EMERGING BACTERIAL DISEASES OF FRUIT TREES AND SOME OTHER CROPS THAT ARE OR MAY BECOME A THREAT FOR SOUTHERN EUROPE: NOTES ON EPIDEMIOLOGY, RISKS, PREVENTION AND MANAGEMENT ON FIRST OCCURRENCE

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ABSTRACT

Bacterial diseases of fruit trees are difficult to control (both chemically and biologically), mostly only by preventive measures such as hygiene, healthy planting material, good cultural practices and avoidance of risk planting sites. Moreover bacteria may easily spread by (surface) water, planting material and contaminated implements/machines and by a-specific or specific insect vectors. Most important risk factors for the introduction of bacterial diseases into southern Europe are imported infected planting material and (infected) insect vectors. In this contribution the epidemiology, management and main risks of three emerging bacterial diseases approaching southern Europe, their causal organisms and vectors will be highlighted, especially 1) Leaf scorch and leaf scald diseases of grape and diverse fruit and ornamental trees, caused by *Xylella fastidiosa*. For this pathogen, although not yet confirmed from Europe or the Mediterranean basin, local possible vectors such as *Cicadella viridis* and *Philaenus spumarius* occur. In less detail 2) Citrus huanglongbin or Citrus greening, caused by the heat tolerant '*Candidatus' Liberobacter asiaticus* and and heat sensitive *'Candidatus' L. africanus* (both forms and respective psyllid vectors *Diaphorina citri* and *Trioza erytreae* are present on the Arabian peninsula, with recent reports of huanglongbin occurring in Iran, Mali, Ethiopia and Somalia and *T. erytreae* already present on some Atlantic Ocean Islands) and 3) Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri*, the so-called Asiatic, most severe form, is present in Irak, Iran, Oman, Somalia UAE, Saudi-Arabia, Yemen and Reunion. Outbreaks and/or risk and possible emerging character of some other bacterial pathogens: *Acidovorax citrulli*, causing bacterial fruit blotch of cucurbits, with recent outbreaks in Greece, Hungary, Israel and Italy, *Pantoea ananatis* on maize and onion, *P. stewartii* subsp. *stewartii* on maize, *Pseudomonas syringae* pv. *actinidiae* on kiwifruit, *Xanthomonas arboricola* pv. *pruni* on stone fruits, with recent outbreaks in Switzerland and in the Netherlands (on *Prunus laurocerasus* - cherry-laurel), '*Candidatus' Phytoplasma phoeniculum* on almond, destroying thousands of trees in Lebanon. Since initial risk avoiding and management measures following an introduction are more or less similar for the above mentioned pathogens, they will be highlighted for *Xylella fastidiosa*. Some considerations on the (effectiveness of) quarantine regulations for plant pathogenic bacteria will also be presented.

Keywords: *Acidovorax citrulli*, '*Candidatus' Liberobacter asiaticus*, '*Candidatus' L. Africanus*, '*Candidatus' Phytoplasma phoeniculum*, emerging bacterial diseases, *Pseudomonas*, *Xanthomonas*

IZVLEČEK

POJAV NOVIH BAKTERIJSKIH BOLEZNI SADNEGA DREVJA IN NEKATERIH DRUGIH RASTLIN, KI SO ALI LAHKO POSTANEJO GROŽNJA ZA JUŽNO EVROPO:

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EPIDEMIOLOGIJA, TVEGANJE, PREPREČEVANJE IN UPRAVLJANJE OB PRVEM POJAVU

Bakterijske bolezni sadnega drevja težko nadzorujemo tako kemično kot tudi biotično. Zaradi tega za njihov nadzor večinoma uporabljamo le preventivne ukrepe. Taki ukrepi so higiena zdravega sadilnega materiala, dobra praksa gojenja in izogibanje sajenja na območjih, kjer je tveganje za okužbo veliko. Dodatno težavo predstavlja enostaven prenos bakterij s specifičnimi žuželčjimi prenašalci, površinskimi vodami, sadilnim materialom in okuženim orodjem. Najpomembnejša dejavnika tveganja za vnos bakterijskih bolezni v južno Evropo sta uvoz okuženega sadilnega materiala in okuženi prenašalci. V tem prispevku bodo izpostavljeni epidemiologija, upravljanje in glavna tveganja treh novih bakterijskih bolezni, ki se približuje južni Evropi, skupaj z njihovimi povzročitelji in prenašalci. Podrobno bodo predstavljeni ožigi listov vinske trte, sadnega in okrasnega drevja, ki jih povzroča *Xylella fastidiosa*. Njena možna, a še ne potrjena prenašalca v Evropi in Sredozemlju sta *Cicadella viridis* in *Philaenus spumarius*. Predstavljena bo bolezen huanglongbin ali zelenenje citrusov, ki jo povzroča proti vročini tolerantna bakterija '*Candidatus*' *Liberobacter asiaticus* in na vročino občutljiva '*Candidatus*' *L. africanus*. Obe obliki bakterije sta skupaj s prenašalcema, bolšicama (Psyllidae) *Diaphorina citri* in *Trioza erytreae*, ki živita na Arabskem polotoku. V zdajšnjem času so znana poročila o pojavljanju bolezni huanglongbin iz Irana, Malija, Etiopije in Somalije. *T. erytreae* se že pojavlja na nekaterih otokih v Atlantskem oceanu. V prispevku bo predstavljen tudi rak citrusov, ki ga povzroča *Xanthomonas axonopodis* pv. *citri*. Njegova najnevarnejša oblika se pojavlja v Iraku, Iranu, Omanu, Somaliji, Združenih arabskih emiratih, Savdski Arabiji, Jemnu in na Reunionu. Predstavljeni bodo izbruhi in/ali tveganja ter morebitn znamenja, ki kažejo na možne izbruhe nekaterih drugih patogenih bakterij: *Acidovorax citrulli*, ki povzroča bakterijsko lisavost na plodovih buč, z nedavnimi izbruhi v Grčiji, na Madžarskem, Izraelu in Italiji; *Pantoea ananatis* na koruzi in čebuli; *P. stewartii* subsp. *stewartii* na koruzi; *Pseudomonas syringae* pv. a*ctinidiae* na kiviju; *Xanthomonas arboricola* pv. *pruni* na koščičarjih, z nedavnimi izbruhi v Švici in na Nizozemskem na lovorikovcu; '*Candidatus*' *Phytoplasma phoeniculum*, ki je uničila na tisoče mandljevih dreves v Libanonu. Ker so izogib začetnemu tveganju in ukrepi, ki pojavu sledijo, podobni za vse naštete patogene, bodo poudarjeni pri bakteriji *Xylella fastidiosa*. V prispevku bodo predstavljena tudi razmišljanja o učinkovitosti karantenskega nadzora rastlinskih patogenih bakterij.

Ključne besede: *Acidovorax citrulli,* '*Candidatus*' *Liberobacter asiaticus,* '*Candidatus*' *L. Africanus,* '*Candidatus*' *Phytoplasma phoeniculum*, nove bakterijske bolezni v Evropi, *Pseudomonas*, *Xanthomonas*

1 INTRODUCTION: EMERGING BACTERIAL DISEASES ON FRUIT TREES

Bacterial diseases are often a major constraint on productivity of fruit trees. Yield losses, which may reach 50% and tree death result from infestations caused by established pathogens such as *Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae* and pv. *morsprunorum*, *Agrobacterium tumefaciens*, *Xanthomonas arboricola* pv. *pruni,* Phytoplasma mali*,* Grapevine flavescence dorée phytoplasma, Bois noir of grapevine*, Phytoplasma pyri and* P. prunorum *(European stone fruit yellows).* Quarantine pathogens are sometimes spreading out of contained loci, presenting present a long-term threat to other EU countries (e.g. *Xanthomonas arboricola* pv. *pruni* spreading out of France and Italy) and others are an emerging threat outside Europe (*Xylella fastidiosa*).

Bacterial diseases of fruit trees are difficult to control (both chemically and biologically), mostly only by preventive measures such as hygiene, healthy planting material, good cultural practices and avoidance of risk planting sites. Moreover they may easily spread by (surface) water, planting material and contaminated implements/machines and by a-specific or specific insect vectors. Most important risk factors for the introduction of bacterial diseases into the Mediterranean basin are imported infected planting material and (naturally) spreading (infected) insect vectors. Therefore early detection and correct identification/diagnosis are of utmost importance also. In a recent review current classic and molecular methods for detection and identification of bacterial pathogens of fruit trees and nuts, including *Xylella fastidiosa*, have been presented and summarized, (Janse, 2010; for a minireview on *Xylella fastidiosa* see Janse and Obradovic, 2010). In this contribution the epidemiology and main risks in the framework of prevention and management in case of first occurrence of some emerging bacterial diseases approaching the Mediterranean basin, their causal organisms and vectors will be highlighted, especially 1) Citrus huanglongbin or Citrus greening, caused by the heat tolerant *"Candidatus"Liberobacter asiaticus* and and heat sensitive *"Candidatus" L. africanus*, both forms and respective psyllid vectors *Diaphorina citri* and *Trioza erytreae* are present on the Arabian peninsula, with recent reports of huanglongbin occurring in Iran, Mali, Ethiopia and Somalia and *T. erytreae* already present on some Atlantic Ocean Islands. Furthermore in less detail 2) Leaf scorch and leaf scald diseases of diverse fruit and ornamental trees, caused by *Xylella fastidiosa*. For this pathogen, although not yet confirmed from Europe or Mediterranean basin local possible vectors such as *Cicadella viridis* and *Philaenus spumarius* occur; 3) Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (syn. *X. axonopodis* pv. *citri*), the so-called Asiatic, most severe form, present in Irak, Iran, Oman, Somalia, UAE, Saudi-Arabia, Yemen and Réunion.

Outbreaks and possible emerging character of some other bacterial pathogens (e.g. *Xanthomonas citri* pv. *mangiferaeindicae* approaching like *X. c.* subsp. *citri* the Mediterranean basin and the devastating *Phytoplasma phoeniculum* that occurs on almond in Lebanon) will also be reported, but not discussed in detail. Since initial management and risk avoiding and initial management measures following an introduction are more or less similar for the three above mentioned pathogens, they will be highlighted for HLB. It has been shown that ornamental and wild hosts may play an important role in spreading of the disease and maintaining the pathogen and its vectors in the environment. These plants should be included in surveys. Rapid and reliable diagnosis remains a key issue, as well as breeding for resistance. It will be argued that the three main diseases addressed in this presentation are emerging threats, with real risks of introduction and in some cases closely approaching the Mediterranean basin. The aim of this contribution is creating awareness of the risks of these diseases, enabling prevention, early detection and proper actions once introduction would occur.

2 CITRUS HUANGLONGBIN OR HLB (CITRUS GREENING), CAUSED BY ¸ *"Candidatus" Liberibacter* species

2.1 General information

Huanglongbin or HLB (Chinese for yellow shoot disease) of Citrus is caused by a non-culturable, fastidious, phloem-inhabiting, Gram-negative bacterium belonging to the α-Proteobacteria and to the genus "*Candidatus*" Liberibacter. This disease is also known as Citrus greening, yellow dragon disease, mottle leaf disease, likubin or vein phloem degeneration. It is one of the most destructive diseases of all cultivated citrus, for which at present no effective control is present yet and in all areas where the disease occurred, citrus production declined. It was properly described for the first time in China in 1956 by Lin, who also determined that the disease was graft-transmissible. Its origin is not clear, probably Asia (India or China) or Africa (Gottwald *et al*., 2007). For a thorough general review please see Bové (2006) and on its epidemiology Gottwald (2010). There have now three different

Liberibacter species been described, based on 16S rRNA sequences that can cause Huanglongbin:
a) "*Candidatus" Liberibacter asiaticus* (Las), originally described as *Liberobacter asiaticum*, the most aggressive species, heat tolerant, stands temperatures above 30°C, disease caused by this species mainly in lowlands. It is transmitted by the heat tolerant psyllid *Diaphorina citri* Kuwayama and is widespread in Asia, the Arabian peninsula, Mauritius and Réunion island, and since 2004 in Brazil Sao Paulo State
b) "*Candidatus" Liberibacter africanus* (Laf), described as *Liberobacter africanum*, less aggressive, heat-sensitive, disease suppressed after longer exposure to temperatures above 30°C, therefore occurring in the tropics at elevations higher than 700 m. It is widespread in Africa and occurs in Somalia, Ethiopia, Cameroon, Réunion, Mauritius, and Yemen. Transmitted by the heat-sensitive psyllid *Trioza erytreae.*
c) "*Candidatus" Liberibacter americanus* (Lam), that is closely related to Las, but heat-sensitive, in Brazil, transmitted by the psyllid *D. citri*.

One subspecies has been described recently, viz. "*Candidatus"* Liberibacter africanus subsp. *capensis*" isolated from a symptomless ornamental rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. In the article describing this subspecies, the genus name *Liberobacter* was changed into *Liberibacter* (Garnier *et al*., 2000). The complete genome sequence of Laf is available (Duan *et al*., 2009) and recently differences between Asiatic (China) and North American strains of Las were reported (Chen *et al*., 2010).

2.2 Some biological details of the psyllid vectors

Both *Diaphorina citri* and *Trioza erytreae* feed on phloem and are experimentally able to transmit Asian and African HLB. Multiplication in the vector has not been clearly demonstrated, the bacteria, however, can be found in larger amounts in haemolymph and salivary glands. For *T. erytreae* there is evidence that transovarial transmission occurs. Other psyllids that occur on citrus have not been found to transmit the bacteria. Psyllids like to feed on new flush and the period of its formation forms a risk period for transmission. Although *D. citri* does not tolerate frost very well it survives frosty days of up to -5°C frost in Florida (Bové, 2006; Brlansky and Rogers, 2007; Gottwald, 2010; Gottwald *et al*., 2007; Halbert and Manjunath, 2004; Manjunath *et al*., 2007).

Table 1: Geographical distribution of bacteria and vectors

Pathogen or vector or host	**Country or geographical region**
***"Candidatus"* Liberibacter asiaticus (Las)** in all countries mentioned transmitted by *D. citri*	§ S.E. Asia: Cambodia, China (including Hong Kong), Indonesia, southern islands of Japan, Laos, Malaysia, Myanmar, Philippines, Taiwan, east Timor, Thailand, and Vietnam. § Indian subcontinent: Bangladesh, Bhutan, India, Nepal, and Pakistan. § W. Asia: Iran (Faghihi *et al*., 2008) § Indian Ocean: Comoros Islands, Madagascar, Mauritius, Reunion and Sri Lanka. § Saudi Arabian peninsula: Saudi Arabia, Yemen in SW along the Red Sea § Africa: N. Ethiopia, De Bac *et al*., 2010 § S. America: Brazil, mainly Sao Paulo State, since 2004 § Caribbean: Cuba (Martinez *et al*., 2009); Dominican Republic (Matoz *et al*., 2009); Belize, 2009 (Manjunath *et al*., 2010 § N. America: USA (Florida, since 2005, Louisiana, 2008, Georgia and South Carolina, 2009

***"Candidatus"* Liberibacter. Africanus (Laf)** in all countries mentioned transmitted by *T. erytreae*	§ Africa: Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Malawi, Rwanda, Somalia, South Africa, Swaziland, Tanzania, and Zimbabwe § Indian Ocean: Mauritius and Reunion § Saudi Arabian peninsula: Saudi Arabia, Yemen
Las and Laf in countries mentioned both vectors *D. citri* and *T. erytreae* present	§ Ethiopia, Mauritius, Réunion, Saudi Arabia and Yemen have both species of vectors and both pathogens
***Candidatus"* L. americanus (Lam)**	§ Brazil § Lam has been reported (but not confirmed) in one of 97 citrus leaf samples from eight provinces of southern China (Lou *et al*., 2008)
Diaphorina citri	§ S.E. Asia: Cambodia, China (including Hong Kong), Indonesia, southern islands of Japan, Macau, Malaysia, Myanmar, Philippines, Taiwan, Thailand, and Vietnam. § Indian subcontinent: Afghanistan, Bangladesh, Bhutan, India, Nepal, and Pakistan. § Indian Ocean: Comoros Islands, Madagascar, Mauritius, Reunion and Sri Lanka. § Saudi Arabian peninsula: Saudi Arabia, Yemen, Oman § S. America: Argentina (since 1984 in NE, since 2006 in NW, Ramallo *et al*., 2008), Brazil (since 1940's), Venezuela § C. America: Honduras, Belize, Costa Rica § Caribbean: Cuba (1999), Haiti (2000), Guadeloupe (1998 (Etienne *et al*., 1998), Bahamas in 1999, Cayman Islands in 2000, Virgin Islands and Dominican Republic in 2001, and Puerto Rico in 2002 (Halbert and Nunez, 2004). § North America: USA (Florida, since 1998, Alabama, Georgia, Mississippi, South Carolina, Louisiana, isolated California (USDA 2010), Texas; 2001 (French *et al*., 2001), Mexico (Trujillo *et al*., 2008) § Pacific Ocean: Hawaii, Maui Conant *et al*., 2007
Trioza erytreae	§ Africa: Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Malawi, Nigeria, Rwanda, Somalia, South Africa, Sudan, Swaziland, Uganda, Tanzania, and Zimbabwe § Indian ocean: Madagascar, Mauritius and Reunion § Saudi Arabian peninsula: Saudi Arabia, Yemen § Atlantic ocean: Canary islands (Tenerife, La Gomera, La Palma and El Hierro) since 2002 (Perez Padron & Hernandez 2002), Madeira (since 1994) and Porto Santo Island , see Fernandes & Franquinho 2001, St. Helena (also see EPPO 2005d; Natural hosts: Anonymous, 2007; CABI/EPPO 1998a and b; Eppo 2005c and d, Beattie *et al*., 2008; Bové 2006; Gottwald *et al*., 2007
Rutaceae *Liberibacter species and vector hosts*	Most *Citrus* species and forms are or can be host of *Liberibacter* species. Sweet oranges, mandarins and tangelos (*C. reticulata* x *C. paradisi)* are generally most susceptible, with most severe symptoms. Lemons, grapefruits, *C. limonia, C. limettioides*, rough lemons, kumquats (*Fortunella* spp.) and citrons show less severe symptoms. Weak symptoms occur only on limes and pummelos (*C. grandis*). However, in Brazil and the USA all commercial *Citrus* species have a very similar susceptibility. Other confirmed hosts are *Limonia acidissima*, *Murraya paniculata* or orange jasmine (often used as ornamental and for hedges) and the related or similar *M. exotica,* furthermore *M. (Bergera) koenigii*, *Severinia buxifolia* and *Vepris lanceolata* (= *V. undulata* = *Toddalia lanceolata*). *Rutaceae,* including all cultivated and wild forms as mentioned above are hosts for *D. citri* and *T. erytreae* ans also *Clausena anisata* (*C. inaequalis*) and *Zanthoxilum capense* (*Fagara capensis*).

2.3 Symptoms

Symptoms may be confused with other diseases and disorders. More typically first symptoms are one or more yellowing shoots. On leaves not well-defined yellow areas develop (so called blotchy mottle symptom, that also may occur on fruits) which are asymmetrical when the two leaf-halves are compared. Veins may become corky, giving the leaves a thicker appearance. Later yellow spots may intensify, and may look very similar to zinc deficiency. Fruits are often smaller, asymmetrically misshaped (lopsided) and show typically green remaining areas, especially at the stylar end when ripening. When infected fruits are cut, yellow-brown vascular bundles and necrotic seeds may be observed. Severe and final stages include severe leaf and fruit drop, twig and stem die-back and eventually tree death (especially when infected by Las).

2.4 Detection and Diagnosis

In the early years detection and diagnosis was mainly based on symptoms, electron microscopy and biological indexing. A monoclonal antiserum was developed but proved to be too specific. PCR (classical, nested, real-time, qualitative real-time and multiplex) is now the main confirmatory test and is routinely used in many areas facilitating also detection of latent infections and in insects (Benyon *et al*., 2008; Li *et al*., 2006 and 2007; Teixeira *et al*., 2008). A species-specific multiplex TaqMan (real-time) PCR for Las, Laf and Lam with COX primers/probe as internal control for the host plant cytochrome oxidase gene has been developed by Okuda *et al*., 2005. For a very sensitive single tube combination of nested PCR and Taqman (real-time) PCR, see Lin *et al*., 2010.

2.5 Epidemiology

HLB epidemics develop rather slow in time (several to more than 12 years), but the disease may spread quite rapidly in an orchard (in several years trees may show severe symptoms and within 7-10 years orchards may become unproductive, depending on the age of the trees and the presence and numbers of vectors. A complicating factor is that many trees apparently may already be latently infected for a considerable time before symptoms become obvious. The number of latent infected trees may be two- to manifold as compared to symptomatic trees, which hinders early detection of HLB and frustrates eradication campaigns. Spread of vectors and disease has a tree to tree pattern although also further spread (a few kilometres) from an infection focus may be possible. Psyllids migrate mostly when host plants are flushing. Real long distance dispersal is by infected planting material or by infected psyllids accidentally taken by man (on plant material or otherwise). Seed transmission seems to be possible but has not been definitively proven. The presence of HLB in a tree may be sector-wise and in the symptomless part the bacteria are often not detectable. In how far psyllids can be carried by high air streams and spread over larger distances is still unknown but its occurrence cannot be excluded. Seed transmission of virulent bacteria has not been demonstrated yet.

2.6 Damage and losses

Since resistance against HLB has not been found or created yet, it is a dangerous and devastating disease. Yield is reduced, mainly by reduced growth and fruit drop and fruit quality impaired. Yield losses may be 30-100% and within 7-10 years after planting orchards may lose productivity completely. It has been calculated that c 100 million trees have been affected and destroyed in Southeast Asia, India, Arabian Peninsula, and South Africa leading to decline of the Citrus cultivation in these areas. In south-western Saudi Arabia, sweet orange and mandarin practically disappeared already during the 1970s. Since 2004, c. 1 million trees have been destroyed in Brazil due to HLB infection. In Florida HLB was detected for the first time in 2005 and by 2009 was detected in most areas where citrus was grown and eradication efforts given up (Bové, 2006; Gottwald, 2010) and the select agent status for all three species of *Liberibacter* by USDA APHIS abandoned.

2.7 Main risk factors

HLB and its vectors have not yet been reported from the European mainland or from the Mediterranean basin and there are no local psyllid vectors known. Long distance spread of *Liberibacter* spp. and vectors is possible and will mainly be with planting material. In Kenya infected breeding material from abroad was suspected to be the source of initial infection (Magomere *et al*., 2009). Note that HLB is approaching the Mediterranean basin mainly from two sides: a) From Saudi-Arabia, where both Laf and Las are present and could move further north b) From Africa, where Laf and its vector are already present as high as Ethiopia and Somalia and Las in North Ethiopia. c) From Iran, where Las has been reported in 2009. *Murraya paniculata* (orange jasmine), a popular landscape plant, played an important role in spread of HLB and *D. citri* in Florida (and probably also to other states), also via garden centres (Manjunath *et al*., 2008). Murraya species are sold (also in bonsai form) to the European market, often with an Asian origin, for quite some years.

2.8 Prevention and management following first introduction

Once established, HLB bacteria and vectors are very difficult to control. Main strategies are geographical isolation and certification/indexing programs for budwood sources and nursery production in insect proof greenhouses, chemical and/or biological vector control and intensive surveying (by visual inspection and lab test) with removal and destruction of infected trees (both visually and latently infected trees) where possible. Healthy budwood can be obtained by a shoot-tip grafting, or alternatively by heat treatment, e.g. water-saturated hot air at 49°C for 50 min and fumigation of budwood against HLB vectors is possible. Visual inspections in the groves should be very intensive and performed from moving inspection platforms. Chemical sprays, for vector control, to be effective, should also be performed very frequently. Antibiotic treatment has been abandoned completely; it was not effective and not human and environment friendly. Biological control was effective in Reunion Island, probably mainly because hyperparasites of the parasite were absent with hymenopterous ectoparasites *Tamarixia dryi* for Laf and *T. radiates* for Las. Genetic resistance development has started, but is still in its infancy (Grosser *et al*., 2008) HLB bacteria and their vectors should be prevented from entering the Mediterranean basin by strict inspection and laboratory testing of imported breeding material from risk areas. In the European union already the importation of citrus planting material from Third (non-EU) countries is prohibited (Annex III A of Directive 2000/29/EC) and EPPO (EPP), 1990) that importation of plants for planting and cut branches of citrus from countries where HLB or its vectors occur should be prohibited. When such material is imported it should be fumigated and quarantined for at least 2 years. *Liberibacter* spp. and *D. citri* and *T. erytreae* are quarantine organisms on the EPPO A1 list and EC Annex II/Ia list. *Murraya* spp. and ornamental citrus spp. already present in garden centres or in plantations should be surveyed, including vector sampling. In suspect areas this could also been done in (young) citrus orchards. Importations from risk areas of these plants should be strictly inspected and laboratory tested. There should be proactive training of diagnosticians, surveyors, nursery managers and producers. Production in citrus nurseries should be in secure, insect proof greenhouses. Once an introduction would have occurred, infected trees should be destroyed and vectors controlled by chemical insecticides.

3 LEAF SCORCH AND LEAF SCALD DISEASES OF DIVERSE FRUIT AND ORNAMENTAL TREES, CAUSED BY *Xylella fastidiosa*

3.1 General

The bacterium *Xylella fastidiosa* is a xylem-inhabiting, vector-transmitted, very slow growing, Gram-negative bacterium. It was cultured and properly described for the first time in 1987 in the USA as the cause of Pierce's disease (PD) of grapevine, *Vitis vinifera* (the disease observed already in 1884) and as the cause of phony peach disease (PPD) in peach, *Prunus persica* (disease observed in 1890 in the USA). In 1993 in Brazil *X. fastidiosa* was found to be the cause of citrus variegated chlorosis (CVC) or citrus X disease of *Citrus*. Moreover it was found that the bacterium also causes a number of so-called leaf scorch diseases in *Prunus* spp. (incl. almond leaf scorch or ALS in *P. armeniaca* and plum leaf scald or PLS in *P. domestica*), *Acer* spp., *Carya illinoinensis* (pecan), *Coffea arabica* (CLC, in Brazil isolated in 1995 and also pathogenic to *Citrus*), *Hedera helix*, *Morus rubra*, *Nerium oleander* (OLS), *Platanus occidentalis*, *Quercus* spp., *Ulmus americana*. Furthermore in *Medicago sativa*, alfalfa (alfalfa dwarf) and *Vinca major* (wilting symptoms). Many wild plants may carry the pathogen with, but more often without, showing symptoms, such as grasses, sedges and trees. All these diseases are not seed-borne and occur mainly in tropical/subtropical areas, although leaf scorch diseases also occur in much colder climate, e.g. oak leaf scorch in Eastern North America up to Canada. *Xylella fastidiosa* is a quarantine organism on the EPPO A1 list and EC Annex II/Ia list.
Several pathogenic varieties have been described, that are often host-specific (e.g., the PD strain will not cause disease if introduced to peach or plum). The following subspecies have been described:
a) ***Xylella fastidiosa*** subsp. ***fastidiosa*** (erroneously named *X. f.* subsp. *piercei*) – PD and LSA, strains from cultivated grape, alfalfa, almond (two), and maple;
b) ***X. fastidiosa*** subsp. ***multiplex*** – PPD, PLS, strains from peach, elm, plum, pigeon grape, sycamore, and almond;
c) ***X. fastidiosa*** subsp. ***pauca* –** CVC**,** strains from citrus and probably those of coffee (CLC)
d) ***X. fastidiosa*** subsp. ***sandyi* –** strains from *Nerium oleander* (OLS);
e) ***X. fastidiosa*** subsp. ***tashke* –** strains from the ornamental tree *Chitalpa tashkentensis*.

3.2 Hosts

A full host list can be found on http://www.cnr.berkeley.edu/xylella/. Some hosts relevant for the Mediterranean basin are: *Nerium oleander* (OLS), *Platanus occidentalis* (sycamore), *Quercus* spp. (oak), *Ulmus americana* (elm tree), *Ambrosia artemisiifolia* (ragweed), *Morus alba* (white mulberry), *Prunus angustifolia* (chickasaw plum) and *Rhus* spp. In Taiwan a pear leaf scorch was described in 1990 on *Pyrus pyrifolia* (Japanese pear), especially variety Hengshan and *P. serotina* (Asian pear), which was found to be caused by a bacterium very similar (but different from strains from North and South America in serological characteristics and in housekeeping gene sequences) to *X. fastidiosa* (Leu and Su, 1993; Chen *et al.*, 2006). Hosts such as Asian pear were recently introduced in Central Europe (Romania) and Japanese pears or nashis, were planted as a novelty crop in southern Europe especially in the 1980's.

3.3 Symptoms and Transmission

In general early symptoms are a slight chlorosis or bronzing along leaf margin or tip that intensifies and that may become water-soaked before browning and drying. These symptoms are first found on a few branches, later on almost all foliage (so-called leaf scorch or scald symptoms). The affected area is delineated by a narrow chlorotic band that becomes especially clear in autumn. A premature defoliation may take place with new malformed leaves formed. Abnormally shaped fruit may also be formed and stems may show internal and external discoloration, dieback and abnormal growth, leading to eventual death of the host. Vectors are mainly sharpshooters and froghoppers or spittlebugs (Cicadellidae) that lack a latent period, and have no transstadial or transovarial transmission of the bacterium.
The pathogen shows persistence in the vector adults and ability to multiply in the foregut. In North America main vectors (for PD unless indicated) are *Cuerna costalis* (PPD), *Draculacephala minerva* (green sharpshooter) important also in ALS in California; *Graphocephala atropunctata* (blue-green sharpshooter), most important before GWSS; *G. versuta* (PPD); *Hordnia circellata*, most efficient; *Homalodisca vitripennis* (formerly) *coagulate* (glas-winged sharpshooter or GWSS); *H. insolita* (PPD), *Oncometopia nigricans*, *O. orbona* (PPD), *Xyphon* (formerly *Carneocephala*) *fulgida* (red-headed sharpshooter). CVC vectors in Brazil are *Acrogonia terminalis* that lays eggs externally on leaves, *Dilobopterus costalimai* and *Oncometopia fascialis*. Local possible vectors for Europe are *Cicadella viridis* and *Philaenus spumarius* (meadow spittle bug).

3.4 Risks

X. fastidiosa is an emerging threat in the Southwest USA, mainly due to recent establishment of the glassy-winged sharpshooter (GWSS, *H. vitripennis*), providing much more efficient transmission than local vectors and leading to very serious outbreaks of PD in grapevine, ALS and OLS. In Central and South America *X. fastidiosa* has become very noxious due to the rapid expansion (most likely via distribution of infected planting material) of CVC in *Citrus*, leading to more than a third of all trees in the area having symptoms of CVC and CLC in coffee.
For Europe there are until now only a few unconfirmed reports of finding *X. fastidiosa* viz. from Kosovo in grapevine and Turkey or disease symptoms observation only from France in grapevine. Since *X. fastidiosa* has more than 150 hosts and many of them, including *Vitis*, were and are imported (often as planting material), risk of introduction (especially in latent form) must not be underestimated.
Absence of the diseases caused by *X. fastidiosa* will mainly be due to the absence of suitable vectors. However, introduction of the pathogen and (infected) vectors with plant material can certainly not be excluded. Moreover, also local Cicadellidae (see above) could become (potential) vectors. Therefore, *X. fastidiosa* has the A1 quarantine status in the EPPO region and *H. vitripennis* (GWSS) that has a very large host range and also feeds on almond, peach and plum, was recently put on the EPPO alert list. As in the more Northern parts of the USA, *Vitis* varieties in Europe are very susceptible to *X. fastidiosa* and this is really a risk when a vector would become established that could survive the winters in Southern Europe and would also become established in wild hosts (e.g. wild and domestic plums and wild cherry are symptomless reservoirs in the USA) and cause spring infections that are most likely to persist over the years. The same risk holds true for *Citrus* (sweet oranges, mandarins, and tangerines) and other hosts, such as almond, plum and peach that are widely grown in (Southeast and Southwest) Europe, especially in the warmer Mediterranean basin (where a

disease-favourable combination of warm nights, regular rainfall/high humidity and long growing season, is present).
The conclusion is that *X. fastidiosa* is a real and emerging threat for Europe, not only for *Vitis* and *Citrus* but also for stone fruits (almond, peach and plum) and oleander (e.g. GWSS likes to feed on oleander), that is difficult to prevent from entering and difficult to control once established, deserving more attention than up till now. Resistance in European grapes is scarce or even absent. Vector control proved not to be very effective in the USA. Cultural practices to keep plants in optimum condition are of importance, but not sufficient and the use of avirulent strains for cross-protection is still in its infancy.

4 CITRUS CANKER, CAUSED BY *Xanthomonas citri* SUBSP. *CITRI (X. axonopodis* pv. *citri)*

4.1 General

A spot disease, showing corky lesions on leaves, fruits and twigs on *Citrus* spp. was first described by Stevens in 1914 and the causal bacterium, now named *Xanthomonas citri* subsp. citri by Hasse in 1915 from the USA. This disease was observed, however, earlier from Asia and originates most probably from China. Symptom formation and spread of the bacterium are enhanced by the activities of the citrus leaf miner, *Phyllocnistis citrella* though the insect is not a true vector. Grapefruit (*C. paradisi*), Mexican/Key lime (*C. aurantiifolia*), lemon (*C. limon*) and *C. hystrix* were found to be most susceptible. *X. citri* subsp. *citri*, causing this so-called Asian citrus canker, is a Gram-negative bacterium with 1 polar flagellum, forming yellow colonies on agar media. Over the years several much related diseases and pathogenic bacteria have been described from Citrus and some other Rutaceae spp. in Asia and North and South America:
X. citri pv. *citri* (syn. *X. citri* subsp. *citri*, *X. axonopodis* pv. *citri*) formerly group A or Asiatic strain is most agressive. Hosts are Mexican lime and Tahiti lime (*C. latifolia)*, but not infecting the susceptible species grapefruit (*C. paradise)*. So-called A^{w} strains were described from Florida, that infect Mexican lime and *C. macrophylla* (Bui Thi Ngoc *et al.*, 2009 and 2010**)**.
X. citri pv. *aurantifolii* (syn. *X. axonopodis* pv. *aurantifolii*, *X. fuscans* pv. *aurantifolii*), causing relatively mild disease in Citrus in Mexico and S. America. It was also reported from Swingle citromelo rootstock (*C. paradisi*) from Brazil (Jaciani *et al*., 2009).
X. citri pv. *bilvae* (syn. *X. campestris* pv. *bilvae*), an ill-defined pathogen, causing shot-hole disease and canker on Bael or Aegle marmelos, belonging to the Rutaceae, described from India in 1953 (Bui Thi Ngoc *et al., 2010).*
X. alfalfae subsp. *citrumelonis* (syn. *X. campestris* pv. *citrumelo*, causing citrus bacterial spot, Florida, USA (Schaad *et al*., 2005).
Only *X. c.* subsp. *citri* causing Asian citrus canker (A, A* and A^{w} strains) is described here. *X. c.* subsp. *citri* is a quarantine organism on the EPPO A1 list and EC Annex II/Ia list.

4.2 Geographical distribution

X. citri pv. *citri* originates from and widespread in Asia, including Georgia, Iran, Iraq, Oman, Saudi Arabia, UAE and Yemen. Australia (eradicated), Argentina, Belau, Brazil, Caroline Islands, Cocos Islands, Comoros, Congo Democratic Republic, Ivory Coast, Fiji, Gabon, Madagascar, Mauritius, Mozambique (eradicated), Netherlands Antilles, New Zealand (eradicated), Micronesia, Palau, Papua New Guinea, Paraguay, Réunion, Seychelles, South Africa (eradicated), Uruguay, USA (CABI/EPPO, 2006).

Recent reports: Somalia (Balestra *et al*., 2008) Mali (Traoré, 2008), Ethiopia (A* strains, Derso *et al*., 2009)
Phyllocnystis citri occurs in nearly all citrus growing areas of the world. In Europe it has established in the Iberian Peninsula, Corsica, Italy, Greece since 1994 and also Montenegro.

4.3 Hosts

X. citri pv. *citri c*ultivated hosts are *Aegle marmelos* (golden apple), *Casimiroa edulis* (white sapote), *Citrus aurantiifolia* (lime), *C. aurantium* (sour orange), C. *hystrix* (mauritius bitter orange), *C. junos* (yuzu), *C. limetta* (sweet lemon tree), *C. limon* (lemon), *C. madurensis* (calamondin), *C. maxima* (pummelo), C. *medica* (citron), C. *natsudaida*i (natsudaidai), C. *reshni* (Cleopatra mandarin), *C. reticulata* (mandarin), *C. reticulata* x *Poncirus trifoliata* (citrumelo), *C. sinensis* (navel orange), *C. sunki* (sour mandarin), *C. tankan* (tankan mandarin), Citrus unshiu (satsuma), *Citrus* x *paradisi* (grapefruit), *Eremocitrus glauca* (Australian desert lime), *Limonia acidissima* (elephant apple), *Poncirus trifoliata* (trifoliate orange or Japanese bitter orange). Minor hosts are *Fortunella japonica* (round kumquat), *F. margarita* (oval kumquat). Wild hosts are *Ageratum conyzoides* (billy goat weed) *Severinia buxifolia* (box orange or boxthorn) and *Swinglea glutinosa* (*Aegle decandra, Limonia glutinosa).*

4.4 Symptoms and transmission

Small spots are first visible on upper leaf surface are formed on leaves, shoots, twigs and fruits and these spots become raised pustules or blister-like eruptions. Later the lesions become up to 10 mm in size and brown and necrotic, with a depressed centre and sometimes surrounded by a yellow halo. On fruits these lesions can be mistaken for scale insects (e.g. the California red scale, *Aonidiella aurantii*). The bacterium is a wound parasite and the citrus leaf miner (*Phyllocnistis citrella*) contributes to the spread and disease severity. Citrus canker is especially epidemic and damaging on seedlings and young trees, especially after storms (hurricanes) with rain under warm weather conditions, but due to dependence on these weather conditions epidemics are sporadic. Full-grown trees show much less disease and damage. The bacterium can survive in a latent form and on diseased shoots and discoloured bark tissue of the trunk and may reoccur suddenly after several years (sometimes even as long as 10 years).

4.5 Risks and damage

Heavy losses have been reported in epidemics due to leaf and premature fruit drop and fruits with spots that cannot be marketed or start to rot and have to be destroyed. Furthermore quarantine measures such as burning of trees and destruction of fruits may add to these losses. In severe cases almost 100% of the fruits and leaves of young, susceptible trees may be infected and their growth delayed for a number of years. The direct Government costs in the USA for the eradication activities from 1995 to 2006 were calculated to be more than $1.3 billion. From 2006 to 2009, together with costs for control of Citrus huanglongbin it was calculated $90 million. The citrus acreage in Florida decreased since 1996 with c. 33 % (Lowe, 2010). In January 2006, USDA determined that canker had become so widespread in Florida that eradication was no longer feasible. Use of healthy planting material and use of other measures, including weather forcasting in an integrated way have been applied in the control of Citrus canker with some success. Resistance has been found especially in *C. mitus* (calamondin) and *Fortunella* (kumquat). *C. reticulata* (mandarin) is tolerant.

It should be remembered, that epidemics of citrus canker on mature plants are sporadic and very dependent on weather conditions (rainstorms, hurricanes). These weather conditions are less prevalent in the Mediterranean basin. On the other hand it should also be realized that the citrus leaf miner is already widespread in this area. Seed transmission has not been observed. Long distance dispersal is by infected planting material or infected fruit. In the past ornamental Citrus has been imported from Asia into Europe and *X. citri* pv. *citri* has been intercepted on this material (pers. exp.). The risk of dispersal by infected fruit has been evaluated very small to absent when fruits have been disinfected before shipment (Gottwald *et al*., 2009). Without disinfection survival chances in symptomatic fruits are apparently also small (Shiotani *et al*., 2009), but not impossible (findings of *X. citri* sp. *citri* on imported fruits by Dutch Plant Protection Service, pers. exp). Since *X. citri* pv. *citri* is endemic and spreading into countries surrounding the Mediterranean basin, many of those also already have problems with huanglongbin, and the citrus leaf miner widespread within the area, the conclusion must be that this pathogen is a real and emerging threat.

4.6 Control

Use of healthy planting material and use of other measures in an integrated way have been applied in the control of Citrus canker with some success. Weather forecasting has been tried in Japan. Resistance has been found especially in *C. mitus* (calamondin) and *Fortunella* (kumquat), however *C. reticulata* (mandarin) is tolerant.

5 SOME OTHER BACTERIAL DISEASES THAT MAY BECOME A THREAT FOR FRUIT TREES IN THE MEDITERRANEAN BASIN

5.1 Black spot of mango – *Xanthomonas citri* pv. *mangiferaeindicae*

A leaf spot and canker disease, called bacterial black spot of mango (*Mangifera indica*) was described by Doidge in South Africa in 1909 and named *Bacillus mangiferae* (later *Xanthomonas mangiferaeindicae*, and *X. campestris (axonopodis*) pv. *mangiferaeindicae*. Ah-You *et al*, 2009 showed that this bacterium and a related pathogen from cashew (*Anacardium occidentale*), both plants belong to the Anacardiaceae as *X. citri* subsp. *mangiferaeindicae* and *X. citri* pv. *anacardii*. Other (rare) hosts are ambarella (*Spondias dulcis*, syn. *S. cytherea*) and Brazilian pepper (*Schinus terebinthifolius*), both belonging to the Anacardiaceae.
Symptoms start as small water-soaked spots that become later raised and black necrotic, sometimes surrounded by a narrow yellow halo. On fruits the water-soaked spots become star-shaped and crack and often show exuding gum. Severe infection under influence of rain storms may lead to premature leaf and fruit drop, twig cankers and twig death.
When other diseases and pests are controlled black spot is a limiting disease in mango production, because it is very difficult to control. In most susceptible cultivars up to 100 % fruit loss may occur. Many commercial cultivars are very susceptible. In 1996 and 1997, severe black spot epidemics were observed in many mango producing areas of South Africa, causing almost 100% fruit loss on the most susceptible cultivars and c. $1million economic loss. There is production of mango in Europe and Mediterranean basin (e.g. Spain, Italy, Israel, Portugal). Black spot was observed in Australia, Comoro Islands, many areas in S. and E. Africa and Asia, Mauritius, New Caledonia, Reunion, Taiwan, and the United Arab Emirates (Gagnevin & Pruvost, 2001). Discrimination of strains from mango and some related hosts and from different geographic origin (Asia, Africa and Brazil) was possible using RFLP (Gagnevin *et al.*, 1997). Large distance dissemination of the disease is thought to be by infected planting material. Epiphytic/endophytic populations of the pathogen occur

(Pruvost *et al.*, 2009). Seed transmission has not been demonstrated. Most cultivars are susceptible to highly susceptible.

5.2 Almond witches'broom - "Candidatus" Phytoplasma phoenicium

This devastating disease of almond (*Prunus amygdalus*), showing small yellow leaves on proliferating shoots that wither and die in later stages, was reported from Lebanon by Choueiri *et al.*, 2001. In a few years time more than 100.000 trees were killed in different areas in Lebanon, and the phytoplasma was also found on peach and nectarine. It was recently also reported to be destructive from Iran (Zirak *et al.*, 2009). Specific leafhopper vectors are suspected, but not yet fully determined. This pathogen has not yet been placed on quarantine lists, but certainly deserves attention as an emerging threat for almond.

5.3 Bacterial blight of pomegranate - *Xanthomonas axonopodis* pv. *punicae*

A bacterial disease on pomegranate (*Punica granatum*) was observed for the first time in 1952 in New Delhi, India and described by Hingorani and Sing (1959) as *Xanthomonas punicae* (later classified as *X. campestris* pv. *punicae* and *X. axonopodis* pv. *punicae*). First symptoms are watersoaked spots on leaves and fruits. On leaves spots become necrotic and during coalesce severe leaf drop may occur. On fruits spots may cause fruit cracking and fruit drop. On branches black necrotic spots occur that may crack and weaken them. Pomegranate is produced mainly by India (50%), Iran (35%), former Russian states and in the Mediterranean basin most in Spain (2.5%), Morocco, Egypt and Turkey. Bacterial blight has developed into a very serious disease, till so far only found and emerging in India, where it leads to very heavy losses, up to 100% (Kumar *et al.*, 2006) in many pomegranate producing areas. Distribution is by rain splash, insects and tools and for long distance infected plant material. Like *X. citri* subsp. *citri* and subsp. *mangifeaeindicae*, humid stormy weather conditions are important in epidemic development. Since these two pathogens have already spread from more eastern parts of Asia, *X. a. punicae* may be seen also as a threat that may soon show its presence closer to the Mediterranean basin

5.4 Bacterial blight of guava – *Erwinia psidii*

A vascular disease from guava (*Psidium guyajava*) was described as bacterial blight from Brazil in 1987 and found to be caused by the bacterium *Erwinia psidii* (Neto *et al.*, 1987) where it caused a major disease problem in the main production areas of South-eastern and Central Regions of Brazil (Tokeshi *et al.*, 1980). There are local and systemic symptoms: on leaves large necrotic lesions at leaf margins with a translucent halo, giving a leaf scorch appearance or small water-soaked spots, sometimes with a chlorotic halo, that later become necrotic and when they coalesce may cause leaf drop. When bacteria reach the xylem they spread into branches, trunk and roots. In severe cases there is defoliation and death of trees. Symptoms on fruits are not common. This disease could be of importance to guava producing countries such as Egypt that is with 230.000 tons fruit production, the 5th guava producer in the world after India, Pakistan, Brazil and Mexico. In Brazil pathogen distribution occurs often by contaminated planting material (Marques *et al.*, 2007; Teixeira *et al.*, 2008a)

5.5 Bacterial spot of passion fruit – *Xanthomonas campestris* pv. *passiflorae*

A destructive disease, with symptoms on leaves of watersoaked, greasy lesions of irregular shape surrounded by chlorotic areas, which, when they coalesce will cause leaf necrosis and greasy spots on the fruits which make them unsuitable for consumption and industrial processing, was described by Pereira in 1969 already from Brazil. It has an emerging character in Brazil and has become a major problem in the passion fruit production. Hosts are *Passiflora alata* Curtis, *P. amethystina* J.C. Mikan, *P. coccinea* Aubl., *P. edulis, P. edulis* f. *flavicarpa* Degenes, *P. maliformis* L., *P. nitida* H. B. & K. and *P. serrato-digitata* L. (Torres Filho & Ponte,1994)

Concomitant with the expansion of passion fruit production, the incidence of disease is also rising (Torres Filho & Ponte, 1994). Bacteriosis caused by *X. campestris* pv. *passiforae* is a major disease limitation in the development of cultivated areas in Brazil. The disease, designated premature death, has been increasing since the late 1970s (Torres Filho & Ponte, 1994). Infection occurs through natural openings and lesions and results in systemic invasion of the whole plant. In the leaves, the disease cause necrosis and soaked, greasy lesions of irregular shape surrounded by chlorotic areas. The fruit is also affected, with the appearance of hard greasy spots making it unsuitable for consumption and industrial processing.

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