EURL
European Reference Laboratory
for **Bacteria** in plants

Work programme 2019/2020


Photo: NVWA

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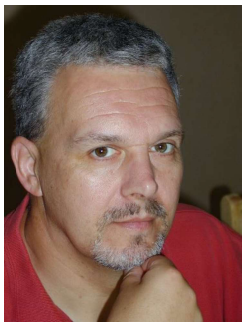
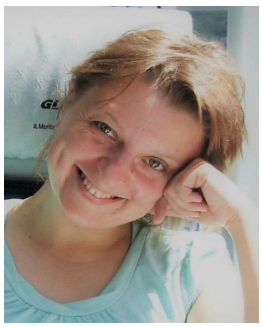
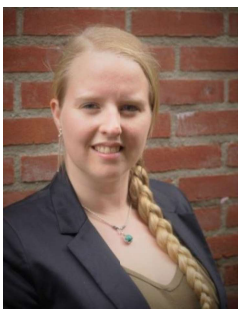
EPPO Panel Heads of Laboratories, 10/09/2019, Paris


Johan van Vaerenbergh
and ILVO team


Tanja Dreo
and NIB team


Stefania Loreti
and CREA team

EURL-Bacteriology Consortium


Saskia Bosman (NVWA)


Maria Bergsma-Vlami
and NVWA team

Aim of EURL in Bacteriology

- Achieve harmonization in testing and an overall high level of diagnostics at National Reference Laboratories (NRLs)

- Focus for 2019-2020 on designated priority pests among organisms listed as union quarantine pests (taxonomy according to 2000/29/EC) :

  - *Xylella fastidiosa* (Wells et al.), IAII b, (XYLEFA)
  - *Ralstonia solanacearum* (Smith) Yabuuchi et al., IAII b, (RALSSO) causal agent of bruin rot of potato and bacterial wilt of ornamentals
  - *Clavibacter michiganensis* subsp. *sepedonicus*, (Spieckermann et Kotthoff) Davis et al., IAII b, (CORBSE) – causal agent of ring rot of potato
  - *Xanthomonas citri* pv. *citri*, IAI b, (XANTCI)
  - *Xanthomonas citri* pv. *aurantifolii*, IAI b, (XANTAU)

  Causal agents of citrus canker disease

  - *Candidatus* Liberibacter spp., causal agent of Huanglongbing disease of citrus/citrus greening, IAI b, (LIBEAF, LIBEAM, LIBEAS)

Official Control Regulation (EC/2017/625; OCR)

Article 92– 101 EURLs & NRLs

Responsibilities and tasks of EURLs (art 94)

- Improvement + harmonization of tests
- Develop annual/multi-annual WP + report (Reg. 652/2014)
- Scientific + technical assistance COM + NRLs (incl. training courses)
- Assistance during outbreaks

+

- Publish list of NRLs
- Provide reference materials to NRLs
- Proficiency tests: inform COM + MS on results and follow up.
- Cooperate to develop new methods
- Collaborate with third countries, with EFSA and EMA


7.4.2017 EN Official Journal of the European Union L 95/1

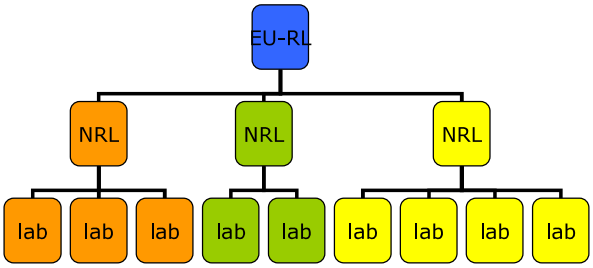
(Legislative acts)

REGULATIONS

REGULATION (EU) 2017/625 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 15 March 2017

Structure of labs in OCR



Requirements of NRLs

- Staff:
  - Impartial, confidentiality
  - Suitably and training + support
  - International Standards
- Infrastructure, equipment and products
  - According to needs
  - Emergency situations

+

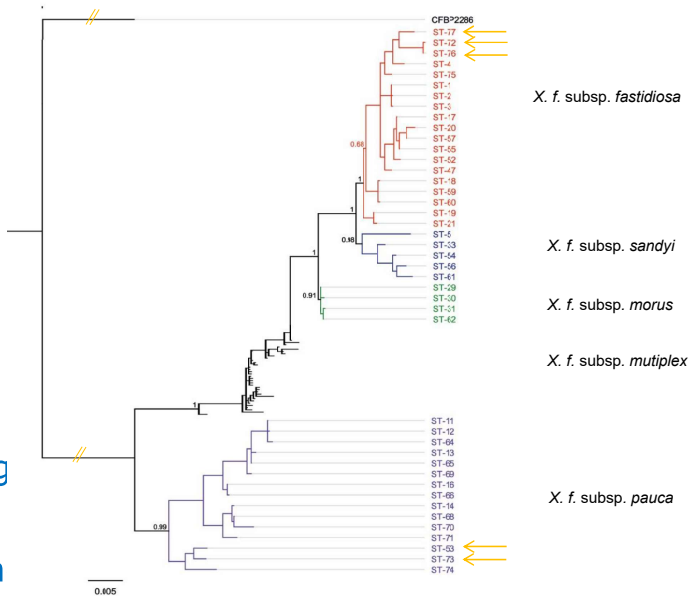
- Scope of accreditation*
- Equipped with relevant biosecurity standards
- CA shall organise audits
- CA shall withdraw the designation if doesn't comply with**:
  - ISO 17025
  - Obligations
  - Expected results in proficiency tests

* Both official labs and NRLs shall have an ISO 17025 accreditation for all the methods for analyses performed as official laboratory

**Art. 37-39 on official labs

Characteristics of *Xylella fastidiosa* (Wells et al.)

- Pathways of spread:
  - Plants for planting, over 350 plants
  - Transmission by vectors
- Different *Xf* subspecies
- Often no symptoms (latency)
- In general, plants show drying, scorching wilting, eventually followed by plant death colonises the xylem (restrict water movem



Bergsma-Vlami et al., 2017, Plant Pathology

Distribution of *Xylella fastidiosa* (Wells et al.)

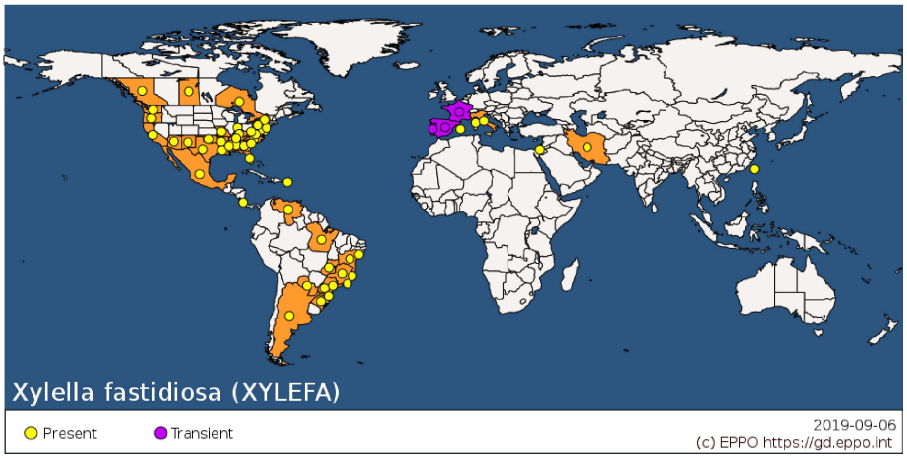


Figure 3: *X. fastidiosa* infected symptomatic plants recorded in the European outbreaks: (A) and (B) olive trees in Apulia, Italy; (C) and (D) almonds in the Balearic Islands (Spain), (E) *Polygala myrtifolia* in Spain, (F) grapevine in Spain, (G) wild olive in Spain (pictures from Italy: courtesy of Donato Boscia, Institute for Sustainable Plant Protection; National Research Council of Italy, from Spain: courtesy of Juan A. Navas-Cortés; Instituto de Agricultura Sostenible and Conselleria de Medi Ambient, Agricultura i Pesca, Direcció General d'Agricultura i Ramaderia, Government of the Balearic Islands, Spain)

High number of outbreaks in the EU since 2013

Decision (EU) 2015/789 (Emergency measures)
as amended by Decisions (EU) 2017/2352 and (EU) 2018/927

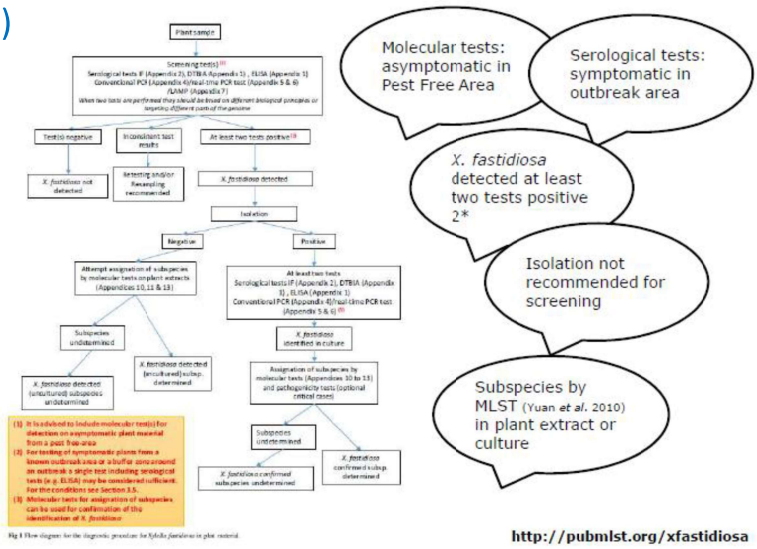
EFSA Journal 2018;16(7):5357, 61 pp.

EPPO diagnostic protocol on *Xylella fastidiosa* PM7/24(3)

- Expert Working Group (AT, FR, IT, NL, SL, ES)
- Many tests described in PM7/24

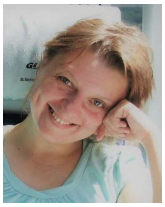
ELISA, IF
Conventional PCR (Minsavage et al., 1994)
Real-time PCR (Harper et al., 2010; erratum 2013)
Real-time PCR (Ouyang et al., 2013)
Real-time PCR (Li et al., 2013)
Real-time PCR tests (Francis et al., 2006)
Real-time LAMP test, (Harper et al., 2010; erratum 2013)

Multilocus Sequence Typing (MLST) (Yuan et al., 2010)
Conventional PCR (Pooler & Hartung, 1995) (pauca only)
Conventional simplex/multiplex PCR
(Hernandez-Martinez et al., 2006)

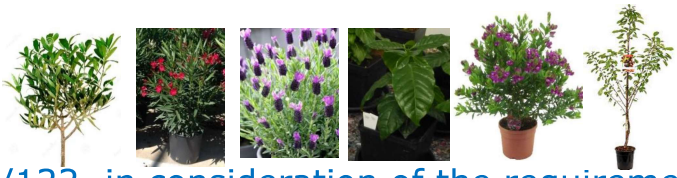


Validation data in http://dc.eppo.int/validationlist.php

Proficiency test (PT) EURL-Bacteriology 2019



Tanja Dreo
and NIB team



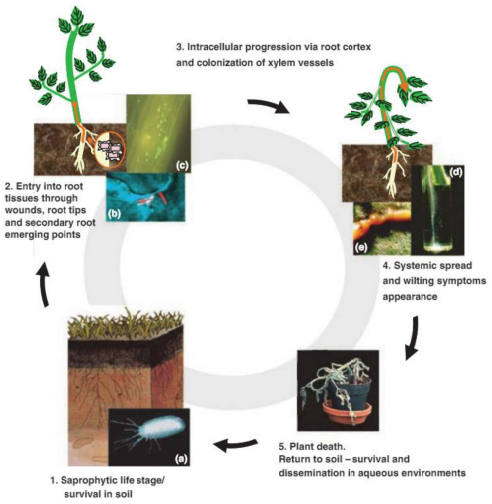
- *Xylella fastidiosa* (EU) 2018/927
- EPPO guidelines PM7/122, in consideration of the requirements of ISO 17043 for PTs
- 14 DNA extract samples and 2 controls (PAC,NAC)
- QuickPick™ SML Plant DNA kit (Bio-Nobile)
- Different subspecies at different concentration levels included

- 28 Member States: total number of registrated NRLs: 22 (7/9/2019)

EURL recommended tests: participants are guided by the Commission Database of Validated Tests for the Identification of the *Xylella fastidiosa* and its subspecies as referred to in Article 3(2) of Commission Implementing Decision (EU) 2015/789 and PM7/024.

Characteristics of *Ralstonia solanacearum* (Smith) Yabuuchi et al.

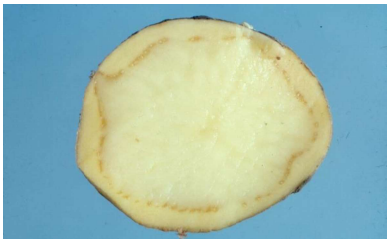
- Soilborne bacterium
- More than 450 plant species and 50 botanical families, also in waterways in the EU.
- In the past epidemics in crop fields of tomato, potato, tobacco, banana, groundnut and ginger.
- Causal agent of bacterial wilt and brown rot of potato, often latent infections!
- Spread from mother plants to their progeny, i.e., in potato seed tubers, rhizomes, and cuttings

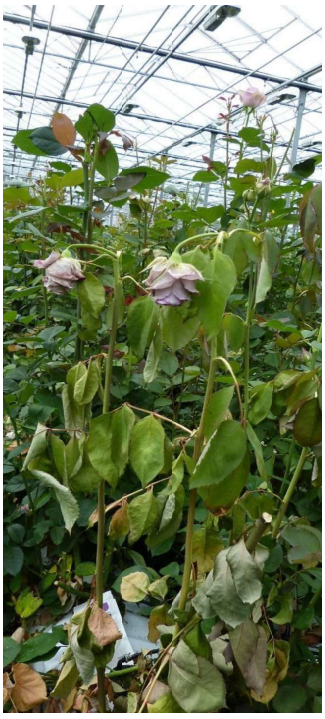


Genin (New Phytologist, 2010, 187, 920-928)

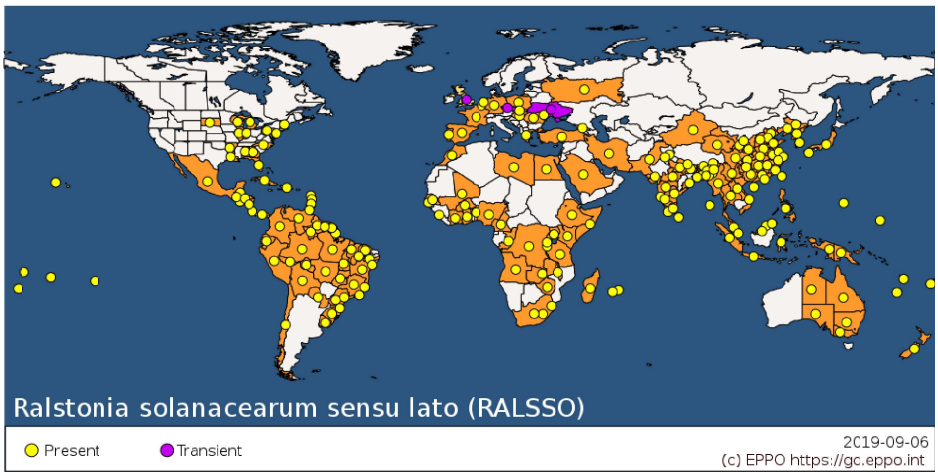
Symptoms

- Brown rot potato
- Wilting (rose, eggplant, pelargonium)
- Bacterial exudation
- Internal vascular discoloration





Distribution of *Ralstonia solanacearum* (Smith) Yabuuchi et al.



RSSC

phylotype I Asia

phylotype II America **(Brown rot potato)**

phylotype III Africa

phylotype IV Indonesia, Australia, Philippines

occurs worldwide under various climatic conditions

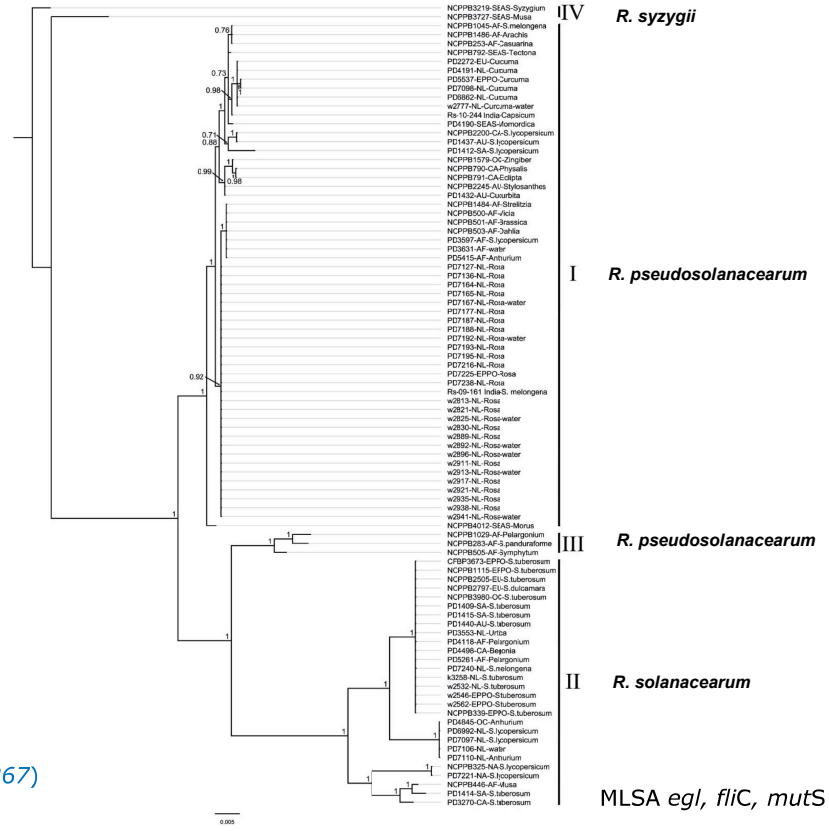
Reclassification of *Ralstonia solanacearum* (Smith) Yabuuchi et al.

According to Safni et al., 2014:

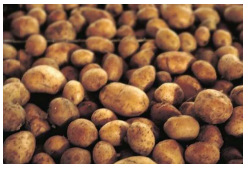
1. *R. pseudosolanacearum* (phylotype I and III)
2. *R. solanacearum* (phylotype II)
3. *R. syzygii* (phylotype IV)
   - *R. s. subsp. indonesiensis*
   - *R. s. subsp. celebensis*
   - *R. s. subsp. syzygii*

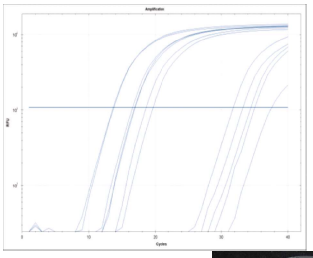
These species are considered in the EURL work programme

(*Bergsma-Vlami et al., 2018, Plant Disease 102, 2258-2267*)



Official testing for *Ralstonia solanacearum* (Smith) Yabuuchi et al.



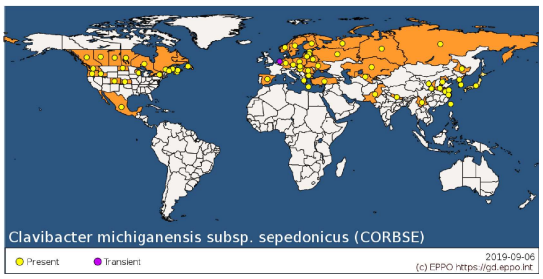


Real-time PCR (Weller et al., 2000)
PT in 2020

EC (2006) Council directive 2006/63/EC. Official Journal of the European Communities L-206, 1–106.

Characteristics of *Clavibacter michiganensis subsp sepedonicus*

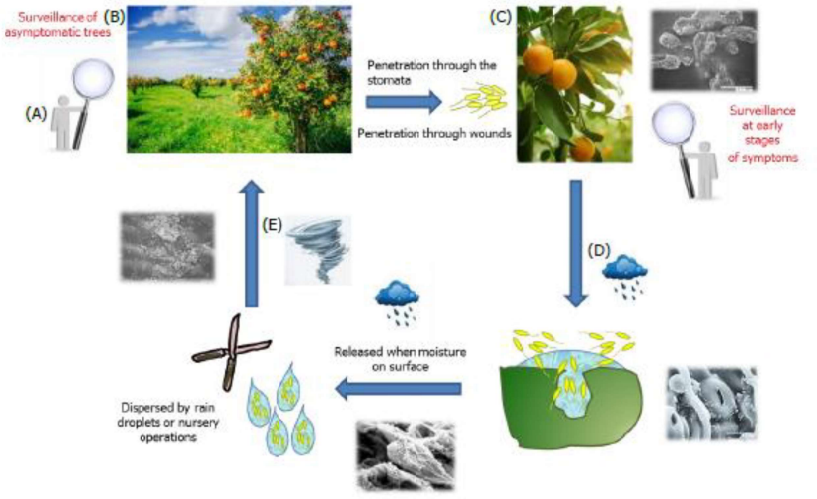
- Causal agent of ring rot of potato, often latent infections!
- Some cultivars rarely show symptoms although the pathogen multiplies within them (symptomless carriers)
- Reclassification to *Clavibacter sepedonicus* comb. nov. (Li et al., 2018)
- Prevalent in cool, northern latitudes
- Wilting and tuber rotting in field and store



- Officially approved test methods described in the Commission Directive 2006/56/EG (Ring rot).
- Addition of real time PCR i.e of Gudmestad *et al.*, 2009 or Schaad *et al.*, 1999 –PT in 2020

Characteristics of *Xanthomonas citri* pv *citri* and pv *aurantifolii*

Citrus canker is mostly a leaf and fruit-spotting disease, but when conditions are highly favorable, infections cause defoliation, shoot dieback and fruit drop.



EFSA Pest survey card on *Xanthomonas citri* pv. *citri* and pv. *aurantifolii* 2019:EN-1587. 25 pp.

EPPO Panel Heads of Laboratories, 10/09/2019, Paris

Distribution of *Xanthomonas citri* pv *citri* and pv *aurantifolii*

Taxonomy		Reference
Xanthomonas campestris		Dye and Lelliott, 1974
Xanthomonas axonopodis		Vauterin et al., 1995
X. citri subsp. *citri*	*X. fuscans* subsp. *aurantifolii*	Schaad et al., 2006
X. citri pv. *citri* (subsp. *citri*) *X. citri* pv. *aurantifolii* (subsp. *aurantifolii*)		Ah-You et al., 2009 Rodriguez et al., 2012 Constantin et al., 2016

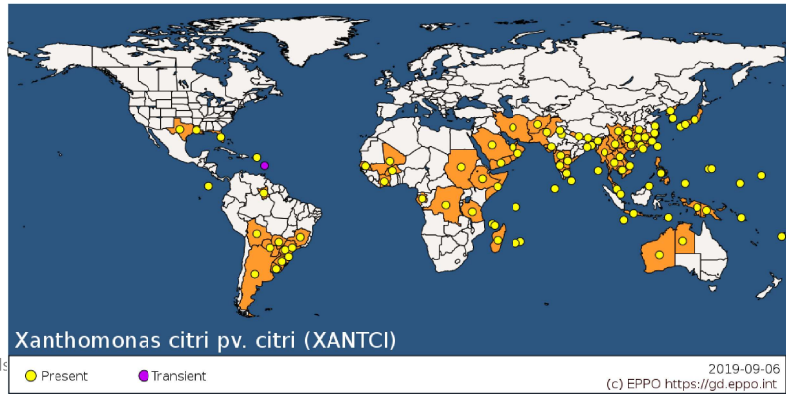
Species	***Xanthomonas citri***			
Pathovar[a]	*citri*		*aurantifolii*	
Pathotype	A	A* (A^w)	B	C
Disease	Asiatic canker		South American canker	
Host range	*Citrus* spp.[b] Several other rutaceous genera	***C. aurantifolia*** *C. macrophylla* (*C. latifolia*) (*C. sinensis*, *C. paradisi*)[c]	***C. aurantifolia*** *C. limon* *C. aurantium* *C. limonia* *C. limettioides* (*C. sinensis*)	***C. aurantifolia*** (*P. trifoliata* x *C. paradisi*)

Bold characters: main host species in field conditions; in brackets: host species rarely infected in the field.

(a): A pathovar is an infra-species taxon. 'The term pathovar is used to refer to a strain or set of strains with the same or similar characteristics, differentiated at infrasubspecific level from other strains of the same species or subspecies on the basis of distinctive pathogenicity to one or more plant hosts' (Young et al., 1991, 2001).

(b): With differential host susceptibility among species and/or cultivars. Many commercial cultivars range from susceptible to very susceptible (Gottwald et al., 2002).

(c): Reported for strains originating from Iran (Escalon et al., 2013).



EFSA Pest survey card on *Xanthomonas citri* pv. *citri* and pv. *aurantifolii* 2019:EN-1587. 25 pp.

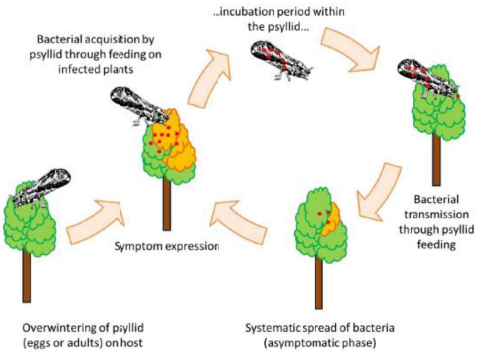
EPPO Panel Heads

Testing for *Xanthomonas citri* pv *citri* and pv *aurantifolii*

- Special requirements (Directive 2000/29/EC) for **the fruit** of Citrus L., Fortunella, Poncirus etc and also **for plants** of Microcitrus, Naringi and Swinglea, other than fruit and seeds, originating in third countries.
- *X. citri* pv. *citri* and pv. *aurantifolii* are clearly distinguished species following **laboratory testing**.
- IPPC 27 protocol 6 on *Xanthomonas citri* subsp. *citri* (2014)
  - Serological detection (IF and ELISA)
  - Molecular detection and identification:
    - Conventional PCR Hartung *et al.* (1993)
    - Conventional PCR Cubero and Graham (2002)
    - Conventional PCR Coletto-Filho *et al. (*2006)
    - Conventional PCR Park *et al.* (2006)
    - Real time PCR Llop *et al.* (1999)
  - But most published primers lack specificity

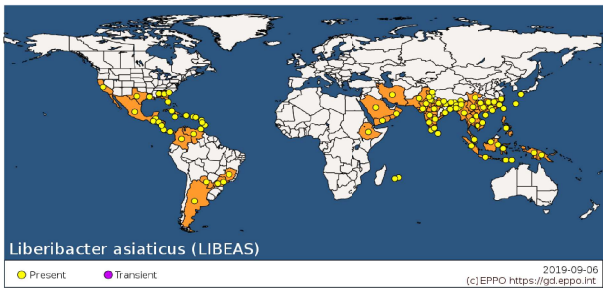
Characteristics of '*Candidatus* Liberibacter spp.'

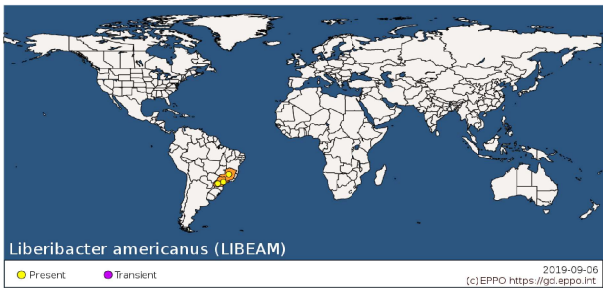
- All commercially cultivated Citrus is susceptible to the disease
- Also found in other Rutaceae plants, in particular on orange jasmine (*Murraya paniculata*)
- Transmition by vectors *Diaphorina citri* (the Asian citrus psyllid) and *Trioza erytreae* (the African citrus psyllid)
- Non culturable, testing by molecular methods
- Detection is difficult, irregular distribution in trees

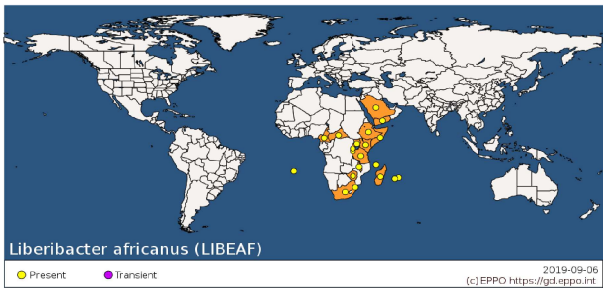


EFSA Pest survey card on Huanglongbing and its vectors 2019:EN-1574. 23 pp.

Distribution of '*Candidatus* Liberibacter spp.'







The most widely distributed HLB pathogen is Las which is present in Asia, North America, South America and Africa

Testing for '*Candidatus* Liberibacter spp.'

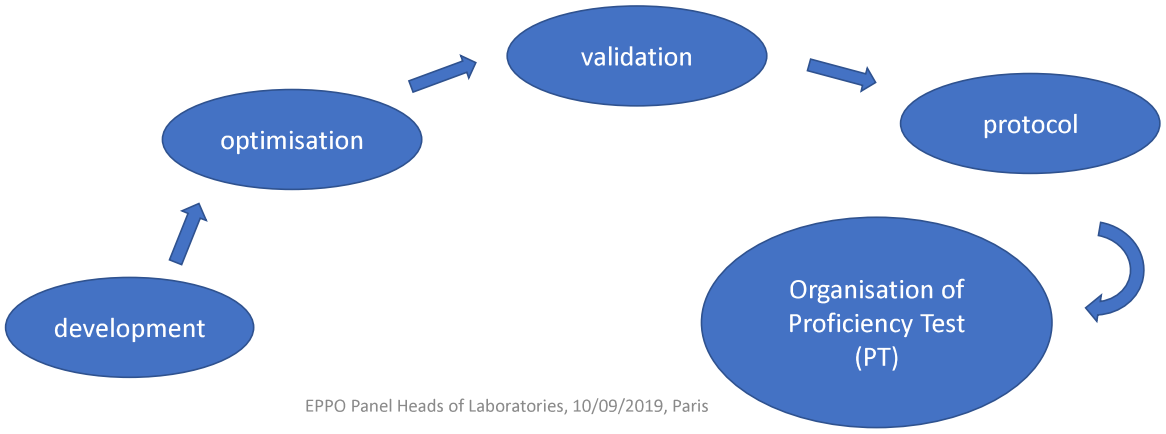
- PM 7/121 (1) 'Candidatus Liberibacter africanus', 'Candidatus Liberibacter americanus' and 'Candidatus Liberibacter asiaticus' (2014)

- Real-time PCR according to Bertolini et al. (2010, 2014)
- Real-time PCR according to Li et al. (2006)
- Real-time PCR according to Morgan et al. (2012)
- Conventional PCR according to Jagoueix et al. (1996)
- Conventional PCR according to Teixeira et al. (2005a,b)
- Conventional PCR according to Hocquellet et al., (1999)

- EUPHRESCO A-232 on HLB: a TPS organised by ANSES in La Réunion comparing the tests in green.

Planning EURL-Bacteriology

	2019	2019-2020	2019-2021
	Xylella fastidiosa	Cms and Rsol	Citrus pathogens
Reference material	NIB	ILVO/NVWA	ILVO/NVWA/CREA
Protocol*	NIB	ILVO/NVWA	ALL
Samples	NIB	ILVO	ILVO/NVWA
Proficiency Test & Reporting	NIB	ILVO	to be decided



Activities EURL-Bacteriology 2019

- **Compilation list of NRLs**
- Questionnaire (response by 15-09)—up to now only 5 MS have responded!!!
  - NRL capabilities, in particular related to priority pests (facilities, experience)
  - Availability of collections
  - Specific demands and/or needs
- Proficiency Test on *Xylella fastidiosa* (registration by 06/09; samples by 23-27/09; results by 16/10) – up to now 22 of the 28 NRLs have been registered
- Annual Workshop (Wageningen, 12/13-11)
- Theoretical Training (Wageningen, 13-11)

Thank you for your attention

Photo: NVWA