Final Meeting Project 266505 FP7-ERANET EUPHRESCO II

Epidemiology and diagnosis of potato phytoplasmas and "Candidatus Liberibacter solanacearum" and their contribution to risk management in potato and other crops (PHYLIB)

Edinburgh, 1-2 October, 2014

Experimental transmission of 'Ca. Liberibacter solanacearum' by Bactericera trigonica

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Experimental transmission of 'Ca. Liberibacter solanacearum' by *Bactericera trigonica*

Field surveys: Looking for potential vectors of 'Ca. Liberibacter solanacearum' in Spain

Transmission trials: evaluating the threat of B. trigonica to other crops

On going research

Field surveys
Looking for potential vectors of 'Ca. Liberibacter solanacearum'

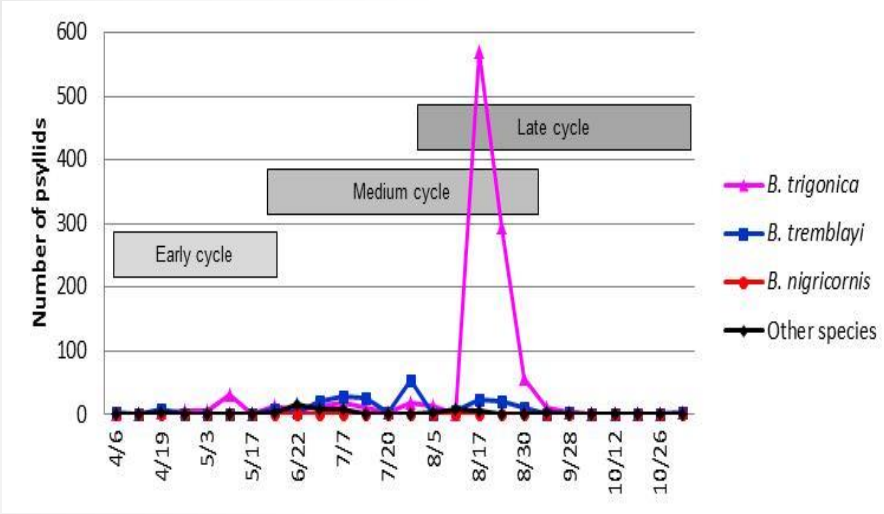
- 16 commercial plots
- Seasonal surveys in carrot and celery crops between 2011-2012
- Occasional surveys carrot & potato 2012-2014

- Estimating number of insects landing
- Identification of psyllid species visiting crops by sticky leaves method (MARROQUIN ET AL, 2014)

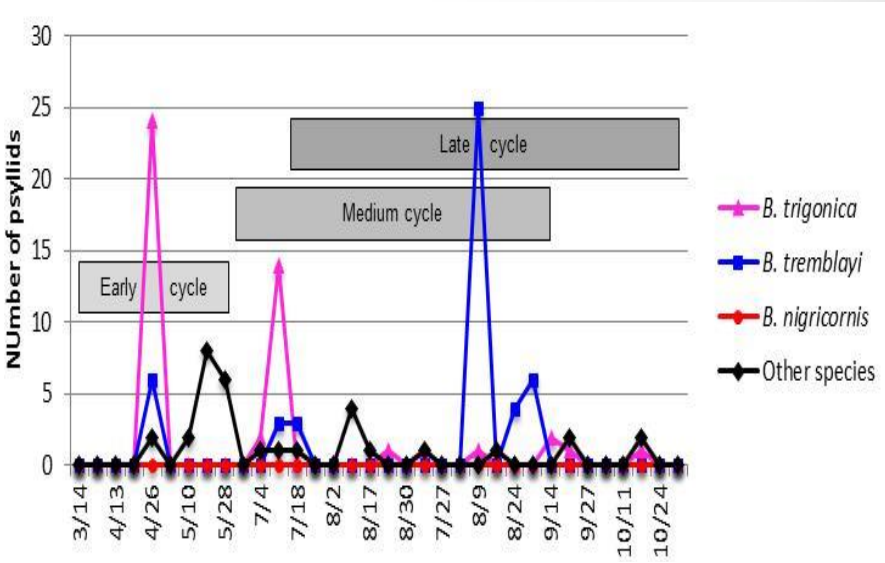
Detection of '*Ca*. L. solanacearum' targets in psyllid species real-time PCR Kit CaLsol/100 Plant Print Diagnostics (TERESANI ET AL., 2014)

Psyllid species captured in seasonal surveys

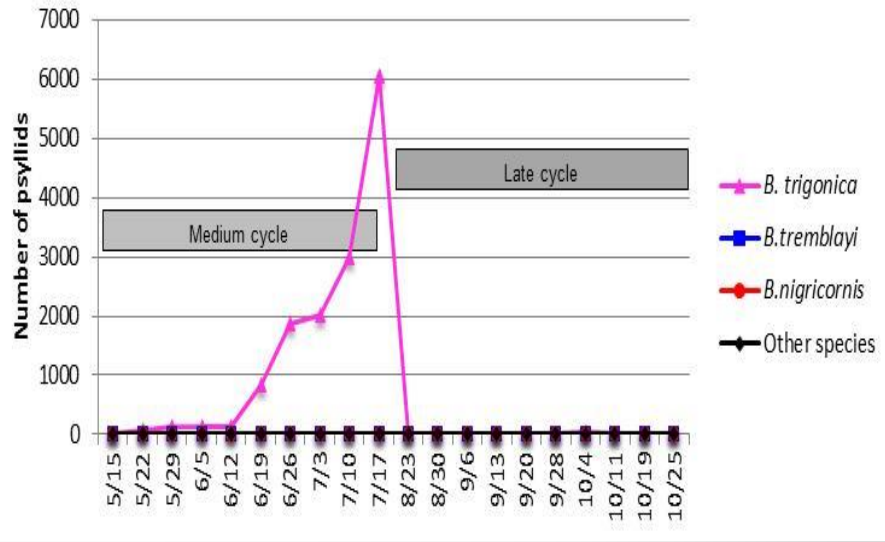
Celery, Villena, 2011



Celery, Villena, 2012



Carrot ,Tenerife, 2012



Other species: *T. urticae*, *Bactericera* sp., *Cacopsylla* sp., *Ctenarytaina* sp., *Psylla* sp. and *Trioza* sp.

Number of specimens carrying the bacterium

Crop	Location	Year	B. trigonica	B. tremblayi	B. nigricornis	Other [a]	Total
Celery	Villena	2011	43+/1085	13+/225	0+/2	0+/61	56+/1,373
		2012	1+/48	1+/46	0	0+/32	2+/126
Carrot	Tenerife	2012	50+/214	0+/0	0+/0	3+/27[b]	53+/241 (14,401)

'*Candidatus* Liberibacter solanacearum' positive psyllid specimens/total of analyzed psyllid specimens (total captured)

[b] The three positive specimens belong to *Bactericera* sp.

Psyllid species	Carrot			Potato	
	2012		2013	2012	2014
	La Rioja	Villena	La Rioja	Tenerife	Valencia
B. trigonica	0+/0[a]	0+/26	210+/476	35+/102	0+/7
B. tremblayi	24+/25	0+/2	33+/44	0+/0	0+/0
B. nigricornis	36+/38	0+/0	0+/0	0+/0	0+/2
Other	0+/0	0+/0	50+/70[b]	0+/17	0+/2
Total	60+/63	0+/28	293+/590	35+/119	0+/11

[b] Other means non-identified specimens, probably *B. trigonica,* they were damaged in key morphological characteristics caused during the process of recovering from the sticky plant.

Conclusions

- *B. trigonica, B. tremblayi* and *B. nigricornis* were found as current visitors of the surveyed crops in continental Spain.
- *B. trigonica* is the predominant species in the Canary Islands in agreement with a previous reports (Alfaro-Fernández *et al*., 2012a & Font *et al*., 1999).
- Ca. L.solanacearum detection in *B. trigonica*, *B. tremblayi*, *B. nigricornis* & *Bactericera* sp.

B. trigonica and *B. nigricornis* associated with most temperate climates

Switzerland, Hungary, Italy, Greece, Portugal ….
Norway, Sweden, Russia , Hungary, Northern Africa ….

B. tremblayi is associated with Mediterranean climate

France, Italy, Greece, Turkey …

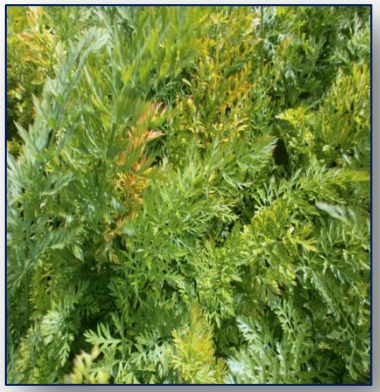
Transmission of CaLsol by Bactericera trigonica

1.- Electrical Penetration Graphs (EPG) studies

2.- Transmission studies under laboratory conditions

3.- Transmission studies under greenhouse conditions

4.- Reproduction in carrot, celery, potato and tomato



Transmission of CaLsol by Bactericera trigonica

Controlled environment room: 25±2ºC, 70±5% humidity, photoperiod of 16:8 (L:D)

Source of insects

Donor plants

Receptor plants

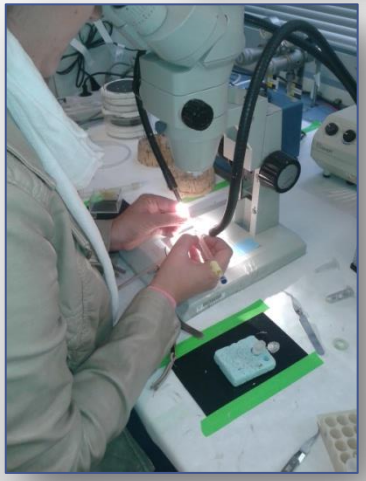
- potato (*Solanum tuberosum* L.) cv. Terrenta,
- tomato (*Solanum lycopersicum* L.) cv. Robin,
- carrot (*Daucus carota* L.) cv. Bangor
- celery (*Apium graveolens* L.) cv. Monterrey

Detection of 'Ca. L. solanacearum'

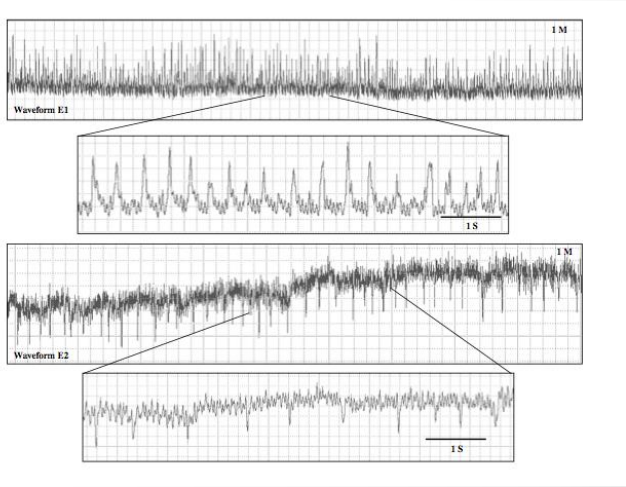
- Crude plant extract CTAB protocol (Murray & Thompson, 1980)
- Detection of '*Ca*. L. solanacearum' targets in psyllid species real-time PCR Kit CaLsol/100 Plant Print Diagnostics (Teresani et al., 2014)

Electrical Penetration Graphs (EPG) studies

DC-EPG device inside a Faraday cage



- 15 adult psyllids
- Each insect 8h recorded
- EPG data acquisition Probe 3.0 software for Windows
- Variables processed using Sarriá *et al*. (2009).



EPG waveforms

Feeding behavior of Bactericera trigonica

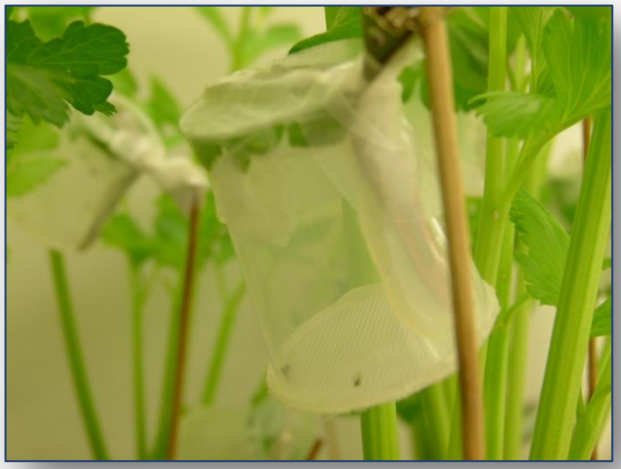
Non-sequential variables	Carrot	Celery	Potato	Tomato
	Mean ± SE			
Number of E1	2.80 ± 1.85 a	4.00 ± 3.76 a	0.13 ± 0.35 b	0.00 ± 0.00 b
Number of E2	1.87 ± 1.59 a	2.80 ± 3.32 a	0.00 ± 0.00 b	0.00 ± 0.00 b
Total duration of E	4389.00 ± 5010.17 a	3923.00 ± 6528.95 a	9.68 ± 26.58 b	0.00 ± 0.00 b
Total duration of E1	854.00 ± 1226.07 a	1127.00 ± 1477.59 a	9.68 ± 26.58 b	0.00 ± 0.00 b
Total duration of E2	3535.00 ± 4789.00 a	2797.00 ± 5901.00 a	0.00 ± 0.00 b	0.00 ±0.00 b
% of probing spent in E1	5.29 ± 7,70 a	6.35 ± 10.85 a	0.16 ± 0.47 b	0.00 ± 0.00 b
% of probing spent in E2	17.40 ± 21.20 a	11.12 ± 21.04 a	0.00 ± 0.00 b	0.00 ± 0.00 b
Number of probes	10.00 ± 4.70 a	9.13 ± 6.08 a	7.47 ± 4.29 a	5.86 ± 5.90 a

Values with different letters are significantly different ($P < 0.05$ using Mann–Whitney U test).

- E1 correlates with salivation into phloem sieve elements - inoculation
- E2 ingestion from phloem - acquisition

Transmission studies under laboratory conditions

Restricted leaf exposure trials.



- 10 adult psyllids/clip cage
- 4 IAP
- 10 replications/host plant

Whole plant exposure trials

- 10 adult psyllids/plant
- 14d IAP
- 10 replications/host plant

- 1 adult psyllids/plant
- 24 h or 14d IAP
- 30 replications/host plant

Transmission of ‘Ca. L. solanacearum’ by B. trigonica

Restricted leaf exposure trials.

IAP[a]	Carrot	Celery	Potato	Tomato
24hours	6+/10[c]	2+/10	1+/10	6+/10
3 days	10+/10	3+/10	1+/10	5+/10
7 days	10+/10	6+/10	2+/10	4+/10
Aprox. 14 days	7+/10	8+/10	3+/10	2+/10
1month	7+/10	10+/10	0+/10	0+/10

(CaLsol+/total)

Transmission of ‘Ca. L. solanacearum’ by B. trigonica

Whole plant exposure trials

	CaLsol[b]+/total (% of transmission)		
	1 B. trigonica		10 B. trigonica
	IAP[a] 24h	IAP aprox. 14d	IAP aprox. 14d
Carrot	59+/87[c] (67.8%)	46+/70 (65.7%)	8+/10 (80%)
Celery	19+/90 (21.1%)	15+/90 (16.6%)	10+/10 (100%)
Potato	On going	On going	1+/10 (10%)
Tomato	5+/83 (6.0%)	4+/59 (6.8%)	1+/10 (10%)

Conclusions

- *B. trigonica* is able to inoculate and acquire the CaLsol when feeding on carrot and celery plants.

- *B. trigonica* could infect potato and tomato if the insect previously has acquired the bacterium in another infected host (i.e. carrot or celery).

B. trigonica is predominant in carrot and celery crops in Spain and is frequently found in potato fields growing in the vicinity this situation could constitute a threat

Current studies

Transmission studies: cages

Greenhouse conditions

Reproduction in carrot, celery, potato and tomato

climatic chambers at 25°C, 70% humidity and a photoperiod of 14:10 h (D:L).

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