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'*Ca*. Liberibacter solanacearum': a carrot seedborne bacterium

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Development of a real-time PCR protocol for direct detection of '*Ca.* Liberibacter solanacearum'

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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PROTOCOL FOR CaLsol DETECTION IN SEEDS (coated or not)

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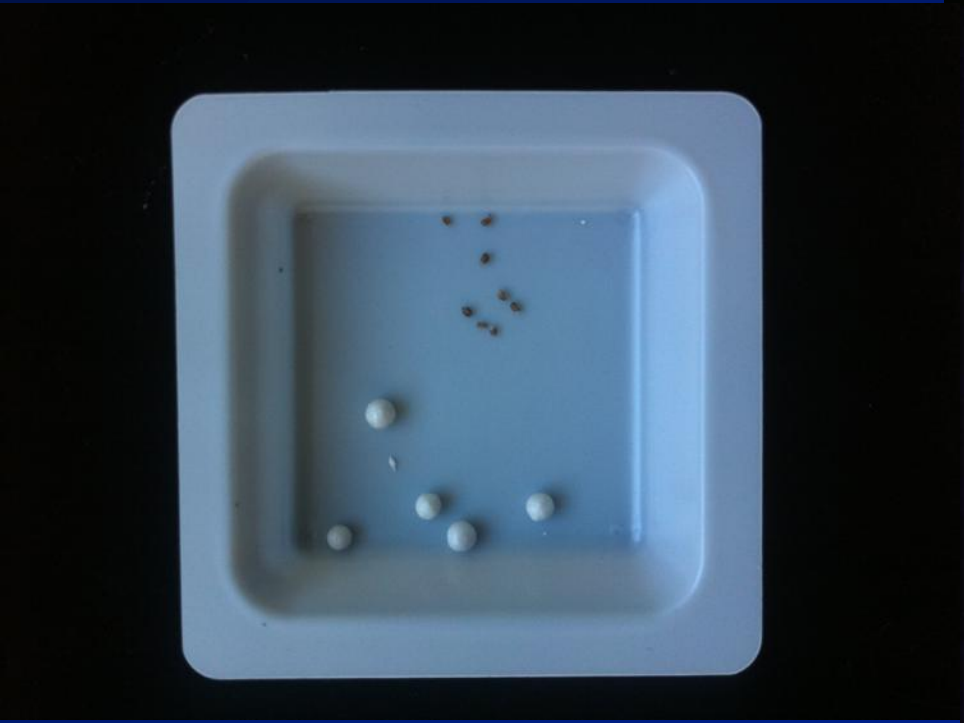


- 1 g (approx. 500 carrot seeds)

- comminuted in PBS, pH 7,2 into a plastic bag

- DNA purification with CTAB

- Real-time PCR

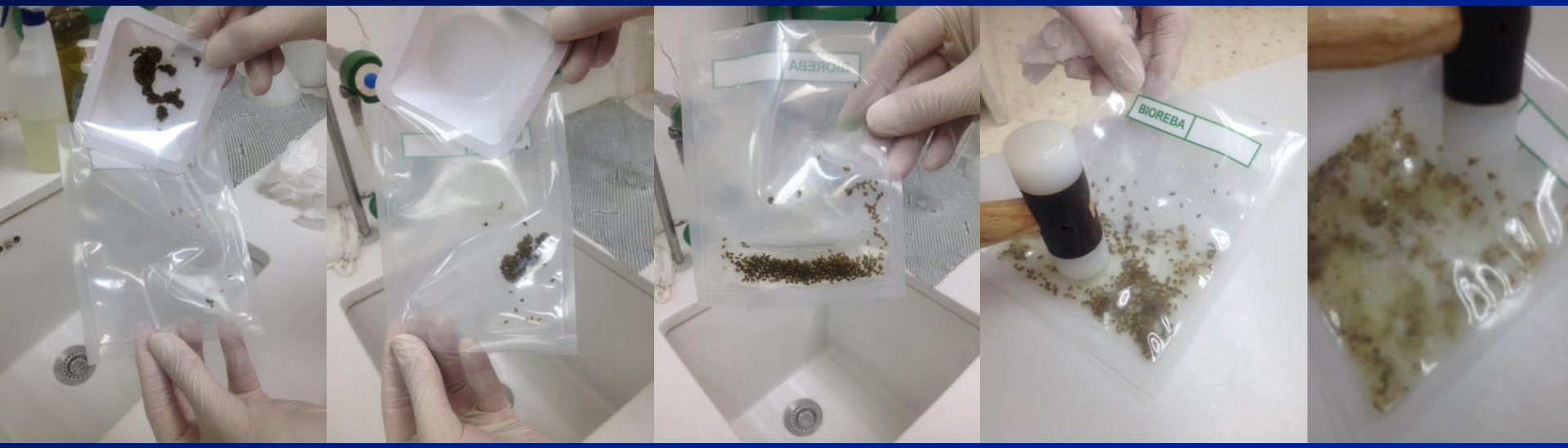
- 1 (individual) carrot seed

- Squashed into an Eppendorf tube in 50 µL of PBS, pH 7,2

- Spot 5 µL on Whatman 3MM paper

- Real-time PCR

Simple preparation of a seed extract with a sieve, water, buffer, plastic bag and a hammer



280 analysed commercial seed lots in 2013 + 2014 (using 1 g / seeds) by real-time PCR for '*Ca*. L. solanacearum'

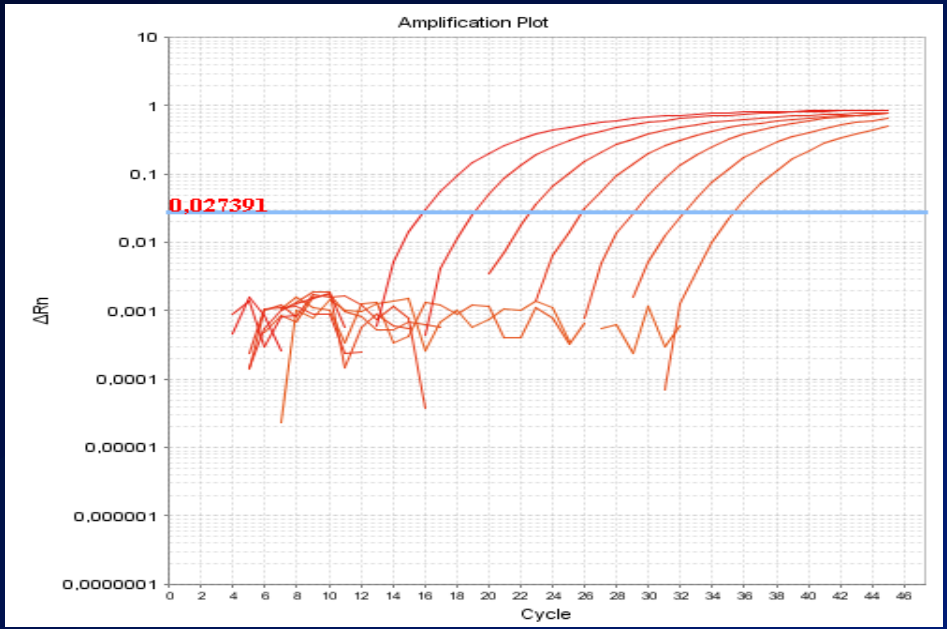
Botanical species	Analysed seed lots	Positive seed lots	% CaLsol detection
Carrot	60+94	27+24	33 %
Celery	6+17	0+0	0 %
Parsnip	29+40	14+18	46 %
Leek	10+24	0+0	0 %
TOTAL	280	83	-

(i.e. 27 out of 60 carrot seed lots tested positive in 2013 for CaLsol)

% CaLsol detection in individual carrot seeds from positive seed lots

Carrot cultivar	Positives/total tested	% CaLsol detection
Amsterdam	14/30	46%
Bangor	29/50	58%
Bolero	10/50	20%
CAC 3075	28/30	93%
Carboli	42/50	87%
Elegance	38/50	76%
Exelso	12/50	24%
Maestro 1	8/50	16%
Maestro 2	30/50	60%
Maestro 3	43/50	86%
Músico	48/50	96%
Newhall	25/50	50%
Soprano	29/50	58%
Yaya	21/50	42%

Quantification of total '*Ca.* L. solanacearum' cells in individual carrot seeds



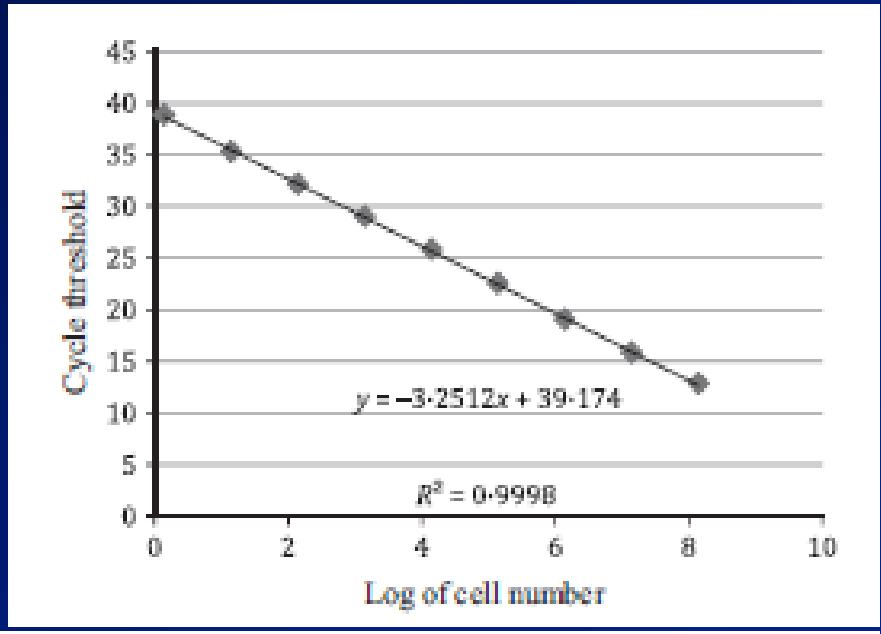


Table 2 Real-time PCR analysis and quantification of '*Candidatus* Liberibacter solanacearum' in individual seeds from three different carrot seed lots

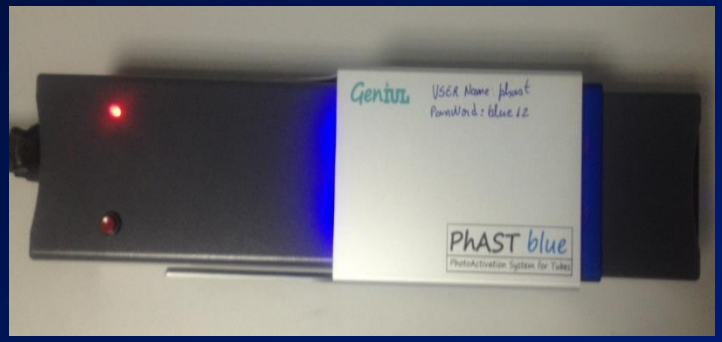
Seed lot code	No. of positive seeds/No. of analysed seeds	Estimated % of positive seeds	Log ('*Ca.* L. solanacearum' cells/carrot seed) range	Log average ± SD (estimate no. of cells per seed)
11/2012	14/30	47	0·04–3·70	1·68 ± 1·15 (48 ± 14·4)
12/2012	22/30	73	0·59–4·55	2·32 ± 0·82 (210 ± 6·7)
10/2012	28/30	93	0·02–1·73	0·69 ± 0·52 (4·8 ± 3·3)

Bertolini *et al*. (2014). Plant Pathology. DOI:10.1111/ppa.12245

Quantification of live (viable) '*Ca*. L. solanacearum' cells in 1 g bulk samples of carrot seed

(Bertolini *et al*. (2014) Plant Pathology. DOI:10.1111/ppa.12245)

Propidium monoazide and real-time PCR according to Teresani *et al*. (2014)



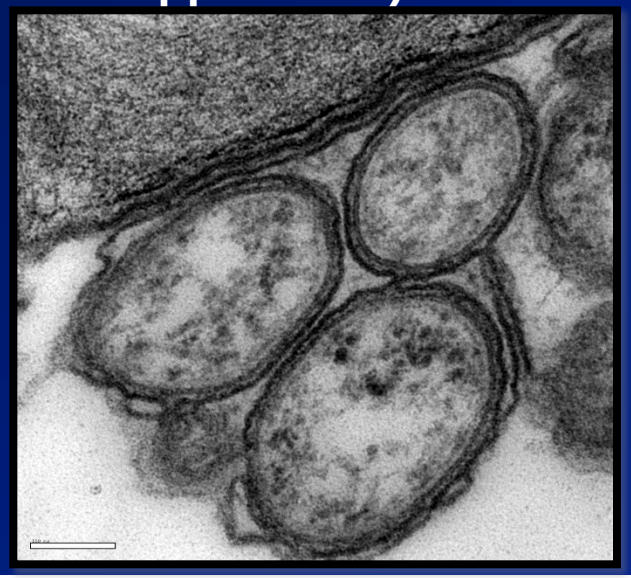


Table 3 Real-time PCR analysis and quantification of '*Candidatus* Liberibacter solanacearum' cells in carrot seeds with propidium monoazide (live cells) and without propidium monoazide treatment (dead and live cells)

Treatment	C_t range	Log ('*Ca*. L. solanacearum' cells/g of seeds) range	Log average ± SD (estimate no. of cells per g of seeds)[a]
With PMA	26·0–31·1	2·06–3·37	2·88 ± 0·32 (760 ± 2·1)
Without PMA	20·9–25·5	3·50–4·64	4·15 ± 0·28 (14 400 ± 1·9)

[a]Average log cells ± standard error from 20 repetitions. Treatments are significantly different ($P < 0·05$ using ANOVA).

Carrot seedlings from positive lots (cv Maestro, CAC 3075 and Amsterdam) were grown in a P2 insect-proof greenhouse. Complete plants or leaves were collected until **5 months** and tested by real-time PCR (Teresani *et al*., 2014)

After 90 days seedlings were transplanted to a 3 liters pot
(1 g of leaves analyzed)

120 days

150 days

Assessment of transmission of ‘Ca. L. solanacearum’ from seeds to seedlings

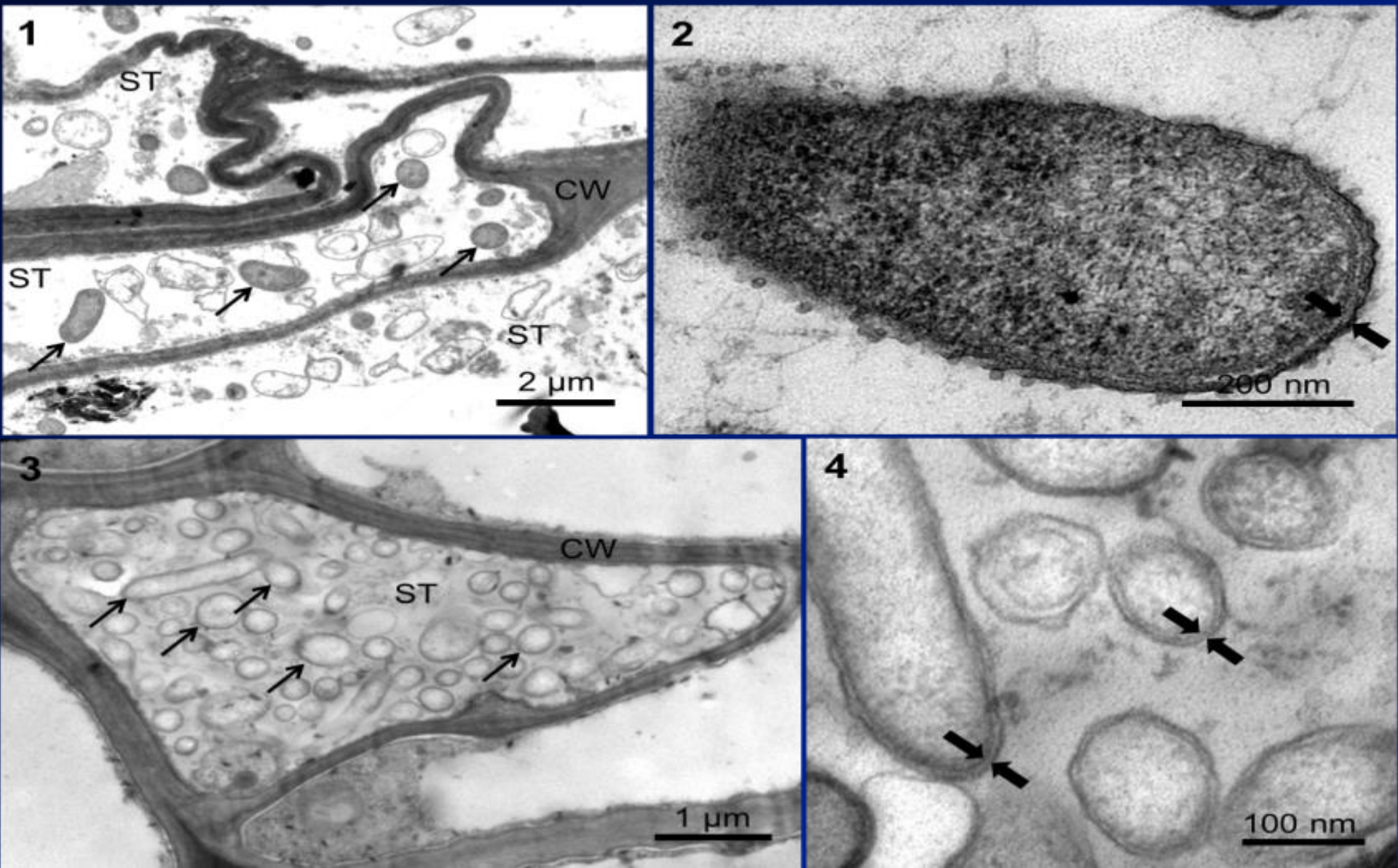
Table 4 Detection of ‘*Candidatus* Liberibacter solanacearum’ by real-time PCR in carrot seedlings grown from positive seed lots in a P2 level containment greenhouse

			Seedlings					
			Days post-germination					
Seed lot code	Cultivar	Seeds	30	60	90	120	150	Total
10/2012	CAC 3075	9/10	0/100	0/100	6/78	7/52	13/49	26/379
11/2012	Amsterdam	5/10	1/100	0/100	5/100	0/95	15/100	21/495
12/2012	CAC 3075	9/10	0/100	0/100	0/63	5/100	42/100	47/463
30/2012	Maestro	16/100	0/100	2/100	5/100	5/100	12/100[a]	24/500
27/2011	Maestro	0/100	0/200	0/200	0/200	0/200	0/200	0/1000

[a]Symptoms observed.

Bertolini *et al*. (2014) Plant Pathology. DOI:10.1111/ppa.12245

Transmission electron microscopy: Carrot seeds (1, 2) and seedlings (3, 4)



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

Thank you very much for
your attention!