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Pest categorisation of Witches' broom disease of lime (*Citrus aurantifolia*) phytoplasma

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Abstract

The EFSA Panel on Plant Health performed a pest categorisation for the Witches' broom disease of lime (*Citrus aurantifolia*) phytoplasma for the EU territory. The pest has been reported in a few countries in the Middle East and is not known to occur in the EU. The disease is caused by a well-defined phytoplasma strain in the '*Candidatus* Phytoplasma *aurantifolia*' species, for which efficient molecular detection assays are available. The most important known natural host is *Citrus aurantifolia,* which is only grown for ornamental purposes in the EU. Sweet limes, rough lemon and trifoliate orange are also naturally infected by that phytoplasma. The latter can be transmitted by grafting also to some citrus species. Other citrus species were reported to be resistant; however, their susceptibility has been assessed only by symptom observations, and the possible presence of phytoplasmas in symptomless plants cannot be ruled out. The phytoplasma is transmitted by the leafhopper *Hishimonus phycitis*, which is not known to occur in the EU. There is no information on the vector status of other phloem feeding insects of citrus present in the EU. The pest is listed in Annex IIAI of Directive 2000/29/EC. The main pathways for entry, plants for planting and the vector insect, are closed by existing legislation on import of citrus plants. Nevertheless, should the pest enter, it could establish and spread. In countries where Witches' broom disease of lime (WBDL) is present, it has significant impact. The main knowledge gaps concern (1) and vertical transmission of the phytoplasma to *H. phycitis* eggs (2) lack of information regarding susceptibility of citrus crops grown in the EU (3) status of potential insect vectors in the EU. Therefore, the WBDL phytoplasma meets the criteria assessed by EFSA for consideration as a potential Union quarantine pest.

Keywords: Witches' broom disease of lime, phytoplasma, citrus, *Citrus aurantifolia*, *Candidatus* phytoplasma *aurantifolia*

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Table of contents

1. Introduction

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

1.1.1. Background

Council Directive 2000/29/EC[1] on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community establishes the present European Union plant health regime. The Directive lays down the phytosanitary provisions and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union. In the Directive's 2000/29/EC annexes, the list of harmful organisms (pests) whose introduction into or spread within the Union is prohibited, is detailed together with specific requirements for import or internal movement.

Following the evaluation of the plant health regime, the new basic plant health law, Regulation (EU) 2016/2031[2] on protective measures against pests of plants, was adopted on 26 October 2016 and will apply from 14 December 2019 onwards, repealing Directive 2000/29/EC. In line with the principles of the above mentioned legislation and the follow-up work of the secondary legislation for the listing of EU regulated pests, EFSA is requested to provide pest categorizations of the harmful organisms included in the annexes of Directive 2000/29/EC, in the cases where recent pest risk assessment/pest categorisation is not available.

1.1.2. Terms of Reference

EFSA is requested, pursuant to Article 22(5.b) and Article 29(1) of Regulation (EC) No 178/2002[3], to provide scientific opinion in the field of plant health.

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

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

For the above mentioned groups, each covering a large number of pests, the pest categorisation will be performed for the group and not the individual harmful organisms listed under 'such as' notation in the Annexes of the Directive 2000/29/EC. The criteria to be taken particularly under consideration for these cases, is the analysis of host pest combination, investigation of pathways, the damages occurring and the relevant impact.

Finally, as indicated in the text above, all references to 'non-European' should be avoided and replaced by 'non-EU' and refer to all territories with exception of the Union territories as defined in Article 1 point 3 of Regulation (EU) 2016/2031.

[1] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169/1, 10.7.2000, p. 1–112.

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

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

1.1.2.1. Terms of Reference: Appendix 1

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

Annex IIAI

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

Aleurocantus spp.
Anthonomus bisignifer (Schenkling)
Anthonomus signatus (Say)
Aschistonyx eppoi Inouye
Carposina niponensis Walsingham
Enarmonia packardi (Zeller)
Enarmonia prunivora Walsh
Grapholita inopinata Heinrich
Hishomonus phycitis
Leucaspis japonica Ckll.
Listronotus bonariensis (Kuschel)
Numonia pyrivorella (Matsumura)
Oligonychus perditus Pritchard and Baker
Pissodes spp. (non-EU)
Scirtothrips aurantii Faure
Scirtothrips citri (Moultex)
Scolytidae spp. (non-EU)
Scrobipalpopsis solanivora Povolny
Tachypterellus quadrigibbus Say
Toxoptera citricida Kirk.
Unaspis citri Comstock

(b) Bacteria

Citrus variegated chlorosis
Erwinia stewartii (Smith) Dye
Xanthomonas campestris pv. *oryzae* (Ishiyama) Dye and pv. *oryzicola* (Fang. et al.) Dye

(c) Fungi

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

(d) Virus and virus-like organisms

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

Annex IIB

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

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

(b) Bacteria

Curtobacterium flaccumfaciens pv. flaccumfaciens (Hedges) Collins and Jones

(c) Fungi

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

1.1.2.2. Terms of Reference: Appendix 2

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

Annex IAI

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

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

1) *Carneocephala fulgida* Nottingham
2) *Draeculacephala minerva* Ball
3) *Graphocephala atropunctata* (Signoret)

Group of Tephritidae (non-EU) such as:

1) *Anastrepha fraterculus* (Wiedemann)
2) *Anastrepha ludens* (Loew)
3) *Anastrepha obliqua* Macquart
4) *Anastrepha suspensa* (Loew)
5) *Dacus ciliatus* Loew
6) *Dacus curcurbitae* Coquillet
7) *Dacus dorsalis* Hendel
8) *Dacus tryoni* (Froggatt)
9) *Dacus tsuneonis* Miyake
10) *Dacus zonatus* Saund.
11) *Epochra canadensis* (Loew)
12) *Pardalaspis cyanescens* Bezzi
13) *Pardalaspis quinaria* Bezzi
14) *Pterandrus rosa* (Karsch)
15) *Rhacochlaena japonica* Ito
16) *Rhagoletis completa* Cresson
17) *Rhagoletis fausta* (Osten-Sacken)
18) *Rhagoletis indifferens* Curran
19) *Rhagoletis mendax* Curran
20) *Rhagoletis pomonella* Walsh
21) *Rhagoletis suavis* (Loew)

(c) Viruses and virus-like organisms

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

1) Andean potato latent virus
2) Andean potato mottle virus
3) Arracacha virus B, oca strain
4) Potato black ringspot virus
5) Potato virus T
6) non-EU isolates of potato viruses A, M, S, V, X and Y (including Yo, Yn and Yc) and Potato leafroll virus

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

1) Blueberry leaf mottle virus
2) Cherry rasp leaf virus (American)
3) Peach mosaic virus (American)
4) Peach phony rickettsia
5) Peach rosette mosaic virus
6) Peach rosette mycoplasm
7) Peach X-disease mycoplasm
8) Peach yellows mycoplasm
9) Plum line pattern virus (American)
10) Raspberry leaf curl virus (American)
11) Strawberry witches' broom mycoplasma
12) Non-EU viruses and virus-like organisms of *Cydonia Mill., Fragaria L., Malus Mill., Prunus L., Pyrus L., Ribes L., Rubus L.* and *Vitis L.*

Annex IIAI

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

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

1) *Margarodes vitis* (Phillipi)
2) *Margarodes vredendalensis* de Klerk
3) *Margarodes prieskaensis* Jakubski

1.1.2.3. Terms of Reference: Appendix 3

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

Annex IAI

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

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

(b) Fungi

Ceratocystis fagacearum (Bretz) Hunt
Chrysomyxa arctostaphyli Dietel
Cronartium spp. (non-EU)
Endocronartium spp. (non-EU)
Guignardia laricina (Saw.) Yamamoto and Ito
Gymnosporangium spp. (non-EU)
Inonotus weirii (Murril) Kotlaba and Pouzar
Melampsora farlowii (Arthur) Davis
Mycosphaerella larici-leptolepis Ito et al.
Mycosphaerella populorum G. E. Thompson
Phoma andina Turkensteen
Phyllosticta solitaria Ell. and Ev.
Septoria lycopersici Speg. var. *malagutii* Ciccarone and Boerema
Thecaphora solani Barrus
Trechispora brinkmannii (Bresad.) Rogers

(c) Viruses and virus-like organisms

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

(d) Parasitic plants

Arceuthobium spp. (non-EU)

Annex IAII

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

Meloidogyne fallax Karssen
Popillia japonica Newman
Rhizoecus hibisci Kawai and Takagi

(b) Bacteria

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

(c) Fungi

Melampsora medusae Thümen
Synchytrium endobioticum (Schilbersky) Percival

Annex I B

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

Leptinotarsa decemlineata Say
Liriomyza bryoniae (Kaltenbach)

(b) Viruses and virus-like organisms

Beet necrotic yellow vein virus

1.2. Interpretation of the Terms of Reference

Witches' broom (MLO) is one of a number of pests listed in the appendices to the Terms of Reference (ToR) to be subject to pest categorisation to determine whether it fulfils the criteria of a quarantine pest or those of a Union regulated non-quarantine pest (RNQP) for the area of the European Union (EU), excluding Ceuta, Melilla and the outermost regions of Member States (MSs) referred to in Article 355(1) of the Treaty on the Functioning of the European Union (TFEU), other than Madeira and the Azores.

'Witches' broom' is an unclear term as it is not the name of a particular disease, but it describes symptoms that can be attributed to many different pests. Directive 2000/29/EC considers Witches' broom (MLO) only on 'plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds', and therefore, for the purpose of this pest categorisation, the PLH Panel considers only the phytoplasma strains that cause witches' broom symptoms on *Citrus aurantifolia.* The causal agent, which is now known to be a phytoplasma, corresponds to the Witches' broom disease of lime (WBDL) phytoplasma. From here on the PLH Panel will refer to this as WBDL phytoplasma. This is one specific strain within the '*Candidatus* Phytoplasma aurantifolia' species.

2. Data and methodologies

2.1. Data

2.1.1. Literature search

A literature search on Witches' broom (MLO) was conducted at the beginning of the categorisation in the ISI Web of Science bibliographic database, using the scientific name of the pest (Witches' broom disease of lime phytoplasma and '*Candidatus* phytoplasma aurantifolia') as search terms. Relevant papers were reviewed, and further references and information were obtained from experts, from citations within the references and grey literature.

2.1.2. Database search

Pest information, on host(s) and distribution, was retrieved from the EPPO Global Database (EPPO, 2017).

Data about import of commodity types that could potentially provide a pathway for the pest to enter the EU and about the area of hosts grown in the EU were obtained from EUROSTAT.

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

2.2. Methodologies

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

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

Table 1 presents the Regulation (EU) 2016/2031 pest categorisation criteria on which the Panel bases its conclusions. All relevant criteria have to be met for the pest to potentially qualify either as a quarantine pest or as a RNQP. If one of the criteria is not met, the pest will not qualify. Note that a pest that does not qualify as a quarantine pest may still qualify as a RNQP which needs to be addressed in the opinion. For the pests regulated in the protected zones only, the scope of the categorisation is the territory of the protected zone; thus, the criteria refer to the protected zone instead of the EU territory.

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

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

Criterion of pest categorisation	Criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Criterion in Regulation (EU) 2016/2031 regarding protected zone quarantine pest (articles 32–35)	Criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest
Identity of the pest (Section 3.1)	Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?	Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?	Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?
Absence/presence of the pest in the EU territory (Section 3.2)	Is the pest present in the EU territory? If present, is the pest widely distributed within the EU? Describe the pest distribution briefly.	Is the pest present in the EU territory? If not, it cannot be a protected zone quarantine organism.	Is the pest present in the EU territory? If not, it cannot be a regulated non-quarantine pest (a regulated non-quarantine pest must be present in the risk assessment area).

Criterion of pest categorisation	Criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Criterion in Regulation (EU) 2016/2031 regarding protected zone quarantine pest (articles 32–35)	Criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest
Regulatory status (Section 3.3)	If the pest is present in the EU but not widely distributed in the risk assessment area, it should be under official control or expected to be under official control in the near future.	The protected zone system aligns with the pest-free area system under the International Plant Protection Convention (IPPC). The pest satisfies the IPPC definition of a quarantine pest that is not present in the risk assessment area (i.e. protected zone).	Is the pest regulated as a quarantine pest? If currently regulated as a quarantine pest, are there grounds to consider its status could be revoked?
Pest potential for entry, establishment and spread in the EU territory (Section 3.4)	Is the pest able to enter into, become established in and spread within the EU territory? If yes, briefly list the pathways.	Is the pest able to enter into, become established in and spread within the protected zone areas? Is entry by natural spread from EU areas where the pest is present possible?	Is spread mainly via specific plants for planting rather than via natural spread or via movement of plant products or other objects? Clearly state if plants for planting is the main pathway
Potential for consequences in the EU territory (Section 3.5)	Would the pests' introduction have an economic or environmental impact on the EU territory?	Would the pests' introduction have an economic or environmental impact on the protected zone areas?	Does the presence of the pest on plants for planting have an economic impact, as regards the intended use of those plants for planting?
Available measures (Section 3.6)	Are there measures available to prevent the entry into, establishment within or spread of the pest within the EU such that the risk becomes mitigated?	Are there measures available to prevent the entry into, establishment within or spread of the pest within the protected zone areas such that the risk becomes mitigated? Is it possible to eradicate the pest in a restricted area within 24 months (or a period longer than 24 months where the biology of the organism so justifies) after the presence of the pest was confirmed in the protected zone?	Are there measures available to prevent pest presence on plants for planting such that the risk becomes mitigated?
Conclusion of pest categorisation (Section 4)	A statement as to whether (1) all criteria assessed by EFSA above for consideration as a potential quarantine pest were met and (2) if not, which one(s) were not met.	A statement as to whether (1) all criteria assessed by EFSA above for consideration as potential protected zone quarantine pest were met, and (2) if not, which one(s) were not met.	A statement as to whether (1) all criteria assessed by EFSA above for consideration as a potential regulated non-quarantine pest were met, and (2) if not, which one(s) were not met.

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

3. Pest categorisation

3.1. Identity and biology of the pest

3.1.1. Identity and taxonomy

Is the identity of the pest established? **YES**

Witches' broom disease of lime is caused by a strain of '*Candidatus* Phytoplasma aurantifolia', a wall-less bacterium within the family Acholeplasmataceae (Martini et al., 2014). All phytoplasmas are classified as *'Ca.* Phytoplasma sp'. due to the fact that they cannot be grown in axenic culture, and therefore, the current rules for bacterial classification cannot be completely fulfilled.

The phytoplasma was first detected in the Sultanate of Oman in the 1970s (Bové, 1986) and it was assigned to the '*Ca.* Phytoplasma aurantifolia' species (Zreik et al., 1995). The 16S rRNA gene sequence from the Oman isolate was produced (GenBank Accession Number U15442), and later compared to those of other isolates from the United Arab Emirates, Iran and others from the Sultanate of Oman, showing low genetic diversity (Al-Abadi et al., 2016).

In the absence of a fully sequenced reference genome, all analysed Witches' broom (WBDL) phytoplasmas form a homogeneous cluster within the same 16S rRNA-based phytoplasma classification (16SrII group).

3.1.2. Biology of the pest

The WBDL phytoplasma lives and multiplies in the phloem of infected plants. Infected lime trees show small light green to yellow leaves and short internodes, dense branching and reduced flowering and fruiting. Symptoms of the disease usually appear in lime trees that are at least 2 years old (Al-Sadi et al., 2012), and some of the infected trees may die within 6–12 years after first symptom appearance (Al-Sadi et al., 2012; Al-Ghaithi et al., 2016). Symptom severity positively correlates with tree age (Al-Yahyai et al., 2015).

The WBDL phytoplasma is transmitted to lime in a persistent and propagative manner by the leafhopper *H. phycitis,* as infected field collected individuals successfully transmitted the disease to *Citrus reticulata* hybrids (Salehi et al., 2007). According to the available databases of Fauna Europaea and EPPO Global Database, the known vector is not present in the EU, although a species from the same genus (*Hishimonus hamatus*) has been reported in Slovenia (Seljak, 2013) and, in 2014 in Italy.[4] However, the ability of *H. hamatus* to transmit WBDL is unknown.

Small fruited acid lime (*C. aurantifolia*) is the most severely and widely affected species, but in the UAE, sweet limes (*Citrus limetta* and *Citrus limettioides*) also showed severe symptoms (Bové and Garnier, 2000). Rough lemon (*Citrus jambhiri*) and hardy orange (*Poncirus trifoliata*) were found infected by WBDL (EPPO, 2006, Al-Yahyai et al., 2012), and grapefruit *(Citrus paradisi*) and citron (*Citrus medica*) were also reported to be infected in Iran (Azadvar et al., 2015; Najafinia and Azadvar, 2016), although the genetic identity of the phytoplasma involved was not confirmed.

Witches' broom (WBDL) can also be transmitted by grafting and, as with all other phytoplasmas, it is not known to be transmitted by mechanical inoculation.

There is no confirmed evidence that the phytoplasma is seed transmitted, but seed from infected plants was shown to have lower germination rates than that from uninfected plants (Faghihi et al., 2011).

3.1.3. Intraspecific diversity

Phytoplasmas cannot be grown in axenic conditions, and therefore, their classification is based on the sequence of their 16S rRNA gene. Witches' broom (WBDL) belongs to the 16SrII group of the phytoplasma classification system. Since 2004, phytoplasmas sharing less than 97.5% similarity among their 16S rRNA gene sequences have been ascribed to different '*Candidatus* Phytoplasma species'. Phytoplasmas that share more than 97.5% similarity of their 16S rRNA gene sequences may also be ascribed to different '*Ca*. Phytoplasma' species, when they are characterised by distinctive biological, phytopathological and genetic properties (IRPCM, 2004).

[4] http://www.fitosanitario.re.it/pubblicazioni-e-notiziar/notiziario-fitopatologico/notiziario-fitopatologico/dicembre-2014/cicaline-crescono-hishimonus-hamatus/

WBDL phytoplasma is a member of the '*Ca*. *P.* aurantifolia' species, and isolates from areas where the disease is present show low levels of genetic diversity (Al-Abadi et al., 2016). Phytoplasmas from this same '*Ca.* Phytoplasma' species that are closely related to WBDL (showing more than 97.5 % similarity at the 16S rRNA gene sequence level), have been reported from wild herbaceous hosts (*Chenopodium morale* L., *Plantago lanceolata* L., *Convolvulus arvensis*) and the leafhopper *Empoasca decipiens* from Saudi Arabia (Alhudaib et al., 2009), vegetables (cabbage, grapevine, faba bean), ornamentals (*Zinnia elegans*) and the leafhopper *Orosius albicinctus* from Iran (Mirzaie et al., 2007; Salehi et al., 2016; Hemmati and Nikooei, 2017), jasmine from Oman (Al-Zadjali et al., 2007) and bamboo from India (Yadav et al., 2016). However, the WBDL phytoplasma has not yet been detected in weeds or other herbaceous plants. All WBDL phytoplasmas isolated from citrus and *H. phycitis* group together in a distinct cluster upon phylogenetic analyses based on three different genomic regions (16S rRNA, immunodominant membrane protein *imp* and secY) (Siampour et al., 2013). *H. phycitis* has very strict biological relationships with citrus and this close association is probably the reason for the low genetic diversity of WBDL phytoplasmas (Khan et al., 2002).

3.1.4. Detection and identification of the pest

Are detection and identification methods available for the pest? **Yes**

Witches' broom (WBDL) phytoplasma can be detected by PCR amplification of total plant DNA extracts. The following primer pairs have been suggested as specific for Witches' broom (WBDL) detection: AdF1/R1 (Al-Zadjali et al., 2007) and P1 (forward) (Weisburg et al., 1989) and WB3 (reverse; Zreik et al., 1995), although both primer pairs may amplify other strains of the '*Ca*. *P.* aurantifolia' species (M. Siampour, personal communication). Recent evidence suggests either *imp* gene or group II introns as powerful targets for differentiating Witches' broom (MLO) from closely related phytoplasmas (M. Siampour, unpublished results). Indeed, *imp*-based real-time PCR primers IMP3-F/IMP3-R (Askari et al., 2011) have been used for quantitative, specific amplification of WBDL phytoplasma.

3.2. Pest distribution

3.2.1. Pest distribution outside the EU

The pest is reported from a few countries in Asia and is not known to occur in the EU (Figure 1).

Last updated: 2017-09-13

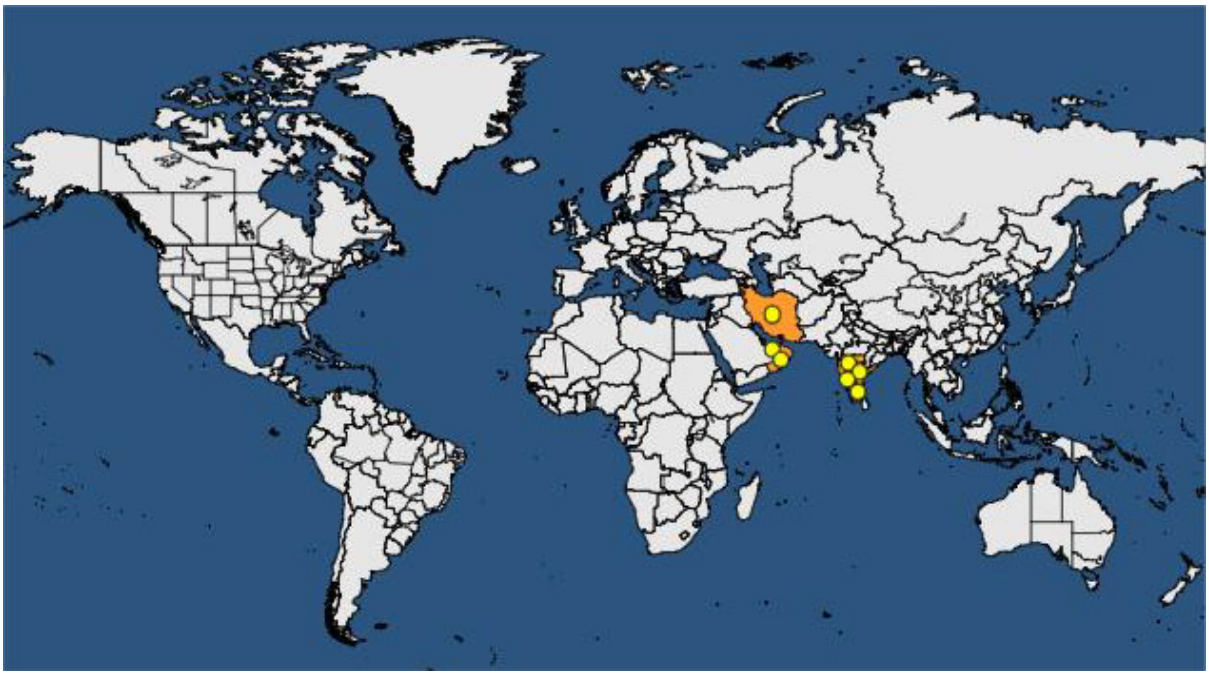

Figure 1: Global distribution of Witches' broom (WBDL) (extracted from EPPO Global Database, accessed 14 September 2017)

The PLH panel considers that the assignation of the Witches' broom (WBDL) from India (Ghosh et al., 2013) requires further confirmation (Table 2).

Table 2: Global distribution of Witches' broom (WBDL) (extracted from EPPO Global Database, accessed 14 September 2017)

Continent	Country	Status - EPPO GD
Asia	India (Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu)	Present, restricted distribution No details
Asia	Iran	Present, restricted distribution
Asia	Oman	Present, widespread
Asia	Saudi Arabia	Absent, unreliable record
Asia	United Arab Emirates	Present, widespread

3.2.2. Pest distribution in the EU

Is the pest present in the EU territory? If present, is the pest widely distributed within the EU? **No**

Witches' broom disease of lime (WBDL) phytoplasma is not known to occur in the EU, and therefore, it does not meet the criterion of 'presence' to qualify as a Union RNQP.

3.3. Regulatory status

3.3.1. Legislation addressing Witches' broom pest (Directive 2000/29/EC)

Witches' broom (MLO) is considered only in annex II of Council Directive 2000/29/EC as detailed in Table 3.

Table 3: Witches' broom (MLO) in Council Directive 2000/29/EC

Annex II, Part A	Harmful organisms whose introduction into, and spread within, all member states shall be banned if they are present on certain plants or plant products	
Section I	Harmful organisms not known to occur in the community and relevant for the entire community	
(d)	Virus and virus-like organisms	
	Species	**Subject of contamination**
15.	Witches' broom (MLO)	Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds

3.3.2. Legislation addressing plants and plant parts on which on which Witches' broom MLO is regulated (Directive 2000/29/EC)

Council Directive 2000/29/EC also regulates the introduction from third countries and the movement within the EU of plants from *Citrus*, *Poncirus*, *Fortunella* genera and their hybrids, as well as plant products from those species. Such measures, detailed in Table 4, contribute to reduction of risks of introduction and spread of Witches' broom MLO. The introduction on *Citrus*, *Poncirus*, *Fortunella* plant genus and their hybrids is prohibited.

Table 4: Regulated hosts and commodities that may involve Witches' broom (MLO) in Annexes III, IV and V of Council Directive 2000/29/EC

Annex III, Part A	Plants, plant products and other objects the introduction of which shall be prohibited in all Member States	
	Description	**Country of origin**
16.	Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds	Third countries
Annex IV, Part A	Special requirements which must be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within all member states	
Section I	Plants, plant products and other objects originating outside the community	
	Plants, plant products and other objects	**Special requirements**
16.1	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, originating in third countries	The fruits shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark.
Annex V	Plants, plant products and other objects which must be subject to a plant health inspection (at the place of production if originating in the community, before being moved within the community — in the country of origin or the consignor country, if originating outside the community) before being permitted to enter the community	
Part A	Plants, plant products and other objects originating in the Community	
I.	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community and which must be accompanied by a plant passport	
1.4	Plants of *Fortunella* Swingle, *Poncirus* Raf. and their hybrids and of *Citrus* L., other than fruit and seeds.	
1.5	Plants of *Citrus* L. and their hybrids other than fruit and seeds.	
1.6	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf. and their hybrids with leaves and peduncles	
Part B	Plants, plant products and other objects originating in territories, other than those territories referred to in part A	
I.	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community	
3.	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids [. . .]	

3.3.3. Legislation addressing potential vectors of WBDL (Directive 2000/29/EC)

Council Directive 2000/29/EC also regulates the introduction from third countries of *Hishomonus phycitis* (sic), an insect known to occur only in Asian countries and known to be a vector of WBDL (Table 5).

The vector name is misspelt in Appendix II AI of 2000/29 EC as *Hishomonus phycitis* (sic); the same spelling is used in Appendix 1 of the current ToR. So as to avoid the perpetuation of the spelling error, this pest categorisation will use the scientifically recognised name *Hishimonus phycitis* (EFSA PLH Panel, 2017).

Table 5: Regulated potential vector of Witches' broom (MLO) in Annexe II of Council Directive 2000/29/EC

Annex II, Part A Section I	Harmful organisms whose introduction into, and spread within, all member states shall be banned if they are present on certain plants or plant products Harmful organisms not known to occur in the community and relevant for the entire community (a) Insects, mites and nematodes, at all stages of their development	
	Species	**Subject of contamination**
(a)	Insects, mites and nematodes, at all stages of their development	
16.	*Hishomonus phycitis*	Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf. and their hybrids, other than fruit and seeds

3.4. Entry, establishment and spread in the EU

3.4.1. Host range

Small fruited acid lime (*C. aurantifolia*) is the most important citrus species naturally infected by the WBDL phytoplasma (Zreik et al., 1995). Sweet limes (*C. limetta* and *C. limettioides*; Bové, 1986), rough lemon (*C. jambhiri*) and trifoliate orange (*P. trifoliata*) can also be naturally infected by WBDL (EPPO, 2006, Al-Yahyai et al., 2012).

The WBDL phytoplasma can be transmitted by grafting to other citrus species such as alemow (*Citrus macrophylla*), dayalap (*Citrus excelsa*), the shrub Ichang papeda (*Citrus ichangensis*), kaffir lime (*Citrus hystrix*), Meyers lemon (*Citrus meyeri*) and mandarin lime (*Citrus limonia*) as well as to some citrus hybrids (e.g. *P. trifoliata* x *C sinensis*, *C. sinensis* × *P. trifoliate*) (EPPO, 2006; Al-Yahyai et al., 2012).

The following species were reported to be resistant to WBDL: bitter orange (*Citrus aurantium*), mandarin orange (*C. reticulata)*, lemon (*Citrus limon)*, Mountain citron (*Citrus halimii)*, yuzu (*Citrus junos)*, Tahiti lime (*Citrus latifolia)*, Cleopatra mandarin (*Citrus reshni)*, pomelo (*Citrus maxima)*, kumquat (*Citrus japonica)*, oval kumquat (*Fortunella margarita)*, Australian round lime (*Microcitrus australis*) and box orange (*Severinia buxifolia)* (EPPO, 2006; Moghal et al., 1998 as reviewed in Al-Yahyai et al., 2012). However, susceptibility of different citrus has been based only on symptom observations; therefore, the possible presence of phytoplasmas in symptomless plants cannot be ruled out which increases the uncertainty.

Grapefruit (*C. paradisi*), orange (*C. sinensis*), mandarin (*Citrus nobilis*) and clementine (*Citrus clementina*) are not believed to be susceptible to the WBDL (EPPO, 2006). However, witches' broom symptoms have been reported on grapefruit and orange in Iran (Najafinia and Azadvar, 2016), but the lack of a molecular identification of the pathogen associated with the symptoms makes it impossible to conclude on the susceptibility of these two citrus species to WBDL.

3.4.2. Entry

The Witches' broom disease of lime (WBDL) phytoplasma could only enter the EU *via* the introduction of infected plants or vectors. However, entry *via* plants for planting of its host species is a closed pathway under current EU regulations, as the import of citrus plants for planting from third countries is prohibited.

Is the pest able to enter into the EU territory?

Yes, WBDL phytoplasma could enter on plant for planting material and in its vector insect. Nevertheless, present regulation on the import of plant for planting material and on *H. phycitis* as a regulated insect close those pathways

Entry *via* infected adults of *H. phycitis* leafhoppers is unlikely, as these move and leap away from plants when disturbed; therefore, it is highly improbable that these mobile stages would remain on host plant material as it is handled along a pathway (EFSA PLH Panel, 2017 *Hishimonous phycitis* pest categorisation).

Should it be discovered that WBDL is vertically transmitted in the vector (infected female passes phytoplasma to infect eggs, i.e. transovarial transmission), the introduction of infected *H. phycitis* eggs on non-regulated commodities such as amaranth may be a route of entry for the phytoplasma into the EU.

Between 1995 and 2017, there were no records in the Europhyt database of interception of plants because of the presence of Witches' broom disease of lime WBDL or of *H. phycitis* leafhoppers.

Shall the existing ban of introduction of citrus plants remain, uncertainties only exist due to the potential introduction of infected vector eggs on non-regulated commodities.

3.4.3. Establishment

3.4.3.1. EU distribution of main host plants

Is the pest able to become established in the EU territory? **YES,** with uncertainties

Small fruited acid lime (*C. aurantifolia*), the major known host of WBDL, is not grown for commercial purposes in the EU at present. Currently, the substitute for lime fruit grown in Europe is Tahiti lime (*C. latifolia*). As far as it is known that *C. latifolia* is not susceptible to Witches' broom disease of lime (WDBDL) (EPPO, 2006; Mariano Cambra, personal communication, 2017).

In EU, grapefruit (*C. paradisi*), orange (*C. sinensis*), mandarin (*C. nobilis*) and clementine (*C. clementina*) are the most economically important citrus crops in the Mediterranean area, and these species are not believed to be susceptible to the WBDL phytoplasma (EPPO, 2006). As for other citrus EU-grown hybrids, some are known to be susceptible; but for others, the information is currently uncertain/unknown. Additional information on susceptibility is in Section 3.1.2.

3.4.3.2. Climatic conditions affecting establishment

For the WBDL phytoplasma, there are no ecoclimatic limitations, besides those applying to the host and the insect vector.

C. aurantifolia is not grown commercially in the EU because the climate is not suitable for fruit production, but it is present in private collections, as climatic conditions of warm areas around the Mediterranean sea allow its cultivation.

The *H. phycitis* vector is not known to be present in the EU, although according to the last pest categorisation, 'parts of southern EU MSs especially warmer areas around the Mediterranean coast' might support vector establishment (EFSA PLH Panel, 2017).

3.4.4. Spread

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

How: (1) grafting with the infected scions; (2) vector transmission;

Should it be introduced into the EU, despite the existing bans concerning movement from third countries of citrus plants for planting as well as leafhopper vector, WBDL would be able to spread within the Mediterranean area of the EU by:

- Movement within EU of plants for planting (e.g: scions from infected mother plants, infected rootstocks) that can freely circulate within the EU.
- The known vector, should it also be introduced and become established (EFSA PLH Panel, 2017 *H. phycitis* pest categorisation).
- Currently unknown vectors feeding on Citrus plants. This is unlikely, as the only other deltocephaline leafhopper that is found on citrus species in the EU is *Penthimiola belli*, so far restricted to Portugal (Zina et al., 2013), and not known as a phytoplasma vector. Nevertheless, uncertainty may be high because of knowledge gaps.

3.5. Impacts

Would the pests' introduction have an economic or environmental impact on the EU territory?
Yes, with uncertainties

In the Sultanate of Oman, where the disease is widespread and is a serious threat to lime production, about 68% of the surveyed farms had infected trees in 2015 (Al-Yahyai et al., 2015). In this country, lime cultivation area has lost 50% of its acreage compared to 1990, mainly due to tree loss caused by WBDL (Al-Yahyai et al., 2012). The combined effect of WBDL with other abiotic stresses (drought and salinity) has resulted in the recent decline of lime production in the country (Al-Yahyai et al., 2012).

As the most important host plant is not currently commercially grown in the EU, and the vector species *H. phycitis* is not known to occur in the EU, the potential impact of the disease is should be limited. Nevertheless, considering (i) the citrus crops already grown in the EU or in the near future, (ii) the limited information regarding the susceptibility of some of these crops and (iii) the lack of information on the ability of other European phloem feeding insects to transmit the phytoplasma, the potential impact of the disease might increase, although the uncertainty is high.

3.6. Availability and limits of mitigation measures

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

YES: entry into the EU of (1) citrus plants for planting and (2) the known insect vector are prohibited

The present ban on the introduction of citrus, which is the host of both the pest and the vector, is an efficient mitigation measure.

Citrus certification scheme could include detection of WBDL phytoplasma.

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

While the current measures are effective for prevention of entry of the pathogen and its vector on the citrus hosts, there remains the possibility that the vector could be introduced on alternative host(s) (See Section 3.4.2).

The probability that an infected plant remains asymptomatic at early stages of infection limits the possibility of detection of WBDL phytoplasma.

3.6.2. Control methods

In countries where the disease is present, use of the following control strategies has proven effective (Najafinia and Azadvar, 2016).

- Removal of infected tree with clear symptom, and of newly emerged symptomatic branches on tree;
- Chemical or mechanical control of weeds;
- Vector control with systemic insecticides;

3.7. Uncertainty

The Panel identified four main sources of uncertainty in the present opinion:

- Vertical transmission of the phytoplasma to *H. phycitis* eggs;
- WBDL vectoring capacity of EU phloem feeding insects and of recently EU-introduced *H. hamatus* species;
- Lack of information regarding susceptibility of citrus varieties and species grown in the EU;

These uncertainties primarily affect three aspects of the present pest categorisation: the potential alternative routes of entry of WBDL into the EU, the efficiency and extent to which the disease would be able to spread, and the impact it would have if introduced in the EU.

4. Conclusions

Citrus aurantifolia, the major host plant of WBDL phytoplasma, is mainly grown for ornamental purposes in the EU. Some other Citrus species which are susceptible, or may be susceptible, are also grown. In countries where WBDL is present, it has significant impact. It is unknown whether phloem feeding insects present in the EU would vector WBDL phytoplasma. The known vector *H. phycitis* is not known to occur in the EU. Large uncertainties remain because of lack of knowledge regarding the host range of WBDL phytoplasma and the vectoring capacities of insects that are endemic in the EU.

WBDL phytoplasma **meets the criteria assessed by EFSA for consideration as** potential Union quarantine pest (Table 6).

Table 6: The Panel's conclusions on the pest categorisation criteria defined in Regulation (EU) 2016/2031 on protective measures against pests of plants (the number of the relevant sections of the pest categorisation is shown in brackets in the first column

Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest	Key uncertainties
Identity of the pest (Section 3.1)	The identity of the pest is well established; it can be identified with reliable and sensitive molecular diagnostic techniques.	The identity of the pest is well established; it can be identified with reliable and sensitive molecular diagnostic techniques.	
Absence/presence of the pest in the EU territory (Section 3.2)	The pest is **not known to occur** in the EU territory.	The pest is not known to occur in the EU territory, therefore does not qualify as a RNQP.	
Regulatory status (Section 3.3)	WBDL and its vector *Hishomonus phycitis* are currently regulated under Directive 2000/29/EC.	WBDL and its vector *Hishomonus phycitis* are currently regulated under Directive 2000/29/EC.	
Pest potential for entry, establishment and spread in the EU territory (Section 3.4)	Yes, the pest could enter the EU on plants or with the vector, although these pathways are closed because of existing bans for citrus and *H. phycitis*. Should the pest enter the EU, it could establish and spread. Natural spread depends on the availability of susceptible plants and effective vector species.	Yes, the pest could enter the EU on plants or with the vector. Nevertheless, these pathways are closed because of existing bans for citrus and H. phycitis. Should the pest enter the EU, it could establish and spread. Natural spread depends on the availability of susceptible plants and effective vector species.	Vertical transmission of the phytoplasma to *H. phycitis* eggs; WBDL vectoring capacity of EU phloem feeding insects and of recently EU-introduced *H. hamatus* species Lack of information regarding susceptibility of citrus varieties and species grown in the EU;
Potential for consequences in the EU territory (Section 3.5)	The most important host species is mainly grown for non-commercial purposes in the EU, but some other potential host species are more widely grown. The known vector is not known to occur in the EU but the vector status of other EU phloem feeding insects is unknown. These aspects make the likely impact uncertain.	The most important host and the vector species are not known to occur in the EU which makes the likely impact limited. However, if other citrus crops are susceptible and other vector insects exist, it would be difficult to predict the potential future impact of the disease.	WBDL vectoring capacity of EU phloem feeding insects and of recently EU-introduced *H. hamatus* species Lack of information regarding susceptibility of citrus varieties and species grown in the EU
Available measures (Section 3.6)	Prohibition on the introduction of *Citrus* plants for planting, certification scheme.	Prohibition on the introduction of *Citrus* plants for planting, certification scheme.	

Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union regulated non-quarantine pest	Key uncertainties
Conclusion on pest categorisation (Section 4)	*Citrus aurantifolia,* the major host plant of WBDL phytoplasma, is mainly grown for ornamental purposes in the EU. Some other Citrus species which are susceptible, or may be susceptible, are also grown. In countries where WBDL is present it has significant impact. It is unknown whether phloem feeding insects present in the EU would vector WBDL phytoplasma. The known vector *Hishimonus phycitis*, is not known to occur in the EU. Large uncertainties remain because of lack of knowledge regarding the host range of WBDL phytoplasma and the vectoring capacities of insects that are endemic in the EU. WBDL phytoplasma **meets the criteria assessed by EFSA for consideration as** potential Union quarantine pest.	WBDL does not meet the presence on the territory criterion and therefore **does not qualify as a Union RNQP.**	
Aspects of assessment to focus on/scenarios to address in future if appropriate	The main knowledge gaps concern 1) vertical transmission of the phytoplasma to *H. phycitis* eggs; 2) WBDL vectoring capacity of EU phloem feeding insects and of recently EU-introduced *H. hamatus* species; 3) Lack of information regarding susceptibility of citrus varieties and species grown in the EU;		

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Abbreviations

EPPO	European and Mediterranean Plant Protection Organization
EU MS	European Union Member State
FAO	Food and Agriculture Organization
IPPC	International Plant Protection Convention
PLH	EFSA PLH Panel
RNQP	regulated non-quarantine pest
TFEU	Treaty on the Functioning of the European Union
ToR	Terms of Reference
WBDL	Witches' broom disease of lime phytoplasma