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Current situation of '*Candidatus* Liberibacter solanacearum' in Spain

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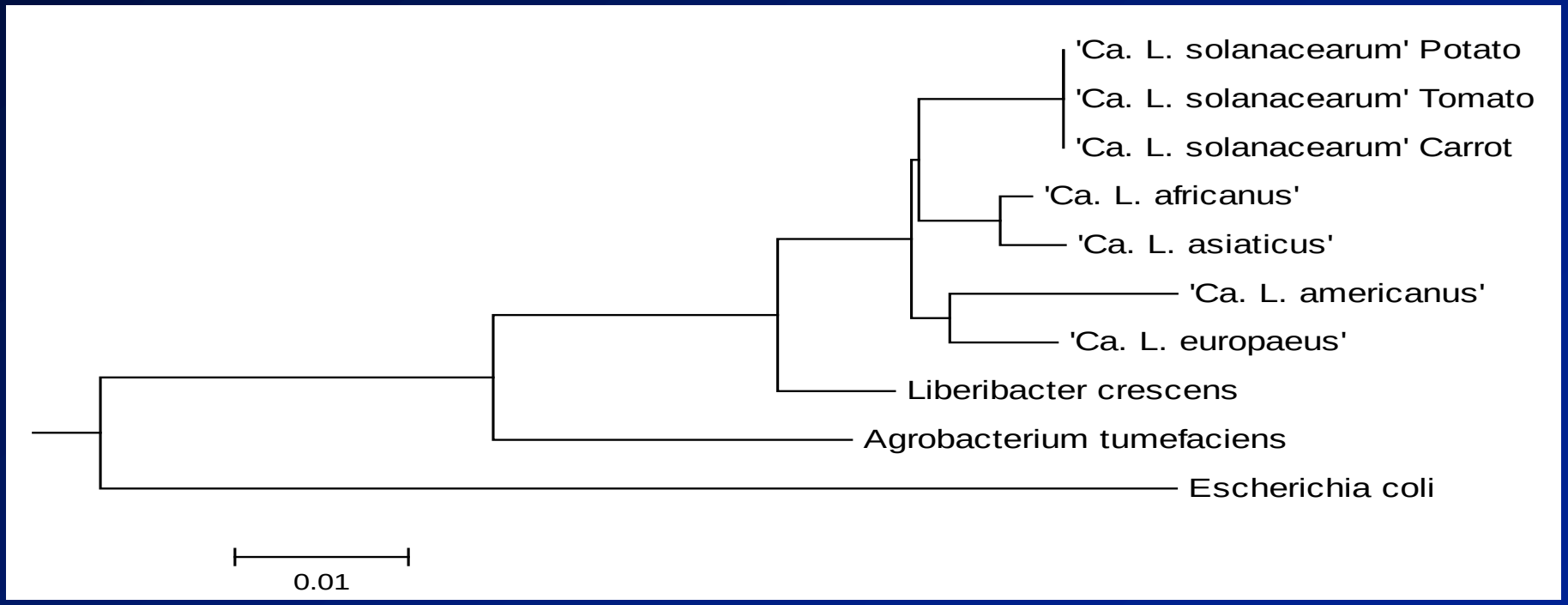
‘*Ca*. Liberibacter’ genus

- ‘*Ca*. Liberibacter’ is a genus in the family *Rhizobiaceae* , class α–Proteobacteria, that lives in intimate association with eukaryotic cells.

- Gram negative, non-culturable bacteria, restricted to the plant’s phloem and insect’s hemolymph.



- Transmitted by graft, cuscuta, infected potato tubers, carrot seeds and by arthropod vectors (psyllid species) in a persistent, circulative manner.

Plant Pathology (2014) Doi: 10.1111/ppa.12245

Transmission of '*Candidatus* Liberibacter solanacearum' in carrot seeds

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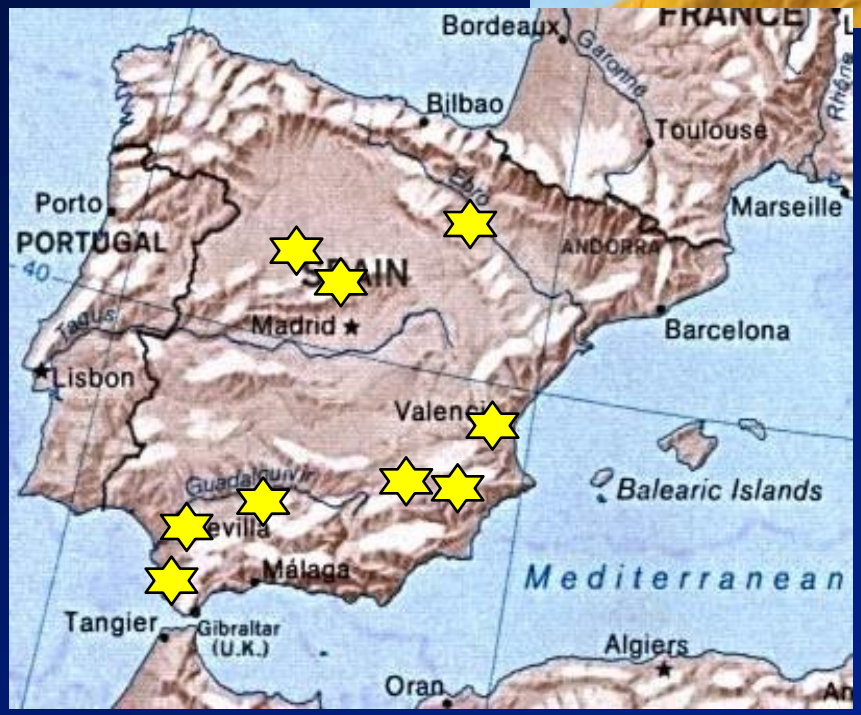
A protocol for the specific detection and quantification of '*Candidatus* Liberibacter solanacearum' in carrot seeds using real-time PCR was developed. The bacterium was detected in 23 out of 54 carrot seed lots from 2010 to 2014, including seeds collected from diseased mother plants. The average total number of '*Ca*. L. solanacearum' cells in individual seeds ranged from 4·8 ± 3·3 to 210 ± 6·7 cells per seed from three seed lots, but using propidium monoazide to target live cells, 95% of the cells in one seed lot were found to be dead. Liberibacter-like cells were observed in the phloem sieve tubes of the seed coat and in the phloem of carrot leaf midrib from seedlings. The bacterium was detected as early as 30 days post-germination, but more consistently after 90 days, in seedlings grown from PCR positive seed lots in an insect-proof P2 level containment greenhouse. Between 12% and 42% of the seedlings from positive seed lots tested positive for '*Ca*. L. solanacearum'. After 150 days, symptoms of proliferation were observed in 12% of seedlings of cv. Maestro. '*Candidatus* Liberibacter solanacearum' haplotype E was identified in the seeds and seedlings of cv. Maestro. No phytoplasmas were detected in seedlings with symptoms using a real-time assay for universal detection of phytoplasmas. The results show that to prevent the entry and establishment of the bacterium in new areas and its potential spread to other crops, control of '*Ca*. L. solanacearum' in seed lots is required.

Keywords: cell viability, detection, electron microscopy, quantification, real-time PCR, seedborne bacterium

Importance of carrot (*Daucus carota*) in Spain



Approx. 9,000 ha (74.000 t)

Carrot is cultivated along the year in open field in areas coexisting with celery, leek, potato, and other horticultural crops (even citrus).

The main cvs are: Maestro, Bangor, Soprano, Romance,…

Carrot is mainly cultivated in three cycles (early, medium and late) along the year.

Early: March to July
Medium: May to December
Late: July to January next year

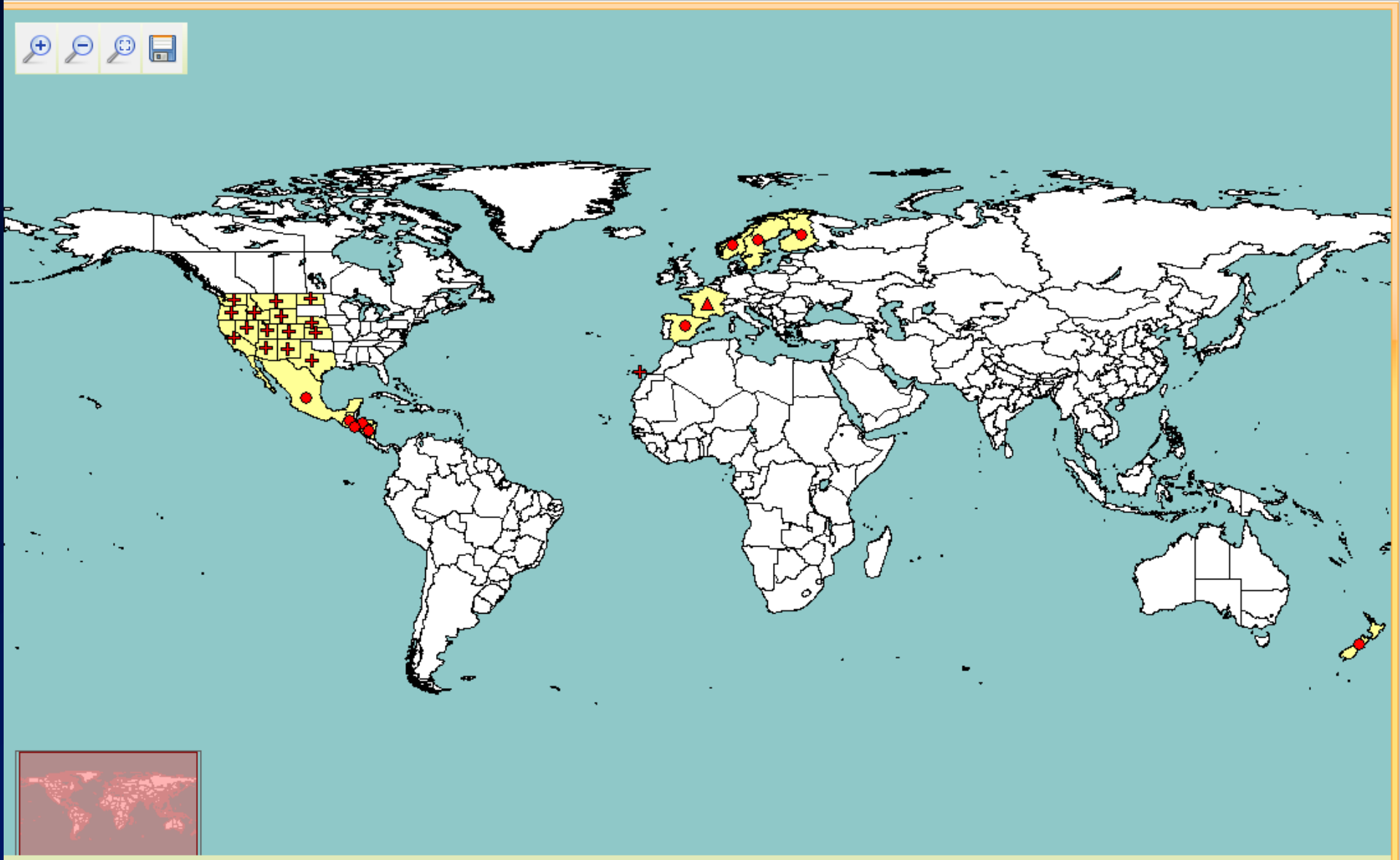
It is commercialized for the industry and after selection in packing houses for fresh consumption

2007: Vegetative disorders were noticed for the first time in carrots in Canary Islands and in mainland Spain.

Symptoms: curling, yellowing, abnormal proliferation and purple discoloration of leaves, stunted growth of shoots and roots, proliferation of secondary roots and filiform root a the end.

Liberibacter solanacearum (LIBEPS)

Close



Legend

- Present (national record)
- Present (subnational record)
- Transient

Diseases caused (associated) by '*Ca*. Liberibacter solanacearum' (2)

3) Yellows decline (Carrot)

Haplotypes C, D and E

- Detected in Finland (2010), Spain (2012), France (2014) and Morocco (2014) and should be present where carrots are grown.
- Vectored by *Trioza apicalis* (Findland) and *B. trigonica* and probably by *B. tremblayi* and *B. nigricornis* (Spain).

4) Vegetative Disorders (Carrot, celery and parsnip)

Haplotypes C, D and E

- **Detected in Spain (2014).**

- Vectored by *B. trigonica* and probably by *B. tremblayi* and *B. nigricornis* (Spain).

Symptomatology in celery crops in 2008

•Abnormal number of shoots •Curling of stems •Yellowing

Commercial production losses were up to 70%

Association of '*Candidatus* Liberibacter solanacearum' with a Vegetative Disorder of Celery in Spain and Development of a Real-Time PCR Method for Its Detection

Gabriela R. Teresani, Edson Bertolini, Ana Alfaro-Fernández, Carmen Martínez, Francisco André Ossamu Tanaka, Elliot W. Kitajima, Montserrat Roselló, Susana Sanjuán, Juan Carlos Ferrándiz, María M. López, Mariano Cambra, and María Isabel Font

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ABSTRACT

Teresani, G. R., Bertolini, E., Alfaro-Fernández, A., Martínez, C., Tanaka, F. A. O., Kitajima, E. W., Roselló, M., Sanjuán, S., Ferrándiz, J. C., López, M. M., Cambra, M., and Font, M. I. 2014. Association of '*Candidatus* Liberibacter solanacearum' with a vegetative disorder of celery in Spain and development of a real-time PCR method for its detection. Phytopathology 104:804-811.

A new symptomatology was observed in celery (*Apium graveolens*) in Villena, Spain in 2008. Symptomatology included an abnormal amount of shoots per plant and curled stems. These vegetative disorders were associated with '*Candidatus* Liberibacter solanacearum' and not with phytoplasmas. Samples from plant sap were immobilized on membranes based on the spot procedure and tested using a newly developed real-time polymerase chain reaction assay to detect '*Ca.* L. solanacearum'. Then, a test kit was developed and validated by intralaboratory assays with an accuracy of 100%. Bacterial-like cells with typical morphology of '*Ca.* Liberibacter' were observed using electron microscopy in celery plant tissues. A fifth haplotype of '*Ca.* L. solanacearum', named E, was identified in celery and in carrot after analyzing partial sequences of 16S and 50S ribosomal RNA genes. From our results, celery (family Apiaceae) can be listed as a new natural host of this emerging bacterium.

CaLsol Haplotypes detected in Spain (D and E)

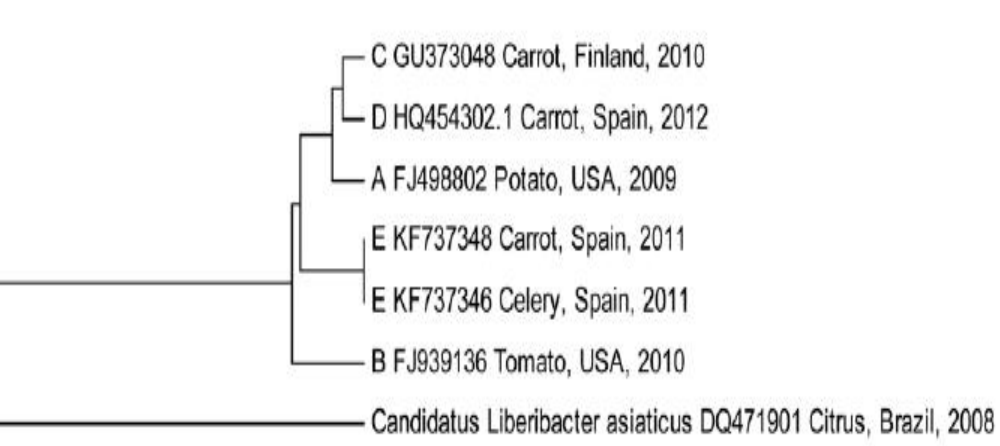

TABLE 4. Single-nucleotide polymorphism differences between haplotypes[a]

Gene region, description	Haplotypes A	B	C	D	E
16S rRNA					
116 C>T	C	C	C	T	C
151 A>G	A	A	A	A	G
212 T>G	T	C	T	T	T
581 T>C	T	C	T	T	T
959 C>T	C	C	C	C	T
1039 A>G	A	A	G	G	A
1073 G>A	G	G	G	A	G
ISR-23S					
1620 A>G	A	A	A	A	G
1632 G>A	A	A	A	A	G
1648 G>A	G	G	G	G	A
1742 A>G	A	A	A	G	A
1748 C>T	C	C	C	T	C
1858_1859insG	–	G	G	–	–
1859_1860insT	–	T	–	–	–
1860_1861delT	T	T	T	–	T
1873 A>G	A	A	A	A	G
1920 T>C	T	T	C	T	T
50S rRNA					
583 G>C	G	G	C	G	G
622 A>G	A	A	A	G	A
640 C>T	C	C	T	C	C
669 G>C	G	C	G	G	G
689 C>T	C	C	C	T	T
691 G>T	G	T	T	G	G
700 A>G	A	A	A	G	A
712 G>T	G	T	G	G	G
722 G>A	G	G	G	G	A
749 C>A	C	C	C	A	C
780_781insA	–	–	A	A	A
785 G>A	G	A	G	G	G
849 T>C	T	T	T	C	C
909 T>C	T	C	C	C	C
920 T>C	T	C	C	C	T
922_923insTGT	–	–	TGT	–	–
955 G>T	G	G	T	G	G
987 T>G	T	G	G	G	G
993 A>G	A	A	G	A	A
1041 G>A	G	A	A	G	G
1049 A>G	A	G	A	A	A
1068 C>T	C	C	C	C	C
1107 G>A	G	A	G	G	G
1110_1111insC	–	–	C	–	–
1122 G>A	G	A	A	A	A
1143 G>A	G	A	G	G	G

[a] The reference sequence for the 16S and 23S ribosomal RNA (rRNA) genes is EU 812559.1 and for 50S rRNA gene is EU 834131.1. Nucleotide numbers count from the beginning of the reference sequence (haplotypes A, B, C, and D), as previously described by Nelson et al. (30,31).

‘*Candidatus* Liberibacter solanacearum’ in carrot, celery and parsnip (associated to yellows decline, psyllid yellows and vegetative disorders).

High inoculum (prevalence) in *Apiaceae* due to carrot seed transmission.

The disease is naturally transmitted by *B. trigonica* (proved vector) and very probably by *B. tremblayi, B. nigricornis* and *Bactericera sp.*

Development of a real-time PCR protocol and validation by a Performance study of a complete kit for direct detection of '*Ca.* Liberibacter solanacearum' (without DNA purification)

Phytopathology 104, 804-811. (2014)

e-Xtra*

Association of '*Candidatus* Liberibacter solanacearum' with a Vegetative Disorder of Celery in Spain and Development of a Real-Time PCR Method for Its Detection

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CaLsol detection

PLANT MATERIAL (leaves or seeds)

NA PURIFICATION
(CTAB, Qiagen, MOBIO…)

DIRECT SAMPLE PREPARATION METHODS
(No RNA/DNA extraction)

USING CRUDE EXTRACTS

DILUTION

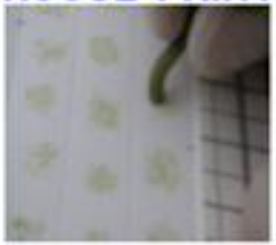
5 µl diluted in extraction buffer

SPOT

5 µl loaded on membrane

WITHOUT CRUDE EXTRACTS

TISSUE-PRINT



Several tissue sections printed in membrane

SQUASH

Aphid squashed on membrane

100 µl of **Water**
or
Glycine buffer

RELEASE OF TARGETS

REAL-TIME PCR

Performance study (Ring-Test)

- **27 Laboratories / 17 countries:** Austria, Argentina, Bolivia, Brazil, Costa Rica, France, Greece (2), Hungary, Israel, Italy, New Zealand, The Netherlands, Spain (9), Turkey, UK, Uruguay and USA (2)

- **10 blind samples** (5 positives and 5 negatives) immobilized on paper one month before the performance study and stored at room temp.

- **Total 53 results / 530 data** (in 4 laboratories no amplification occurred and some laboratories make repetitions).

- **A complete kit** sent by courier at room temperature in November 2012 (travelling days at room temperature: 3-7).

PlantPrint Diagnòstics, SL.

COMPLETE KIT® FOR "*Candidatus* Liberibacter" spp. ASSOCIATED TO CITRUS HLB DISEASE. RAPID SCREENING TEST BY REAL-TIME PCR

Kit CaLspp/100 components:

1) 2 vials (white cap) containing 50 pieces (0.2 cm²/piece) of Whatman 3MM paper.
2) 1 package containing 2 paper membranes (7x13cm) (Whatman 3MM).
3) 1 vial (blue cap) containing 1mL distilled sterile water DN/RNases free for master mix preparation.
4) 1 tube x 12 mL of distilled water DN/RNases free for sample preparation.
5) 2 vials (yellow cap) containing lyophilized complete master mix (universal primers and TaqMan FAM/TAMRA probe) for "*Ca.* Liberibacter" spp. amplification (each tube contains enough master mix for 50 reactions using 9 µL/reaction).
6) 2 vials (red cap) containing 1 immobilized positive control spotted on a piece of membrane (5 µL per spot of "*Ca.* Liberibacter asiaticus" infected plant crude extract).
7) 2 vials (green cap) containing 1 immobilized negative control spotted on a piece of membrane (5 µL per spot of healthy plant crude extract).
8) [illegible] protocol.

® Complete kit. Spanish-EU Patent N° 2.377.690 (201001157/08-09-2010). IVIA-FUNDECITRUS.
E. Bertolini, M. Cambra, P. Serra, M.M. López, N. Duran-Vila, J. Ayres and J.M. Bové. Direct procedure for specific detection of "Ca. Liberibacter" spp. by immobilized targets and real-time PCR [illegible] kit for its detection.

Calculation of diagnostic parameters

	Disease	No disease	
Positive	232 (TP)	2 (FP)	234
Negative	33 (FN)	263 (TN)	296
	265	265	530

- **Sensitivity:** 0.87
- **Specificity:** 0.99
- **Accuracy:** 93 %

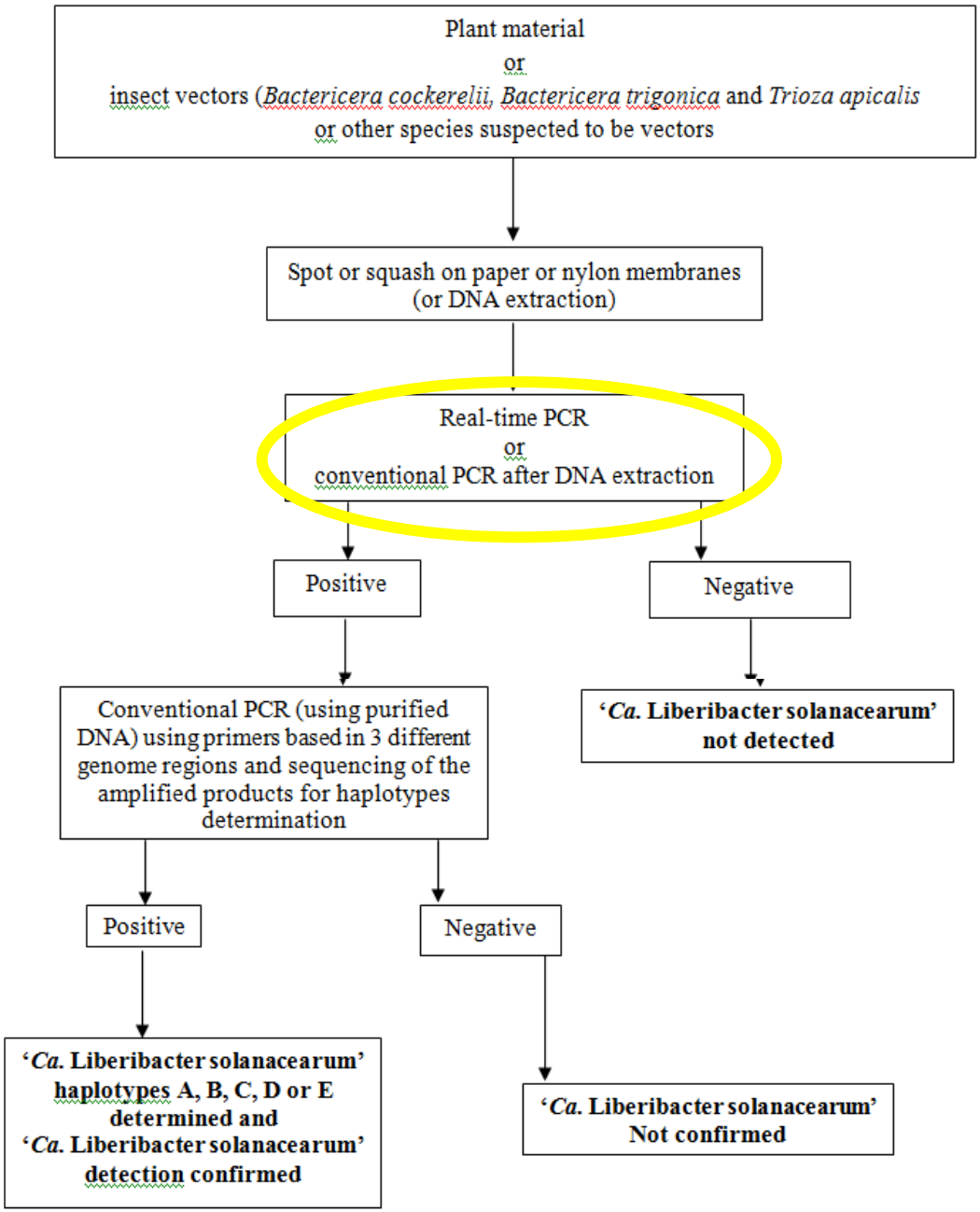
(TP+TN / N)

EPPO/OEPP (2014)

Flow diagram for detection of '*Ca.* L. solanacearum' spp.

(In press)

Fig. 1: Flow diagram for the detection of '*Ca.* Liberibacter solanacearum' in plant material.



Risk assessment:

- CaLsol has probably been introduced by carrot seeds in Spain and transmitted by psyllid vectors among carrots and from carrot to celery and parsnip. The bacterium should be present in all areas growing carrot.

- There is a real risk of natural transmission to solanaceous hosts (potato, tomato, aubergine and pepper) from infected carrot.

- We are studying the ability of different psyllid species in feeding in the phloem of different psyllid plant hosts and no hosts (monitored by Electrical Penetration Graph-EPG).

- Experimentally *Bactericera trigonica* is able to transmit CaLsol from carrot to carrot and celery (high efficiency) and from carrot to potato and tomato (low efficiency, but with detected infection in plants), showing the risk of natural transmission from carrot to solanaceous crops. In fact CaLsol was detected in symptomatic potato tubers.

DETECCIÓN EN PATATA EN ESPAÑA DE UN HAPLOTIPO DE *'Candidatus* Liberibacter solanacearum' NO DESCRITO EN SOLANÁCEAS

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INTRODUCCIÓN

La enfermedad conocida como "zebra chip" de la patata produce bandas oscuras en el interior del tubérculo y causa importantes pérdidas económicas. Está asociada a los haplotipos A y B de *'Candidatus* Liberibacter solanacearum', descritos en América (EEUU, Méjico y Centroamérica) y Nueva Zelanda. Estos haplotipos, no detectados en Europa, han sido incluidos en la lista A1 de la OEPP/EPPO (OEPP/EPPO 2013).

En Europa se han descrito nuevos haplotipos (C, D y E) en zanahoria y apio, que están presentes en España (Teresani *et al.*, 2014). La bacteria se transmite eficazmente por semilla de zanahoria (Bertolini *et al.*, 2014) y de forma persistente circulativa por diferentes especies de psílidos (Munyaneza *et al.*, 2010; Alfaro-Fernández *et al.*, 2012).

El objetivo de este trabajo es evaluar el riesgo de transmisión natural de *'Ca.* L. solanacearum' a patata a partir de cultivos de zanahoria y apio infectados. Para ello se analizaron 1.987 lotes de patata de siembra y consumo de diferentes orígenes geográficos.

MATERIAL Y MÉTODOS

- Detección Fase A: 215 lotes de 200 tubérculos; análisis de extractos congelados procedentes de prospecciones oficiales; PCR en tiempo real directo (kit CaLsol/100, Plant Print Diagnostics SL) basado en Teresani *et al.* (2014).
- Detección Fase B: 1.772 lotes de 200 tubérculos; inspección visual de sección perpendicular en ombligo; selección de tubérculos con síntomas; extracción ADN de tejido sintomático (Murray y Thompson, 1980); PCR en tiempo real (kit anterior).
- Determinación de haplotipo según Nelson *et al.* (2011). Se ha amplificado (PCR convencional) DNA de *'Ca.* Liberibacter solanacearum' en tres regiones distintas (16S DNA, 16S/23S ISR y 50S DNA). Las secuencias obtenidas han sido analizadas y comparadas con las secuencias de haplotipos descritos utilizando el programa Geneious. Los polimorfismos de un solo nucleótido (SNPs) fueron visualmente identificados y anotados. Se construyó un cladograma con el programa MEGA.

RESULTADOS Y DISCUSIÓN

- Detección Fase A: los análisis de extractos dieron resultado negativo en los 215 lotes. - Detección Fase B: en la inspección visual de tubérculos de 1.772 lotes se seleccionaron 12 lotes con síntomas sospechosos de "zebra chip" (Figs. 1, 2 y 3), con una prevalencia siempre inferior al 1,5% de los tubérculos/lote sintomático. En los análisis mediante PCR en tiempo real, se obtuvo resultado positivo en 9 lotes (cvs: Fontane, Arran Banner, Kennebec (4) y Red Pontiac (3), procedentes de las provincias de Ávila (1), Palencia (1) y Burgos (7) (puntos rojos en Fig. 4).

Figuras 1, 2 y 3 – Síntomas de "zebra chip" en los tubérculos analizados (Arran Banner (1) y Kennebec (2 y 3))

- Haplotipo: se identificó el haplotipo E, descrito en zanahoria y apio. En la Tabla 1 se muestran los SNPs del haplotipo E comparado con los haplotipos A, B, C y D. En el cladograma de la Fig. 5 se observa la divergencia de los distintos haplotipos.

- Al sembrar tubérculos sintomáticos no se produjo brotación o dieron lugar a plantas débiles con anomalías vegetativas que produjeron escasos tubérculos sintomáticos de pequeño tamaño (Figs. 6 y 7).

- Los resultados indican que el haplotipo E de apiáceas ha infectado naturalmente a patata, sugiriendo que otros haplotipos (no de solanáceas), pueden ocasionalmente infectar patata. Hasta el momento el hallazgo no ha tenido repercusión epidemiológica ni económica, pero supone una amenaza si no se controla la prevalencia de la bacteria en apiáceas que pueden servir de donantes para vectores ya presentes.

Figura 4 – Distribución de los casos detectados en Castilla y León

Tabla 1 Polimorfismo de nucleótidos de los distintos haplotipos de '*Ca.* Liberibacter solanacearum'

Gen	Descripción	Haplotipos				
		A	B	C	D	E
16S rRNA	116 C>T	C	C	C	T	C
	151 A>G	A	A	A	A	G
	212 T>G	T	C	T	T	T
	581 T>C	T	C	T	T	T
	959 C>T	C	C	C	C	T
	1039 A>G	A	A	G	G	A
	1073 G>A	G	G	G	A	G
ISR-23S	1620 A>G	A	A	A	A	G
	1632 G>A	A	A	A	A	G
	1648 G>A	G	G	G	G	A
	1742 A>G	A	A	A	G	A
	1748 C>T	C	C	C	T	C
	1858_1859insG	-	G	G	-	-
	1859_1860insT	-	T	-	-	-
	1860_1861delT	T	T	T	-	T
	1873 A>G	A	A	A	A	G
	1920 T>C	T	T	C	T	T
50S rRNA	583 G>C	G	G	C	G	G
	622 A>G	A	A	A	G	A
	640 C>T	C	C	T	C	C
	669 G>C	G	C	G	G	G
	689 C>T	C	C	C	T	T
	691 G>T	G	T	T	G	G
	700 A>G	A	A	A	G	A
	712 G>T	G	T	G	G	G
	722 G>A	G	G	G	G	A
	749 C>A	C	C	C	A	C
	780_781insA	-	-	A	A	A
	785 G>A	G	A	G	G	G
	849 T>C	T	T	T	C	C
	909 T>C	T	C	C	C	C
	920 T>C	T	C	C	C	T
	922_923insTGT	-	-	TGT	-	-
	955 G>T	G	G	T	G	G
	987 T>G	T	G	G	G	G
	993 A>G	A	A	G	A	A
	1041 G>A	G	A	A	G	G
	1049 A>G	A	G	A	A	A
	1107 G>A	G	A	G	G	G
	1110_1111insC	-	-	C	-	-
	1122 G>A	G	A	A	A	A
	1143 G>A	G	A	G	G	G

Figura 5– Cladograma de secuencias del gen 16S de los haplotipos de '*Ca.* Liberibacter solanacearum'

Figura 6– Falta de brotación y anomalías vegetativas al sembrar tubérculos afectados (bandeja izquierda), comparados con sanos (bandeja derecha).

Figura 7– Plantas muy débiles y filiformes, ausencia de tuberización o tubérculos sintomáticos de pequeño tamaño.

REFERENCIAS

-Alfaro-Fernández et al. (2012), Plant Dis. 96:581

-Munyaneza *et al.* (2010), J. Econ. Entomol. 103:1060-1070

-Nelson *et al.*, (2011), Eur. J. Plant Pathol. 130:5–12

- OEPP/EPPO Bulletin 43 (2), 197–201

- Bertolini *et al.* (2014), Doi: 10.1111/ppa.12245

- Murray HG, Thompson WF (1980), Nucleic Acids Res 8:4321-4325

- Nelson *et al.* (2012), Eur J Plant Pathol. 135:633-639

- Teresani *et al.* (2014). Phytopathology 104:8, 804-811

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Gibraltar (U.K.)
Algiers
Oran

Castilla y Leon
Leon
Palencia
Burgos
Zamora
Valladolid
Soria
Segovia
Salamanca
Avila

Analysis (2013-2014) of potato seed lots (200 navel tubers/lot) according to EU protocol for testing quarantine bacteria. (Tested by Plant Print Diagnòstics CaLsol/100 kit)

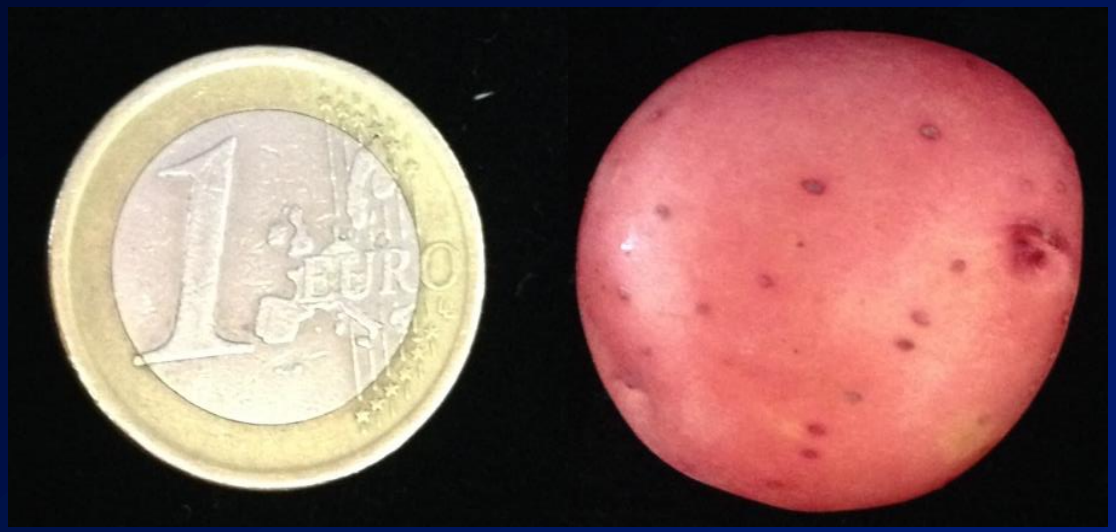
Phase A: 215 frozen extracts = negative real-time PCR.

Phase B: 1,772 visually inspected potato lots (section in the navel area) = 12 lots showed symptomatic tubers.
9 tested positive against CaLsol using real-time PCR.

The symptomatic tubers in positive potato lots (1,5%) were grown in pots. The plants than emerged from positive tubers were weak and phyliphorm. When tubers were produced (in few plants) they were symptomatic and small.

Currently the disease is not epidemic and it was not observed in the field (only sporadically in tubers). The natural spread is associated to a vector with poor efficiency to transmit haplotype E to potato (may be *B. trigonica*).

Potato cvs found positive : Fontane (1), Arran Banner (1), Kennebec (4) y Red Pontiac (3)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Mitigation strategies (I):

1) In order to prevent the introduction of the bacterium in new areas, to reduce the inoculum in carrot fields and to mitigate the spread to other potential hosts: control of the bacterium in seed lots (that are sold in Europe and worldwide) is required.

Therefore, investigations are required into carrot seed production in pest-free areas or under insect-proof facilities, to ensure seed is free from '*Ca*. L. solanacearum'. In addition, research is needed into seed treatments that could inactivate the bacterium in infected seed.

2) Strategies are required to reduce the populations of psyllid vector species and the spread of '*Ca*. L. solanacearum' to other crops (i.e. horticultural mineral oils, kaolin…).

Mitigation strategies (II):

3) In Spain extensive areas (in cooperation with Agricola Villena Coop. V., the main producer of fresh carrots) were established in 2014 using carrot seed lots that tested negative for CaLsol by real-time PCR (Teresani et al., 2014). No prevalence of CaLsol was observed in the first cycle of cultivation (March to July). Only a prevalence of 1 to 2 % of the plants is detected in the second cycle of cultivation (May to December, prevalence estimated at the middle of September).

4) Already the Committee on Sanitary and Phytosanitary Measures of the World Trade Organization (G/SPS/N/AUS/345 August 21th, 2014) notified in Australia as an emergency measure: the mandatory analysis by real-time PCR of carrot seed lots (20,000 seeds/lot) and *in vitro* carrot and celery plants, before the entry to the country.

5) A modification of the quarantine status of CaLsol (including non-solanaceous haplotypes) is expected in the EU to protect socio-economically important solanaceous crops.

Thank you very much for your attention!