climates at optimal temperatures in the range 27–32 °C (Jagoueix *et al.*, 1996). Cell multiplication in plant tissues is	P	**Uruguay** Important information *Category : TECHNICAL*

		partially limited at 38º C (Lopes et al., 2009a). It is present in Asia, Africa, Oceania and North and South America (Bové, 2006; da Graça, 2010; CABI, 2021).		
30	47	**'*Candidatus* Liberibacter asiaticus'**, transmitted by *Diaphorina citri*, is heat tolerant and induces symptoms in warm climates at optimal temperatures in the range 27–32 °C (Jagoueix *et al.*, 1996)~~.~~. Cell multiplication in plant tissues is partially limited at 38°C (Lopes et al., 2009a). It is present in Asia, Africa, Oceania and North and South America (Bové, 2006; da Graça, 2010; CABI, 2021).	P	**Brazil** Important information *Category : TECHNICAL*
31	48	**'*Candidatus* Liberibacter africanus'** is transmitted by *Trioza erytreae*, is heat-sensitive and causes symptoms between 22 °C and 24 °C (Jagoueix, Bové and Garnier, 1994), with no symptoms appearing at 27–30 °C (da Graça, 1991). It is present in Asia (Saudi Arabia and Yemen) and Africa (Ascension, Saint Helena and Tristan da Cunha; Burundi; Cameroon; the Central African Republic; the Comoros; Ethiopia; Kenya; Madagascar; Malawi; Mauritius; Mayotte; Réunion; Rwanda; Somalia; South Africa; Swaziland; the United Republic of Tanzania; and Zimbabwe) (Bové, 2006; da Graça, 2010; CABI, 2021). ~~'*Candidatus* Liberibacter africanus subsp. capensis' has been reported in South Africa on an ornamental rutaceous tree, *Calodendrum capense* (Garnier *et al.*, 2000).~~	P	**Australia** Suggest deleting sentence due to out of date information and South Africa being listed in the sentence prior refering to the species. It is considered that species level is enough information and subspecies is too detailed for the purpose. Justification: Information provided is not up-to-date. Since the report of this subspecies, four new subspecies (LafCL, LafV, LafT and LafZ) have been reported in South Africa (Robert et al. 2015; Robert& Pietersen 2017). Including the subspecies information is not essential and might be misleading as the exact taxonomic position of these subspecies in relation to Laf has not been fully clarified (Robert& Pietersen 2017). References: Roberts R, Steenkamp ET, Pietersen G. Three novel lineages of 'Candidatus Liberibacter africanus' associated with native rutaceous hosts of Trioza erytreae in South Africa. Int J Syst Evol Microbiol. 65(2):723-731. Roberts R, Pietersen G. A novel subspecies of 'Candidatus Liberibacter africanus' found on native Teclea gerrardii (Family: Rutaceae) from South Africa. Antonie Van Leeuwenhoek. 110(3):437-444. *Category : TECHNICAL*
32	48	**'*Candidatus* Liberibacter africanus'** is transmitted by *Trioza erytreae*, is heat-sensitive and causes symptoms between 22 °C and 24 °C (Jagoueix, Bové and Garnier, 1994), with no symptoms appearing at 27–30 °C (da Graça, 1991). It is present in Asia (Saudi Arabia and Yemen) and Africa (Ascension, Saint Helena and Tristan da Cunha; Burundi; Cameroon; the Central African Republic; the Comoros; Ethiopia; Kenya; Madagascar; Malawi; Mauritius; Mayotte; Réunion; Rwanda; Somalia; South Africa; Swaziland; the United Republic of Tanzania; and Zimbabwe) (Bové, 2006; da Graça, 2010; CABI, 2021). '*Candidatus* Liberibacter africanus subsp. capensis' has been reported in South Africa on an ornamental rutaceous tree, *Calodendrum capense* (Garnier *et al.*, 2000).	C	**EPPO** CLaf has not been detected recently in la Réunion Island and its possible presence is under further investigation. Official information from France will be provided and the pest status will be updated in the EPPO Global Database as soon as this information is available. *Category : SUBSTANTIVE*

33	48	**'*Candidatus* Liberibacter africanus'** is transmitted by *Trioza erytreae*, is heat-sensitive and causes symptoms between ~~22 °C~~ 22°C and ~~24 °C~~ 24°C (Jagoueix, Bové and Garnier, 1994), with no symptoms appearing at ~~27–30 °C~~ 27–30°C (da Graça, 1991). It is present in Asia (Saudi Arabia and Yemen) and Africa (Ascension, Saint Helena and Tristan da Cunha; Burundi; Cameroon; the Central African Republic; the Comoros; Ethiopia; Kenya; Madagascar; Malawi; Mauritius; Mayotte; Réunion; Rwanda; Somalia; South Africa; Swaziland; the United Republic of Tanzania; and Zimbabwe) (Bové, 2006; da Graça, 2010; CABI, 2021). '*Candidatus* Liberibacter africanus subsp. capensis' has been reported in South Africa on an ornamental rutaceous tree, *Calodendrum capense* (Garnier *et al.*, 2000).	P	**EPPO** Typo (three spaces deleted). *Category : EDITORIAL*
34	49	**'*Candidatus* Liberibacter americanus'** was described as a new species when it was first found in 2004 in São Paulo, Brazil (Teixeira *et al.*, 2005a, 2005b, 2005c; Bové, 2006). It is also transmitted by *Diaphorina citri* (Yamamoto *et al*., 2006). '*Ca.* L. americanus' is ~~less~~ heat ~~tolerant than '~~sensitive, with cell multiplication in plant tissues partially affected at 32°C and highly affected at 35°C and 38°C (Lopes et al., 2009a)~~*Ca.* L. asiaticus'.~~ Similarly, Gasparoto~~*et al.*) found that temperatures above 32 °C negatively affected the multiplication of '*Ca.* L. americanus' in infected plants, whereas '*Ca.* L. asiaticus' was affected only by temperatures above 38 °C. Similarly, Gasparoto~~ *et al.* (2012) found that '*Ca.* L. americanus' did not infect plants maintained at night/day temperature conditions ~~of27/32~~ of 27/32 °C, but infection by '*Ca.* L. asiaticus' occurred at all the studied temperatures.	P	**COSAVE** To give clarification. Better do not compare species but to provide information regarding the temperature range of each species separatelly. *Category : TECHNICAL*
35	49	**'*Candidatus* Liberibacter americanus'** was described as a new species when it was first found in 2004 in São Paulo, Brazil (Teixeira *et al.*, 2005a, 2005b, 2005c; Bové, 2006). It is also transmitted by *Diaphorina citri* (Yamamoto *et al*., 2006). '*Ca.* L. americanus' is less heat tolerant than '*Ca.* L. asiaticus'. *et al.*) found that temperatures above ~~32 °C~~ 32°C negatively affected the multiplication of '*Ca.* L. americanus' in infected plants, whereas '*Ca*. L. asiaticus' was affected only by temperatures above ~~38 °C~~38°C. Similarly, Gasparoto *et al.* (2012) found that '*Ca*. L. americanus' did not infect plants maintained at night/day temperature conditions ~~of27/32 °C~~of 27/32°C, but infection by '*Ca.* L. asiaticus' occurred at all the studied temperatures.	P	**EPPO** Typos (3 spaces deleted and a space added). *Category : EDITORIAL*
36	49	**'*Candidatus* Liberibacter americanus'** was described as a new species when it was first found in 2004 in São Paulo, Brazil (Teixeira *et al.*, 2005a, 2005b, 2005c; Bové, 2006). It is also transmitted by *Diaphorina citri* (Yamamoto *et al*., 2006). '*Ca.* L. americanus' is ~~less~~ heat ~~tolerant than '~~sensitive, with cell multiplication in plant tissues partially affected at 32ºC and highly affected at 35ºC and 38ºC (Lopes	P	**Uruguay** To give clarification. Better do not compare species but to provide information regarding the temperature range of each species separatelly *Category : TECHNICAL*

		et al., 2009a)~~*Ca.* L. asiaticus'~~. Similarly, Gasparoto~~*et al.*) found that temperatures above 32 °C negatively affected the multiplication of '*Ca.* L. americanus' in infected plants, whereas '*Ca.* L. asiaticus' was affected only by temperatures above 38 °C. Similarly, Gasparoto~~ *et al.* (2012) found that '*Ca.* L. americanus' did not infect plants maintained at night/day temperature conditions ~~of27/32~~ of 27/32 °C, but infection by '*Ca.* L. asiaticus' occurred at all the studied temperatures.		
37	49	**'*Candidatus* Liberibacter americanus'** was described as a new species when it was first found in 2004 in São Paulo, Brazil (Teixeira *et al.*, 2005a, 2005b, 2005c; Bové, 2006). It is also transmitted by *Diaphorina citri* (Yamamoto *et al*., 2006). '*Ca.* L. americanus' is ~~less~~ heat ~~tolerant than~~ 'sensitive, with cell multiplication in plant tissues partially affected at 32°C and highly affected at 35 and 38°C (Lopes et al., 2009a).~~*Ca.* L. asiaticus'.~~ Similarly, Gasparoto~~*et al.*) found that temperatures above 32 °C negatively affected the multiplication of '*Ca.* L. americanus' in infected plants, whereas '*Ca.* L. asiaticus' was affected only by temperatures above 38 °C. Similarly, Gasparoto~~ *et al.* (2012) found that '*Ca.* L. americanus' did not infect plants maintained at night/day temperature conditions of27/32 °C, but infection by '*Ca.* L. asiaticus' occurred at all the studied temperatures.	P	**Brazil** To give clarification. Better do not compare species but to provide information regarding the temperature range of each species separatelly *Category : SUBSTANTIVE*
38	50	Huanglongbing is a disease ~~limited to~~ that affects *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C.* ×*aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella japonica*) (kumquat), *C. medica* (citrons), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya paniculata* (Garnier *et al.*, 2000, Lopes *et al.*, 2010) and *Atalantia* (syn. *Severinia*) *buxifolia* (Deng *et al*., 2008) also harbour '*Ca.* Liberibacter' species and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., 2000), Jagoueix *et al*., 1996). Other hosts may be viewed at .	P	**Australia** Suggest wording change to remove statement that HLB only affects Citrus and genera under Rutaceae. This is due to HLB having been detected in Plants i.e. tobacco/tomato of other families, outside of Rutaceae and is hence not limited to Citrus and other genera of Rutaceae. Reference: https://www.horticulture.com.au/globalassets/hort-innovation/resource-assets/ny11001-huanglongbing.pdf *Category : TECHNICAL*
39	50	Huanglongbing is a disease limited to *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C.* ×*aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella* ~~*japonica*~~*japonica* ~~)~~ (kumquat), *C. medica* (citrons), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya paniculata* (Garnier *et al.*, 2000, Lopes *et al.*, 2010) and *Atalantia* (syn. *Severinia*) *buxifolia* (Deng *et al*., 2008) also	P	**Colombia** It is important to mention in the text the approximate incubation period of HLB in the plant and its uneven distribution in the tree. This information is of great importance when sampling and detecting HLB. This paragraph can go after paragraph 50 attached or separated. Consequently, the sampling of each citrus tree or other species should be the most representative possible of its leaf area to

		harbour '*Ca.* Liberibacter' species and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., 2000), Jagoueix *et al*., 1996). Other hosts may be viewed at . The incubation period for HLB within citrus trees ranges from a few months to one or more years (Gottwald, 2010). At about 3 months after inoculation, Clas was detected in 70% of inoculated sweetorange and grapefruit seedlings (Folimonova & Achor, 2010), and severe asymmetrical yellowing of leaves was clearly observed 5–6 months after grafting. In a similar study (Coletta-Filho et al., 2010), Clas bacterium was detected in 60% of the 'Valencia' trees 1 month after inoculation, and typical HLB symptoms (chlorosis of leaves) were observed 6–8 months after inoculation. Quantification of the bacterium using qPCR showed that the Clas bacterium was present in differentparts of the infected plant; however, it was unevenly distributed (Tatineni et al., 2008). Consequently, the sampling of each citrus tree or other species should be the most representative possible of its leaf area to increase the probability of detecting and identifying the causative agent of HLB in each laboratory analysis.		increase the probability of detecting and identifying the causative agent of HLB in each laboratory analysis. *Category : TECHNICAL*
40	50	Huanglongbing is a disease limited to *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C.* ×*aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella japonica*) (kumquat), *C. medica* (citrons), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya paniculata* (Garnier *et al.*, 2000, Lopes *et al.*, ~~2010)~~2010, Cifuentes Arenas et. al., 2019) and *Atalantia* (syn. *Severinia*) *buxifolia* (Deng *et al*., 2008) also may harbour '*Ca.* Liberibacter' species but at lower titers than in citrus plants, and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., ~~2000)~~2000, Jagoueix *et al*., 1996). Other hosts may be viewed at .	P	**COSAVE** To improve information. "may harbour", because not always it happens. *Category : TECHNICAL*
41	50	Huanglongbing is a disease limited to *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C.* ×*aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella japonica*) (kumquat), *C. medica* (citrons), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya paniculata* (Garnier *et al.*, 2000, Lopes *et al.*, 2010) and	C	**EPPO** Links to the other species should be added as well as the link is only on Candidatus Liberibacter asiaticus https://gd.eppo.int/taxon/LIBEAF/hosts https://gd.eppo.int/taxon/LIBEAM/hosts *Category : TECHNICAL*

		Atalantia (syn. *Severinia*) *buxifolia* (Deng *et al*., 2008) also harbour '*Ca.* Liberibacter' species and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., 2000), Jagoueix *et al*., 1996). Other hosts may be viewed at .		
42	50	Huanglongbing is a disease limited to *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C.* ×*aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella japonica*) (kumquat), *C. medica* (citrons), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya ~~paniculata~~ paniculata* (Garnier *et al.*, 2000~~,~~; Lopes *et al.*, 2010) and *Atalantia* (syn. *Severinia*) *buxifolia*~~–~~ (Deng *et al*., 2008) also harbour '*Ca.* Liberibacter' species and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., ~~2000)~~2000;~~,~~ Jagoueix *et al*., 1996). Other hosts may be viewed at .	P	**EPPO** Typos. *Category : EDITORIAL*
43	50	Huanglongbing is a disease limited to *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C. ~~×aurantium~~ aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella japonica*) (kumquat), *C. medica* (~~citrons)~~citron), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya paniculata* (Garnier *et al.*, 2000, Lopes *et al.*, 2010) and *Atalantia* (syn. *Severinia*) *buxifolia*~~–~~ (Deng *et al*., 2008) also harbour '*Ca.* Liberibacter' species and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., 2000), Jagoueix *et al*., 1996). Other hosts may be viewed at .	P	**New Zealand** add space to align with the format of other names. needs to be singular to align with the other common names remove additional space *Category : EDITORIAL*
44	50	Huanglongbing is a disease limited to *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C.* ×*aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella japonica*) (kumquat), *C. medica* (citrons), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya paniculata* (Garnier *et al.*, 2000, Lopes *et al.*, ~~2010)~~2010, Cifuentes Arenas et. al., 2019) and *Atalantia* (syn. *Severinia*) *buxifolia* (Deng *et al*., 2008) also may harbour '*Ca.* Liberibacter' species but at lower titers	P	**Uruguay** To improve information. "may harbour", because not always it happens *Category : TECHNICAL*

		than in citrus plants, and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., ~~2000)~~2000, Jagoueix *et al*., 1996). Other hosts may be viewed at .		
45	50	Huanglongbing is a disease limited to *Citrus* and a few other genera of Rutaceae. The disease is present in *C. aurantiifolia* (lime), *C.* ×*aurantium* (sour orange), *C. limonia* Osbeck (Rangpur lime), *C. limon* L. (lemon), *C. limettioides* (Palestinian sweet lime), *C. japonica* (syn. *Fortunella japonica*) (kumquat), *C. medica* (citrons), *C. paradisi* (grapefruit), *C. paradisi* × *C. reticulata* (tangelo), *C. reticulata* (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *Poncirus trifoliata* (trifoliate orange) (da Graça, 1991). The rutaceous trees *Calodendrum capense* (Cape chestnut), *Murraya paniculata* (Garnier *et al.*, 2000, Lopes *et al.*, ~~2010)~~2010, Cifuentes Arenas *et al*., 2019) and *Atalantia* (syn. *Severinia*) *buxifolia* (Deng *et al*., 2008) also may harbour '*Ca.* Liberibacter' species but at lower titers than in citrus plants, and support populations of *Trioza erytreae* and *Diaphorina citri* (Garnier *et al*., 2000), Jagoueix *et al*., 1996). Other hosts may be viewed at .	P	**Brazil** To improve information. MAY HARBOUR: Because not always it happens. *Category : SUBSTANTIVE*
46	51	The psyllids reported as being the vectors of the HLB agents persist and multiply on other rutaceous plants including *A. buxifolia*, *Atalantia missionis*, *Citrus inodora*, *Citrus* ×*virgata* Mabb 'Sydney Hybrid', *Citropsis gabunensis*, *Citropsis schweinfurthii*, *Clausena anisum-olens*, *Limonia acidissima*, *Naringi crenulata* (Barkley and Beattie, 2008), *Swinglea glutinosa* (Garnier and Bové, 1993)~~, –~~ , and *Vepris lanceolata* (Gottwald *et al*., 2007).	P	**EPPO** Typo (one space deleted) *Category : EDITORIAL*
47	51	The psyllids reported as being the vectors of the HLB agents persist and multiply on other rutaceous plants including *A. buxifolia*, *Atalantia missionis*, *Citrus inodora*, *Citrus* ×*virgata* Mabb 'Sydney Hybrid', *Citropsis gabunensis*, *Citropsis schweinfurthii*, *Clausena anisum-olens*, *Limonia acidissima*, *Naringi crenulata* (Barkley and Beattie, 2008), *Swinglea glutinosa* (Garnier and Bové, 1993), and *Vepris lanceolata* (Gottwald *et al*., 2007).	C	**EPPO** For more information, see Table 2 & 3 p23-24 in the HLB PRA from Anses: https://www.anses.fr/fr/system/files/SANTVEG2016SA0235Ra.pdf *Category : TECHNICAL*
48	52	To date, psyllids are the only group of insects known to transmit '*Ca.* Liberibacter' spp. (Cen *et al*., 2012). The bacterium can multiply in the body of the insect vectors (Aubert, 1987 and Jagoueix, Bové and Garnier, 1997). Pelz-Stelinsky *et al*. (2010) reported that transmission of '*Ca.* L. asiaticus' from parent to offspring (transovarial) occurred at a rate of 2–6%, as opposed to absence of transovarial transmission reported by Hung et al. (2004) .	P	**COSAVE** To improve and update the information. *Category : TECHNICAL*
49	52	To date, psyllids are the only group of insects known to transmit '*Ca.* Liberibacter' spp. (Cen *et al*., 2012). The bacterium can multiply in the body of the insect vectors (Aubert, ~~1987 and~~ 1987; Jagoueix, Bové and Garnier, 1997). Pelz-Stelinsky *et al*. (2010) reported that transmission of '*Ca.* L. asiaticus' from parent to offspring (transovarial) occurred at a rate of 2–6%.	P	**EPPO** Typo. *Category : EDITORIAL*

50	52	To date, psyllids are the only group of insects known to transmit '*Ca.* Liberibacter' spp. (Cen *et al*., 2012). The bacterium can multiply in the body of the insect vectors (Aubert, 1987 and Jagoueix, Bové and Garnier, 1997). Pelz-Stelinsky *et al*. (2010) reported that transmission of '*Ca.* L. asiaticus' from parent to offspring (transovarial) occurred at a rate of 2–6%, as opposed to absence of transovarial transmission reported by Hung et al. (2004).	P	**Uruguay** To improve and update the information *Category : TECHNICAL*
51	52	To date, psyllids are the only group of insects known to transmit '*Ca.* Liberibacter' spp. (Cen *et al*., 2012). The bacterium can multiply in the body of the insect vectors (Aubert, 1987 and Jagoueix, Bové and Garnier, 1997). Pelz-Stelinsky *et al*. (2010) reported that transmission of '*Ca.* L. asiaticus' from parent to offspring (transovarial) occurred at a rate of ~~2–6%~~2–6% as opposed to absence of transovarial transmission reported by Hung et al. (2004).	P	**Brazil** To improve and update the information *Category : SUBSTANTIVE*
2. Taxonomic information				
52	55	**Synonym:** '*Candidatus* Liberobacter africanus' Jagoueix *et al.*, 1994	C	**EPPO** EPPO has as other scientific names Liberibacter africanum, Liberibacter africanus *Category : TECHNICAL*
53	55	**Synonym:** '*Candidatus* Liberobacter africanus' Jagoueix *et al.*, 1994	C	**New Zealand** The Jagoueix et al. Paper called these Liberobacter africanum and asiaticum and this is mentioned below. However, the text below discusses the i/o change in Liberibacter but not the us/um change for africanum. *Category : TECHNICAL*
54	56	**Name:** '*Candidatus* Liberibacter americanus' Texeira *et al.*, 2005	C	**EPPO** EPPO has as other scientific name Liberibacter americanus *Category : TECHNICAL*
55	58	**Synonym:** '*Candidatus* Liberobacter asiaticus' Jagoueix *et al.*, 1994	C	**EPPO** EPPO has as other scientific names Liberibacter asiaticum, Liberibacter asiaticus *Category : TECHNICAL*
56	59	**Taxonomic position:** Bacteria, Proteobacteria, Alpha-Proteobacteria, Rhizobiales, ~~Rhizobiacea~~Phyllobacteriaceae	P	**China** The taxonomic status of 'Candidatus Liberibacter' spp. was phyllobacteriaeae. *Category : SUBSTANTIVE*
57	60	**Disease names:** Huanglongbing (HLB) or citrus greening. The common name "huanglongbing" is currently widely adopted in the scientific literature (CABI, 2021).	C	**EPPO** It would be interesting to indicate the translation of Huanglongbing: "Yellow shoot disease". Citrus greening is not recognize anymore as the name of the disease. *Category : TECHNICAL*
58	61	In 1994, the International Committee for Systematic Bacteriology recommended that, as proposed by Murray and Schleifer (1994), a '*Candidatus*' designation be used as an interim taxonomic status, to provide a proper allocation of sequence-based potential new taxa at the genus and species level (Murray and Schleifer,	P	**Australia** Suggest deleting sentence as including the subspecies information is not essential and might be misleading due to the exact taxonomic position of these subspecies in relation to Laf not being fully clarified.

		1994; Murray and Stackebrant, 1995). Jagoueix, Bové and Garnier (1994) proposed that this new group in the alpha subdivision of the Proteobacteria should be referred to by the name "liberobacter" (from the Latin *liber* [bark] and *bacter* [bacteria]). Subsequently, two "*Candidatus* species", Liberobacter asiaticum and Liberobacter africanum, were recognized based on polymorphism in the 16S rDNA nucleotide sequences. Later, the spelling was corrected to 'Liberibacter' to conform to the Latin convention of using the connecting vowel "i" rather than "o" (Garnier *et al*., 2000)~~. Garnier .*et al.* (2000) proposed, based on serological differences and phylogenetic analysis, the name I'*Ca.* Liberibacter africanus subsp. capensis' for an isolate obtained from *Calodendrum capense*. I~~n 2004, a new species was discovered in Brazil that failed to amplify with primers designed for '*Ca.* Liberibacter asiaticus' and '*Ca.* Liberibacter africanus' in PCR and the new strain was named '*Ca.* L. americanus' Teixeira *et al.* (2005c).		*Category : TECHNICAL*
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60	61	In 1994, the International Committee for Systematic Bacteriology recommended that, as proposed by Murray and Schleifer (1994), a '*Candidatus*' designation be used as an interim taxonomic status, to provide a proper allocation of sequence-based potential new taxa at the genus and species level (Murray and Schleifer, 1994; Murray and Stackebrant, 1995). Jagoueix, Bové and Garnier (1994) proposed that this new group in the alpha subdivision of the Proteobacteria should be referred to by the name ~~"liberobacter"~~ "Liberobacter" (from the Latin *liber* [bark] and *bacter* [bacteria]). Subsequently, two "*Candidatus* species",	P	**EPPO** Typo. *Category : EDITORIAL*

		Liberobacter asiaticum and Liberobacter africanum, were recognized based on polymorphism in the 16S rDNA nucleotide sequences. Later, the spelling was corrected to 'Liberibacter' to conform to the Latin convention of using the connecting vowel "i" rather than "o" (Garnier *et al*., 2000). Garnier *et al.* (2000) proposed, based on serological differences and phylogenetic analysis, the name '*Ca.* Liberibacter africanus subsp. capensis' for an isolate obtained from *Calodendrum capense*. In 2004, a new species was discovered in Brazil that failed to amplify with primers designed for '*Ca.* Liberibacter asiaticus' and '*Ca.* Liberibacter africanus' in PCR and the new strain was named '*Ca.* L. americanus' Teixeira *et al.* (2005c).		
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62	61	In 1994, the International Committee for Systematic Bacteriology recommended that, as proposed by Murray and Schleifer (1994), a '*Candidatus*' designation be used as an interim taxonomic status, to provide a proper allocation of sequence-based potential new taxa at the genus and species level (Murray and Schleifer, 1994; Murray and Stackebrant, 1995). Jagoueix, Bové and Garnier (1994) proposed that this new group in the alpha subdivision of the Proteobacteria should be referred to by the name "liberobacter" (from the Latin *liber* [bark] and *bacter* [bacteria]). Subsequently, two "*Candidatus* species", Liberobacter asiaticum and Liberobacter africanum, were recognized based on polymorphism in the 16S rDNA nucleotide sequences. Later, the spelling was corrected to 'Liberibacter' to conform to the Latin convention of using the connecting vowel "i" rather than "o" (Garnier	P	**Uruguay** For better clarification. Species rather than strain, because Candidatus are species, not strains *Category : TECHNICAL*

		et al., 2000). Garnier *et al.* (2000) proposed, based on serological differences and phylogenetic analysis, the name '*Ca.* Liberibacter africanus subsp. capensis' for an isolate obtained from *Calodendrum capense*. In 2004, a new species was discovered in Brazil that the target DNA failed to amplify with primers designed for '*Ca.* Liberibacter asiaticus' and '*Ca.* Liberibacter africanus' in PCR and the new ~~strain~~ species was named '*Ca.* L. americanus' Teixeira *et al.* (2005c).		
63	61	In 1994, the International Committee for Systematic Bacteriology recommended that, as proposed by Murray and Schleifer (1994), a '*Candidatus*' designation be used as an interim taxonomic status, to provide a proper allocation of sequence-based potential new taxa at the genus and species level (Murray and Schleifer, 1994; Murray and Stackebrant, 1995). Jagoueix, Bové and Garnier (1994) proposed that this new group in the alpha subdivision of the Proteobacteria should be referred to by the name "liberobacter" (from the Latin *liber* [bark] and *bacter* [bacteria]). Subsequently, two "*Candidatus* species", Liberobacter asiaticum and Liberobacter africanum, were recognized based on polymorphism in the 16S rDNA nucleotide sequences. Later, the spelling was corrected to 'Liberibacter' to conform to the Latin convention of using the connecting vowel "i" rather than "o" (Garnier *et al*., 2000). Garnier *et al.* (2000) proposed, based on serological differences and phylogenetic analysis, the name '*Ca.* Liberibacter africanus subsp. capensis' for an isolate obtained from *Calodendrum capense*. In 2004, a new species was discovered in Brazil that the target DNA failed to amplify with primers designed for '*Ca.* Liberibacter asiaticus' and '*Ca.* Liberibacter africanus' in PCR and the new ~~strain~~ specie was named '*Ca.* L. americanus' Teixeira *et al.* (2005c).	P	**Brazil** For better clarification. Species rather than strain, because Candidatus are species, not strains. *Category : SUBSTANTIVE*
3. Detection				
64	63	Huanglongbing was diagnosed in the late twentieth century by conventional procedures such as electron microscopic examination and by bioassays on indicator plants. The '*Candidatus* Liberibacter' species associated with HLB have not yet been cultured *in vitro*, but methods based on the polymerase chain reaction (PCR) amplification of sequences from genes, such as the 16S ribosomal (r)RNA gene and the *rplKAJL-rpoBC* gene cluster, are considered efficient and sensitive for the detection of liberibacters in HLB-infected plant tissue and psyllids.	C	**Costa Rica** It would be convenient to specify the reference that corroborates the indicated. *Category : SUBSTANTIVE*
65	63	Huanglongbing was diagnosed in the late twentieth century by ~~conventional~~ procedures such as electron microscopic examination and by bioassays on indicator plants. The '*Candidatus* Liberibacter' species associated with HLB have not yet been cultured *in vitro*, but methods based on the polymerase chain reaction (PCR) amplification of sequences from genes, such as the 16S ribosomal (r)RNA gene (Li et al, 2006) and the *rplKAJL-rpoBC* gene ~~cluster~~cluster (Hocquellet et al., 1999;	P	**EPPO** As for LAMP test below references should be given. *Category : TECHNICAL*

		Teixera et al., 2005), are considered efficient and sensitive for the detection of liberibacters in HLB-infected plant tissue and psyllids.		
66	63	Huanglongbing was diagnosed in the late twentieth century by conventional procedures such as electron microscopic examination and by bioassays on indicator plants. The '*Candidatus* Liberibacter' species associated with HLB have not yet been cultured *in vitro*, but methods based on the polymerase chain reaction (PCR) amplification of sequences from genes, such as the 16S ribosomal (r)RNA gene and the *rplKAJL-rpoBC* gene cluster, are considered efficient and sensitive for the detection of liberibacters in HLB-infected plant tissue and psyllids.	C	**EPPO** Regarding the issue of culture of these bacteria, the revised EPPO DP makes the following statement Historically 'Ca. Liberibacter' species were considered non-culturable bacteria. Although, four reports (Davis et al., 2008; Sechler et al., 2009; Ha et al., 2019; Mandadi et al., 2020) refer to the cultivation of HLB-related 'Ca. Liberibacter' species, this needs more confirmation. We believe that this reflects better the reality of the state of the art. *Category : SUBSTANTIVE*
67	63	Huanglongbing was diagnosed in the late twentieth century by conventional procedures such as electron microscopic examination and ~~by~~ bioassays on indicator plants. The '*Candidatus* Liberibacter' species associated with HLB have not yet been cultured *in vitro*, but methods based on the polymerase chain reaction (PCR) amplification of sequences from genes, such as the 16S ribosomal (r)RNA gene and the *rplKAJL-rpoBC* gene cluster, are ~~considered~~ efficient and sensitive for the detection of liberibacters in HLB-infected plant tissue and psyllids.	P	**New Zealand** *Category : EDITORIAL*
68	64	The use of PCR to detect '*Ca.* Liberibacter' spp. in a vector is a very useful tool for surveillance because it allows detection of the pathogen in the insect before the appearance of the ~~symptoms~~symptoms in the plant. Indeed, Nguyen, Le and Nguyen (2003) showed that HLB-infected psyllids contain a higher titre of the bacterium than HLB-infected plant tissue. . Molecular detection is the method that may detect the bacterium in a single adult or in the third, fourth and fifth instars of the psyllid (Manjunath *et al*., 2008). ~~Nguyen, Le and Nguyen (2003) showed that HLB-infected psyllids contain a higher titre of the bacterium than HLB-infected plant tissue.~~	P	**EPPO** Sentence better placed here *Category : TECHNICAL*
69	64	The use of PCR to detect '*Ca.* Liberibacter' spp. in a vector is ~~a~~ very useful ~~tool~~ for surveillance because it allows detection of the pathogen in the insect before the appearance of ~~the~~ symptoms. Molecular detection is the method that may detect the bacterium in a single adult or in the third, fourth and fifth instars of the psyllid (Manjunath *et al*., 2008). Nguyen, Le and Nguyen (2003) showed that HLB-infected psyllids contain a higher titre of the bacterium than HLB-infected plant tissue.	P	**New Zealand** not necessary to describe PCR as a tool *Category : EDITORIAL*
70	65	Loop mediated isothermal amplification (LAMP) has been adapted for the sensitive detection of '*Ca.* L. asiaticus' (Okuda *et al.*, 2005~~,~~; Rigano *et al.*, 2014; Keremane	P	**EPPO** Two typos. *Category : EDITORIAL*

		et al., 2015; Choi *et al.*, 2018). Such LAMP-based methods are performed at a constant temperature, can be used on crude DNA extractions, and have shown promise for on-site diagnostics. However, these methods have not yet been well validated for routine diagnosis of '*Ca.* L~~.~~. asiaticus' and hence are not included in this diagnostic protocol.		
71	65	~~Loop-mediated~~ Loop-mediated isothermal amplification (LAMP) has been adapted for the sensitive detection of '*Ca.* L. asiaticus' (Okuda *et al.*, 2005, Rigano *et al.*, 2014; Keremane *et al.*, 2015; Choi *et al.*, 2018). Such LAMP-based methods are performed at a constant temperature, can be used on crude DNA extractions, and have shown promise for on-site diagnostics. However, these methods have not yet been well validated for routine diagnosis of '*Ca.* L. asiaticus' and hence are not included in this diagnostic protocol.	P	**New Zealand** compound modifier needed *Category : EDITORIAL*
72	66	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.The following diagram allows laboratories to be given tools, using various methodologies referenced in the document to arrive at the detection and identification of the causative agent of HLB disease.Detection for asymptomatic and symptomatic in citrus plant tissue and insect vector samplesOption 1Generic molecular tests for Ca. Liberibacter spp. (Section 3.4.4.1). Real- time PCRResults. Positive (Option 2). Negative (not detected).Option 2More specific molecular methods for the detection of Ca. Liberibacter species. (Section 3.4.3 and 3.4.4). Conventional and real-time PCRResults. Positive (Ca. Liberibacter Las, Laf and/or Lam detected -Convencional PCR (using purified DNA) based in different gene for Las, Laf and/or Lam.). Negative (not detected). IDENTIFICATION Sequence of the amplified products and sequence analysis. (Section 4).Results. Positive (Ca. Liberibacter Las, Laf and/or Lam confirmed). Negative (not confirmed).[1] Fig. 1 Flow diagram for the detection of 'Ca. Liberibacter' spp. in samples. Laf = 'Ca. Liberibacter africanus', Lam = 'Ca. Liberibacter americanus' and Las = 'Ca. Liberibacter asiaticus'.[2] * Identification may not be necessary for all samples that test positive for Ca. Liberibacter spp., on certain circumstances, for example, in areas facing a confirmed outbreak of Ca. Liberibacter spp.	P	**Colombia** Flow chart for a better understanding of the detection of the pathogen. The protocol has several techniques for the detection of Ca. Liberibacter spp., So it is necessary to guide the researcher in the detection options for the pathogen. The diagram allows laboratories to be given tools, using various methodologies referenced in the document to arrive at the detection and identification of the causative agent of HLB. THE SYSTEM DOES NOT ALLOW TO ENTER IMAGES SO IT WAS NOT POSSIBLE TO INCLUDE THE IMAGE. IS THERE ANY OTHER WAY WE CAN SEND IT? *Category : TECHNICAL*
3.1 Symptoms				
73	67	**3.1 Symptoms**	C	**Kenya** To include disease symptoms on citrus nursery stock (seedlings), and more information on the symptoms as they appear on the diseased fruits (for example, the colour change, hardness of the

				fruits or sponginess appearance on the fleshy part of the fruits after cutting. *Category : TECHNICAL*
74	68	Inspection is important for detection in symptomatic plants and is a routine method for the surveillance of HLB in areas where the disease is not ~~present~~present and a necessary measure to subsidize decision on erradicating deseased plants. Yellow shoots and blotchy mottle symptoms on leaves are typical symptoms on HLB-infected trees and can be used on site as part of an initial diagnosis. However, symptoms can be confused with nutritional disorders (zinc, iron or manganese deficiencies) or with other diseases (e.g. Australian citrus dieback ('*Candidatus* Phytoplasma' sp.), citrus blight, *Citrus tristeza virus*, stubborn disease of citrus ~~(~~(*Spiroplasma citri*) and gummosis (*Phytophthora* spp.)). '*Ca*. Liberibacter' spp. can also be present in the host plant at a very low concentration and can be unevenly distributed in the host plant, resulting in sparse symptoms that are easy to miss.	P	**COSAVE** To improve information *Category : SUBSTANTIVE*
75	68	Inspection is important for detection in symptomatic plants and is a routine method for the surveillance of HLB in areas where the disease is not present. Yellow shoots and blotchy mottle symptoms on leaves are typical symptoms on HLB-infected trees and can be used on site as part of an initial diagnosis. However, symptoms can be confused with nutritional disorders (zinc, iron or manganese deficiencies) or with other diseases (e.g. Australian citrus dieback ('*Candidatus* Phytoplasma' sp.), citrus blight, *Citrus tristeza virus*, stubborn disease of citrus ~~(~~(*Spiroplasma citri*)). '*Ca*. Liberibacter' spp. can also be present in the host plant at a very low concentration and ~~can be~~ is unevenly distributed in the host plant, resulting in sparse symptoms that are easy to miss.	P	**EPPO** Typo (one space deleted). This also have a great impact on disease detection, as one has to be careful when sampling plants, in order to get specific parts that are known to harbour high titre of HLB target cells. *Category : SUBSTANTIVE*
76	68	Inspection is important for detection in symptomatic plants and is a routine method for the surveillance of HLB in areas where the disease is not present. Yellow shoots and blotchy mottle symptoms on leaves are typical symptoms on HLB-infected trees and can be used on site as part of an initial diagnosis. However, symptoms can be confused with nutritional disorders (zinc, iron or manganese deficiencies) or with other diseases (e.g. Australian citrus dieback ('*Candidatus* Phytoplasma' sp.), citrus blight, *Citrus tristeza virus*, stubborn disease of citrus ~~(~~(*Spiroplasma citri*)). '*Ca*. Liberibacter' spp. can also be present in the host plant at a very low concentration and can be unevenly distributed in the host plant, resulting in sparse symptoms that are easy to miss.	P	**New Zealand** remove space *Category : EDITORIAL*
77	68	Inspection is ~~important for detection in symptomatic plants and is~~ a routine method for the surveillance of HLB both in areas where the ~~disease~~ pest is not present or where it is present, as a necessary measure for early detection and subsidize	P	**Brazil** For clarification and improve the information *Category : SUBSTANTIVE*

		decision on erradicating diseased plants. Yellow shoots and blotchy mottle symptoms on leaves are typical symptoms on HLB-infected trees and can be used on site as part of an initial diagnosis. However, symptoms can be confused with nutritional disorders (zinc, iron or manganese deficiencies) or with other diseases (e.g. Australian citrus dieback (‘*Candidatus* Phytoplasma’ sp.), citrus blight, *Citrus tristeza virus*, stubborn disease of citrus ~~(~~(*Spiroplasma citri*) and gummosis (*Phytophthora* spp.)). ‘*Ca*. Liberibacter’ spp. can also be present in the host plant at a very low concentration and can be unevenly distributed in the host plant, resulting in sparse symptoms that are easy to miss.		
78	69	Symptoms of HLB develop slowly. Infected trees gradually decline in vigour and yield and remain stunted or eventually die (Figure 1). The disease develops irregularly, so individual trees may show a mixture of normal and diseased sectors (Figure 2 and 3). This mixture within the same tree is a diagnostic characteristic (see).	C	**China** Pictures here in this ISPM are not typical and China NPPO would like to provide. *Category : SUBSTANTIVE*
79	70	Symptoms first appear as leaf yellowing, followed by mottling and chlorosis in one shoot or sector of the tree. Later, leaf symptoms resemble nutritional deficiencies (zinc, copper or nitrogen) but may vary depending on the bacterial ~~strain~~specie. The larger leaves on the base of branches turn yellow along the main and secondary veins and later change to a “blotchy mottle”, with the two halves of the leaf being asymmetrical in terms of the pattern of yellow and green. This is the most characteristic foliar symptom (see).	P	**COSAVE** Candidatus are species not strains *Category : TECHNICAL*
80	70	Symptoms first appear as leaf yellowing, followed by mottling and chlorosis in one shoot or sector of the tree. Later, leaf symptoms resemble nutritional deficiencies (zinc, copper or nitrogen) but may vary depending on the bacterial strain. The larger leaves on the base of branches turn yellow along the main and secondary veins and later change to a “blotchy mottle”, with the two halves of the leaf being asymmetrical in terms of the pattern of yellow and green. This is the most characteristic foliar symptom (see).	C	**EPPO** Is there a publication associated? *Category : TECHNICAL*
81	70	Symptoms first appear as leaf yellowing, followed by mottling and chlorosis in one shoot or sector of the tree. Later, leaf symptoms resemble nutritional deficiencies (zinc, copper or ~~nitrogen)~~ nitrogen), but may vary depending on the bacterial strain. The larger leaves on the base of branches turn yellow along the main and secondary veins and later change to a “blotchy mottle”, with the two halves of the leaf being asymmetrical in terms of the pattern of yellow and green. This is the most characteristic foliar symptom (see).	P	**EPPO** A comma added. *Category : EDITORIAL*
82	70	Symptoms first appear as leaf yellowing, followed by mottling and chlorosis in one shoot or sector of the tree. Later, leaf symptoms resemble nutritional deficiencies (zinc, copper or nitrogen) but may vary depending on the bacterial ~~strain~~species. The larger leaves on the base of branches turn yellow along the main and	P	**Uruguay** Candidatus are species not strains *Category : TECHNICAL*

		secondary veins and later change to a “blotchy mottle”, with the two halves of the leaf being asymmetrical in terms of the pattern of yellow and green. This is the most characteristic foliar symptom (see).		
83	70	Symptoms first appear as leaf yellowing, followed by mottling and chlorosis in one shoot or sector of the tree. Later, leaf symptoms resemble nutritional deficiencies (zinc, copper or nitrogen) but may vary depending on the bacterial ~~strain~~specie. The larger leaves on the base of branches turn yellow along the main and secondary veins and later change to a “blotchy mottle”, with the two halves of the leaf being asymmetrical in terms of the pattern of yellow and green. This is the most characteristic foliar symptom (see).	P	**Brazil** Candidatus are species not strains *Category : SUBSTANTIVE*
84	71	As the discoloration spreads away from the veins, the leaves become pale to light yellow with unevenly distributed dark green patches. Leaves on weak terminal twigs are small, upright and show a variety of chlorotic patterns. Infected fruits have a bitter and salty taste and a reduced Brix ratio, are smaller and of poor quality, often fail to develop normal fruit colour (colour inversion), and ~~can~~ often fall prematurely (see).	P	**EPPO** Revised change by bouhot-delduc on 11 Aug 2021 19:01 *Category : EDITORIAL*
85	71	As the discoloration spreads away from the veins, the leaves become pale to light yellow with unevenly distributed dark green patches. Leaves on weak terminal twigs are small, upright and show a variety of chlorotic patterns. Infected fruits have a bitter and salty taste and a reduced Brix acid ratio, are smaller and of poor quality, often fail to develop normal fruit colour (colour inversion), and can fall prematurely (see).	P	**New Zealand** This should be either a Brix acid ratio or, Brix value, not a Brix ratio. *Category : TECHNICAL*
86	72	The columella is curved, causing the fruit to be distorted and lopsided (see). Seeds in the affected fruit are usually ~~abortive~~aborted.	P	**COSAVE** Clarification *Category : TRANSLATION*
87	72	The columella is curved, causing the fruit to be distorted and lopsided (see). Seeds in the affected fruit are usually ~~abortive~~aborted.	P	**Uruguay** Clarification *Category : EDITORIAL*
88	72	The columella is curved, causing the fruit to be distorted and lopsided (see). Seeds in the affected fruit are usually ~~abortive~~aborted.	P	**Brazil** Clarification *Category : TRANSLATION*
3.2 Sampling and sample preparation				
89	75	Huanglongbing is a systemic disease of citrus, and ‘*Ca.* L. asiaticus’ has been detected in bark tissue, leaf midrib, roots, and different floral and fruit parts of infected citrus trees (Tatineni *et al.*, 2008).	C	**EPPO** These reference give more insights: 1. Li, W., L. Levy, and J. S. Hartung. 2009. Quantitative distribution of 'Candidatus Liberibacter asiaticus' in citrus plants with citrus huanglongbing. Phytopathology 99:139-144. 2. Louzada, E. S., O. E. Vazquez, W. E. Braswell, G. Yanev, M. Devanaboina, and M. Kunta. 2016. Distribution of 'Candidatus Liberibacter asiaticus' above and below ground in Texas citrus.

				Phytopathology 106:702-709. 3. Teixeira, D. C., C. Saillard, C. Couture, E. C. Martins, N. A. Wulff, S. Eveillard-Jagoueix, P. T. Yamamoto, A. J. Ayres, and J. M. Bové. 2008. Distribution and quantification of Candidatus Liberibacter americanus, agent of huanglongbing disease of citrus in Sao Paulo State, Brasil, in leaves of an affected sweet orange tree as determined by PCR. Mol. Cell. Probes 22:139-150. *Category : TECHNICAL*
3.2.1 Symptomatic material				
90	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). Tissue prints of the petioles or the basal part of the leaves on membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4°C and if no decay has occurred. The midribs of collected leaves are excised and processed for DNA extraction because the leaf midribs are enriched in phloem vessels and as consequence have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).	C	**EPPO** the same information is provided in sentence starting with 'The midribs.... it should be merged also with the other comment stating that it is the 2/3rd of the leave. *Category : TECHNICAL*
91	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). Tissue prints of the petioles or the basal part of the leaves on membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4°C and if no decay has occurred. The midribs of collected leaves are excised and processed for DNA extraction because the leaf midribs are enriched in phloem vessels and as consequence have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).	C	**EPPO** In the next paragraph some temperature indication is given. We believe this is for the cool box. Athough the temerature should be indicated as 'approximative' (see comment below) we believe the same information should be given here. *Category : TECHNICAL*
92	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). Tissue prints of the petioles or the basal part of the leaves on	C	**EPPO** Consider changing

		membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4°C and if no decay has occurred. The midribs of collected leaves are excised and processed for DNA extraction because the leaf midribs are enriched in phloem vessels and as consequence have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).		EPPO PM7/121 (2) states "Appropriate sample selection is critical for 'Ca. Liberibacter' spp. detection: each host tree should be sectioned into quadrants; each quadrant should be sampled to give a total of ~15 leaves per tree in order to get at least ~1 g of petiole and midribs from symptomatic or symptomless trees to be analyzed" NAPPO 2012 states "In symptomatic trees, samples are taken from 1 – 4 branches with symptomatic leaves or fruit." *Category : TECHNICAL*
93	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). Tissue prints of the petioles or the basal part of the leaves on membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4 °C and if no decay has occurred. The midribs of collected leaves are excised and processed for DNA extraction because the leaf midribs are enriched in phloem vessels and as consequence have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).	C	**EPPO** The first 2/3rd; and including the petiole. *Category : TECHNICAL*
94	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). Tissue prints of the petioles or the basal part of the leaves on membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at ~~4 °C~~ 4°C and if no decay has occurred. The midribs of collected leaves are excised and processed for DNA extraction because the leaf midribs are enriched in phloem vessels and as consequence have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*

		Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).		
95	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). In particular, the leaves should with typical symptoms. Tissue prints of the petioles or the basal part of the leaves on membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4 °C and if no decay has occurred. The midribs of collected leaves are excised and processed for DNA extraction because the leaf midribs are enriched in phloem vessels and as consequence have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).	P	**China** 'Ca. Liberibacter' spp. are unevenly distributed in the host plant, collecting symptomatic leaves can avoid missed inspections. *Category : TECHNICAL*
96	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). Tissue prints of the petioles or the basal part of the leaves on membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4 °C and if no decay has occurred. The midribs of collected leaves are excised and processed for DNA extraction because the leaf midribs are enriched in phloem vessels and ~~as consequence~~consequently have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).	P	**New Zealand** *Category : EDITORIAL*
97	77	An appropriate sample from a symptomatic tree consists of five to ten leaves (NAPPO, 2012). Tissue prints of the petioles or the basal part of the leaves on membranes can also be used, as described by Bertolini *et al.* (2014) and Siverio *et al.* (2017) (see section 3.4.1). The leaf samples are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves can be processed at any time up to three weeks after collection if kept in sealed plastic bags or other sealed containers at 4 °C and if no decay has occurred. The midribs of collected leaves are excised and	C	**Brazil** Shouldn't be at -4°C? *Category : TECHNICAL*

		processed for DNA extraction because the leaf midribs are enriched in phloem vessels and as consequence have a higher titre of '*Ca.* Liberibacter' cells. The older leaves and longer infected plants yield a higher titre of Liberibacter DNA (Nguyen, Le and Nguyen, 2003). There is also a higher titre of bacterial DNA in sieve tube cells of citrus leaf midribs than in lamina tissue (da Graça, 1991; Wang *et al.*, 2006).		
3.2.2 Asymptomatic material				
98	79	An appropriate sample from a symptomless tree consists of at least ten mature leaves collected from around the canopy of a tree (EPPO, 2014). For small trees (e.g. in a nursery), three to four leaves per tree are collected. The sampled leaves are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves should be kept in sealed plastic bags or sealed containers at approximately 4 °C and processed as soon as possible.	P	**EPPO** *Category : TECHNICAL*
99	79	An appropriate sample from a symptomless tree consists of at least ten mature leaves collected from around the canopy of a tree (EPPO, 2014). For small trees (e.g. in a nursery), three to four leaves per tree are collected. The sampled leaves are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves should be kept in sealed plastic bags or sealed containers at 4°C and processed as soon as possible.	C	**EPPO** Consider changing EPPO PM7/121 (2) states "Appropriate sample selection is critical for 'Ca. Liberibacter' spp. detection: each host tree should be sectioned into quadrants; each quadrant should be sampled to give a total of ~15 leaves per tree in order to get at least ~1 g of petiole and midribs from symptomatic or symptomless trees to be analyzed" NAPPO 2012 states "If symptoms are not present in a suspect tree, samples are taken from one year-old branches with 5 – 10 leaves from the upper portion of each of the four quadrants of the tree. If branches are not present, as in the case of small nursery trees, 1 – 12 mature leaves are taken from each tree" For the temperature 'approximately should be added before 4°C. precise temperatures can cause issues with accreditation bodies *Category : TECHNICAL*
100	79	An appropriate sample from a symptomless tree consists of at least ten mature leaves collected from around the canopy of a tree (EPPO, 2014). For small trees (e.g. in a nursery), three to four leaves per tree are collected. The sampled leaves are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves should be kept in sealed plastic bags or sealed containers at ~~4 °C~~ 4°C and processed as soon as possible.	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
101	79	An appropriate sample from a symptomless tree consists of at least ten mature leaves collected from around the canopy of a tree (EPPO, 2014). For small trees (e.g. in a nursery), three to four leaves per tree are collected. The sampled leaves	C	**Brazil** Shouldn't be at -4°C? *Category : TECHNICAL*

		are placed in a labelled plastic bag (one bag per tree), stored in a cool box while in the field, and refrigerated as soon as possible. Leaves should be kept in sealed plastic bags or sealed containers at 4 °C and processed as soon as possible.		
3.2.3 Psyllids				
102	80	**3.2.3 Psyllids**	C	**China** Add pictures about Psyllids and China NPPO would like to provide. *Category : SUBSTANTIVE*
103	81	The preparation of the specimen or specimens consists of placing the adults or nymphs in a labelled vial and then either processing them for DNA extraction immediately or preserving them in ~~70%~~ more than 95% ethanol. The insects may also be squashed onto membranes (see section 3.4.2).	P	**China** If the psyllids are used for DNA extraction, it is suggested to store them in more than 95% alcohol to ensure the success of subsequent DNA extraction *Category : SUBSTANTIVE*
3.3 Biological detection (graft transmission)				
104	83	Biological indexing ~~is a reliable technique~~ has proven value for '*Ca*. Liberibacter' species detection despite the low rate of graft transmission and is suitable as a screening test for use by diagnosticians who have experience with symptom observation. The indicators used commonly are *C. sinensis* (sweet orange) or *C. reticulata* (mandarin) for '*Ca*. L. asiaticus', sweet orange or (*Citrus* x *tangelo* (Orlando tangelo) for '*Ca*. L. africanus', and *C. sinensis* or *C. reticulata* × *C. sinensis* (Murcott tangor) for '*Ca*. L. americanus' (Lopes and Frare, 2008; NAPPO, 2012). *Catharanthus roseus* (periwinkle) may also be used: in this host, HLB can multiply and is present at a higher titre than in citrus plants after transmission by *Cuscuta campestris* (dodder) (Garnier and Bové, 1983), with the symptoms developing after three months at 25 °C (Bové, 2006; Nguyen, Le and Nguyen, , 2003).	P	**Australia** Biological indexing is not considered a reliable sole measure to be effective for managing the risk of HLB. Australia, for example, does not accept biological indexing as a sole measure to manage the risk of HLB associated with citrus nursery stock imports. Suggested reword is to avoid the assumption that biological indexing is the best method for 'Ca. Liberibacter' species detection. *Category : TECHNICAL*
105	83	Biological indexing is a reliable technique for '*Ca*. Liberibacter' species detection despite the low rate of graft transmission and is suitable as a screening test for use by diagnosticians who have experience with symptom observation. The indicators used commonly are *C. sinensis* (sweet orange) or *C. reticulata* (mandarin) for '*Ca*. L. asiaticus', sweet orange or ~~(~~*Citrus* x *tangelo* (Orlando tangelo) for '*Ca*. L. africanus', and *C. sinensis* or *C. reticulata* × *C. sinensis* (Murcott tangor) for '*Ca*. L. americanus' (Lopes and Frare, 2008; NAPPO, 2012). *Catharanthus roseus* (periwinkle) may also be used: in this host, HLB can multiply and is present at a higher titre than in citrus plants after transmission by *Cuscuta campestris* (dodder) (Garnier and Bové, 1983), with the symptoms developing after three months at ~~25 °C~~ 25°C (Bové, 2006; Nguyen, Le and Nguyen, ~~,~~ 2003).	P	**EPPO** Typos (one parenthesis, one space and one comma deleted). *Category : EDITORIAL*
106	83	Biological indexing is a reliable technique for '*Ca*. Liberibacter' species detection despite the low rate of graft transmission and is suitable as a screening test for use by diagnosticians who have experience with symptom observation. The indicators	C	**New Zealand** the font should be consistent with other scientific names *Category : EDITORIAL*

		used commonly are *C. sinensis* (sweet orange) or *C. reticulata* (mandarin) for '*Ca*. L. asiaticus', sweet orange or (*Citrus* x *tangelo* (Orlando tangelo) for '*Ca*. L. africanus', and *C. sinensis* or *C. reticulata* × *C. sinensis* (Murcott tangor) for '*Ca*. L. americanus' (Lopes and Frare, 2008; NAPPO, 2012). *Catharanthus roseus* (periwinkle) may also be used: in this host, HLB can multiply and is present at a higher titre than in citrus plants after transmission by *Cuscuta campestris* (dodder) (Garnier and Bové, 1983), with the symptoms developing after three months at 25 °C (Bové, 2006; Nguyen, Le and Nguyen, , 2003).		
107	84	There are several recommendations for selecting plant material for grafting onto indicator plants. According to Lopes *et al*. (2009), the best inoculum is from symptomatic branches (particularly those showing symptoms within the previous 12 months) that are suspected to be infected by any '*Ca.* Liberibacter' spp. The selected branch piece is cut into segments, each 2–4 cm long, and the segments are grafted onto the ~~opposite side~~ stem of the indicator ~~twig~~potted plant. After inoculation, the graft is protected with polyethylene tape, ~~the grafted indicator twig is protected with a polyethylene bag,~~ and the plants are maintained in a greenhouse. The grafted indicator plants are then inspected ~~regularly (one or twice a month)~~regularly. The first symptoms usually appear ~~at~~ three to four ~~or five~~ months after inoculation with a light yellowing of the mature apical ~~leaves (similar to~~leaf ~~manganese or iron deficiencies)~~ and progress to ~~the appearance of~~ blotchy ~~mottled leaves showing a diffuse~~ mottling (diffuse and asymmetrical ~~light chlorosis~~ chlorosis) and eventually vein thickning after 6 to 12 months (Lopes and Frare, 2008; EPPO, 2014).	P	**COSAVE** Better clarification of the method and symptoms. Information provided by the author. *Category : TECHNICAL*
108	84	There are several recommendations for selecting plant material for grafting onto indicator plants. According to Lopes *et al*. (2009), the best inoculum is from symptomatic branches (particularly those showing symptoms within the previous 12 months) that are suspected to be infected by any '*Ca.* Liberibacter' spp. The selected branch piece is cut into segments, each 2–4 cm long, and the segments are grafted onto the opposite side of the indicator twig. After inoculation, the graft is protected with polyethylene tape, the grafted indicator twig is protected with a polyethylene bag, and the plants are maintained in a greenhouse. The grafted indicator plants are then inspected regularly (one or twice a month). The first symptoms appear at four or five months after inoculation with yellowing of the apical leaves (similar to manganese or iron deficiencies) and progress to the appearance of blotchy mottled leaves showing a diffuse and asymmetrical light chlorosis after 6 to 12 months (Lopes and Frare, 2008; EPPO, 2014).	C	**EPPO** the EPPO Standard PM 7/121 recommends 3-5 cm. *Category : TECHNICAL*

109	84	There are several recommendations for selecting plant material for grafting onto indicator plants. According to Lopes *et al*. (2009), the best inoculum is from symptomatic branches (particularly those showing symptoms within the previous 12 months) that are suspected to be infected by any '*Ca.* Liberibacter' spp. The selected branch piece is cut into segments, each 2–4 cm long, and the segments are grafted onto the ~~opposite side~~ stem of the indicator ~~twig~~potted plant. After inoculation, the graft is protected with polyethylene tape, ~~the grafted indicator twig is protected with a polyethylene bag,~~ and the plants are maintained in a greenhouse. The grafted indicator plants are then inspected ~~regularly (one or twice a month)~~regularly. The first symptoms usually appear ~~at~~ three to four ~~or five~~ months after inoculation with a light yellowing of the mature apical ~~leaves (similar to~~leaf a ~~manganese or iron deficiencies) and~~ nd progress to the ~~appearance of~~ blotchy ~~mottled leaves showing a diffuse~~ mottling (diffuse and asymmetrical ~~light chlorosis~~ chlorosis) and eventually vein thickning after 6 to 12 months (Lopes and Frare, 2008; EPPO, 2014).	P	**Uruguay** Better clarification of the method and symptoms. Information provided by the author *Category : TECHNICAL*
110	84	There are several recommendations for selecting plant material for grafting onto indicator plants. According to Lopes *et al*. (2009), the best inoculum is from symptomatic branches (particularly those showing symptoms within the previous 12 months) that are suspected to be infected by any '*Ca.* Liberibacter' spp. The selected branch piece is cut into segments, each 2–4 cm long, and the segments are side grafted onto the ~~opposite side~~ stem of the indicator ~~twig~~potted plant. After inoculation, the graft is protected with polyethylene tape, ~~the grafted indicator twig is protected with a polyethylene bag,~~ and the plants are maintained in a greenhouse. The grafted indicator plants are then inspected ~~regularly (one or twice a month)~~regularly. The first symptoms usually appear ~~at~~ three to four ~~or five~~ months after inoculation with a light yellowing of the mature apical ~~leaves (similar to~~leaf ~~manganese or iron deficiencies)~~ and progress to ~~the appearance of~~ blotchy ~~mottled leaves showing a diffuse~~ mottling (diffuse and asymmetrical ~~light chlorosis~~ chlorosis) and eventually vein thickning after 6 to 12 months (Lopes and Frare, 2008; EPPO, 2014).	P	**Brazil** Better clarification of the method and symptoms. Information provided by the author *Category : TECHNICAL*
111	85	Leaf grafting is performed using a 3 × 12 mm section of the midrib part of the leaf, placed into a T-cut in the bark of an indicator seedling (Roistacher, 1991). The grafted plants are kept at 20–25 °C for '*Ca*. Liberibacter africanus' and at 25–32 °C for '*Ca*. Liberibacter asiaticus' (EPPO, 2014). It has been demonstrated that '*Ca*. L. asiaticus' is transmitted more efficiently than '*Ca*. L. americanus' and reaches a higher titre in the infected plant (Hall *et al*., ~~2012)~~2012) (Lopes et. al., 2009).	P	**COSAVE** Please, confirm Hall et al. 2012 *Category : EDITORIAL*

112	85	Leaf grafting is performed using a 3 × 12 mm section of the midrib part of the leaf, placed into a T-cut in the bark of an indicator seedling (Roistacher, 1991). The grafted plants are kept at ~~20–25 °C~~ 20–25°C for '*Ca*. Liberibacter africanus' and at ~~25–32 °C~~ 25–32°C for '*Ca*. Liberibacter asiaticus' (EPPO, 2014). It has been demonstrated that '*Ca*. L. asiaticus' is transmitted more efficiently than '*Ca*. L. americanus' and reaches a higher titre in the infected plant (Hall *et al*., 2012).	P	**EPPO** Typos (two spaces deleted). *Category : EDITORIAL*
113	85	Leaf grafting is performed using a ~~3~~ 3mm × 12 mm section of the midrib part of the leaf, placed into a T-cut in the bark of an indicator seedling (Roistacher, 1991). The grafted plants are kept at 20–25 °C for '*Ca*. Liberibacter africanus' and at 25–32 °C for '*Ca*. Liberibacter asiaticus' (EPPO, 2014). It has been demonstrated that '*Ca*. L. asiaticus' is transmitted more efficiently than '*Ca*. L. americanus' and reaches a higher titre in the infected plant (Hall *et al*., 2012).	P	**New Zealand** to reduce ambigulty *Category : EDITORIAL*
114	85	Leaf grafting is performed using a 3 × 12 mm section of the midrib part of the leaf, placed into a T-cut in the bark of an indicator seedling (Roistacher, 1991). The grafted plants are kept at 20–25 °C for '*Ca*. Liberibacter africanus' and at 25–32 °C for '*Ca*. Liberibacter asiaticus' (EPPO, 2014). It has been demonstrated that '*Ca*. L. asiaticus' is transmitted more efficiently than '*Ca*. L. americanus' and reaches a higher titre in the infected plant (Hall *et al*., ~~2012)~~2012, Lopes et al., 2009).	P	**Uruguay** Please confirm Hall et al, 2012 *Category : EDITORIAL*
115	85	Leaf grafting is performed using a 3 × 12 mm section of the midrib part of the leaf, placed into a T-cut in the bark of an indicator seedling (Roistacher, 1991). The grafted plants are kept at 20–25 °C for '*Ca*. Liberibacter africanus' and at 25–32 °C for '*Ca*. Liberibacter asiaticus' (EPPO, 2014). It has been demonstrated that '*Ca*. L. asiaticus' is transmitted more efficiently than '*Ca*. L. americanus' and reaches a higher titre in the infected plant (Hall *et al*., ~~2012)~~2012) (Lopes et al., 2009).	P	**Brazil** Please, confirm Hall et al. *Category : EDITORIAL*
3.4 Molecular detection				
116	87	Conventional PCR is relatively sensitive and specific, particularly when symtomatic samples are intended to be diagnosed. This test can lead to false negative results when ~~Although conventional PCR is relatively sensitive and specific, this test can lead to false negative results when~~ the concentration of the bacterium is too low to detect, for instance in newly infected trees with a low concentration and uneven distribution of the pathogen (Bové, 2006). Consequently, conventional PCR should only be used on plants exhibiting symptoms and is not reliable for the detection of '*Ca.* Liberibacter' spp. in symptomless plants. However, real-time PCR may be useful in programmes for the production of certified citrus nursery trees and in post-entry quarantine. Li *et al.* (2006), Teixeira	P	**COSAVE** For better clarification and improve information. *Category : TECHNICAL*

		et. al., 2008a and Bertolini *et al.* (2014) have reported that real-time PCR can detect '*Ca.* Liberibacter' spp. in symptomless samples of infected plants and is more convenient for early ~~detection~~detection than conventional PCR.		
117	87	~~Although conventional~~ Conventional PCR is relatively sensitive and specific, ~~this~~ particularly when symptomatic samples are intended to be diagnosed. This test can lead to false negative results when the concentration of the bacterium is too low to detect, for instance in newly infected trees with a low concentration and uneven distribution of the pathogen (Bové, 2006). Consequently, conventional PCR should only be used on plants exhibiting symptoms and is not reliable for the detection of '*Ca.* Liberibacter' spp. in symptomless plants. However, real-time PCR may be useful in programmes for the production of certified citrus nursery trees and in post-entry quarantine. Li *et al.* (2006) and Bertolini *et al.* (2014) have reported that real-time PCR can detect '*Ca.* Liberibacter' spp. in symptomless samples of infected plants and is more convenient for early ~~detection~~detection than conventional PCR.	P	**Uruguay** For better clarification and improve information *Category : TECHNICAL*
118	87	Conventional PCR is relatively sensitive and specific, particularly when symptomatic samples are intended to be diagnosed. This test can lead to false negative results when ~~Although conventional PCR~~ the concentration of the bacterium is ~~relatively sensitive and specific, this test can lead~~ too low to ~~false negative results when~~ detect, for instance in newly infected and assymptomatic trees with a low concentration and uneven distribution~~the concentration of the bacterium is too low to detect, for instance in newly infected trees with a low concentration and uneven distribution~~ of the pathogen (Bové, 2006). Consequently, conventional PCR should only be used on plants exhibiting symptoms and is not reliable for the detection of '*Ca.* Liberibacter' spp. in symptomless plants. However, real-time PCR may be useful in programmes for the production of certified citrus nursery trees and in post-entry quarantine. Li *et al.* (2006), Teixeira *et al.* (2008a) and Bertolini *et al.* (2014) have reported that real-time PCR can detect '*Ca.* Liberibacter' spp. in symptomless samples of infected plants and is more convenient for early ~~detection~~detection than conventional PCR.	P	**Brazil** For better clarification and improve information *Category : SUBSTANTIVE*
3.4.1 Nucleic acid extraction from plant material				
119	88	**3.4.1 Nucleic acid extraction from plant material**	C	**EPPO** A new and rapid method can be used for DNA extraction, and consists of grinding plant midribs and petioles with a 2% NaOH solution and diluting the liquid phase at 1/50 for molecular amplification. Validation data available and tested with Li et al. 2006 qPCR. Cf EPPO PM7/121 revised version *Category : TECHNICAL*

120	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% β-mercaptoethanols is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at 65 °C. The resulting extract is centrifugated at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and centrifugated at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at −20 °C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at −20 °C.	C	**COSAVE** A foot note associated with each brand name should be included as agreed in other DPs for example DP 26. The foot note should read: "The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable" *Category : TECHNICAL*
121	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% ~~β-mercaptoethanols~~ β-mercaptoethanol is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at 65 °C. The resulting extract is centrifugated at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and centrifugated at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at −20 °C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at −20 °C.	P	**EPPO** β-mercaptoethanol (witthout "s") *Category : EDITORIAL*
122	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% β-mercaptoethanols is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at 65°C. The resulting extract is centrifugated at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is	C	**EPPO** Add until use *Category : TECHNICAL*

		then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and centrifugated at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at −20°C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at −20°C.		
123	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% β-mercaptoethanols is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at 65°C. The resulting extract is centrifugated at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and centrifugated at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at −20°C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at −20°C.	C	**EPPO** (24:1 (v/v)) *Category : TECHNICAL*
124	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% β-mercaptoethanols is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at 65°C. The resulting extract is centrifugated at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and centrifugated at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at −20°C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at −20°C.	C	**EPPO** As it is written, it seems that the buffer is added after the homogenization of the sample. Instead, it should be present during homogenization. In addition, it should be specified that β-mercaptoethanol should be added to the buffer immediately before use *Category : TECHNICAL*

125	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% β-mercaptoethanols is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at ~~65 °C~~65°C. The resulting extract is centrifugated at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and centrifugated at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at ~~−20 °C~~ −20°C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at ~~−20 °C~~−20°C.	P	**EPPO** Typos (three spaces deleted). *Category : EDITORIAL*
126	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% β-mercaptoethanols is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at 65 °C. The resulting extract is ~~centrifugated~~ centrifuged at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and ~~centrifugated~~ centrifuged at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at −20 °C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at −20 °C.	P	**New Zealand** Both spellings exist, but centrifugated is rare *Category : EDITORIAL*
127	90	**CTAB extraction.** The plant tissue (500 mg midribs) is disrupted either by use of commercially available equipment (a Fastprep (MP Biomedicals) instrument or a Mini-Beadbeater (BioSpec) instrument) or manually by grinding with a mortar and pestle or by crushing the tissue in a plastic bag. Cetyl trimethyl ammonium bromide (CTAB) buffer (3 mL) containing 0.2% β-mercaptoethanols is added and stirred. After this, 2 mL homogenate is transferred to a microtube and incubated, if possible with shaking, for at least 15 min at 65 °C. The resulting extract is centrifugated at 3 000 *g* for 5 min in a microcentrifuge and 1 mL supernatant is	C	**Uruguay** A footnote associated with each brand name should be included as agreed in other DPs, for example DP 26. The footnote should read "The use of names of reagents, chemicals or equipment in this diagnostic protocol implies no approval of them to the exlusion of others that may also be suitable" *Category : TECHNICAL*

		then put in a 2 mL microtube with 1 mL chloroform-isoamyl alcohol solution (24:1), mixed and centrifugated at 14 000 *g* for 5 min. The aqueous phase is transferred to a new microtube, mixed with 0.6 volume of cold isopropanol and kept at −20 °C for 30 min before centrifugation at 14 000 *g* for 20 min. The supernatant is discarded and the pellet washed twice with 70% ethanol and resuspended in 100 µL sterile distilled water. The resulting extracts can be stored at −20 °C.		
128	91	**Commercial kits.** After using any of the disruption methods described above in relation to CTAB extraction, DNA extraction is carried out using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions.	C	**COSAVE** See comment in paragraph 90 *Category : TECHNICAL*
129	91	**Commercial kits.** After using any of the disruption methods described above in relation to CTAB extraction, DNA extraction is carried out using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions.	C	**EPPO** only one kit is mentioned. Delete 's' or should the DNeasy kit be mentioned as an example opening the possibility for other kits? *Category : TECHNICAL*
130	91	**Commercial kits.** After using any of the disruption methods described above in relation to CTAB extraction, DNA extraction is carried out using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions.	C	**Uruguay** See comment in paragraph 90 *Category : TECHNICAL*
131	92	**Plant tissue print.** The plant tissue print method is a rapid, direct method of sample preparation (Bertolini *et al*., 2008) that can be done under field conditions and has demonstrated its efficiency when combined with the real-time PCR detection method of Bertolini *et al*. (2014). The tissue print method is performed by pressing five to ten fresh, manually detached, citrus leaf petioles onto an area (0.5 cm^2) of a positively charged nylon or 3MM filter paper membrane (Bertolini *et al.*, 2008). The tissue printed membrane is cut out and inserted into microcentrifuge tubes containing either 100 µL distilled water, 0.5% Triton X-100 or glycine buffer (0.1 M glycine, 0.05 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA)). Samples are then incubated at 100°C at for 10 min as described by Bertolini *et al.* (2014), vortexed and placed on ice until use.	C	**EPPO** "…inserted, with tweezers, into microcentrifuge tubes…" *Category : TECHNICAL*
132	92	**Plant tissue print.** The plant tissue print method is a rapid, direct method of sample preparation (Bertolini *et al*., 2008) that can be done under field conditions and has demonstrated its efficiency when combined with the real-time PCR detection method of Bertolini *et al*. (2014). The tissue print method is performed by pressing five to ten fresh, manually detached, citrus leaf petioles onto an area (0.5 cm^2) of a positively charged nylon or 3MM filter paper membrane (Bertolini *et al.*, 2008). The tissue printed membrane is cut out and inserted into microcentrifuge tubes containing either 100 µL distilled water, 0.5% Triton X-100 or glycine buffer (0.1 M glycine, 0.05 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA)). Samples are then incubated at 100°C at for 10 min as described by Bertolini *et al.* (2014), vortexed and placed on ice until use.	C	**EPPO** From Bertolini et al., 2014: "Samples were then incubated at 100°C or at room temperature for 10 min, vortexed and placed on ice until use." EPPO EPPO PM7/121 (2), for plant material no incubation is reported. *Category : TECHNICAL*

133	92	**Plant tissue print.** The plant tissue print method is a rapid, direct method of sample preparation (Bertolini *et al*., 2008) that can be done under field conditions and has demonstrated its efficiency when combined with the real-time PCR detection method of Bertolini *et al*. (2014). The tissue print method is performed by pressing five to ten fresh, manually detached, citrus leaf petioles onto an area (0.5 cm^2) of a positively charged nylon or 3MM filter paper membrane (Bertolini *et al.*, 2008). The tissue printed membrane is cut out and inserted into microcentrifuge tubes containing either 100 µL distilled water, 0.5% Triton X-100 or glycine buffer (0.1 M glycine, 0.05 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA)). Samples are then incubated at 100 °C at for 10 min as described by Bertolini *et al.* (2014), vortexed and placed on ice until use.	C	**EPPO** delete *Category : EDITORIAL*
134	92	**Plant tissue print.** The plant tissue print method is a rapid, direct method of sample preparation (Bertolini *et al*., 2008) that can be done under field conditions and has demonstrated its efficiency when combined with the real-time PCR detection method of Bertolini *et al*. (2014). The tissue print method is performed by pressing five to ten fresh, manually detached, citrus leaf petioles onto an area (0.5 cm^2) of a positively charged nylon or 3MM filter paper membrane (Bertolini *et al.*, 2008). The tissue printed membrane is cut out and inserted into microcentrifuge tubes containing either 100 µL distilled water, 0.5% Triton X-100 or glycine buffer (0.1 M glycine, 0.05 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA)). Samples are then incubated at ~~100 °C~~ 100°C at for 10 min as described by Bertolini *et al.* (2014), vortexed and placed on ice until use.	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
3.4.2 Nucleic acid extraction from the psyllid vectors				
135	94	**Manjunath *et al*. (2008).** In this method, the psyllids (up to 50) are air-dried for 10 min, transferred to a 1.5 mL microtube containing 300 µL extraction buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 2% Sodium dodecyl sulphate) and 20 units of Proteinase K (New England Biolabs), ground finely and incubated either at 50 °C for 3 h or 37 °C overnight. An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) is added, vortexed and the aqueous phase transferred to a second tube containing 300 µL chloroform- isoamyl alcohol (24:1) and the extraction procedure is repeated. The aqueous phase is ethanol precipitated and the resulting DNA pellet is dissolved in 20–50 µL sterile water and stored at −20 °C.	C	**COSAVE** See comment in paragraph 90 *Category : TECHNICAL*
136	94	**Manjunath *et al*. (2008).** In this method, the psyllids (up to 50) are air-dried for 10 min, transferred to a 1.5 mL microtube containing 300 µL extraction buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 2% Sodium dodecyl sulphate) and 20 units of Proteinase K (New England Biolabs), ground finely and incubated either at ~~50 °C~~ 50°C for 3 h or ~~37 °C~~ 37°C overnight. An equal volume	P	**EPPO** Typos (three spaces deleted). *Category : EDITORIAL*

		of phenol-chloroform-isoamyl alcohol (25:24:1) is added, vortexed and the aqueous phase transferred to a second tube containing 300 µL chloroform- isoamyl alcohol (24:1) and the extraction procedure is repeated. The aqueous phase is ethanol precipitated and the resulting DNA pellet is dissolved in 20–50 µL sterile water and stored at ~~−20 °C~~20°C.		
137	94	**Manjunath *et al*. (2008).** In this method, the psyllids (up to 50) are air-dried for 10 min, transferred to a 1.5 mL microtube containing 300 µL extraction buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 2% Sodium dodecyl sulphate) and 20 units of Proteinase K (New England Biolabs), ground finely and incubated either at 50 °C for 3 h or 37 °C overnight. An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) is added, vortexed and the aqueous phase transferred to a second tube containing 300 µL chloroform–isoamyl alcohol (24:1) and the extraction procedure is repeated. The aqueous phase is ethanol precipitated and the resulting DNA pellet is dissolved in 20–50 µL sterile water and stored at −20 °C.	P	**New Zealand** remove space *Category : EDITORIAL*
138	94	**Manjunath *et al*. (2008).** In this method, the psyllids (up to 50) are air-dried for 10 min, transferred to a 1.5 mL microtube containing 300 µL extraction buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 2% Sodium dodecyl sulphate) and 20 units of Proteinase K (New England Biolabs), ground finely and incubated either at 50 °C for 3 h or 37 °C overnight. An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) is added, vortexed and the aqueous phase transferred to a second tube containing 300 µL chloroform- isoamyl alcohol (24:1) and the extraction procedure is repeated. The aqueous phase is ethanol precipitated and the resulting DNA pellet is dissolved in 20–50 µL sterile water and stored at −20 °C.	C	**Uruguay** See comment in paragraph 90 *Category : TECHNICAL*
139	95	**Bertolini *et al.* (2014).** In this method, individual psyllids are inmobilized and squashed on nylon or paper membranes with the bottom end of a microcentrifuge tube. Pieces of membrane harbouring the squashed samples are inserted into microtubes containing 100 µL distilled water, 0.5% Triton X-100 or glycine buffer (0.1 M glycine, 0.05 M NaCl, 1 mM EDTA). Samples are then incubated at 100°C for 10 min as described by Bertolini *et al.* (2014), vortexed and placed on ice until used for real-time PCR.	C	**EPPO** From Bertolini et al., 2014: "Samples were then incubated at 100°C or at room temperature for 10 min, vortexed and placed on ice until use." In EPPO EPPO PM7/121 (2) for psyllid is reported only 10 min at room temperature. *Category : TECHNICAL*
140	95	**Bertolini *et al.* (2014).** In this method, individual psyllids are inmobilized and squashed on nylon or paper membranes with the bottom end of a microcentrifuge tube. Pieces of membrane harbouring the squashed samples are inserted into microtubes containing 100 µL distilled water, 0.5% Triton X-100 or glycine buffer (0.1 M glycine, 0.05 M NaCl, 1 mM EDTA). Samples are then incubated at ~~100 °C~~	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*

		100°C for 10 min as described by Bertolini *et al.* (2014), vortexed and placed on ice until used for real-time PCR.		
141	96	**NAPPO (2012).** In this method, one to five adult psyllids are placed into a microfuge tube and homogenized in the tube with a micropestle. DNA extraction is then carried out using the commercial kit Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions.	C	**COSAVE** See comment in paragraph 90 *Category : TECHNICAL*
142	96	**NAPPO (2012).** In this method, one to five adult psyllids are placed into a microfuge tube and homogenized in the tube with a micropestle. DNA extraction is then carried out using the commercial kit Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions.	C	**Uruguay** See comment in paragraph 90 *Category : TECHNICAL*
3.4.3 Conventional PCR				
143	97	**3.4.3 Conventional PCR**	C	**Japan** In this section, two conventional PCR method to detect Ca. L. asiaticus and Ca. L. africanus are introduced as "3.4.3.1 Jagoueix et al. (1996)" and "3.4.3.2 Hocquellet et al. (1999)". There is, however, more sensitive method to detect Ca. L. asiaticus published by Fujikawa et al. (2012) *. It is considered that this method is more useful in the Asian region and the American continent where only Ca. L. asiaticus is present among Ca species. So, we propose to add the method of Fujikawa et al. (2012) in this section. * Fujikawa T, Iwanami T. Sensitive and robust detection of citrus greening (huanglongbing) bacterium "Candidatus Liberibacter asiaticus" by DNA amplification with new 16S rDNA specific primers. Molecular and Cellular Probes 26 (2012) 194-197 *Category : TECHNICAL*
144	98	Conventional PCR has proven to be a reliable, specific and sensitive technique for detecting '*Ca.* Liberibacter' species in HLB-infected symptomatic trees. Li, Hartung and Levy (2007) determined that there were no significant differences in sensitivity among the conventional PCR methods listed below. All conventional PCR methods can detect 10^{-2} dilutions of DNA extracts obtained from 200 mg of midribs from infected plants. ~~Wang *et al*. (2006) quantified the detection sensitivity of conventional PCR as 439 fg/µL DNA extract from infected plants.~~	P	**China** The target of the PCR is the DNA of 'Candidatus Liberibacter' spp. in the plant tissue. *Category : SUBSTANTIVE*
145	100	Jagoueix *et al.* (1996) used three primers in the same PCR mixture: OA1, OI1 and OI2c (Teixeira *et al.*, 2005a, 2005b). The primer sequences, which are based on the 16S rDNA sequences, are as follows:	C	**EPPO** Should the order rather be "...OI1, OA1..." because of the order used in paragraphs 101 and 102 and in paragraph 104"? *Category : TECHNICAL*
146	101	OI1 (forward):5´- GCG CGT ATG CAA TAC GAG CGG CA –- 3´	P	**EPPO** Typo ("–" replaced with "-" for consistency). *Category : EDITORIAL*

147	101	OI1 (forward):5´- GCG CGT ATG CAA TAC GAG CGG CA – 3´	C	**United States of America** According to updated CLas genome sequences (Duan et al, 2009, and whole genome sequences of other CLas strains deposited in NCBI GenBank), a missing nucleotide "G" should be added in the primer sequences of "[101]OI1 (forward)" and "[247]HLBas (forward primer)" for specific amplifycation of partial 16S rDNA of 'Ca. L. asiaticus'. [101]OI1 (forward):5´- GCG CGT ATG C"G"AA TAC GAG CGG CA – 3´ *Category : TECHNICAL*
148	104	The primer pair OI1/OI2c amplifies '*Ca.* L. asiaticus' and '*Ca.* L. africanus'; the primer pair OA1/OI2c preferentially amplifies '*Ca.* L. africanus'. The sequence of the reverse primer OI2c is the same for all three '*Ca.* Liberibacter' species associated with HLB. The sequences of the forward primer OA1 for '*Ca.* L. africanus' and OI1 for '*Ca.* L. asiaticus' and *'Ca.* L. africanus' are identical except that GCA in OI1 is replaced by TTT in OA1.	C	**EPPO** In Teixeira et al., 2005a it is reported that the sequence of the reverse primer OI2c is not the same for all three species. It is the same for 'Ca. L. asiaticus' and 'Ca. L. africanus'. In 'Ca. L. americanus' sequence there are three different nucleotides. *Category : TECHNICAL*
149	104	The primer pair OI1/OI2c amplifies '*Ca.* L. asiaticus' and '*Ca.* L. africanus'; the primer pair OA1/OI2c preferentially amplifies '*Ca.* L. africanus'. The sequence of the reverse primer OI2c is the same for all three '*Ca.* Liberibacter' species associated with HLB. The sequences of the forward primer OA1 for '*Ca.* L. africanus' and OI1 for '*Ca.* L. asiaticus' -and *'Ca.* L. africanus' are identical except that GCA in OI1 is replaced by TTT in OA1.	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
150	104	The primer pair OI1/OI2c amplifies '*Ca.* L. asiaticus' and '*Ca.* L. africanus'; the primer pair OA1/OI2c preferentially amplifies '*Ca.* L. africanus'. The sequence of the reverse primer OI2c is the same for all three '*Ca.* Liberibacter' species associated with HLB. The sequences of the forward primer OA1 for '*Ca.* L. africanus' and OI1 for '*Ca.* L. asiaticus' -and *'Ca.* L. africanus' are identical except that GCA in OI1 is replaced by TTT in OA1.	P	**New Zealand** *Category : EDITORIAL*
151	105	Although Jagoeix *et al.* (1996) determined that the primer pair OI1/OI2c detects '*Ca.* L. asiaticus' and '*Ca.* L. africanus', this primer pair does not detect '*Ca.* L. americanus' (Li, Hartung and Levy, 2007). ~~No amplification was obtained when this primer pair was tested on *Acinetobacter lwoffi*, . *Agrobacterium tumefaciens*, *Citrus tristeza virus*, *Escherichia coli*, '*Candidatus* Phytoplasma aurantifolia' (lime witches broom phytoplasma), '*Candidatus* Phytoplasma solani' (stolbur phytoplasma), *Spiroplasma citri*, *Xanthomonas campestris*, and *Xylella fastidiosa*. The sensitivity of the method was not quantified, but although amplifications were obtained from 20 mg of infected midribs they were not obtained when lesser amounts of infected midribs were mixed with 1 g of healthy midrib tissue.~~	P	**Japan** If the method is specific for detecting the target species of this protocol, there is no need to describe that other certain species are not detected. It is better to simplify the description to avoid unnecessary misunderstanding (e.g. other species than the written species here can be detected?). *Category : SUBSTANTIVE*

152	105	Although Jagoueix *et al.* (1996) determined that the primer pair OI1/OI2c detects '*Ca.* L. asiaticus' and '*Ca.* L. africanus', this primer pair does not detect '*Ca.* L. americanus' (Li, Hartung and Levy, 2007). No amplification was obtained when this primer pair was tested ~~on~~ on *Acinetobacter lwoffi*, *Agrobacterium tumefaciens*, *Citrus tristeza virus*, *Escherichia coli*, '*Candidatus* Phytoplasma aurantifolia' (lime witches broom phytoplasma), '*Candidatus* Phytoplasma solani' (stolbur phytoplasma), *Spiroplasma citri*, *Xanthomonas campestris*~~,~~ and *Xylella fastidiosa*~~.~~ . The sensitivity of the method was not quantified, but although amplifications were obtained from 20 mg of infected midribs they were not obtained when lesser amounts of infected midribs were mixed with 1 g of healthy midrib tissue.	P	**EPPO** Typos (two spaces and a comma deleted). *Category : EDITORIAL*
153	105	Although Jagoueix *et al.* (1996) determined that the primer pair OI1/OI2c detects '*Ca.* L. asiaticus' and '*Ca.* L. africanus', this primer pair does not detect '*Ca.* L. americanus' (Li, Hartung and Levy, 2007). No amplification was obtained when this primer pair was tested ~~on~~ on *Acinetobacter lwoffi*, *Agrobacterium tumefaciens*, *Citrus tristeza virus*, *Escherichia coli*, '*Candidatus* Phytoplasma aurantifolia' (lime witches broom phytoplasma), '*Candidatus* Phytoplasma solani' (stolbur phytoplasma), *Spiroplasma citri*, *Xanthomonas campestris*, and *Xylella fastidiosa*~~.~~ . The sensitivity of the method was not quantified, but although amplifications were obtained from 20 mg of infected ~~midribs~~ midribs, they were not obtained when lesser amounts of infected midribs were mixed with 1 g of healthy midrib tissue.	P	**New Zealand** remove space requires a comma *Category : EDITORIAL*
154	131	~~94 °C~~ 94°C for 2 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
155	135	~~92 °C~~ 92°C for 60 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
156	137	~~72 °C~~ 72°C for 90 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
157	139	~~72 °C~~ 72°C for 10 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
158	146	***3.4.3.2 Conventional PCR using the primers of Hocquellet* et al. *(1999)***	C	**EPPO** in the EPPO PM 7/121 the PCR conditions (temperatures reaction volume) have been optimized for their use in duplex PCR with Teixeira et◦al. (2005a) *Category : TECHNICAL*
159	146	***3.4.3.2 Conventional PCR using the primers of Hocquellet* et al. *(1999)***	C	**EPPO** These primers can be used in duplex PCR with GB1 and GB3 primers by Teixeira et al 2005. The duplex PCR was reported in

				Cellier et al., 2020 "even though no 'Ca. L. americanus' DNA samples were tested". EURL bacteriology, coordinated by Maria Vlami (NVWA), performed in 2020 a Test Performance Study (TPS) with the duplex setting on 'Candidatus Liberibacter americanus' and 'Candidatus Liberibacter asiaticus' organised by NVWA. In EPPO 7/121(2) only the duplex PCR is indicated: "Appendix 5 Duplex Conventional PCR adapted from Teixeira et al. (2005) and Hocquellet et al. (1999)" *Category : TECHNICAL*
160	147	Hocquellet *et al.* (1999) designed the primers A2 and J5 specifically to detect '*Ca*. L. asiaticus' and '*Ca*. L. africanus'~~. No amplifications were obtained when this method was used on *A. tumefaciens*, *A. lwoffi*, *E. coli*, *Xanthomonas axonopodis* pv. *citri*, *X. fastidiosa S. citri*, '*Candidatus* Phytoplasma aurantifolia', and '*Candidatus* Phytoplasma solani' (stolbur phytoplasma). These primers do not detect '*Ca.* L. americanus' (Li, Hartung and Levy, 2007).~~	P	**Japan** If the method is specific for detecting the target species of this protocol, there is no need to describe that other certain species are not detected. It is better to simplify the description to avoid unnecessary misunderstanding (e.g. other species than the written species here can be detected?) *Category : SUBSTANTIVE*
161	147	Hocquellet *et al.* (1999) designed the primers A2 and J5 specifically to detect '*Ca*. L. asiaticus' and '*Ca*. L. africanus'. No amplifications were obtained when this method was used on *A. tumefaciens*, *A. lwoffi*, ~~.~~*E. coli*, *Xanthomonas axonopodis* pv. *citri*, *X.* ~~.~~*fastidiosa*,~~*fastidiosa* .~~ *S. citri*, '*Candidatus* Phytoplasma aurantifolia', and '*Candidatus* Phytoplasma solani' (stolbur phytoplasma). These primers do not detect '*Ca.* L. americanus' (Li, Hartung and Levy, 2007).	P	**EPPO** Typo (one space deleted and one comma added). *Category : EDITORIAL*
162	147	Hocquellet *et al.* (1999) designed the primers A2 and J5 specifically to detect '*Ca*. L. asiaticus' and '*Ca*. L. africanus'. No amplifications were obtained when this method was used on *A. tumefaciens*, *A. lwoffi*, *E. coli*, Xanthomonas citri pv. citri~~*Xanthomonas axonopodis* pv. *citri*~~, *X. fastidiosa S. citri*, '*Candidatus* Phytoplasma aurantifolia', and '*Candidatus* Phytoplasma solani' (stolbur phytoplasma). These primers do not detect '*Ca.* L. americanus' (Li, Hartung and Levy, 2007).	P	**China** The new name of Xanthomonas axonopodis pv. citri(Xanthomonas citri subsp. citri) is Xanthomonas citri pv. citri. *Category : SUBSTANTIVE*
163	147	Hocquellet *et al.* (1999) designed the primers A2 and J5 specifically to detect '*Ca*. L. asiaticus' and '*Ca*. L. africanus'. No amplifications were obtained when this method was used on *A. tumefaciens*, *A. lwoffi*, ~~.~~*E. coli*, *Xanthomonas axonopodis* pv. *citri*, *X. fastidiosa S. citri*, '*Candidatus* Phytoplasma aurantifolia', and '*Candidatus* Phytoplasma solani' (stolbur phytoplasma). These primers do not detect '*Ca.* L. americanus' (Li, Hartung and Levy, 2007).	P	**New Zealand** *Category : EDITORIAL*
164	165	Primer J5 (forward)	C	**EPPO** Shouldn't it be "reverse" instead of "forward" (please see paragraph 150)? *Category : TECHNICAL*

165	174	~~94 °C~~ 94°C for 2 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
166	178	~~92 °C~~ 92°C for 20 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
167	180	~~62 °C~~ 62°C for 20 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
168	182	~~72 °C~~ 72°C for 45 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
169	184	~~72 °C~~ 72°C for 10 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
170	193	***3.4.3.3 Conventional PCR using the primers of Teixeira* et al. *(2005a)***	C	**EPPO** in the EPPO PM 7/121 the PCR conditions (temperatures reaction volume) have been optimized for their use in duplex PCR with Hocquellet et al. (1999) *Category : TECHNICAL*
171	193	***3.4.3.3 Conventional PCR using the primers of Teixeira* et al. ~~*(2005b)*~~*(2005a)***	P	**EPPO** *Category : EDITORIAL*
172	197	The primer pair GB1/GB3 detects only '*Ca*. L. americanus' and not '*Ca*. L. asiaticus' or '*Ca*. L. africanus'~~. No amplification .was obtained when the method was used on *Phytophthora citricola* and *Phytophthora citrophthora*, *X. axonopodis* pv. *citri* strain A, *X. fastidiosa*, (Li, Hartung and Levy, 2007).~~	P	**Japan** If the method is specific for detecting the target species of this protocol, there is no need to describe that other certain species are not detected. It is better to simplify the description to avoid unnecessary misunderstanding (e.g. other species than the written species here can be detected?) Li, Hartung and levy, 2007 includes only the data that L. americanus is detected, but does not include the data that other two species are not detected. So, it is not appropriate to put the paper as a sole reference. Delete it or replace it with "Teixeira et al. 2005a". *Category : SUBSTANTIVE*
173	197	The primer pair GB1/GB3 detects only '*Ca*. L. americanus' and not '*Ca*. L. asiaticus' or '*Ca*. L. africanus'. No amplification was obtained when the method was used on *Phytophthora citricola* and *Phytophthora citrophthora*, *X. axonopodis* pv. *citri* strain A, *and X. fastidiosa*~~,~~ (Li, Hartung and Levy, 2007).	P	**EPPO** Two typos. *Category : EDITORIAL*
174	212	Primer GB3 (forward)	C	**EPPO** Shouldn't it be "reverse" instead of "forward" (please see paragraph 196)? *Category : TECHNICAL*
175	221	~~94 °C~~ 94°C for 2 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*

176	225	~~94 °C~~ 94°C for 45 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
177	227	~~64 °C~~ 64°C for 45 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
178	229	~~72 °C~~ 72°C for 60 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
179	231	~~72 °C~~ 72°C for 10 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
3.4.4 Real-time PCR				
180	240	Li, Hartung and Levy (2007) reported that real-time PCR could detect down to 10^{-5} dilutions of DNA extracts obtained from 200 mg of midribs from infected plants. ~~Wang *et al.* (2006) quantified the detection sensitivity of real-time PCR as 4.39 fg/µL DNA extract from infected plants.~~ The real-time PCR method of Bertolini *et al.* (2014) showed similar sensitivity for '*Ca.* Liberibacter' spp. detection, as did the method of Li *et al.* (2006).	P	**China** The target of the PCR is the DNA of 'Candidatus Liberibacter' spp. in the plant tissue. *Category : SUBSTANTIVE*
181	242	This ~~multiplex,~~ real-time PCR method allows the detection of each of the three '*Ca.* Liberibacter' species in plant tissue and in psyllids. It is based on combinations of three species-specific forward primers, a reverse primer common to all three '*Ca.* Liberibacter' species and a TaqMan probe that anneals to the amplicon of each of the three species associated with HLB. The method can be further multiplexed with internal controls for plant and psyllid tissue. Li *et al.* (2006) observed no substantial differences in Ct values when internal and target primers and probes were multiplexed for the detection of '*Ca.* Liberibacter' spp.	P	**COSAVE** This method is not multiplex *Category : TECHNICAL*
182	242	This ~~multiplex,~~ real-time PCR method allows the detection of each of the three '*Ca.* Liberibacter' species in plant tissue and in psyllids. It is based on combinations of three species-specific forward primers, a reverse primer common to all three '*Ca.* Liberibacter' species and a TaqMan probe that anneals to the amplicon of each of the three species associated with HLB. The method can be further multiplexed with internal controls for plant and psyllid tissue. Li *et al.* (2006) observed no substantial differences in Ct values when internal and target primers and probes were multiplexed for the detection of '*Ca.* Liberibacter' spp.	P	**Uruguay** The method is not multiplex *Category : TECHNICAL*
183	242	This ~~multiplex,~~ real-time PCR method allows the detection of each of the three '*Ca.* Liberibacter' species in plant tissue and in psyllids. It is based on combinations of three species-specific forward primers, a reverse primer common to all three '*Ca.* Liberibacter' species and a TaqMan probe that anneals to the amplicon of each of the three species associated with HLB. The method can be	P	**Brazil** This method is not multiplex *Category : TECHNICAL*

		~~further~~ multiplexed with internal controls for plant and psyllid tissue. Li *et al.* (2006) observed no substantial differences in Ct values when internal and target primers and probes were multiplexed for the detection of '*Ca.* Liberibacter' spp.		
184	243	Li *et al.* (2006) determined that the primer–probe set HLBaspr (HLBas/HLBp/HLBr) detects '*Ca.* L. asiaticus' and the primer–probe set HLBafpr (HLBaf/HLBp/HLBr) detects '*Ca.* L. africanus'. The primer–probe set HLBaspr can detect '*Ca.* L. africanus' and HLBafpr can detect '*Ca.* L. asiaticus', but with higher Ct values. The primer–probe set HLBampr (HLBam/HLBp/HLBr) detects '*Ca.* L. americanus' but not '*Ca.* L. africanus' or '*Ca.* L. asiaticus'. ~~No amplification was obtained when the method was used on *Citrus tristeza virus* and *Curtobacterium flaccumfaciens* strain ER1/6, *P. citricola* I 22F3, *P. citrophthora* I 1E4, *X. fastidiosa*, *X. axonopodis* pv. *citri* strain A, (Li *et al.*, 2006).~~	P	**Japan** If the method is specific for detecting the target species of this protocol, there is no need to describe that other certain species are not detected. It is better to simplify the description to avoid unnecessary misunderstanding (e.g. other species than the written species here can be detected?) *Category : SUBSTANTIVE*
185	243	Li *et al.* (2006) determined that the primer–probe set HLBaspr (HLBas/HLBp/HLBr) detects '*Ca.* L. asiaticus' and the primer–probe set HLBafpr (HLBaf/HLBp/HLBr) detects '*Ca.* L. africanus'. The primer–probe set HLBaspr can detect '*Ca.* L. africanus' and HLBafpr can detect '*Ca.* L. asiaticus', but with higher Ct values. The primer–probe set HLBampr (HLBam/HLBp/HLBr) detects '*Ca.* L. americanus' but not '*Ca.* L. africanus' or '*Ca.* L. asiaticus'. No amplification was obtained when the method was used on *Citrus tristeza virus* and *Curtobacterium flaccumfaciens* strain ER1/6, *P. citricola* I 22F3, *P. citrophthora* I 1E4, *X. fastidiosa*, *X. axonopodis* pv. *citri* strain A, (Li *et al.*, 2006).	C	**Australia** Suggest replacing HLBas (forward) with another primer (CLas-4G) as it has been shown to improve qPCR sensitivity compared with HLBas (forward) Reasoning Bao et al (2020) found that the Hibas primer was missing nucleotide G between C and A (TCGAGCGCGTATGC-AATACG). Although the missing G did not affect the sensitivity of the test in detecting huanglongbing (HLB) at high bacterial titres, this primer was found to be slightly less sensitive than those having this G at low titres. Therefore, the correct CLas-4G primer, which includes this G (AGTCGAGCGCGTATGCGAAT) was proposed to prevent potential false-negative results. Reference: Bao et al. 2020, Plant Disease, 104:527-532 *Category : TECHNICAL*
186	243	Li *et al.* (2006) determined that the primer–probe set HLBaspr (HLBas/HLBp/HLBr) detects '*Ca.* L. asiaticus' and the primer–probe set HLBafpr (HLBaf/HLBp/HLBr) detects '*Ca.* L. africanus'. The primer–probe set HLBaspr can detect '*Ca.* L. africanus' and HLBafpr can detect '*Ca.* L. asiaticus', but with higher Ct values. The primer–probe set HLBampr (HLBam/HLBp/HLBr) detects '*Ca.* L. americanus' but not '*Ca.* L. africanus' or '*Ca.* L. asiaticus'. No amplification was obtained when the method was used on *Citrus tristeza virus* and *Curtobacterium flaccumfaciens* strain ER1/6, *P. citricola* I 22F3, *P. citrophthora* I 1E4, *X. fastidios a*, and *X. axonopodis* pv. *citri* strain A (Li *et al.*, 2006).	C	**EPPO** Is a publication available. This has never been observed in French laboratories *Category : TECHNICAL*
187	243	Li *et al.* (2006) determined that the primer–probe set HLBaspr (HLBas/HLBp/HLBr) detects '*Ca.* L. asiaticus' and the primer–probe set HLBafpr	P	**EPPO** Two typos.

		(HLBaf/HLBp/HLBr) detects '*Ca.* L. africanus'. The primer–probe set HLBaspr can detect '*Ca.* L. africanus' and HLBafpr can detect '*Ca.* L. asiaticus', but with higher Ct values. The primer–probe set HLBampr (HLBam/HLBp/HLBr) detects '*Ca.* L. americanus' but not '*Ca.* L. africanus' or '*Ca.* L. asiaticus'. No amplification was obtained when the method was used on *Citrus tristeza virus* and *Curtobacterium flaccumfaciens* strain ER1/6, *P. citricola* I 22F3, *P. citrophthora* I 1E4, *X. fastidiosa*, and *X. axonopodis* pv. *citri* strain ~~A,~~ A (Li *et al.*, 2006).		*Category : EDITORIAL*
188	243	Li *et al.* (2006) determined that the primer–probe set HLBaspr (HLBas/HLBp/HLBr) detects '*Ca.* L. asiaticus' and the primer–probe set HLBafpr (HLBaf/HLBp/HLBr) detects '*Ca.* L. africanus'. The primer–probe set HLBaspr can detect '*Ca.* L. africanus' and HLBafpr can detect '*Ca.* L. asiaticus', but with higher Ct values. The primer–probe set HLBampr (HLBam/HLBp/HLBr) detects '*Ca.* L. americanus' but not '*Ca.* L. africanus' or '*Ca.* L. asiaticus'. No amplification was obtained when the method was used on *Citrus tristeza virus* and *Curtobacterium flaccumfaciens* strain ER1/6, *P. citricola* I 22F3, *P. citrophthora* I 1E4, *X. fastidios a*, *X. axonopodis* pv. *citri* strain A, (Li *et al.*, 2006).	C	**EPPO** On "Candidatus Liberibacter solanacerum", either. *Category : TECHNICAL*
189	243	Li *et al.* (2006) determined that the primer–probe set HLBaspr (HLBas/HLBp/HLBr) detects '*Ca.* L. ~~asiaticus'~~ asiaticus', and the primer–probe set HLBafpr (HLBaf/HLBp/HLBr) detects '*Ca.* L. africanus'. The primer–probe set HLBaspr can detect '*Ca.* L. africanus' and HLBafpr can detect '*Ca.* L. asiaticus', but with higher Ct values. The primer–probe set HLBampr (HLBam/HLBp/HLBr) detects '*Ca.* L. americanus' but not '*Ca.* L. africanus' or '*Ca.* L. asiaticus'. No amplification was obtained when the method was used on *Citrus tristeza virus* and *Curtobacterium flaccumfaciens* strain ER1/6, *P. citricola* I 22F3, *P. citrophthora* I 1E4, *X. fastidiosa*, *X. axonopodis* pv. *citri* strain A, (Li *et al.*, 2006).	P	**New Zealand** *Category : EDITORIAL*
190	245	The sequences of the four primers and one TaqMan probe, which are based on the 16S rDNA sequences of the three '*Ca.* Liberibacter' species, are as ~~follows:~~ follows (the sequence of the primer HLBas has been updated using the genome information of Ca. Liberibacter asiaticus (Zhou et al., 2011))	P	**COSAVE** To improve information *Category : TECHNICAL*
191	245	The sequences of the four primers and one TaqMan probe, which are based on the 16S rDNA sequences of the three '*Ca.* Liberibacter' species, are as ~~follows~~follows (the sequence of the primer HLBas has been updated using the genome information of Ca Liberibacter asiaticus (Zhou et al., 2011):	P	**Uruguay** To improve information *Category : TECHNICAL*
192	245	The sequences of the four primers and one TaqMan probe, which are based on the 16S rDNA sequences of the three '*Ca.* Liberibacter' species, are as ~~follows~~follows	P	**Brazil** To improve information *Category : TECHNICAL*

		(the sequence of the primer HLBas has been updated using the genome information of *Ca*. Liberibacter asiaticus (Zhou et al., 2011)):		
193	247	HLBas (forward primer): 5' - TCG AGC GCG TAT GCA ATA CG - 3'	C	**Australia** Suggest replacing HLBas (forward) with another primer (CLas-4G) as it has been shown to improve qPCR sensitivity compared with HLBas (forward) Reasoning Bao et al (2020) found that the Hibas primer was missing nucleotide G between C and A (TCGAGCGCGTATGC-AATACG). Although the missing G did not affect the sensitivity of the test in detecting huanglongbing (HLB) at high bacterial titres, this primer was found to be slightly less sensitive than those having this G at low titres. Therefore, the correct CLas-4G primer, which includes this G (AGTCGAGCGCGTATGCGAAT) was proposed to prevent potential false-negative results. Reference: Bao et al. 2020, Plant Disease, 104:527-532 *Category : TECHNICAL*
194	247	HLBas (forward primer): 5' - TCG AGC GCG TAT ~~GCA ATA~~ GCG AAT A CG - 3'	P	**COSAVE** This G was added to the primer sequence based on the sequence of the 16SrDNA genes from Ca. L. asiaticus by Zhou et al., 2011. All subsequent genome confirmed this sequence. *Category : TECHNICAL*
195	247	HLBas (forward primer): 5' - TCG AGC GCG TAT GCA ATA CG - 3'	C	**United States of America** According to updated CLas genome sequences (Duan et al, 2009, and whole genome sequences of other CLas strains deposited in NCBI GenBank), a missing nucleotide "G" should be added in the primer sequences of "[101]OI1 (forward)" and "[247]HLBas (forward primer)" for specific amplification of partial 16S rDNA of 'Ca. L. asiaticus'. [247]HLBas (forward primer): 5' - TCG AGC GCG TAT GC" G"A ATA CG - 3' *Category : TECHNICAL*
196	247	HLBas (forward primer): 5' - TCG AGC GCG TAT ~~GCA ATA CG~~ GCG AAT A CG- 3'	P	**Uruguay** This G was added to the primer sequence based on the sequence of the 16SrDNA genes from Ca. Liberibacter asiaticus by Zhou et al., 2011. All subsequent genome confirmed this sequence *Category : TECHNICAL*
197	247	HLBas (forward primer): 5' - TCG AGC GCG TAT ~~GCA~~ GCG A ATA CG - 3'	P	**Brazil** A "G" was added to the primer sequence based on the sequence of the 16SrDNA genes from Ca. L. asiaticus by Zhou et al., 2011. All subsequent genome confirmed this sequence *Category : TECHNICAL*
198	251	HLBaf (forward primer): 5'- CGA GCG CGT ATT TTA TAC GAG CG –3'	P	**EPPO** the sequence of the HLBaf primer is not complete.

				Category : TECHNICAL
199	258	COXp probe: 5'-TET-CAG ATG CTT ACG CTG-BHQ1-3'	C	**United States of America** The internal control primers and probe to target wingless gene (WG) of psyllid (Li et al., 2008) were adopted in our protocols and are recommended as follows: WG (forward primer): 5'-GCT CTC AAA GAT CGG TTT GAC GG -3' WG (reverse primer): 5'-GCT GCC ACG AAC GTT ACC TTC -3' WG (hydrolysis probe): 5' -TET-TTA CTG ACC ATC ACT CTG GAC GC/BHQ-2 -3' (the 5' reporter dye (TET) may be changed to other fluorescent dyes, e.g., JOE and VIC, according to individual fluorescence filter sets of a real-time instrument) (Reference: Li, W. Duan, Y., Brlansky, R.H. Twieg, E. and Levy, L. 2008. Incidences and population of 'Candidatus Liberibacter asiaticus' in Asian citrus psyllid (Diaphorina citri) on citrus plants affected by Huanglongbing in Florida. Int. Res. Cong. Huanglongbing, Dec. 1-5, 2008, Orlando, Florida). *Category : TECHNICAL*
200	258	CLAas-4G：AGTCGAGCGCGTATGCgAATCOXp probe: 5'-TET-CAG ATG CTT ACG CTG-BHQ1-3'	P	**China** primer used significantly improve the sensitivity and accuracy of detection of CLas in real-time PCR system. *Category : SUBSTANTIVE*
201	267	Other real-time PCR master mixes have been shown to work with this method: for example Go Taq Probe qPCR master mix (Promega) (Cellier *et al.*, 2020) and Path-ID qPCR master mix (Ambion) (EPPO, 2014).	C	**COSAVE** See comment in paragraph 90 *Category : TECHNICAL*
202	267	Other real-time PCR master mixes have been shown to work with this method: for example Go Taq Probe qPCR master mix (Promega) (Cellier *et al.*, 2020) and Path-ID qPCR master mix (Ambion) (EPPO, 2014).	C	**Uruguay** See comment in paragraph 90 *Category : TECHNICAL*
203	289	0.15 µM	C	**EPPO** Optimized to 0.13 in EPPO PM 7/121 *Category : TECHNICAL*
204	297	~~5~~ 1 U	P	**Australia** 1 U was used in the Li et al. (2006) protocol. Reference: As in reference list *Category : EDITORIAL*
205	300	**Cycling parameters**	C	**EPPO** Optimized in EPPO PM 7/121 according to an USDA (California) protocol (contact person was Cynthia Levesque) *Category : TECHNICAL*
206	303	~~95 °C~~ 95°C for 10 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*

207	307	~~95 °C~~ ~~95°C~~ for 20 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
208	307	95 °C for 20 s	C	**United States of America** The denaturation time may be 1 to 3 seconds depending on which real-time PCR instrument and labware are used, e.g. it was 1 s on Cepheid but 3 s on ABI instruments according to our protocols. *Category : TECHNICAL*
209	309	~~58 °C~~ ~~58°C~~ for 40 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
210	310	[i] For a final reaction volume of 25 µL.	C	**EPPO** A volume of 13µl works perfectly. The revised EPPO Standard PM7-121 revision, is referring to an optimized protocol more adapted to current real-time PCR. *Category : TECHNICAL*
211	311	[ii] See page footnote 1.	C	**EPPO** Where is "ii" in the table? *Category : EDITORIAL*
212	311	ii See page footnote 1.	C	**EPPO** Footnote 1 wasn´t found. It does not match to the only one footnote (page 2). We suppose maybe paragraph 267 should be this footnote as it´s text does not link-up with the text above it. *Category : EDITORIAL*
213	313	***3.4.4.2 Real-time PCR using the primers and probes of Bertolini et al. (2014)***	C	**EPPO** In EPPO PM7/121(2) this test is no longer included. In the old version (1) was present, in the versión (2) no longer. "The real-time PCR test described by Bertolini et al. (2010, 2014) is not recommended as a screening test, as it produces false positive results and thus requires confirmation by another test.". From Cellier et al., 2020 "in addition to CLso_1, the Bertolini method repeatedly amplified several non-target DNA samples, leading to an exclusivity score of 25.0%." *Category : SUBSTANTIVE*
214	313	A validated real-time PCR target RNR (Zheng et al, 2016) was adopted and included in our protocols, and it is recommended as follows:A feature of the RNR target is of its five-copy presence in a genome of CLas, presumptively it would be more sensitive than the three-copy 16S target of [247] [254] [253] HLBaspr assay (Li, Hartung and Levy, 2006). In analytical testing side-by-side, the RNR was more specific and sensitive than the 16S HLBaspr method. The Master mix may be the same as in Table 4, but the annealing and elongation temperature is 60 °C for 40 s.RNRf (forward primer): 5'- CAT GCT CCA TGA AGC TAC CC -3'RNRr (reverse primer): 5'- GGA GCA TTT AAC CCC ACG AA -3'RNRp (hydrolysis probe): 5' - (6-FAM) CCT CGA AAT CGC CTA TGC AC (BHQ-1) -3' (Reference: Zheng Zheng, Meirong Xu, Minli Bao, Fengnian Wu, Jianchi Chen, Xiaoling Deng. (2016). Unusual Five Copies and Dual Forms of nrdB in "Candidatus Liberibacter asiaticus": Biological Implications and PCR Detection Application. Scientific Reports | 6:39020 | DOI:	P	**United States of America** *Category : TECHNICAL*

		10.1038/srep39020).***3.4.4.2 Real-time PCR using the primers and probes of Bertolini* et al. *(2014)***		
215	315	The primer sequences, which are based on the 16S rDNA sequences of '*Ca*. Liberibacter' spp., are as follows:	P	**New Zealand** requires a fullstop to be a correct abbreviation *Category : EDITORIAL*
216	320	According to Bertolini *et al.* (2014), the primers and probe used in this method detect all three '*Ca*. Liberibacter' species on *Citrus* spp. The primers CaLsppF and ~~CaL sppR~~CaLsppR, which are based on the sequence of the most conserved region of the '*Ca*. Liberibacter' spp. genome, were found to detect all the tested '*Ca*. Liberibacter' species associated with HLB from different hosts and origins. No cross-reaction was noticed when the method was tried on other graft-transmitted pathogens of citrus. In further evaluation during a comparative performance study by Cellier *et al.* (2020), false positive amplifications from non-target bacteria were observed. Raising the annealing temperature to ~~64~~ 64°C did reduce some of this risk~~. .~~. However, because of the residual risk, positive test results using this method can only be considered reliable if they are confirmed by other HLB-specific PCR detection methods." *Category : EDITORIAL*	P	**EPPO** Typos (three spaces and one quotation mark deleted). *Category : EDITORIAL*
217	320	According to Bertolini *et al.* (2014), the primers and probe used in this method detect all three '*Ca*. Liberibacter' species on *Citrus* spp. The primers CaLsppF and CaL sppR, which are based on the sequence of the most conserved region of the '*Ca*. Liberibacter' spp. genome, were found to detect all the tested '*Ca*. Liberibacter' species associated with HLB from different hosts and origins. No cross-reaction was noticed when the method was tried on other graft-transmitted pathogens of citrus. In further evaluation during a comparative performance study by Cellier *et al.* (2020), false positive amplifications from non-target bacteria were observed. Raising the annealing temperature to 64 °C did reduce some of this risk. However, because of the residual risk, positive test results using this method can only be considered reliable if they are confirmed by other HLB-specific PCR detection methods."	P	**New Zealand** remove quotes as there is no opening quote anywhere in this paragraph. *Category : EDITORIAL*
218	340	~~95 °C~~ 95°C for 10 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
219	344	~~95 °C~~ 95°C for 15 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
220	346	~~60 °C~~ 60°C for 60 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
221	348	ii See page footnote 1.	C	**EPPO** Where is footnote 1? *Category : EDITORIAL*

222	350	A diagnostic kit, HLB 100, for use with immobilized plant tissue prints or vector squashes and this PCR method (with lyophilized master mix), is commercially available from Plant Print Diagnostics1 (). It has been used in surveys in Brazil, Réunion (France) and Spain, among other countries (Bertolini *et al.*, 2014; Siverio *et al.*, 2017).	C	**COSAVE** See comment in paragraph 90 *Category : TECHNICAL*
223	350	A diagnostic kit, HLB 100, for use with immobilized plant tissue prints or vector squashes and this PCR method (with lyophilized master mix), is commercially available from Plant Print Diagnostics1 (). It has been used in surveys in Brazil, Réunion (France) and Spain, among other countries (Bertolini *et al.*, 2014; Siverio *et al.*, 2017).	C	**Uruguay** See comment in paragraph 90 *Category : TECHNICAL*
224	351	***3.4.4.3 Real-time PCR using the primers and probes of Morgan et al. (2012)***	C	**EPPO** A SYBR Green version of this qPCR has also been validated and is as efficient as the probe version. *Category : TECHNICAL*
225	352	This real-time PCR method was developed for detection of '*Ca.* Liberibacter asiaticus' and uses primers based on the internal 100 bp region of the 132 bp full repeat shared by the high copy *hyvI* and *hyvII* genes.	C	**EPPO** In EPPO PM 7/121 this test is used for identification only *Category : TECHNICAL*
226	356	Probe LJ900p_p: FAM-ACA TCT TTC GTT TGA GTA GCT AGA TCA TTG A-Iowa Black FQ	C	**EPPO** In EPPO PM7/121(2) is indicated BHQ1 *Category : TECHNICAL*
227	365	Primer LJ900f_f (forward)	C	**EPPO** In the original article there is a difference in primer concentration (which is reflected in the current EPPO PM 7/121). This should be checked. *Category : TECHNICAL*
228	376	95 °C for 30 s	C	**EPPO** 3min in Morgan's publication. *Category : TECHNICAL*
229	376	~~95 °C~~ 95°C for 30 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
230	380	~~95 °C~~ 95°C for 3 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
231	382	~~62 °C~~ 62°C for 30 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
232	384	[ii] See page footnote 1.	C	**EPPO** Where is footnote 1? *Category : EDITORIAL*
233	388	Inner primers:	C	**EPPO** inner primers should be described after outer primers as outer primers amplify first and inner ones in the second phase. *Category : TECHNICAL*
234	396	Lin *et al.* (2010) evaluated the specificity (analytical specificity) of the method with over 70 strains of '*Ca.* L. asiaticus' from six different countries and against	P	**Japan** If the method is specific for detecting the target species of this protocol, there is no need to describe that other certain species

		several non-target pathogens of citrus including '*Ca.* L. ~~africanus',~~ africanus' and '*Ca.* L. ~~americanus' and '~~americanus'~~*Ca.* L. solanacearum',~~ . Only '~~*S. citri*, *Xanthomonas citri* subsp. *citri*, *X. fastidiosa*,. Only '~~*Ca.* L. asiaticus' was detected. The sensitivity was estimated as 10^3 copies of target DNA. No other performance data are available.		are not detected. It is better to simplify the description to avoid unnecessary misunderstanding (e.g. other species than the written species here can be detected?). *Category : SUBSTANTIVE*
235	396	Lin *et al.* (2010) evaluated the specificity (analytical specificity) of the method with over 70 strains of '*Ca.* L. asiaticus' from six different countries and against several non-target pathogens of citrus including '*Ca.* L. africanus', '*Ca.* L. americanus' and '*Ca.* L. solanacearum', *S. citri*, The new name of Xanthomonas axonopodis pv. citri(Xanthomonas citri subsp. citri) is Xanthomonas citri pv. citri~~*Xanthomonas citri* subsp. *citri*~~, *X. fastidiosa*,. Only '*Ca.* L. asiaticus' was detected. The sensitivity was estimated as 10^3 copies of target DNA. No other performance data are available.	P	**China** The new name of Xanthomonas axonopodis pv. citri(Xanthomonas citri subsp. citri) is Xanthomonas citri pv. citri. *Category : SUBSTANTIVE*
236	396	Lin *et al.* (2010) evaluated the specificity (analytical specificity) of the method with over 70 strains of '*Ca.* L. asiaticus' from six different countries and against several non-target pathogens of citrus including '*Ca.* L. africanus', '*Ca.* L. americanus' and '*Ca.* L. solanacearum', *S. citri*, *Xanthomonas citri* subsp. *citri*, and *X. fastidiosa*,. Only '*Ca.* L. asiaticus' was detected. The sensitivity was estimated as 10^3 copies of target DNA. No other performance data are available.	P	**New Zealand** *Category : EDITORIAL*
237	420	~~50 °C~~ 50°C for 2 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
238	422	~~95 °C~~ 95°C for 10 min	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
239	426	~~95 °C~~ 95°C for 30 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
240	428	~~67 °C~~ 67°C for 45 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
241	430	~~72 °C~~ 72°C for 45 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
242	434	~~95 °C~~ 95°C for 30 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
243	436	~~57 °C~~ 57°C for 45 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*
244	438	~~72 °C~~ 72°C for 45 s	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*

245	440	[ii] See page footnote 1.	C	**EPPO** Where is footnote 1? *Category : EDITORIAL*
3.5 Controls for molecular testing				
246	442	**3.5 Controls for molecular testing**	C	**EPPO** Shouldn't it rather be "3.4.5" because "3.4" is about "Molecular detection". *Category : EDITORIAL*
247	443	For the test result obtained to be considered reliable, appropriate controls —which will depend on the type of test used and the level of certainty required —should be considered for each series of nucleic acid isolations and amplifications of the target nucleic acid. For PCR, a positive nucleic acid control (consisting of the target '*Ca.* Liberibacter' species, e.g. '*Ca.* L. asiaticus') and a negative amplification control (no template control) are the minimum controls that should be used. Additional controls may be used for PCR as described below.	P	**New Zealand** *Category : EDITORIAL*
248	445	**Internal control.** For conventional and real-time PCR, a plant housekeeping gene such as *COX* (Weller *et al.*, 2000; Li *et al.*, 2006) should be used as an internal control to eliminate the possibility of PCR false negatives resulting either from nucleic acid extraction failure or degradation or from the presence of PCR inhibitors. For an internal control for vectors, a primer–probe set based on the glycoprotein gene in psyllids may be used (Manjunath *et al.*, 2008)~~.~~ Tests should be repeated if any contradictory or unclear results are obtained.	P	**Colombia** Consideration in the case of doubtful results. Note in the text in the case of doubtful results, the test must be repeated. *Category : TECHNICAL*
249	448	The positive control should be approximately one-tenth of the amount of leaf tissue used per plant for the DNA extraction. 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 be sequenced so that this sequence can be readily compared with sequences obtained from PCR amplicons of the correct size. Alternatively, synthetic positive controls may be made with a known sequence that, again, can be compared with PCR amplicons of the correct size.	C	**Costa Rica** It would be convenient to specify the reference that corroborates the indicated. *Category : SUBSTANTIVE*
250	449	**Negative extraction control.** This control is used to monitor both contamination during nucleic acid extraction and cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from healthy host plants of the same species but where not available other hosts can be used, such as periwinkle or sweet orange plants grown from seed or healthy psyllids reared on healthy plants.	C	**EPPO** In EPPO PM 7/121 it is indicated that, if uninfected matrix is not available, clean extraction buffer could be used. *Category : TECHNICAL*
3.6 Interpretation of results				
251	450	**3.6 Interpretation of results**	C	**EPPO** should be 3.4.6 (see previous comment and other numbers adjusted) *Category : EDITORIAL*

3.6.1 Conventional PCR				
252	453	the positive control produces the correct size amplicon;	C	**EPPO** What about the internal control when used? There should be an amplification for the test to be considered valid. *Category : TECHNICAL*
3.6.2 Real-time PCR				
253	459	no amplification curve is seen (i.e. Ct value is ~~40)~~ 40 or, if a cut-off value has been defined, Ct value is > cut-off value) either with the negative extraction control or the negative amplification control.	P	**EPPO** Revised change by bouhot-delduc on 20 Sep 2021 15:18 *Category : TECHNICAL*
254	461	A sample will be considered positive if it produces an exponential amplification curve. The cycle cut-off value needs to be verified in each laboratory when implementing the method for the first time. Guidance on how to determine the cycle cut-off value can be found in Chandelier *et al.* (2010). Tests should be repeated if any contradictory or unclear results are obtained.	P	**Colombia** Consideration in the case of doubtful results. Note in the text in the case of doubtful results, the test must be repeated. *Category : TECHNICAL*
4. Identification				
255	462	**4. Identification**	C	**EPPO** Bioassay is included in the EPPO PM 7/121 *Category : TECHNICAL*
256	464	If the outcome is critical (e.g. post-entry quarantine sample, new record), conventional PCRs that amplify the 16S rDNA gene (section 3.4.3) should be performed and the PCR products sequenced. The primers developed by Jagoueix et al. (1996) will amplify a 1160 bp product from *Ca*. L. asiaticus', or '*Ca*. L. africanus' and primers developed by Teixeira et al. (2005b) will amplify a 1027 bp product '*Ca*. L. americanus'. Sanger sequencing of these PCR products should be carried out using each primer to generate two independent DNA sequence reads in alternate directions. These sequences should be aligned to identify conflicting information. Chromatograms should be edited to resolve conflicting signals. If multiple peaks at a nucleotide are observed in the sequences generated using both the forward and reverse primers then the site should be assigned as an ambiguous base (i.e. N = A, C, T or G). The final edited sequence should be at least 900 base pairs (bp) in length for data interpretation. Sequence data can be analysed using the Basic Local Alignment Search Tool (BLASTN), available at the National Center for Biotechnology Information (). For species identification the sequence should be at least 99% match to a published authentic sequence.	C	**Costa Rica** It would be convenient to specify the reference that corroborates the indicated " The final edited sequence should be at least 900 base pairs (bp) in length for data interpretation" *Category : SUBSTANTIVE*
257	464	If the outcome is critical (e.g. post-entry quarantine sample, new record), conventional PCRs that amplify the 16S rDNA gene (section 3.4.3) should be performed and the PCR products sequenced. The primers developed by Jagoueix et al. (1996) will amplify a 1160 bp product from *Ca*. L. asiaticus', or	P	**Colombia** Consideration of the amplification of genes other than 16S for the identification of species of Ca. Liberibacter spp. Possibility of amplifying genes other than those mentioned in this protocol that support the identification of the CLas species, for

		'*Ca*. L. africanus' and primers developed by Teixeira et al. (2005b) will amplify a 1027 bp product '*Ca*. L. americanus'. Sanger sequencing of these PCR products should be carried out using each primer to generate two independent DNA sequence reads in alternate directions. These sequences should be aligned to identify conflicting information. Chromatograms should be edited to resolve conflicting signals. If multiple peaks at a nucleotide are observed in the sequences generated using both the forward and reverse primers then the site should be assigned as an ambiguous base (i.e. N = A, C, T or G). The final edited sequence should be at least 900 base pairs (bp) in length for data interpretation. Sequence data can be analysed using the Basic Local Alignment Search Tool (BLASTN), available at the National Center for Biotechnology Information (). For species identification the sequence should be at least 99% match to a published authentic sequence. In addition, 'Ca. Liberibacter' spp. presents the highest risk of introduction in some countries, conventional PCR that amplify genes other than 16S rDNA and sequence the PCR products to confirm its diagnosis must be also performed.		phytosanitary decision making in each territory or country. The analysis of genes other than the 16S ribosomal, helps to detect false positives due to contamination with ribosomal DNA amplicons and determines the identification of the bacterial species causing the HLB disease. *Category : SUBSTANTIVE*
258	464	If the outcome is critical (e.g. post-entry quarantine sample, new record), conventional PCRs that amplify the 16S rDNA gene (section 3.4.3) should be performed and the PCR products sequenced. The primers developed by Jagoueix et al. (1996) will amplify a 1160 bp product from *Ca*. L. ~~asiaticus',~~ asiaticus' or '*Ca*. L. ~~africanus'~~ africanus', and primers developed by Teixeira ~~et al~~*et al*. (2005b) will amplify a 1027 bp product from '*Ca*. L. americanus'. Sanger sequencing of these PCR products should be carried out using each primer to generate two independent DNA sequence reads in alternate directions. These sequences should be aligned to identify conflicting information. Chromatograms should be edited to resolve conflicting signals. If multiple peaks at a nucleotide are observed in the sequences generated using both the forward and reverse ~~primers~~ primers, then the site should be assigned as an ambiguous base (i.e. N = A, C, T or G). The final edited sequence should be at least 900 base pairs (bp) in length for data interpretation. Sequence data can be analysed using the Basic Local Alignment Search Tool (BLASTN), available at the National Center for Biotechnology Information (). For species identification the sequence should be at least 99% match to a published authentic sequence.	P	**EPPO** Typos and editorial amendments suggested for more clarity. *Category : EDITORIAL*
259	464	If the outcome is critical (e.g. post-entry quarantine sample, new record), conventional PCRs that amplify the 16S rDNA gene (section 3.4.3) should be performed and the PCR products sequenced. The primers developed by Jagoueix et al. (1996) will amplify a 1160 bp product from *Ca*. L. asiaticus', or '*Ca*. L. africanus' and primers developed by Teixeira et al. (2005b) will amplify a	P	**New Zealand** *Category : EDITORIAL*

		1027 bp product '*Ca*. L. americanus'. Sanger sequencing of these PCR products should be carried out using each primer to generate two independent DNA sequence reads in alternate directions. These sequences should be aligned to identify conflicting information. Chromatograms should be edited to resolve conflicting signals. If multiple peaks at a nucleotide are observed in the sequences generated using both the forward and reverse primers then the site should be assigned as an ambiguous base (i.e. N = A, C, T or G). The final edited sequence should be at least 900 base pairs (bp) in length for data interpretation. Sequence data can be analysed using the Basic Local Alignment Search Tool (BLASTN), available at the National Center for Biotechnology Information (). For species identification the sequence should be at least a 99% match ~~to~~ with a published authentic sequence.		
5. Records				
260	467	In cases where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance (ISPM 13 ~~(~~*Guidelines for the notification of non-compliance and emergency action*~~))~~) and where '*Ca*. L. asiaticus', '*Ca*. L. africanus' or '*Ca*. L. americanus' is found in an area for the first time, the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability:	P	**New Zealand** *Category : EDITORIAL*
261	468	The original sample should be kept frozen at ~~–80 °C~~–80°C, or freeze-dried, or dried over calcium chloride and kept at ~~4 °C~~4°C.	P	**EPPO** Typos (two spaces deleted). *Category : EDITORIAL*
262	469	If relevant, DNA extractions should be kept at ~~–20 °C~~–20°C or at ~~–80 °C~~–80°C, and plant extracts spotted on membranes should be kept at room temperature.	P	**EPPO** Typos (two spaces deleted). *Category : EDITORIAL*
263	470	If relevant, PCR amplification products should be kept at ~~–20 °C~~–20°C or at ~~–80 °C~~–80°C.	P	**EPPO** Typos (two spaces deleted). *Category : EDITORIAL*
7. Acknowledgements				
264	477	The first draft of this protocol was written by María M. López (Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain), Solke De Boer (Charlottetown Laboratory, Canadian Food Inspection Agency, Canada), ~~-~~John Hartung (Molecular Plant Pathology Laboratory, Agricultural Research Service, United States Department of Agriculture, United States of America), Rita Lanfranchi (Laboratory of Plant Pest and Disease, SENASA, Argentina (see preceding section)), Takayuki Matsuura (Ministry of Agriculture, Forestry and Fisheries, Japan), Jacek Plazinski (Office of the Chief Plant Protection Officer, Division of Product Integrity, Animal and Plant Health, Australia), Changyong	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*

		Zhou (Citrus Research Institute, Chinese Academy of Agricultural Sciences/Southwest University, Chonging, China).		
8. References				
265	483	**Bertolini, E., Felipe, R.T.A., Sauer, A.V., Lopes, S.A., Arilla, A., Vidal, E., Mourão Filho, F.A.A. *et al.*** 2014. Tissue-print and squash real-time PCR for direct detection of '*Candidatus* Liberibacter' species in citrus plants and psyllid vectors. *Plant Pathology*, 63~~:~~: 1149–1158.	P	**New Zealand** *Category : EDITORIAL*
266	484	**Bertolini, E., Moreno, A., Capote, N., Olmos, A., de Luis, A., Vidal, E., Pérez-Panades, J. & Cambra, M.** 2008. Quantitative detection of *Citrus tristeza virus* in plant tissues and single aphids by real-time RT-PCR. *European Journal of Plant Pathology*, 120:~~,~~ 177–188.	P	**New Zealand** *Category : EDITORIAL*
267	486	**CABI.** 2021. Citrus huanglongbing (greening) disease (citrus greening). Datasheet. In: *Invasive Species Compendium* [online]. Wallingford, UK, CABI. [Cited 15 April 2021]. www.cabi.org/isc/datasheet/16567Bao, M., Zheng,Z., Sun, X., Chen, J.,& Deng, X. 2020. Enhancing PCR capacity to detect 'Candidatus Liberibacter asiaticus' utilizing whole genome sequence information. Plant Disease, 104:527-532	P	**China** Integrity *Category : SUBSTANTIVE*
268	486	**CABI.** 2021. Citrus huanglongbing (greening) disease (citrus greening). Datasheet. ~~In:~~ In *Invasive Species Compendium* [online]. Wallingford, UK, CABI. [Cited 15 April 2021].	P	**New Zealand** *Category : EDITORIAL*
269	487	**Cellier, G., Redondo, C., Cubero, J., Roselló, M., de Andrade, E., Cruz, L., Ince, E. *et al.*** 2020. Comparison of the performance of the main real-time and conventional PCR detection tests for '*Candidatus* ~~Liberibacter'spp~~Liberibacter' spp., plant pathogenic bacteria causing the Huanglongbing disease in *Citrus* spp. *European Journal of Plant Pathology*, 157: 919–941.	P	**EPPO** Typo (one space added). *Category : EDITORIAL*
270	490	**Chandelier, A., Planchon, V. and Oger, R.** 2010. Determination of cycle cut off in real-time PCR for the detection of regulated plant pathogens. *EPPO bulletin*, *40*: 52-58.**Chiyaka C, Singer BH, Halbert SE, Morris JG, Jr, Van Bruggen AHC.** 2012. Modeling huanglongbing transmission within a citrus tree. Proc Natl Acad Sci U S A 109:12213–12218.	P	**Colombia** After paragraph 490. Bibliographic reference of the comment 1. Bibliographic reference of the comment 1. *Category : EDITORIAL*
271	490	**Chandelier, A., Planchon, V. and Oger, R.** 2010. Determination of cycle cut off in real-time PCR for the detection of regulated plant pathogens. *EPPO bulletin*, *40*: ~~52–58~~52–58.	P	**EPPO** Typo ("-" replaced with "–" for the pages). *Category : EDITORIAL*
272	490	**Chandelier, A., Planchon, V. ~~and~~ & Oger, R.** 2010. Determination of cycle cut off in real-time PCR for the detection of regulated plant pathogens. *EPPO ~~bulletin~~Bulletin*, *40*: 52-58.	P	**New Zealand** *Category : EDITORIAL*

273	491	**Choi, C.W., Hyun, J.W., Hwang, R.Y. and Powell, C.A.** 2018. Loop-mediated Isothermal Amplification assay for Detection of Candidatus Liberibacter asiaticus, a Causal Agent of Citrus Huanglongbing. *The Plant Pathology Journal*, 34:499.**Coletta-Filho HD, Carlos EF, Alves KCS, Pereira MAR, Boscariol-Camargo RL et al.** (2010) In planta multiplication and graft transmission of 'Candidatus Liberibacter asiaticus' revealed by real-time PCR. Eur J Plant Pathol 126: 53–60. doi:10.1007/s10658-009-9523-2.	P	**Colombia** After paragraph 491. Bibliographic reference of the comment 2. Bibliographic reference of the comment 2. *Category : EDITORIAL*
274	491	**Choi, C.W., Hyun, J.W., Hwang, R.Y. and Powell, C.A.** 2018. Loop-mediated Isothermal Amplification assay for Detection of ~~Candidatus~~ '*Candidatus* Liberibacter ~~asiaticus~~asiaticus', a Causal Agent of Citrus Huanglongbing. *The Plant Pathology Journal*, 34:499.	P	**EPPO** correct scientific name but perhaps not used in the original reference. *Category : EDITORIAL*
275	491	**Choi, C.W., Hyun, J.W., Hwang, R.Y. ~~and~~ & Powell, C.A.** 2018. Loop-mediated ~~Isothermal Amplification~~ isothermal amplification assay for ~~Detection~~ detection of Candidatus Liberibacter asiaticus, a ~~Causal Agent~~ causal agent of ~~Citrus~~ citrus Huanglongbing. *The Plant Pathology Journal*, 34~~:~~: 499.	P	**New Zealand** *Category : EDITORIAL*
276	492	**Cifuentes-Arenas, J. C., Beattie, G. A, Peña, L., and Lopes, S. A. 2019**. Murraya paniculata and Swinglea glutinosa as short-term transient hosts of 'Candidatus Liberibacter asiaticus' and implications for the spread os huanglongbing. Phytopathology, 109:2064-2073.**Da Graça, J.** 1991. Citrus greening disease. *Annual Review of Phytopathology*, 29: 109–136.	P	**COSAVE** As mentioned in paragraph 50 *Category : EDITORIAL*
277	492	**Cifuentes-Arenas, J. C., Beattie, G. A, Peña, L., and Lopes, S. A. 2019. Murraya paniculata and Swinglea glutinosa as short-term transient hosts of 'Candidatus Liberibacter asiaticus' and implications for the spread os huanglongbing. Phytopathology, 109:2064-2073 Da Graça, J.** 1991. Citrus greening disease. *Annual Review of Phytopathology*, 29: 109–136.	P	**Uruguay** As mentioned in paragraph 50 *Category : TECHNICAL*
278	492	**Cifuentes-Arenas, J.C., Beattie, G.A, Peña, L., and Lopes, S.A.** 2019. Murraya paniculata and Swinglea glutinosa as short-term transient hosts of 'Candidatus Liberibacter asiaticus' and implications for the spread os huanglongbing. Phytopathology, 109:2064-2073**Da Graça, J.** 1991. Citrus greening disease. *Annual Review of Phytopathology*, 29: 109–136.	P	**Brazil** As mentioned in paragraph 50 *Category : EDITORIAL*
279	493	**Da Graça, J.V. ~~2010.~~** ~~Etiology, history and world situation of citrus Huanglongbing.~~ 2010. Etiology, history and world situation of citrus Huanglongbing. *In* Proceedings of the Second International Workshop on Citrus Huanglongbing and the Asian Citrus Psyllid. Mérida, Yucatán, Mexico.	P	**EPPO** Typo (date not in bold). *Category : EDITORIAL*
280	494	~~Deng, X., Lou, Z., Feng, Z., Li, H., Chen, J. and Civerolo, E.L.,~~ **Deng, X., Lou, Z., Feng, Z., Li, H., Chen, J. and Civerolo, E.L.** 2008. First report of ~~'Candidatus~~ '*Candidatus* Liberibacter asiaticus' from *Atalantia buxifolia* in Guangdong, China. *Plant disease* 92: ~~314-314~~314–314.	P	**EPPO** Typos (authors in bold; no comma before the date; correct scientific name but perhaps not used in the original reference; "-" replaced with "–" for the pages). *Category : EDITORIAL*

281	494	Deng, X., Lou, Z., Feng, Z., Li, H., Chen, J. ~~and~~ & Civerolo, E.L., 2008. First report of 'Candidatus Liberibacter asiaticus' from *Atalantia buxifolia* in Guangdong, China. *Plant ~~disease~~Disease*, 92: 314-314.	P	**New Zealand** should be bold *Category : EDITORIAL*
282	495	**Donovan, N.J., Beattie, G.A.C., Chambers, G.A., Holford, P., Englezou, A., Hardy, S., Dorjee, Phuntsho Wangdi, Thinlay & Namgay Om.** 2012. First report of '*Candidatus* Liberibacter asiaticus' in *Diaphorina communis*. *Australasian Plant Disease Notes*, 7:~~,~~ 1–4.	P	**New Zealand** *Category : EDITORIAL*
283	497	**EPPO** (European and Mediterranean Plant Protection Organization). 2014. '*Candidatus* Liberibacter africanus', '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus'. PM 7/121(1). *EPPO Bulletin*, 44(3): 376–389.**Folimonova SY, Achor DS** (2010) Early events of citrus greening (huanglongbing) disease development at the ultrastructural level. Phytopathology 100: 949–958. doi:10.1094/PHYTO-100-9-0949. PubMed: 20701493.	P	**Colombia** After paragraph 497. Bibliographic reference of the comment 2. Bibliographic reference of the comment 2. *Category : EDITORIAL*
284	497	**EPPO** (European and Mediterranean Plant Protection Organization). 2014. '*Candidatus* Liberibacter africanus', '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus'. PM 7/121(1). *EPPO Bulletin*, 44(3): 376–389.	C	**EPPO** see first comment a new version has been published online. *Category : TECHNICAL*
285	501	**Garnier, M., Jagoueix-Eveillard, S., Cronje, P.R. Le Roux, H.F. & Bové, J.M.** 2000. Genomic characterization of a liberibacter present in an ornamental rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. Proposal ~~of"~~ of '*Candidatus* Liberibacter africanus subsp. capensis'. *International Journal of Systematic and Evolutionary Microbiology*, 50: 2119–2125.	P	**EPPO** Typo. *Category : EDITORIAL*
286	503	**Gottwald, T.R., Graça, J.V.D. & Bassanezi, R.B.** 2007. Citrus huanglongbing: the pathogen and its impact. *Plant Health Progress*, 8 [online]. [Cited 15 April 2021].	P	**Colombia** After paragraph 503. Bibliographic reference of the comment 2. Bibliographic reference of the comment 2. *Category : EDITORIAL*
287	504	**Hall, D.G., Richardson, M.L., Ammar, E.-D. & Halbet, S.E.** 2012~~.~~, Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. *Entomologia Experimentalis et Applicata*, 146: 207–223.	P	**EPPO** Typo (one space added). *Category : EDITORIAL*
288	505	**Hung, T.H., Hung, S.C., Chen, C.N., Hsu, M.H., Su, H.J.** 2004. Detection by PCR of *Candidatus Liberibacter asiaticus*, the bacterium causing citrus Huanglongbing in vector psyllids: application to the study of vector-pathogen relationships. Plant Pathology 53: 96-102. doi: https://doi.org/10.1111/j.1365-3059.2004.00948.x**Hocquellet, A., Toorawa, P., Bové, J.-M. & Garnier, M.** 1999. Detection and identification of the two *Candidatus* Liberobacter species associated with citrus huanglongbing by PCR amplification of ribosomal protein genes of the ß operon. *Molecular and Cellular Probes*, 13: 373–379.	P	**COSAVE** Mentioned in paragraph 52 *Category : EDITORIAL*

289	505	**Hocquellet, A., Toorawa, P., Bové, J.-M. & Garnier, M.** 1999. Detection and identification of the two *Candidatus* Liberobacter species associated with citrus huanglongbing by PCR amplification of ribosomal protein genes of the ß operon. *Molecular and Cellular Probes*, 13: 373–379.Hung, T.H., Hung, S.C., Chen, C.N., Hsu, M.H., Su, H.J. 2004. Detection by PCR of Candidatus Liberibacter asiaticus, the bacterium causing citrus Huanglongbing in vector psyllids: application to the study of vector-pathogen relationships. Plant Pathology 53: 96-102. doi: https://doi.org/10.1111/j.1365-3059.2004.00948.x	P	**Uruguay** Mentioned in paragraph 52 *Category : TECHNICAL*
290	506	**Hung, T.H., Hung, S.C., Chen, C.N., Hsu, M.H., Su, H.J.** 2004. Detection by PCR of Candidatus Liberibacter asiaticus, the bacterium causing citrus Huanglongbing in vector psyllids: application to the study of vector-pathogen relationships. Plant Pathology 53: 96-102. doi: https://doi.org/10.1111/j.1365-3059.2004.00948.x**Jagoueix, S., Bové, J.M. & Garnier, M.** 1994. The phloem-limited bacterium of greening disease of citrus is a member of the α subdivision of the *Proteobacteria*. *International Journal of Systematic Bacteriology*, 44: 379–386.	P	**Brazil** As mentioned in paragraph 52 *Category : EDITORIAL*
291	511	**Li, W., Hartung, J. & Levy, L.** 2007. Evaluation of DNA amplification methods for improved detection of "*Candidatus* Liberibacter species" associated with citrus huanglongbing. *Plant Disease*, 91, 51–58.Li W, Levy L, Hartung JS (2009) Quantitative distribution of 'Candidatus Liberibacter asiaticus' in citrus plants with citrus huanglongbing. Phytopathology 99:139–144.	P	**Colombia** After paragraph 511. Bibliographic reference of the comment 1. Bibliographic reference of the comment 1. *Category : EDITORIAL*
292	514	**Lopes, S.A. & Frare, G.F.** 2008. Graft transmission and cultivar reaction of citrus to '*Candidatus* Liberibacter americanus'. *Plant Disease*, 92:121–124.Lopes, S.A. & Cifuentes-Arenas, J.C. 2021. A protocol for successful transmission of 'Candidatus Liberibacter asiaticus' from citrus to citrus using Diaphorina citri. Phytopathology, 105:in press.Lopes, S.A., Frare, G.F., Bertolini, E., Cambra, M., Fernandes, N.G., Ayres, A.J., Marin, D.R., Bové, J.M. 2009a. Liberibacters associated with citrus Huanglongbing in Brazil: 'Candidatus Liberibacter asiaticus' is heat tolerant, 'Ca. L. americanus' is heat sensitive. Plant Disease, 93:257-262.	P	**Uruguay** As mentioned in paragraphs 47 and proposed 86 *Category : TECHNICAL*
293	515	**Lopes, S.A. & Cifuentes-Arenas, J.C**. 2021. A protocol for successful transmission of 'Candidatus Liberibacter asiaticus' from citrus to citrus using Diaphorina citri. Phytopathology, 105:in press.**Lopes, S.A., Frare, G.F., Bertolini, E., Cambra, M., Fernandes, N.G., Ayres, A.J., Marin, D.R., Bové, J.M**. 2009a. Liberibacters associated with citrus Huanglongbing in Brazil: 'Candidatus Liberibacter asiaticus' is heat tolerant, 'Ca. L. americanus' is heat sensitive. Plant Disease, 93:257-262.**Lopes, S.A., Frare, G.F., Camargo, L.E.A., Wulff, N.A., Teixeira, D.C., Bassanezi, R.B., Beattie, G.A.C. & Ayres, A.J.** 2010. Liberibacters associated with orange jasmine in Brazil: Incidence in urban areas and relatedness to citrus liberibacters. *Plant Pathology*, 59: 1044–1053.	P	**COSAVE** As mentioned in paragraphs 47 and proposed 86 *Category : EDITORIAL*

294	515	**Lopes, S.A., Frare, G.F., Camargo, L.E.A., Wulff, N.A., Teixeira, D.C., Bassanezi, R.B., Beattie, G.A.C. & Ayres, A.J.** 2010. Liberibacters associated with orange jasmine in Brazil: Incidence in urban areas and relatedness to citrus liberibacters. *Plant Pathology*, 59: 1044–1053.Lopes, S.A., Luiz, F.Q.B.F., Oliveira, H.T., Cifuentes-Arenas, J.C., and Raiol-Junior, L. 2017. Seasonal variation of 'Candidatus Liberibacter asiaticus' titers in new shoots of citrus in distinct environments. Plant Disease, 101:583-590.	P	**Uruguay** As mentioned in paragraph 46 *Category : TECHNICAL*
295	515	**Lopes, S.A. & Cifuentes-Arenas, J.C.** 2021. A protocol for successful transmission of 'Candidatus Liberibacter asiaticus' from citrus to citrus using Diaphorina citri. Phytopathology, 105:in press.**Lopes, S.A., Frare, G.F., Bertolini, E., Cambra, M., Fernandes, N.G., Ayres, A.J., Marin, D.R., Bové, J.M.** 2009a. Liberibacters associated with citrus Huanglongbing in Brazil: 'Candidatus Liberibacter asiaticus' is heat tolerant, 'Ca. L. americanus' is heat sensitive. Plant Disease, 93:257-262.**Lopes, S.A., Frare, G.F., Camargo, L.E.A., Wulff, N.A., Teixeira, D.C., Bassanezi, R.B., Beattie, G.A.C. & Ayres, A.J.** 2010. Liberibacters associated with orange jasmine in Brazil: Incidence in urban areas and relatedness to citrus liberibacters. *Plant Pathology*, 59: 1044–1053.	P	**Brazil** As mentioned in paragraphs 47 and proposed 86 *Category : EDITORIAL*
296	516	**Lopes, S.A., Luiz, F.Q.B.F., Oliveira, H.T., Cifuentes-Arenas, J.C., and Raiol-Junior, L.** 2017. Seasonal variation of 'Candidatus Liberibacter asiaticus' titers in new shoots of citrus in distinct environments. Plant Disease, 101:583-590.**Manjunath, K.L., Halbert, S.E., Ramadugu, C. Webb, S. & Lee R.F.** 2008. Detection of '*Candidatus* Liberibacter asiaticus' in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. *Phytopathology*, 98: 387–396.	P	**COSAVE** As mentioned in paragraph 46 *Category : EDITORIAL*
297	516	**Manjunath, K.L., Halbert, S.E., Ramadugu, C~~.~~ , Webb, S. & Lee R.F.** 2008. Detection of '*Candidatus* Liberibacter asiaticus' in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. *Phytopathology*, 98: 387–396.	P	**EPPO** Typo (a comma added). *Category : EDITORIAL*
298	516	**Lopes, S.A., Luiz, F.Q.B.F., Oliveira, H.T., Cifuentes-Arenas, J.C., and Raiol-Junior, L.** 2017. Seasonal variation of 'Candidatus Liberibacter asiaticus' titers in new shoots of citrus in distinct environments. Plant Disease, 101:583-590**Manjunath, K.L., Halbert, S.E., Ramadugu, C. Webb, S. & Lee R.F.** 2008. Detection of '*Candidatus* Liberibacter asiaticus' in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. *Phytopathology*, 98: 387–396.	P	**Brazil** As mentioned in paragraph 46 *Category : EDITORIAL*
299	524	**Rigano, L.A., Malamud, F., Orce, I.G., Filippone, M.P., Marano, M.R., Do Amaral, A.M., Castagnaro, A.P. ~~and~~ & Vojnov, A.A.** 2014. Rapid and sensitive detection of ~~Candidatus~~ *Candidatus* Liberibacter asiaticus by loop mediated isothermal amplification combined with a lateral flow dipstick. *BMC microbiology*, 14:~~1-9~~1–9.	P	**EPPO** Typos ("&" for consistency with the other references; shouldn't "Candidatus" be in italics?; "-" replaced with "–" for the pages). *Category : EDITORIAL*
300	530	**Teixeira, D.C., Saillard, C., Eveillard, S, Danet, J.L., da Costa, P.I., Ayres, A.J. & Bove, J.** 2005c. '*Candidatus* Liberibacter americanus', associated with	P	**Uruguay** Mentioned in section 3.4

		citrus huanglongbing (greening disease) in São Paulo State, Brazil. *International Journal of Systematic and Evolutionary Microbiology*, 55: 1857–1862.Teixeira DC, Saillard C, Couture C, Martins E, Wulff NA, Yamamoto PT, Eveillard-Jagoueix S, Ayres AJ & Bové JM. 2008. Candidatus Liberibacter americanus, agent of huanglongbing disease of citrus in Sao Paulo State, Brasil: Distribution and quantification of the liberibacter in leaves of an affected sweet orange tree as determined by PCR methods. Molecular and Cellular Probes 22, 139-150.		*Category : TECHNICAL*
301	530	**Teixeira DC, Saillard C, Couture C, Martins E, Wulff NA, Yamamoto PT, Eveillard-Jagoueix S, Ayres AJ & Bové JM.** 2008. Candidatus Liberibacter americanus, agent of huanglongbing disease of citrus in Sao Paulo State, Brasil: Distribution and quantification of the liberibacter in leaves of an affected sweet orange tree as determined by PCR methods. Molecular and Cellular Probes 22, 139-150.**Teixeira, D.C., Saillard, C., Eveillard, S, Danet, J.L., da Costa, P.I., Ayres, A.J. & Bove, J.** 2005c. '*Candidatus* Liberibacter americanus', associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. *International Journal of Systematic and Evolutionary Microbiology*, 55: 1857–1862.	P	**Brazil** As mentioned in section 3.4 *Category : EDITORIAL*
302	531	**Teixeira DC, Saillard C, Couture C, Martins E, Wulff NA, Yamamoto PT, Eveillard-Jagoueix S, Ayres AJ & Bové JM**. 2008. Candidatus Liberibacter americanus, agent of huanglongbing disease of citrus in Sao Paulo State, Brasil: Distribution and quantification of the liberibacter in leaves of an affected sweet orange tree as determined by PCR methods. Molecular and Cellular Probes 22, 139-150.**Wang Z., Yin, Y., Hu, H., Yuan, Q., Peng, G. & Xia, Y.** 2006. Development and application of molecular based diagnosis for '*Candidatus* Liberibacter asiaticus', the causal pathogen of citrus huanglongbing. *Plant Pathology*, 55: 630–638.	P	**COSAVE** Mentioned in section3.4 *Category : EDITORIAL*
303	532	**Weller, S.A., Elphinstone, J.G., Smith, N.C., Boonham, N. ~~and~~ & Stead, D.** 2000. Detection of *Ralstonia solanacearum* strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Applied and environmental microbiology*, 66: ~~2853-2858~~2853–2858.	P	**EPPO** Typos ("&" for consistency with the other references; "-" replaced with "–" for the pages). *Category : EDITORIAL*
304	533	**Yamamoto, P.T., Felippe, M.R., Garbim, L.F., Coelho, J.H.C., Martins, E.C. & Leite, A.P.R.** 2006. *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllydae): Vector of the bacterium *Candidatus* Liberibacter americanus. *In:* Proceedings of the Huanglongbing-Greening International Workshop. Fundecitrus, Araraquara, p. 96. Ribeirão Preto, Brazil.**Zhou, L., Powell, C. A., Hoffman, M. T., Li, W., Fan, G., Liu, B., Lin, H., and Duan, Y.** 2011. Diversity and plasticity of the intracellular plant pathogen and insect symbiont "Candidatus Liberibacter asiaticus" as revealed by hypervariable prophage genes with intragenic tandem repeats. Appl Environ Microbiol. 77:6663-6673. doi:10.1128/AEM.05111-11:	P	**COSAVE** As mentioned in paragraph 245 *Category : EDITORIAL*

305	533	**Yamamoto, P.T., Felippe, M.R., Garbim, L.F., Coelho, J.H.C., Martins, E.C. & Leite, A.P.R.** 2006. *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllydae): Vector of the bacterium *Candidatus* Liberibacter americanus. *In:* Proceedings of the Huanglongbing-Greening International Workshop. Fundecitrus, Araraquara, p. 96. Ribeirão Preto, Brazil.**Zhou, L., Powell, C. A., Hoffman, M. T., Li, W., Fan, G., Liu, B., Lin, H., and Duan, Y. 2011. Diversity and plasticity of the intracellular plant pathogen and insect symbiont "Candidatus Liberibacter asiaticus" as revealed by hypervariable prophage genes with intragenic tandem repeats. Appl Environ Microbiol. 77:6663-6673. doi:10.1128/AEM.05111-11:**	P	**Uruguay** As mentioned in paragraph 245 *Category : TECHNICAL*
306	533	**Yamamoto, P.T., Felippe, M.R., Garbim, L.F., Coelho, J.H.C., Martins, E.C. & Leite, A.P.R.** 2006. *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllydae): Vector of the bacterium *Candidatus* Liberibacter americanus. *In:* Proceedings of the Huanglongbing-Greening International Workshop. Fundecitrus, Araraquara, p. 96. Ribeirão Preto, Brazil.**Zhou, L., Powell, C. A., Hoffman, M. T., Li, W., Fan, G., Liu, B., Lin, H., and Duan, Y.** 2011. Diversity and plasticity of the intracellular plant pathogen and insect symbiont "Candidatus Liberibacter asiaticus" as revealed by hypervariable prophage genes with intragenic tandem repeats. Appl Environ Microbiol. 77:6663-6673. doi:10.1128/AEM.05111-11:	P	**Brazil** As mentioned in paragraph 245 *Category : EDITORIAL*
9. Figures				
307	538	**Figure 2.** Four-year-old *Citrus sinensis* (orange) tree declining from Huanglonbing. Small upright leaves near shoot tips (where transmission take place), leaf drop/canopy thinning, and dieback. Photo courtesy of Greg McCollum, Agricultural Research Service, United States Department of Agriculture, United States.	P	**EPPO** Typo (one space deleted). *Category : EDITORIAL*