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Candidatus Liberibacter Pest Report to support ranking of EU candidate priority pests

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1. Introduction to the report

This document is one of the 28 Pest Reports produced by the EFSA Working Group on EU Priority Pests under task 3 of the mandate M-2017-0136. It supports the corresponding Pest Datasheet published together on Zenodo¹ and applies the methodology described in the Methodology Report published on the EFSA Journal (EFSA, 2019).

This Pest Report has five sections. In addition to this introduction, a conclusion and references, there are two key sections, sections 2 and 3.

Section 2 first summarises the relevant information on the pest related to its biology and taxonomy. The second part of Section 2 provides a review of the host range and the hosts present in the EU in order to select the hosts that will be evaluated in the expert elicitations on yield and quality losses. The third part of Section 2 identifies the area of potential distribution in the EU based on the pest's current distribution and assessments of the area where hosts are present, the climate is suitable for establishment and transient populations may be present. The fourth part of Section 2 assesses the extent to which the presence of the pest in the EU is likely to result in increased treatments of plant protection products. The fifth part of section 2 reviews additional potential effects due to increases in mycotoxin contamination or the transmission of pathogens.

In Section 3, the expert elicitations that assess potential yield losses, quality losses, the spread rate and the time to detection are described in detail. For each elicitation, the general and specific assumptions are outlined, the parameters to be estimated are selected, the question is defined, the evidence is reviewed and uncertainties are identified. The elicited values for the five quantiles are then given and compared to a fitted distribution both in a table and with graphs to show more clearly, for example, the magnitude and distribution of uncertainty. A short conclusion is then provided.

The report has two appendices. Appendix A contains a host list created by amalgamating the host lists in the EPPO Global Database (EPPO, online) and the CABI Crop Protection Compendium (CABI, 2019). Appendix B provides a summary of the evidence used in the expert elicitations.

It should be noted that this report is based on information available up to the last day of the meeting² that the Priority Pests WG dedicated to the assessment of this specific pest. Therefore, more recent information has not been taken into account.

For *Candidatus* Liberibacter, the following documents were used as key references: Bové, 2006; Gottwald, 2010; Grafton-Cardwell et al., 2013; Wang et al., 2017.

¹ Open-access repository developed under the European OpenAIRE program and operated by CERN, https://about.zenodo.org/

² The minutes of the Working Group on EU Priority Pests are available at http://www.efsa.europa.eu/sites/default/files/wgs/plant-health/wg-plh-EU Priority pests.pdf



2. The biology, ecology and distribution of the pest

2.1. Summary of the biology and taxonomy

This report focuses on *Candidatus* Liberibacter spp. associated with citrus greening (from now on CL). These are nonculturable, gram-negative, phloem-limited bacteria belonging to the Rhizobiaceae family mostly vectored by psyllid insects. Yellow shoots, leaf blotchy mottle, and lopsided fruits with colour inversion and aborted seeds are quite specific symptoms, but they do not necessarily appear together, or can be masked by other factors and be confused with mineral deficiencies or other diseases (Bové, 2006; EPPO, 2014). The symptomatology is however similar to that caused by other phloem-limited pathogens, such as *Spiroplasma citri* or *Candidatus* Phytoplasma probably due to the common consequences given by the disruption of phloem function (Wang et al., 2017). The direction of the systemic infection follows the direction of phloem sap: from sources (leaves) to sinks (roots, tubers, flushes, fruit) with a speed of approximately 2-3 cm/day (Wang et al., 2017).

Infections ultimately lead to dieback, stunted growth and plant death (Bové, 2006; Gottwald et al., 2007; Berk, 2016). When the tree is still alive, the infected fruit are of reduced quality due to the production of secondary metabolites associated with bitter and astringent tastes in orange juice (Dala Paula et al., 2017). The consequences on fruit quality are also dependent on citrus species and cultivar (e.g. experiment by Bassanezi et al. (2009): less pronounced on early and mid-season sweet orange cultivars — cv. Hamlin, Valencia Americana and Westin and cv. Pera respectively — than on late season cv. Valencia). The assessment therefore does not include quality losses, and under yield losses considers together tree decline, reduced number of fruits, reduced fruit size, change of taste.

Latent infections can take years before appearing and the distribution of the bacteria in the plant is uneven (EPPO, 2014). However, Coletta-Filho et al. (2010) observed a direct relationship between the concentration of pathogen and the expression of symptoms.

The location in the vascular tissue makes these pathogens inaccessible by chemical treatments and their spread requires the presence of vectors. This pathogen will survive as long as the infected host or vector survives.

This assessment includes the three following *Candidatus* Liberibacter species, the agents of citrus greening (or Huanglongbing; from now on HLB):

Candidatus africanus	Liberibacter	primarily transmitted by <i>Trioza erytreae</i> (Bové, 2006)	in Africa and the Mascarene islands
Candidatus americanus	Liberibacter	transmitted by <i>Diaphorina</i> citri (Teixeira et al., 2005)	up to now only found in Brazil
Candidatus asiaticus	Liberibacter	transmitted by <i>Diaphorina</i> citri (Saponari et al., 2010)	mainly in Asia, America and more recently in Ethiopia

Diaphorina citri and Trioza erytreae have shown experimentally the capacity to transmit both CL africanus and CL asiaticus (Massonie et al., 1976; Aubert, 1987). Also a small proportion of fourth and fifth instar nymphs are reportedly able to transmit the pathogen (van den Berg et al., 1991–1992), but they are vulnerable to desiccation (Aubert, 1987), therefore with a limited capacity to spread the disease.



Two more psyllid species have been found positive to CL spp: *Diaphorina communis* in Bhutan (Donovan et al., 2012) and *Cacopsylla citrisuga* in China (Cen et al., 2012) but their transmission capacity has not yet been confirmed (Haapalainen, 2014).

CL could be considered in origin an insect endophyte that has adapted to the plant: the vector is the main source of inoculum, making its control essential for the disease management (Gottwald, 2010).

The direct impact of *D. citri* and *T. erytreae*, e.g. the excreted honeydew that coats the outside of fruits and leaves and promotes the growth of sooty mould fungus that inhibits photosynthesis, weakens the plant, and makes fruit unattractive (Martin et al., 2012ab).

No control measures are available against the bacterium and therefore successful eradication programs for CL spp have not yet been found (Polek et al., 2007). However, systemic insecticides can be applied against the vectors (Plant Biosecurity, 2010), but, according to the experience in Florida, a successful eradication of the disease is unlikely to occur where the incidence of HLB is between 50 and 100% (USDA, 2016). Moreover, systemic insecticides may control the vector but be inefficient to avoid CL transmission.

2.2. Host plants

2.2.1. List of hosts

Rutaceae are the natural hosts of CL spp. and all commercial citrus species are susceptible to HLB. All citrus species, including ornamentals, have been shown to harbour the pathogen, e.g. orange jasmine (Murraya paniculata) (Deng et al., 2007), Calodendrum capensis (Nelson et al., 2015), Toddalia lanceolata (Garnier and Bové, 1996). HLB has been experimentally transmitted to several hosts outside the Rutaceae family, including dodder (Cuscuta spp.), Catharanthus roseus and Nicotiana xanthi (Garnier and Bové, 1993), and this would suggest, according to Halbert and Manjunath (2004), a wide physiological host range for the pathogens.

Rootstock selection doesn't influence the disease incidence but can increase the scion tolerance to HLB (Albrecht et al., 2012).

Appendix A provides the full list of hosts.

2.2.2. Selection of hosts for the evaluation

All commercial citrus species are considered equally prone to develop the disease in the EU growing conditions.

2.2.3. Conclusions on the hosts selected for the evaluation

The impacts on all commercial citrus species and varieties were assessed together since they are assumed to have similar susceptibilities to *Candidatus* Liberibacter species.



2.3. Area of potential distribution

2.3.1. Area of current distribution

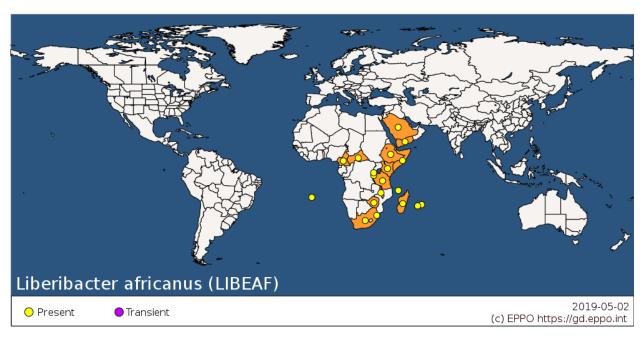


Figure 1 Distribution map of Candidatus Liberibacter africanus from the EPPO Global Database accessed 02/05/2019.

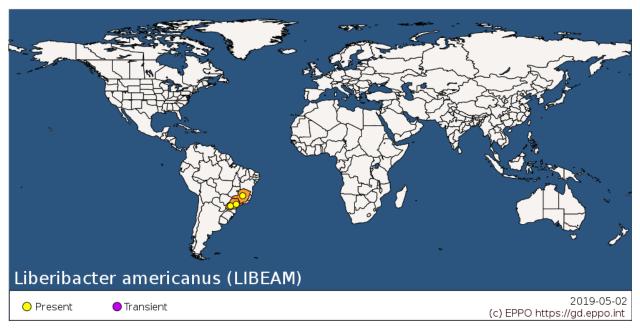


Figure 2 Distribution map of Candidatus Liberibacter americanus from the EPPO Global Database accessed 02/05/2019.



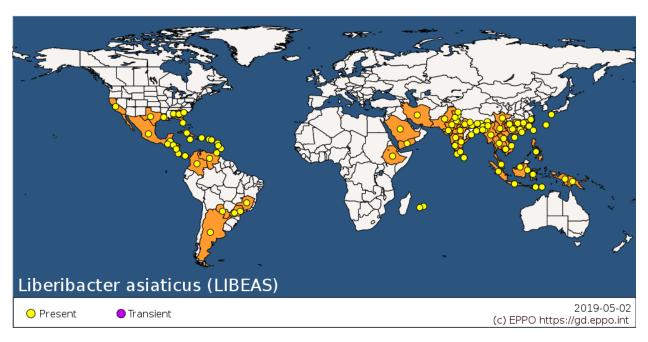


Figure 3 Distribution map of Candidatus Liberibacter asiaticus from the EPPO Global Database accessed 02/05/2019.

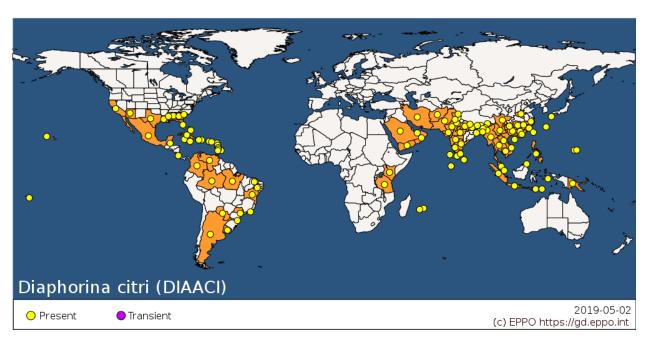


Figure 4 Distribution map of *Diaphorina citri* from the EPPO Global Database accessed 02/05/2019.



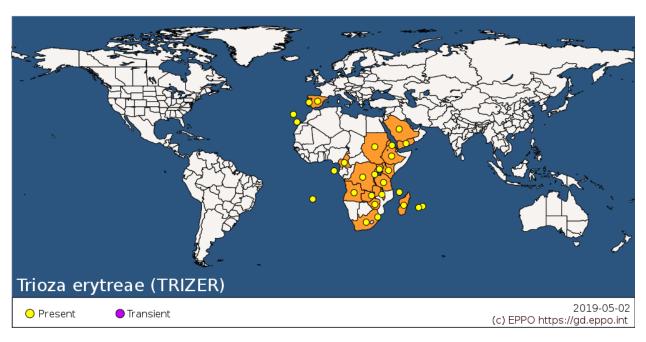


Figure 5 Distribution map of Trioza erytreae from the EPPO Global Database accessed 02/05/2019.

2.3.2. Area of potential establishment

The area of potential distribution takes into account the potential maximum distribution of the three CL species and of the two vectors (*D. citri* and *T. erytreae*).

CL africanus (discovered in Yemen) is heat-sensitive: Bové (2006) indicates that disease symptoms are expressed at 22–24 °C but not above 32 °C.

According to many authors, CL americanus has temperature sensitivities similar to CL africanus, but Teixeira et al. (2005) observed severe symptoms at both cool (22-24 °C) and warm (27-32 °C) conditions, suggesting that CL americanus is a heat-tolerant form (Bové, 2006).

CL asiaticus was discovered in Saudi Arabia but the earliest description of HLB-like symptoms came from central India in the 1700's (Gottwald, 2010). It is heat-tolerant, causing symptoms at both cool and warm (even above 30 °C) temperatures and at low humidity (Bové, 2006; Li et al., 2008). It causes heavy damagealso to the formerly resistant pomelo and kumquat (Tsai et al., 2008).

T. erytreae is heat sensitive and its vectoring capacity is limited to areas with temperatures lower than 32 °C combined with 30% relative humidity (Aubert, 1987). Adults spend 2–3 months on host plants in cooler environments (van den Berg, 1990) and nymphs require a temperature threshold of 10–11 °C to develop (Liu and Tsai, 2000).

T. erytreae was found in Madeira in 1994 and in the Canary Islands in 2002, then in continental Europe, more precisely in Galicia (August 2014) and later (January 2015) in northern Portugal (Siverio et al., 2017).

Green and Catling (1971) showed the combined effects of temperature and humidity (measured as the saturation deficient index) and have mapped the distribution and prevalence of *T. erytreae* in South Africa through time. Only regions or seasons with high humidity and moderate temperatures resulted in high populations of *T. erytreae*. This explains why in South Africa the damage caused by CL africanus infections is particularly severe in cool areas above 600 m, with relative humidity above 25% (Bové et al., 2008).



The effect of climate on the spread of *T. erytreae* is best illustrated in Réunion Island in the Indian Ocean. The climate of Réunion is tropical, with temperatures affected by elevation. The average coastal temperature is between 18–31 °C, with temperatures dropping in the interior; humidity is high. Here, *T. erytreae* co-exists with *D. citri* (da Graça, 1991). *Trioza erytreae* is restricted to altitudes above 500-700 m where the climate is cooler while *D. citri* occurs below this altitude where the climate is warmer (da Graça, 1991; Bové, 2006).

Diaphorina citri thrives in warm environments and is tolerant of high temperatures (Bové, 2006). Depending on the duration of freeze events, a large majority of *D. citri* adults would survive freezing temperatures of 0 to -4 °C and might survive even to < -6.5° C. However, a freeze event severe enough to kill flush leaves (on which oviposition and nymph development occur) would cause mass mortality of immatures, except perhaps of older nymphs that might complete development on stems or mature leaves (Hall et al., 2011). The developmental period under experimental conditions is: 14-15 days at 28 °C and 43.5 days at 18 °C (Liu and Tsai, 2000; Nava et al., 2007), with the optimal temperatures reported at 24–28 °C.

Observations on spatiotemporal dynamics of *D. citri* invasion support the hypothesis that its spread is attributable to habitat suitability more than to wind direction (Bayles et al., 2017). Temperature, photoperiod and rainfall have been proved to produce morphometric variations on *D. citri*, with potential consequences on its spread capacity, as already observed in several insects' taxa (Paris et al., 2017). Also host species plays a role on the psyllid size and shape (Paris et al., 2016). Temperature resulted to be the most relevant factor: adult *D. citri* reared at 20°C were larger than those reared at 30°C and would have developed at approximately half the rate, based on prior studies (Paris et al., 2017).

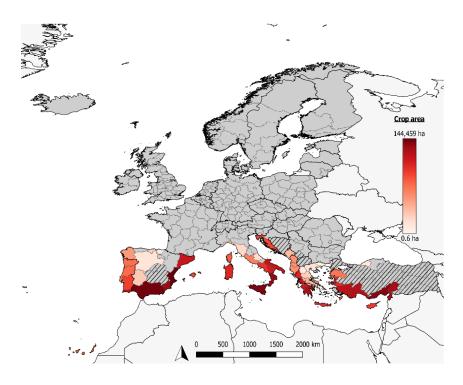


Figure 6 Citrus spp. growing areas. Statistic data of crop area at NUTS 2 level. Areas with lines indicate areas with no data (figure from EFSA PLH Panel, 2019).



In this assessment the area of potential distribution for *Candidatus* Liberibacter species is represented by the sum of the areas of potential establishment for all the CL species and their vectors (Figures 1-5). Since the CL species and their vectors are found in areas comparable to the climates at the limits of citrus production in the EU, the area of potential distribution for is considered to be equivalent to the area of *Citrus* production in the EU (Figure 6).

2.3.3. Transient populations

Candidatus Liberibacter is not expected to form transient populations in the EU (for "transient" see the definition in EFSA, 2019).

2.3.4. Conclusions on the area of potential distribution

The area of potential distribution for *Candidatus* Liberibacter species is equivalent to the area of *Citrus* production in the EU (Figure 7).



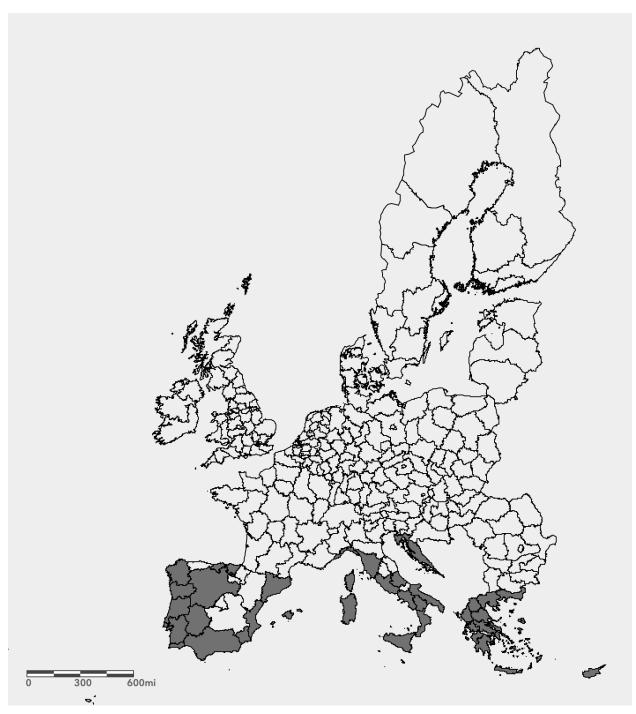


Figure 7 The potential distribution of the pest in the EU NUTS2 regions based on the scenarios established for assessing the impacts of the pest by the EFSA Working Group on EU Priority Pests (EFSA, 2019). This link provides an online interactive version of the map that can be used to explore the data further: https://arcg.is/1Lv5vr

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2.4. Expected change in the use of plant protection products

No curative methods to control CL are available and so control is by preventing trees from becoming infected (Bové, 2006). Once the pathogen reaches a new area of invasion, the only possible action is to limit the number of infected trees by eliminating the inoculum (tree removal) and reducing the vector population (Bové, 2006). Aubert (2008) indicates that chemical protection alone against extremely fertile species such as psyllid vectors may end in a vicious cycle with rising levels of resistance and damage to the environment. In addition, many insecticides presently used to control psyllids are systemic, requiring immigrating psyllids to feed to acquire lethal levels of insecticide and therefore ending up with CL transmission prior to death of vectors (Gottwald, 2010). The use of biocontrol agents has been effective in specific circumstances (e.g. in Reunion Island) (Gottwald, 2010).

Gottwald (2010) summarises the control strategy as follows:

- control of vectors in commercial plantings to reduce transmission;
- removal of infected trees in commercial plantings to reduce inoculum sources;
- geographical isolation and disease certification programs for budwood sources;
- geographical isolation of nursery production;
- all citrus nursery production conducted in secure insect proof screen houses.

In conclusion, based on the table below, this pest belongs to Case "D" and category "2", as an increase in the number of treatments is not expected to be effective in controlling *C.* Liberibacter and integrated strategies will need to be applied.

Table 1: Expected changes in the use of Plant Protection Products (PPPs) following *Candidatus* Liberibacter establishment in the EU in relation to four cases (A-D) and three level score (0-2) for the expected change in the use of PPPs

Expected change in the use of PPPs	Case	PPPs
		indicator
PPPs effective against the pest are not available/feasible in the EU	Α	0
PPPs applied against other pests in the risk assessment area are also effective against the	В	0
pest, without increasing the amount/number of treatments		
PPPs applied against other pests in the risk assessment area are also effective against the	С	1
pest but only if the amount/number of treatments is increased		
A significant increase in the use of PPPs is not sufficient to control the pest: only new	D	2
integrated strategies combining different tactics are likely to be effective		

2.5. Additional potential effects

2.5.1. Mycotoxins

The species is not known to be related to problems caused by mycotoxins.

2.5.2. Capacity to transmit pathogens

The species is not known to vector any plant pathogens.



3. Expert Knowledge Elicitation report

3.1. Yield and quality losses

3.1.1. Structured expert judgement

3.1.1.1. *Generic scenario assumptions*

All the generic scenario assumptions common to the assessments of all the priority pests are listed in the section 2.4.1.1 of the Methodology Report (EFSA, 2019).

3.1.1.2. Specific scenario assumptions

- Vectors do not represent a limitation to the epidemiology of the disease since they are assumed to be present throughout the area of potential establishment of the pathogen
- The impact of the vectors on the host plants is not assessed
- The impact assessment is based on the production cycle of an orchard. Any replacement of infected trees with healthy plants is not taken into account in the assessment of impact
- All commercial citrus species and varieties are assumed to have similar susceptibilities to Candidatus Liberibacter species. The impact assessment therefore takes into account the average losses in the whole EU citrus crop, including most sensitive and the most tolerant hosts
- The incubation period (from infection to symptoms expression) is a maximum of 5 years

3.1.1.3. *Selection of the parameter(s) estimated*

CL infections ultimately lead to dieback, stunted growth and plant death. This is why only yield losses are assessed.

3.1.1.4. Defined question(s)

What is the percentage yield loss in citrus production under the scenario assumptions in the area of the EU under assessment for *Candidatus* Liberibacter, as defined in the Pest Report?

3.1.1.5. Evidence selected

The experts reviewed the evidence obtained from the literature (see Table B.1 in Appendix B) selecting the data and references used as the key evidence for the EKE on impact. A main general point was made on the fact that disease severity is influenced by the climatic conditions.

3.1.1.6. Uncertainties identified

- Most of the available information refers to the incidence
- Most of the available information refers to CL asiaticus, but not much data is available on the other species CL africanus and CL americanus
- Most of the information available refers to D. citri but not to T. erytreae
- HLB and *D. citri* are present in California, but the disease is under eradication and quantitative information on yield losses is lacking



- No indications are available to establish a clear relationship between climatic conditions and level of impact
- Difficult to distinguish between environment and susceptibility effects
- Duration of the incubation and latency periods are not certain, particularly under Mediterranean conditions

3.1.2. Elicited values for yield losses on citrus

What is the percentage yield loss in citrus production under the scenario assumptions in the area of the EU under assessment for *Candidatus* Liberibacter, as defined in the Pest Report?

The five elicited values on yield loss on citrus on which the group agreed are reported in the table below.

Table 2: The 5 elicited values on yield loss (%) on citrus

Percentile	1%	25%	50%	75%	99%
Expert elicitation	20%	50%	70%	80%	95%

3.1.2.1. Justification for the elicited values for yield loss on citrus

Reasoning for a scenario which would lead to high yield loss (99th percentile / upper limit)

The upper value of 95% of yield loss was agreed based on the assumption that there is a latency period of 6 months. In the worst-case scenario, only one out of 20 orchards would still be productive after infection.

Reasoning for a scenario which would lead to low yield loss (1st percentile / lower limit)

The lower value of 20 % was agreed considering that, even in the best case scenario, there is a latency period of around 1.5 year which still brings considerable losses.

Reasoning for a central scenario equally likely to over- or underestimate the yield loss (50th percentile / median)

The median value is quite high (80 %) because it considers the cumulative effect of the latency period and the fact that no replacement is usually made, therefore on a part of infected orchards, below a certain amount of infected plants, there is no removal of them.

Reasoning for the precision of the judgement describing the remaining uncertainties (1st and 3rd quartile / interquartile range)

The precision reflects the fact that there is more certainty around the higher values than to the lower estimates.



3.1.2.2. Estimation of the uncertainty distribution for yield loss

The comparison between the fitted values of the uncertainty distribution and the values agreed by the group of experts is reported in the table below.

Table 3: Fitted values of the uncertainty distribution on citrus

Percentile	1%	2.5%	5%	10%	17%	25%	33%	50%	67%	75%	83%	90%	95%	97.5%	99%
Expert elicitation	20%					50%		70%		80%					95%
Fitted distribution	17.7%	23.8%	29.9%	37.7%	45.0%	52.0%	57.8%	67.8%	76.7%	81.1%	85.7%	89.8%	93.5%	95.7%	97.6%

Fitted distribution: BetaGeneral (3.2128,1.6915,0,1), @RISK7.5

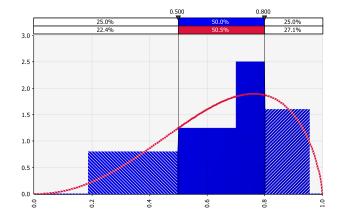


Figure 8 Comparison of judged values (histogram in blue) and fitted distribution (red line) for yield loss on citrus.

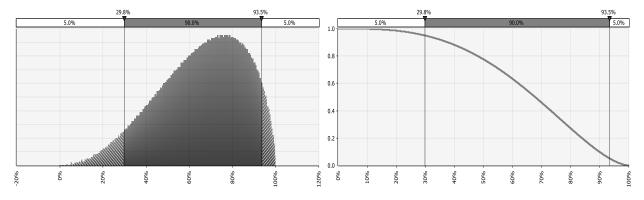


Figure 9 Fitted density function to describe the uncertainties with 90% uncertainty interval (left) and fitted descending distribution function showing the likelihood (y-axis) that a given proportion (x-axis) maybe exceeded (right) for yield loss on citrus.



3.1.3. Conclusions on yield and quality losses

Based on the general and specific scenario considered in this assessment, the proportion (in %) of yield losses on citrus is estimated to be 67.8% (with a 95% uncertainty range of 23.8-95.7%).

Quality losses have not been included in the assessment because considered to be negligible compared to the yield losses.

3.2. Spread rate

3.2.1. Structured expert judgement

3.2.1.1. Generic scenario assumptions

All the generic scenario assumptions common to the assessments of all the priority pests are listed in the section 2.4.2.1 of the Methodology Report (EFSA, 2019).

3.2.1.2. Specific scenario assumptions

- Vectors are present in the EU area of citrus production and their abundance and distribution do not represent a limiting factor to the pathogen spread
- The spread rate is assessed considering the disease transmission by the most efficient vector and its dispersal behavior

3.2.1.3. Selection of the parameter(s) estimated

The spread rate has been assessed as the number of kilometres per year.

3.2.1.4. *Defined question(s)*

What is the spread rate in 1 year for an isolated focus within this scenario based on average European conditions? (units: km/year)

3.2.1.5. Evidence selected

The experts reviewed the evidence obtained from the literature (see Table B.2 in Appendix B) selecting the data and references used as the key evidence for the EKE on spread rate. A main general reference was identified in the data provided by Gottwald et al. (2007).

3.2.1.6. Uncertainties identified

- Extrapolation of behavior of *D. citri* under Mediterranean conditions. Limited information is provided, mainly from California, during the eradication campaign (with effects on spread of the disease)
- Disease spread not accurately assessed by visual inspection of symptoms, due to the long incubation period
- Accuracy of current detection methods when used for cryptic infections



 Climatic conditions, under which observations were collected, are not always comparable with the EU

3.2.2. Elicited values for the spread rate

What is the spread rate in 1 year for an isolated focus within this scenario based on average European conditions? (units: km/year)

The five elicited values on spread rate on which the group agreed are reported in the table below.

Table 4: Summary of the 5 elicited values on spread rate (km/y)

Percentile	1%	25%	50%	75%	99%
Expert elicitation	1.0	12.0	20.0	30.0	40.0

3.2.2.1. *Justification for the elicited values of the spread rate*

Reasoning for a scenario which would lead to wide spread (99th percentile / upper limit)

Gottwald et al. (2007) was mostly considered for this estimate. The paper indicates 20 Km/year as a spread rate based on the dispersal from the wind. However, a point was made that such paper only considers symptomatic plants, therefore the maximum dispersal rate should be increased for the consideration of non-symptomatic plants and also considering the human-mediated spread. A final value of 40 Km (40.000 m) was agreed.

Reasoning for a scenario, which would lead to limited spread (1st percentile / lower limit)

A value of 1 Km (1.000 m) was agreed to be the minimum dispersal rate considering the vector spread (flies) occurring only once a year.

Reasoning for a central scenario, equally likely to over- or underestimate the spread (50th percentile / median)

The median value of 20 Km was agreed based on the evidence provided by Gottwald et al. (2007) and the consideration that, while climatic conditions in Europe might be less favourable to dispersal, there is a need to consider the non-symptomatic plants. Therefore, the average obtained in Brazil can be considered valid for Europe as well.

Reasoning for the precision of the judgement describing the remaining uncertainties (1st and 3rd quartile / interquartile range)

The precision is based on the fact that there is no much uncertainty around the median. Therefore, 12 Km was indicated as 25th percentile and 30 Km for the 75th percentile.



3.2.2.2. Estimation of the uncertainty distribution for the spread rate

The comparison between the fitted values of the uncertainty distribution and the values agreed by the group of experts is reported in the table below.

Table 5: Fitted values of the uncertainty distribution on the spread rate (km/y)

Percentile	1%	2.5%	5%	10%	17%	25%	33%	50%	67%	75%	83%	90%	95%	97.5%	99%
Expert elicitation	1.0					12.0		20.0		30.0					40.0
Fitted distribution	0.9	1.9	3.2	5.5	8.3	11.6	14.7	20.6	26.5	29.6	32.8	35.6	37.9	39.2	40.1

Fitted distribution: BetaGeneral (1.2811,1.2703,0,41), @RISK7.5

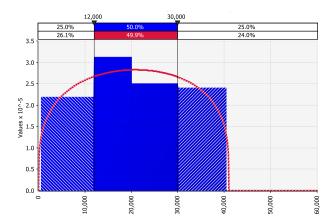


Figure 10 Comparison of judged values (histogram in blue) and fitted distribution (red line) for spread rate.

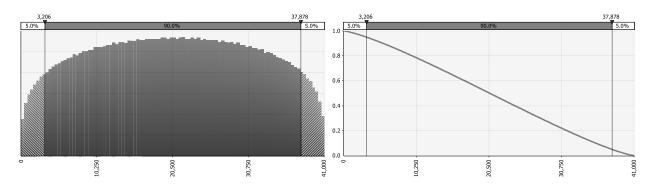


Figure 11 Fitted density function to describe the uncertainties with 90% uncertainty interval (left) and fitted descending distribution function showing the likelihood (y-axis) that a given proportion (x-axis) maybe exceeded (right) for spread rate.



3.2.3. Conclusions on the spread rate

Based on the general and specific scenarios considered in this assessment, the maximum distance expected to be covered in one year by C. Liberibacter is 20 km (with a 95% uncertainty range of 2-39 km).

3.3. Time to detection

3.3.1. Structured expert judgement

3.3.1.1. *Generic scenario assumptions*

All the generic scenario assumptions common to the assessments of all the priority pests are listed in the section 2.4.2.1 of the Methodology Report (EFSA, 2019).

3.3.1.2. Specific scenario assumptions

No specific assumptions are introduced for the assessment of the time to detection.

3.3.1.3. Selection of the parameter(s) estimated

The time for detection has been assessed as the number of months between the first event of pest transfer to a suitable host and its detection.

3.3.1.4. *Defined question(s)*

What is the time between the event of pest transfer to a suitable host and its first detection within this scenario based on average European conditions? (unit: months)

3.3.1.5. Evidence selected

The experts reviewed the evidence obtained from the literature (see Table B.2 in Appendix B) selecting the data and references used as the key evidence for the EKE on time to detection.

3.3.1.6. Uncertainties identified

- Duration of the incubation and latency periods, particularly under Mediterranean conditions
- Accuracy of current methods for pathogen detection uneven distribution of the bacteria in the plant, particularly in mature trees recently infected and asymptomatic
- Disease symptoms are unspecific and can be confused with other systemic diseases or nutrient deficiencies
- Initial outbreaks in California in residential areas with backyard trees, might be not subjected to formal surveys by trained inspectors
- Although the scenario indicates the vectors as present, there is the possible effect of an increase of awareness (with effect on detection probability) considering the risk of presence of HLB in the assessment area



3.3.2. Elicited values for the time to detection

What is the time between the event of pest transfer to a suitable host and its first detection within this scenario based on average European conditions? (unit: months)

The five elicited values on time to detection on which the group agreed are reported in the table below.

Table 6: Summary of the 5 elicited values on time to detection (months)

Percentile	1%	25%	50%	75%	99%
Expert elicitation	9	18	24	36	72

3.3.2.1. Justification for the elicited values of the time to detection

Reasoning for a scenario which would lead to a long time for detection (99th percentile / upper limit)

The upper value of 72 months was agreed by the elicitation experts based on the consideration that time to detection varies quite much in the various assessed literature findings, in overall ranging from 7 months to 13 years. A paper reporting a study carried out in California, reports 7 years to be the maximum time to detection. However, for this study plants imported from Asia were used and therefore it does not represent a natural condition. The experts agreed to decrease this maximum value to 6 years (72 months) to represent the natural conditions in Europe.

Reasoning for a scenario which would lead to a short time for detection (1st percentile / lower limit)

The lower value of 9 months needed for the first detection is based on the assumption that there is a good surveillance of the plants and, if the orchard is in a good condition, it is possible to observe the symptoms at an early stage. Three months would be enough for symptoms expression; however, the experts agreed that, also in the best-case scenario, it is not easy to observe the symptoms. They agreed a minimum of 9 months is needed in average for the shortest time to detection.

Reasoning for a central scenario, equally likely to over- or underestimate the time for detection (50th percentile / median)

The median value of 24 months was agreed representing the sum of the incubation period (18 months) which are needed for a clear symptom expression plus 6 months needed to detect.

Reasoning for the precision of the judgement describing the remaining uncertainties (1st and 3rd quartile / interquartile range)

The precision reflects the fact that the time for lab confirmation is not included in the median period necessary for detection (24 months), so there is a need for 1 more year in order to confirm its presence, thus 36 months is the 75th percentile. More uncertainty exists toward the lower limit (18 months for the 25th percentile).



3.3.2.2. Estimation of the uncertainty distribution for the time to detection

The comparison between the fitted values of the uncertainty distribution and the values agreed by the group of experts is reported in the table below.

Table 7: Fitted values of the uncertainty distribution on the time to detection (months)

Percentile	1%	2.5%	5%	10%	17%	25%	33%	50%	67%	75%	83%	90%	95%	97.5%	99%
Expert elicitation	9					18		24		36					72
Fitted distribution	7.6	9.2	10.8	13.0	15.2	17.6	19.9	24.8	30.9	34.9	40.5	47.5	57.1	67.0	80.7

Fitted distribution: Lognorm (28.211,15.269), @RISK7.5

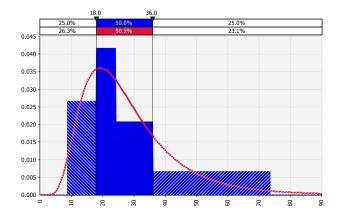


Figure 12 Comparison of judged values (histogram in blue) and fitted distribution (red line) for time to detection.

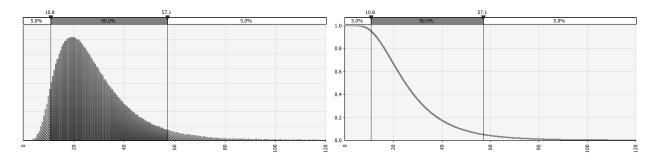


Figure 13 Fitted density function to describe the uncertainties with 90% uncertainty interval (left) and fitted descending distribution function showing the likelihood (y-axis) that a given proportion (x-axis) maybe exceeded (right) for time to detection.



3.3.3. Conclusions on the time to detection

Based on the general and specific scenarios considered in this assessment, the time between the event of pest transfer to a suitable host and its detection is estimated to be around 2 years (with a 95% uncertainty range of 9 months to 5.5 years).

4. Conclusions

Hosts selection

The impacts on all commercial citrus species and varieties were assessed together since they are assumed to have similar susceptibilities to *Candidatus* Liberibacter species.

Area of potential distribution

The area of potential distribution for *Candidatus* Liberibacter species is equivalent to the area of *Citrus* production in the EU.

Increased number of treatments

This pest belongs to Case "D" and category "2", as an increase in the number of treatments is not expected to be effective in controlling C. Liberibacter and integrated strategies will need to be applied.

Yield and quality losses

Based on the general and specific scenario considered in this assessment, the proportion (in %) of yield losses on citrus is estimated to be 67.8% (with a 95% uncertainty range of 23.8-95.7%).

Quality losses have not been included in the assessment because considered to be negligible compared to the yield losses.

Spread rate

Based on the general and specific scenarios considered in this assessment, the maximum distance expected to be covered in one year by *C.* Liberibacter is 20 km (with a 95% uncertainty range of 2-39 km).

Time for detection after entry

Based on the general and specific scenarios considered in this assessment, the time between the event of pest transfer to a suitable host and its detection is estimated to be around 2 years (with a 95% uncertainty range of 9 months to 5.5 years).



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Appendix A – CABI/EPPO host list

The following list, defined in the Methodology Report (EFSA, 2019) as the full list of host plants, is compiled merging the information from the most recent PRAs, the CABI Crop Protection Compendium and the EPPO Global Database. Hosts from the CABI list classified as 'Unknown', as well as hosts from the EPPO list classified as 'Alternate', 'Artificial', or 'Incidental' have been excluded from the list.

Genus	
	Species epithet
Atalantia	buxifolia
Catharanthus	roseus
Citrofortunella	microcarpa
Citroncirus	
Citrus	
Citrus	aurantiifolia
Citrus	aurantium
Citrus	jambhiri
Citrus	latifolia
Citrus	limettioides
Citrus	limon
Citrus	limonia
Citrus	macroptera
Citrus	maxima
Citrus	medica
Citrus	nobilis
Citrus	paradisi
Citrus	reticulata
Citrus	sinensis
Citrus	tangelo
Clausena	indica
Clausena	lansium
Cleome	rutidosperma
Fortunella	
Limonia	acidissima
Murraya	paniculata
Pisonia	aculeata
Poncirus	trifoliata
Rutaceae	
Trichostigma	octandrum
Triphasia	trifolia



Appendix B – Evidence tables

B.1 Summary on the evidence supporting the elicitation of yield and quality losses

Susceptibility	Infection	Symptoms	Impact	Additional information	Reference
	Incidence	Severity	Losses		
Mandarin			Area 1961: 19,330 ha 1965: 12,010 ha (-38%) 1970: 7,080 ha (-63%) 1974: 4,840 ha (-75%)	Entire Philippines production. According to authors the main cause was CL	Altamirano et al., 1976
Sweet orange			1962: 5,750 ha 1965: 5,330 ha (-7%) 1970: 4,600 ha (-20%) 1974: 3,470 ha (-40%)	Entire Philippines production. According to authors the main cause was CL	Altamirano et al., 1976
Pummelo			1962: 6,910 ha 1965: 5,720 ha (-17%) 1970: 5,220 ha (-24%) 1974: 4,200 ha (-39%)	Entire Philippines production. According to authors the main cause was CL	Altamirano et al., 1976
Citrus			CL spp. destroy citrus orchards within 5–8 years where they occur		Gottwald et al., 1989
Citrus	Estimated 4/11 million trees infected		production was virtually eliminated in three major citrus areas	South Africa, mid 1970s	Buitendag and von Broembsen, 1993
Citrus	6/22 adult psyllids sampled from a tree were infected (max psyllid infection rate of 27%)			Brazil, CL americanus However, samples were lots of 10 psyllids and thus the actual infection rate is	Teixeira et al., 2005



				probably lower than 27%	
Citrus			Crop losses of 30–100% have been reported	some production regions of South Africa, between 1932 to 1960	Pretorius and Van Vuuren, 2006
Citrus	Disease incidence: 2 years after plantation (yap): 0.4-20% 3 yap: 26-40% 4 yap: 24% 5 yap: 70% A similar block had an increase from 6% to 27.4% in 9–10 months			São Paulo Brazil orchard surrounded by heavily infected adult trees	Gottwald et al., 2007
Citrus	From 0.2% to 39% incidence in 10 months			Commercial planting in South Florida	Gottwald et al., 2007
Citrus	incidence > 95% in 3–13 years after the first symptoms occur			Fast disease progression	Gottwald et al., 2007
Citrus		Symptoms are more pronounced in somewhat moist, cool conditions and at high elevations.		CL africanus	Schwarz and Green, 1972; Polek et al., 2007
Citrus	the increasing incidence of CL asiaticus suggests the CL americanus is not a good competitor			CL americanus and CL asiaticus at the same location in two orchards of São Paulo State, Brazil	Lopes et al., 2009a and b
8 months-old Valencia sweet orange (C. sinensis)	Bacterial population 10 ³ CN at 30 dpi 10 ⁸ CN at 240 dpi (10,000 times)	10 ⁵ CN g ⁻¹ → yellowed leaves or shoots 10 ⁷ CN g ⁻¹ after 180 dpi → blotchy mottle		Brazil, plants grafted on Rangpur lime (<i>C.</i> <i>limonia</i>) rootstock	Coletta-Filho et al., 2010



Citrus	Disease can reach more than 50% incidence in 3-5 years, whereas in older groves the disease will not reach such high incidence. 3 years after planting, incidence was - 0.96 without any insecticide - 0.74 with conventional insecticides - 0.24 with monthly trunk applications of systemic insecticide	Severe symptoms in trees have been observed 1-5 years after onset of the first symptoms, depending on the age of the tree at the time of infection, but also on the number of infections per tree, which are often multiple.	Younger orchards will express symptoms within 6-12 months after planting, indicating that young, rapidly growing trees, much smaller in canopy volume, have a shorter incubation period. Observations of trees over 10 years of age indicate even slower symptom development.	Excellent paper with relevant information to consider in risk assessment (see table 1 with indicated terminal incidence in different regions according to planting years.	Gottwald, 2010
Citrus		Relationship of the relative yield with HLB severity described by a negative exponential model		Brazil Disease severity and yield were assessed on 949 trees distributed in 11 different blocks from sweet orange cultivars Hamlin, Westin, Pera and Valencia	Bassanezi et al., 2011
Citrus		100%	Up to 19%		Bassanezi et al., 2011
16 citrus scion types	70% after 45 days			Plants in greenhouse exposed to <i>D. citri</i>	McCollum et al., 2016
Citrus			Before 2004: 80 million trees on 748,555 acres (302,929 ha) 2013: 60 million trees on 524,640 acres (212,314 ha) = 30% losses	Florida	USDA, 2016
Grapefruit			From 14 to 5 million trees	Florida	USDA, 2016



Sweet orange	<4.0-80% cumulative disease incidence	~260 plots from 5 farms (2.	São Paulo state,	Belasque et al., 2017
	7.1-25.9% incidence/farm, in spite of similar	5 million trees), under strict	Brazil	
	disease management with high spatial	management (≥4 HLB-tree		
	heterogeneity and high concentration of	removal/year and ≥12		
	affected trees in some plots located in the	insecticide sprays/year)		
	borders.			
	In the majority of plots, especially those			
	presenting higher disease incidences, >95% of			
	HLB-trees occurred up to 200 metres from their			
	borders			

B.2 Summary on the evidence supporting the elicitation of the spread rate

Spread	Additional information	Reference
Fly distances of at least 1.5 km	T. erytreae	Van den Berg and Deacon, 1988
3-5 m	Adult <i>D. citri</i> fly from one tree to the other	Aubert and Xia, 1990
Vertical flight at 7 m followed by wind assisted dispersal of 0.5–1 km	D. citri	Aubert and Xia, 1990
25–30 m	Adults of <i>D. citri</i> when disturbed	Gottwald et al., 1991
> 10 km on seasonal trade winds or hurricanes	It is believed that <i>T. erytreae</i> was introduced from Madeira into the Canary island by the dominant North-South trade winds	Bové, 2006 citing Gonzales Hernandez, 2003
12 miles (19.3 km) per year	D. citri Estimation from the presumed point of introduction to the advancing edge of the epidemic. The most frequent spatial relationship of 1.58 km may well indicate a common or average distance for psyllid dispersal of HLB regionally	Gottwald et al., 2007
There is some evidence for occasional adult mass migrations	D. citri	Hall et al., 2008
	dynamic point model, spatialised over the Australian landscape, for understanding the interactions between <i>D. citri</i> and its Valencia orange host and their responses to increasing temperatures	Aurambout et al., 2009
Fly distances of 8–60 m	D. citri	Hall and Hentz, 2011
The longest continuous flight distance: 978 m for females and 1241 m for males	D. citri using a flight mill	Arakawa and Miyamoto, 2007
	Model analysing how the numbers in each class (i susceptible; ii, infected but not symptomatic; iii infected and symptomatic; iv dead) change over time due to bacterial transmission between trees and psyllids	Jacobsen et al., 2013



	Spatially explicit disease model determining the transmission process among trees (which are either Susceptible, Exposed, Infectious, Detected or Removed) considering the effect of psyllid management, host age. Integration of the model with biological data	Parry et al., 2014
	Model describing how the pattern of HLB spread in a grove depends upon the location within the grove that psyllids initially invade. Integration of the model with biological data	Lee et al., 2015
> 2 km/12 days.	D. citri Japan in the absence of severe weather events, wind direction was not correlated with the number of marked psyllids captured	Lewis-Rosenblum, 2015
	From 2009 (first detection in Tenerife) to 2015, <i>Trioza erythreae</i> colonized all the 7 islands of archipelago	Siverio et al., 2017
Systemic infection: 2-3 cm/day	The direction is that of phloem sap: from sources (leaves) to sinks (roots, tubers, flushes, fruit)	Wang et al., 2017

B.3 Summary on the evidence supporting the elicitation of the time to detection

Category of factors	Case	Effect	Additional information	Reference
Detection methods		Bacterium detected in the fruits even if the population levels were 1000-fold higher in the midribs, leaf blades, bark and roots.	CL asiaticus	Li et al., 2009
		-Incomplete distribution of the bacterium in the tree, could produce false negative -Visual detection inadequate due to long incubation period		Gottwald, 2010
		CL africanus and CL americanus are more similar for symptoms severity and temperature tolerance therefore the two species can be easily confused if identification is only visual		Pietersen et al., 2010
		Generally, the bacterium is detected only in symptomatic fruits.		Plant Biosecurity, 2010
		Difficult to consistently detect the pest through the use of biological assays, the presence of fluorescent substances, light or electron microscopy, or ELISA, presumably because of the low concentration and uneven distribution of the pathogen in hosts and vectors		USDA, 2012
		LAMP is 100 times more sensitive than traditional real time PCR	30 min for 6 samples	Keremane et al., 2015
		Detection techniques comparison		Arredondo Valdés et al., 2016



		Adults of <i>D. citri</i> poorly detected by shoot evaluation conducted 2 to 5 times/month in 1% of trees in each plot. Higher frequency in the most HLB-affected plots where yellow stick cards were used	Sweet orange	Belasque et al., 2017
		When asymptomatic citrus tissues were tested, the tissue printing method gave a higher rate of detection (83%) than the qPCR method (64%)		Ding et al., 2017
		Leaf microbiome profiling and metabolomics are superior to qPCR both for tree diagnoses and individual samples		Levesque et al., 2017
Biology of Dest and Dectors	Vectors life cycle	The duration of the psyllid lifecycle from egg to adult is 15-47 days depending upon food supply and ambient temperature	D. citri	Knapp et al., 2006
		Adults are known to survive 8–9 months over winter on suitable hosts	D. citri	Yang et al., 2006
	Vectors reproduction	217-1305 eggs by a mated female under insectary conditions	T. erytreae	Catling, 1973
		748 eggs laid at 28°C	D citri	Liu and Tsai, 2000
		Extremely fertile vectors: each female lays 1000-2000 eggs in matter of three weeks		Aubert, 2008
	Vectors behaviour	Adults of <i>D. citri</i> survive up to 52.5 (at 30 °C) – 94.5 (at 25 °C) h without feeding if suitable foliage is not available	D. citri	McFarland and Hoy, 2001
		T. erytreae nymphs are embedded in pits or nests on the underside of the leaves which look like bumps on the upper side. The bump remains after the adult emergence, therefore the presence of even one single bump on one single leaf is proof of T. erytreae occurrence. Such bumps are not produced by D. citri		Bové, 2006
		Spatial and temporal distribution of <i>D. citri</i> in Southern California is non-random: statistically significant hotspots of <i>D. citri</i> occurrence identified		Bayles et al., 2017
	Transmission capacity	Adults can acquire the pathogen within 24 h of feeding and after a latent period of an additional 24 h can transmit the disease	T. erytreae	van Vuuren et al., 1986
	, ,	Latent period of 3–4 days if acquisition feeding for 1-4 h no latent period if acquisition feeding for 8-24 hours high transmission rates (23%: the best transmission efficiency observed in literature according to Haapalainen, 2014) within 7 days after a one-day acquisition feeding	T. erytreae adults	van Vuuren and van der Merwe 1992
		30 min feeding for acquisition, 1-21 days of latent period	D. citri	Halbert and Manjunath, 2004
		Psyllids can carry CL asiaticus in either adult or nymphal stages, except in the first instar. The pathogen persists in the adult's body but CL is not transovarially transmitted to the offspring.	D. citri	Hung et al., 2004
		In the absence of control measures, known vectors have been recorded to spread the pathogen to 100% of trees in five years		Bové, 2006
		CL africanus can multiply and survive in the salivary glands of its psyllid vectors for as long as the vector is alive		Gottwald et al., 2007



	Higher titres increase the chances for pathogen acquisition and transmission by the insect vector		Lopes et al., 2009b
	Transmission frequency to sweet orange: - < 38% 6 to 12 months by controlled psyllid inoculation - 50% in 1 year and 100% in min 3 years by natural inoculation in field conditions	D. citri	Albrecht et al., 2014
	CL acquisition by <i>D. citri</i> was positively associated with plant infection level and time since inoculation. Psyllids can acquire CL during the asymptomatic phase	D. citri on sweet orange plants graft inoculated with CL asiaticus	Coletta-Filho et al., 2014
	CL is a circulative and persistent pathogen In the specific population studied by the authors nymphs resulted much more efficient vectors than adults	D. citri	Ammar et al., 2016
	-Young flush become infectious within 15 days after receiving an inoculum of CL asiaticus - Psyllid introduction scenarios to show that entire groves can become infected with up to 12,000 psyllids per tree in less than 1 y, before most of the trees show any symptoms		Lee et al., 2015
	Psyllids transmitted for up to 5 weeks, when submitted to sequential 1-week inoculation access periods IAPs after a 14-day acquisition access period AAP as nymphs. A median latent period (LP50, i.e., acquisition time after which 50% of the individuals can inoculate) of 16.8 and 17.8 days for psyllids that acquired Las as nymphs and adults		Canale et al., 2017
Symptom expression			Gottwald et al., 2007
	Symptom development may be delayed for several months, or as long as 2–3 years after initial infection		Gottwald et al., 2007
	Symptomless leaves are either healthy, and will never give positive PCR reactions, or they are already infected, but the CL titer is still too low to be detected by the PCR methods available. Therefore, CL cannot be detected in symptomless leaves, even though its detection in mottled leaves is straightforward		Bové, 2006
	budwood sourced from asymptomatic trees could present low concentrations of the bacterium		Li et al., 2009
	Plant infection levels increased rapidly over time, saturating at uniformly high levels near 200 days after inoculation	D. citri on sweet orange plants graft inoculated with CL asiaticus	Coletta-Filho et al., 2014
	A newly infected flush can become infectious within 15 days, after transmission. Transmission does not require symptoms expression: infected adult psyllids can transmit the pathogen to the next generation via nymphs feeding on infectious flush	CL asiaticus and <i>D.</i> citri	Lee et al., 2015



	Differences	CL americanus is unable to multiply efficiently in citrus plants	Lopes and Frare, 2008
	among		
	subspecies		
		CL americanus titres in hosts are much lower than CL asiaticus titres. This may result in	Lopes and et al., 2009a
		lower vector acquisition and transmission rates	
		'CL americanus' is less heat tolerant than 'CL asiaticus	Lopes et al., 2009b
Hosts		non-commercial trees are very effective sources of HLB, even when represented by a	Belasque et al., 2017
Hosts		few plants.	