REPORT
EUPHRESCO PHYLIB End of Project Meeting
1-2nd October 2014 at SASA, Edinburgh, UK

Presentations

1. EU PHYLIB project. Welcome to Scotland. John Speirs, Scottish Government (policy), UK.
2. An introduction to SASA. Kevin O'Donnell, Scottish Government (SASA), UK.
3. Occurrence of 'Ca. Liberibacter solanacearum' in Finland; what do we know so far. Anne Nissinen MTT, Agrifood Research, Finland. Some details e.g. on epidemiology, withheld prior to publication
4. Genome sequencing of 'Ca. Liberibacter solanacearum infecting carrots in Finland'. Minna Pirrhonen, University of Helsinki.
5. Transmission tests of 'Ca Liberibacter solanacearum' by carrot seeds. Marianne Loiseau, ANSES, Lab. de la Santé des Vegetaux, Angers, France.
6. Current situation of '*Ca*. Liberibacter solanacearum' in Spain. Mariano Cambra, IVIA, Valencia, Spain.
7. *Ca*. Liberibacter solanacearum': a carrot seedborne bacterium. Gabi Teresani, IVIA, Valencia, Spain.
8. Experimental transmission of '*Ca*. Liberibacter solanacearum' by *Bactericera trigonica.* Estrella Hernández, ICIA, Tenerife, Spain.
9. Overview. Activities of NVWA-NRC, NL Molecular test methods for 'Ca. Liberibacter solanacearum'. N. Tjou Tam-Sin (Leon), NPPO, The Netherlands.
10. Liberibacter research in Belgium, an update. Kris De Jonghe, Institute for Agricultural and Fisheries Research, Plant Science Unit-ILVO, Belgium.
11. Psyllid survey in GB carrot and potato crops and EPG feeding behaviour study. Larisa Collins, Fera, UK.
12. The survey for '*Ca*. Liberibacter solanacearum' and psyllids in Scotland. Fiona Highet, Scottish Government (SASA), UK.
13. 'Ca. Liberibacter solanacearum detection in potato microplants. (Wendy Monger, Scottish Government (SASA), UK.
14. Research progress on potato zebra chip disease in Canada. (Sean) Xiang Li, CFIA, Canada. Presented by Colin Jeffries.
15. Phytoplasma Survey Results of ZMMAE. Aynur Karahan, Plant Protection Central Research Institute, Turkey.
16. Microarrays for discrimination of phytoplasmas. Proof of principle. Catherine Harrison, Fera, UK.
17. Reliability of testing potato microplants for infection with phytoplasmas. Monika Krol, Scottish Government (SASA), UK.
18. Proficiency test results, for the testing of potato infecting phytoplasmas. Carolyn Nisbet, Scottish Government (SASA), UK.

19. Potato Phytoplasma research in Belgium, an update. Kris De Jonghe, Institute for Agricultural and Fisheries Research, Plant Science Unit-ILVO, Belgium
20. Contribution to the PHYLIB project. Activities in Hungary in 2012-2013-2014. Maria Kolber. Presented by Colin Jeffries
21. 'Ca Phytoplasma asteris'. First find in commercial carrot crop in the UK. Carolyn Nisbet, Scottish Government (SASA), UK
22. EUPHRESCO. Where to next. David Kenyon and Mariano Cambra, respectively Scottish Government (SASA), UK and IVIA, Valencia, Spain.

1. *Ca.* Liberibacter solanacearum (Lso) and its vectors

The talks were diverse and covered the bacterium, the vectors and the different crops. Some countries have a clear problem (Finland, France and Spain), the presence of known and probable psyllid vectors in these countries, *Trioza apicalis* (Finland) and *Bactericera trigonica* and probably *B. tremblayi* and *B. nigricornis* (Spain) have spread the disease from the infected carrot to more carrots and other crops e.g. celery, parsnip, potato.

Belgium (and Morocco)

Due to financial constraints only a limited survey has been done of potato crops for Lso (2012, 2013, 2014). Lso was not found. Research station staff and extension workers were trained in the identification of Lso symptoms in carrots. Lso has not been found but '*Candidatus* Phytoplasma asteris (16SrI-C) was detected (see this section Netherlands and section on phytoplasmas, UK). Confirmation of Lso in carrots in Morocco was obtained, with haplotypes D and E present (Tahzima *et al* 2014).

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Canada

Zebra chip in potato is not present in Canada, nor as other haplotypes of Lso. However, a commercial tomato seedling imported into Canada has tested positive for Lso and destroyed. Under the containment conditions, grafted tomato plants harbour 'Ca. Liberibacter solanancearum', but demonstrated little symptoms. When latently infected tomato plant was grafted to potato, symptoms appeared after 3-7 weeks in all 10 potato cvs tested. When infected tomato was grafted to healthy tomato the two cvs used showed a high tolerance to Lso although symptoms appeared in some plants 4 weeks after grafting. All parts of the infected tomato plant including flowers and fruit (peduncle, pericarp, style end, columella placena) tested positive. However, plants grown from the seeds tested negative, suggesting transmission from plant to seed is at very low levels as has been shown with other plant species. In tissue culture, infected tomato plants appear healthy. Lyophilised tomato tissue from stems, petioles and midribs were better than leaf blade tissue for long term storage of Lso as a positive control. Vascular tissue stored at room temperature tested positive after 4 years, whereas leaf blade tissue remained positive for only 6 months.

Finland

The carrot psyllid (*T. apicalis*) is a serious pest of carrots in Finland, and it also acts as a vector of Lso. The typical leaf curling symptom developing in carrots already two days after psyllid feeding was found to be caused by the psyllid and not by Lso infection. The leaf curling symptom is considered as a gall-forming reaction, and a previous study showed that the overwintered female psyllids caused the strongest symptoms. In contrast, the yellow, red or purple discolouration of the leaves was associated with Lso infection, and it appeared late, over a month after inoculation. Both

the psyllid feeding and the Lso infection resulted in a substantial decrease in the carrot root weight. Electron microscopy performed two months after inoculation revealed that the phloem sieve tubes of the infected carrot plants were systemically colonized by rod-shaped bacteria, from the leaf veins to the root tip (Nissinen et al. 2014).

To assess the occurrence of Lso in Southwestern Finland, plant and insect samples were collected from 33 fields in total, including both carrot and potato fields, in 2011, 2012 and 2013. Results on the epidemiology of Lso are withheld prior to publication.

Whole genome sequencing of Lso haplotype C is carried out to determine how similar this carrot disease-associated haplotype is in comparison with the Lso haplotype B, associated with potato "zebra chip" disease. A high degree of genetic similarity between these haplotypes could predict a potential risk of Lso epidemic spreading to potato crop in Finland. Genetic differences, on the other hand, could indicate that these two haplotypes have adapted to different hosts, reducing the risk of a new epidemic. The draft genome assembly of the haplotype C shows that while most of the genome regions are highly similar to the haplotype B, there are also many differences, in both the genome organization and in individual genes. The Lso genome also contains two large prophage regions, which are more variable than the core genome sequence, and it is possible that these regions contain some genes that determine the transmission and/or virulence of the bacteria in different hosts.

France

Seed lots collected from 3 carrot infected plots (Ct values 20-23) were infected with Lso at high levels (Cts 23- 26). No transmission from seed to seedling was shown during the 9 months of this experiment (only one Lso positive sample was detected and this was not reproducible). Seed from these seed lots were also tested by Spain who found 4-10% positive seedlings after 5 months (see section on Spain). When cotyledon, root tissue and coat tissue, from individual seeds was tested, Lso was only found in coat tissue. This has also been found by Spain.

Netherlands

Tomato (glasshouse grown) and carrots (field grown) were surveyed for visual symptoms of disease and the vectors *T. apicalis, B. cockerelli, B. trigonica, B. nigricornis, B. tremblayi.* No potato or carrot psyllids have been found so far. Suspect symptoms were seen in carrot but was found to be caused by '*Candidatus* Phytoplasma asteris'(see this section Belgium and section on phytoplasmas, UK).

Real time PCR platforms (Applied Biosystems (7900HT, 7500), Bio-Rad (CFX96) and Roche (LC480 & LC96)), extraction method and available molecular tests (including chemistry), were evaluated. Reproducibility was better with the Applied Biosystems and Bio-Rad machines than the Roche machines.

Methods for the detection of Lso were chosen from the literature for evaluation based on analytical performance criteria (sensitivity, specificity, selectivity) These were: two conventional PCRs, Ravindran *et al* 2011 (targets adk and ITS); semi-nested (SYBR) real-time from Beard *et al* 2011 (16S rDNA); and the real time assay of Li *et al* 2012 (16S rDNA). Both the Ravindran *et al* 2011 assays were sensitive (although less sensitive than the Li *et al* 2009 real-time assay) and specific. Sequence analysis of the ITS region proved effective for confirmation of Lso in presumptive infected samples from initial screening with the Li *et al* 2009 real-time assay.

Of the real-time PCR assays, the Li *et al* 2009 real-time assay using TaqMan Universal Master Mix (Life Technologies, catalog no. 4318157) was the most sensitive and specific (no false positives or negatives) when testing plant samples (carrot, potato and tomato). Typical exponential curves and the expected qualitative results were obtained. No results were obtained (only background noise could be seen) using Premix Ex Taq (Perfect Real-time) from TaKaRa.

The DNeasy kit from Qiagen was effective for DNA extractions, however, some carrot root extracts required a 10x dilution before the PCR based assay would work, indicating that they contain PCR inhibitors. The use of an automated QuickPick Plant DNA kit (Bionobile) with additionally PVPP purification has proven itself fit for purpose. Expected qualitative results were obtained without indications of PCR inhibitors**.**

Based on the work done, the Crude plant sap extraction (Munyaneza 2010; NVWA optimized), "QuickPick" DNA extraction plnt kit + PVPP prurification", the Bio-Rad (CFX96) platform, and TaqMan Universal Master Mix (Life Technologies, catalog no. 4318157) was selected for use in the NPPO-Netherlands plant diagnostic laboratory.

In the NPPO quarantine glasshouses a Lso collection in tomato and potato plants is maintained for research and reference purposes. Supplementary Lso infected plant material from carrot was made available by Spain (IVIA).

Spain

A real time assay for detection of Lso was developed without DNA purification, and a complete kit evaluated in a ring test involving 27 laboratories. Sensitivity was 0.87, Specificity 0.99 and accuracy 93%.

Lso has been detected in *B. trigonica, B. tremblayi* and *B. nigricornis*. *B. trigonica* and *B. nigricornis* are associated with temperate climates and *B. tremblayi* with Mediterranean climates in Europe. All 3 are found in surveyed crops in continental Spain with *B. trigonica* predominant in carrot and celery in Spain and frequently found in potato fields growing in the vicinity. *B. trigonica* is also the predominant species in the Canary Islands. Electrical penetration graph (EPG) studies showed that showed that *B. trigonica* was able to reach the phloem sieve elements and remain in the phloem when fed on carrot and celery considerably longer than when fed on potato and tomato Also it was unable to ingest from the phloem of potato and tomato. Transmission studies under laboratory conditions showed that *B. trigonica* was able to transmit Lso from carrot to carrot, celery and parsnip (with high efficiency) and from carrot to potato and tomato (low efficiency). Higher numbers of psyllids (from 1 to 10) increased the rate of transmission. Current studies involve the reproduction rates on carrot, celery, potato and tomato.

The symptoms seen in celery are an abnormal number of shoots, curling of stems and yellowing. Symptoms were first seen in Spain in 2008; commercial production losses were up to 70%. The haplotypes found in Spain were D and E. It is believed that Lso was introduced by carrot seeds to Spain and transmitted by psyllid vectors to other carrots and then from carrots to celery and parsnip.

In potato the disease has not yet been observed in the field in growing plants, but has been seen sporadically in tubers. From 2013-2014, 1,772 potato lots were visually inspected; 12 of these were found with symptomatic tubers and 9 of these (cvs Fontane, Arran Banner, Kennebec and Red Pontiac) tested positive for Lso (haplotype E). Transmission to potato was probably by *B. trigonica*, with poor efficiency.

In 2013 and 2014 33% of commercial seed lots of carrot (154 lots) and 46% of parsnip (69 lots), tested Lso positive tested positive. Commercial seed lots of celery (23 lots) and leek (34 lots), tested Lso negative. There were big differences, in the level of Lso infection (16% to 96%) between carrot cultivars. Using propidium monoazide to target live cells, up to 95 % of the cells were found to be dead. Some carrot seed lots that tested Lso positive were germinated and plants tested at 30, 60, 90, 120 and 150 days post germination. Most seed lots did not test positive until 90 days and the number of positives were still increasing at 150 days post germination. Using transmission electron microscopy, '*Ca.* Liberibacter'-like cells were seen in the phloem of the seed coat individual carrot seeds, but the mechanism of transmission to seedlings is currently not known.

Spanish researchers are working with the main carrot producer in Spain Agricola Villena Coop.V, and the carrot seed suppliers to ensure that only carrot seed lots that tested negative for Lso are planted. Observed symptoms in carrots were greatly reduced in 2014.

UK

Trioza apicalis is reported in the UK as far north as York but this information is 40 years old. Given the tolerance of this psyllid to cold temperatures in Scandinavia it may be present throughout the UK. The UK survey for psyllids 2012 (Fera) that included Scotland, was performed in carrot and potato crops to determine whether any known vectors of Lso are present. Growers were sent traps and returned them weekly during the growing season. A total of 53 psyllids were found some of these were *Triozidae* but none were *T. apicalis.* None of the species identified were known vectors of Lso. 2012 was a cold and wet summer with a lot fewer insects than normal.
In Scotland, SASA also surveyed carrot growers 2012-2014 for psyllids (yellow traps and limited sweep netting of carrot, potato and field margins) and Lso symptomatic plants (growers to send plants with symptoms to SASA for diagnosis). No known psyllid vectors were identified. Few responses were obtained from carrot growers for plants with symptoms. Suspect samples found in 2013 were identified as '*Ca.* Phytoplasma asteris' (see Phytoplasma section UK).

EPG studies using *Trioza apicalis* showed that there were no overall differences between time spent in different activities between males and females, only between plants. More time was spent in start of penetration, stylet tip in parenchyma , stylet tip in sieve element, phloem salivation and phloem ingestion when feeding on carrot than potato. One male psyllid was recorded phloem feeding from a potato plant therefore it may be possible for *T. apicalis* to transmit *Ca. L. solanacearum* to potato plants (see section on Finland).

Experiments to determine the most appropriate sampling strategy for potato microplants in potato quarantine showed that to reduce the uncertainty of detection the base part of the microplant should be tested and the top part subcultured for further propagation. In the glasshouse, the base of the potato plant or anything that looks symptomatic should be tested.

2. Potato phytoplasmas and their vectors

Belgium

Potato stolbur like symptoms were seen in the post control potato field at ILVO in 2012 and identified as '*Ca* P solani' based on tuf sequence results. A survey in 2013 on grapevine, potatoes and strawberry and raspberry failed to detect stolbur.

Hungary

Varieties were surveyed for stolbur infection. The first mild symptoms appeared around 50 days after emergence (<1 % of the plants) and increased thereafter. There were differences among varieties in susceptibility to stolbur infection (5-33%) based on symptom expression.

Transmission of potato stolbur from infected tubers to the stems was low. When 84% of the mother tubers were infected.0.5% stolbur transmission occurred. However, in 2012 when less than 5% of mother tubers were infected no transmission occurred (stems tested by nested PCR) despite these tubers producing stems that were initially weak and runner like, indicative of phytoplasma infection. After 2 months it was not possible to visually distinguish these plants from the other apparently healthy plants.

In 2012 and 2013 potato fields were monitored for Auchenorrhyncha species to study their abundance, flight activity and whether they were infected with phytoplasma. Over a 3 month period from beginning of June, 27879 insects from sticky traps were identified and 425 were collected by sweep netting. The insects were identified as belonging to 30 Auchenorrhyncha species: leafhoppers (16), Cixiidea plant hoppers (3), psyllids (3), bugs (6) and aphids (2). Stolbur vectors and potential vectors belong to the Cixiidae of which *Hyalesthes obsoletus* and *R. quinquecostatus* are considered as stolbur vectors. In 2012 adult cixiids flew from the 1st week in June to the 1st week in August. In 2013 the flight period started a week later but continued until the 1st week in September. In both years *Hyalesthes obsoletus* started to fly earlier than *R. quinquecostatus.* In 2012 *Hyalesthes obsoletus* was the most abundant (64%) and in 2013, *R. quinquecostatus* was the most abundant (86%). In both years they were found to be infected by stolbur phytoplasma. Based on these results it is assumed that mainly Cixiids are responsible for the stolbur epidemics in certain years in Hungary.

Turkey

173,000 ha of seed potato are grown mostly in 9 provinces in Turkey. Ware potatoes are grown in all provinces. There is a zero tolerance for potato stolbur in seed potato production. A survey was carried out in 4 provinces in July and August 2013 (Bolu, Kirsehir, Yozgat and Sivas) and 50 samples (26- Bolu, 6-Kirsehir, 6-Yozgat and 12-Sivas) showing symptoms of phytoplasma were taken. Other hosts (eggplant, pepper and tomato) were also tested.

10-20 midribs were taken from each sample and cut into 2-3 mm pieces and frozen at -85^{o}C until use. Extractions were done using Dneasy plant mini kit + CTAB buffer. Detection was by PCR, P1/P7 nested with R16F2n/R16R2 and real time PCR (Christensen primers). Using nested PCR and real time PCR, respectively 18 and 12 samples were found to be positive. Stolbur phytoplasma was detected for the first time in eggplant and pepper

Work is on going. Infected tubers have been planted and covered in insect proof cages. After harvesting the tubers will be tested. Insects have also been collected and will be identified.

UK

The use of microarrays for the detection and discrimination of phytoplasmas was investigated. The array uses multiple targets for each species and multiple probes for each target. The result is a robust system that allows a degree of species discrimination. The target organisms to date are the Phytoplasma groups, I, II, III, IV, V, VI, VII, IX, X, XI, XII, XIII, XXII, XXX and the '*Candidatus* Liberibacter spp', *africanus, americanus, asiaticus, crescens, europaeus* and *solanacearum.* The sensitivity of the assay is high due to the inclusion of a PCR step for the target

genes prior to the microarray detection. The target genes are SecA, 16S and 23S. The designed PCR primers are both universal and specific that result in multiple spots on the array for each species. The PCR is performed as a series of mini multiplex reactions, each containing multiple primer sets that are combined for use on the microarray. The microarray is in a tube format that allows for easy handling and this method can be performed from beginning to end in one day. The assay was more sensitive than real-time for some target species.

Experiments were done to determine the most appropriate sampling method for the reliable detection of phytoplasmas in potato microplants and glasshouse plants in potato quarantine. An attempt to obtain stolbur infected microplants from stolbur infected tubers (supplied by Maria Kolber, Hungary) failed. Although phytoplasma was detected in tuber tissue and eyes (by nested PCR), microplants generated from sprouts (some were hair sprouts, typical of phytoplasam indection) growing from these eyes were negative for phytoplasma.

Instead BLTVA infected microplants (supplied Jim Crosslin, USA) were used in several experiments. Microplants Results from Experiment 1 showed that the number of phytoplasma positive plants decreased on subculture from 10 to 6 and only one was BLTVA positive 4 weeks after planting (it had 2 stems only one of which was positive). In experiment 2, 10/10 microplant subcultures were positive but only 2 plants tested positive 3 weeks after planting in the glasshouse and none tested positive after 8 weeks. In experiment 3, 5/9 microplant subcultures were positive (one plant did not grow), 4/ 10 plants were positive 4 weeks after planting in the glasshouse and these tested positive over a 10 week period. Two other plants tested positive at some time over the 10 week period. BLTVA was detected in progeny tubers in all 6 plants that had tested positive for BLTVA. In summary detection of BLTVA was most reliable when testing microplants, 10 week old glasshouse plants (all stems) and progeny tubers.

A proficiency test (PT) for phytoplasmas was undertaken to evaluate assays currently used by laboratories. Five PHYLIB partners requested to take part. Prior to the preparing the PT, the stability of samples: healthy potato leaf DNA spiked with BLTVA from infected potato plants and '*Ca* phytoplasma asteris' plasmid, at high, medium and low dilutions was checked when stored at 4C, room temperature and 30^{o}C for up to 15 days using real-time PCR (Christensen *et al* 2004) and nested PCR (R16f2/r2 primers nested with fu5/ru3). The samples were relatively stable at all temperatures and over the specified time period. PT samples: *Ca* phytoplasma asteris' plasmid (10^{-1}, 10^{-2}, 10^{-3}); '*Ca* phytoplasma solani' plasmid (10^{-2}, 10^{-3}, 10^{-4});'*Ca* phytoplasma trifolli' plasmid (1:20, 1:50); BLTVA '*Ca* phytoplasma trifolli' from infected glasshouse grown plants (Neat, 10^{-1},10^{-2}); *Paenibacillus* sp.; *Brevundimonas* sp.; *Agrobacterium tumefaciens*; healthy potato leaf DNA) were despatched, 4 September 2014, to be stored in a fridge on arrival and tested within two weeks. A healthy and BLTVA infected microplant was sent for testing. Four of the five laboratories returned their results. Only one laboratory obtained the expected results, with all their assays. Lab 4 included a real-time method (Hodgetts *et al* 2009) which does not detect the plasmid insert used.

As part of the PHYLIB project a survey (based on symptoms) of Scottish carrot growers was carried out. In 2013, four carrots showing Lso symptoms of leaf curling, leaf yellowing and reddening were sent to SASA for testing. They tested negative for Lso but positive for '*Ca* Phytoplasma asteris' This is the finding of of *'Ca* P asteris' infecting a commercial carrot crop in the UK. No leafhoppers or planthoppers were observed within the carrot fields at the time of sampling and a range of non carrot plants from the field edges and also non symptomatic carrots from the field were sampled; these all tested negative for phytoplasma. To examine the possibility that phytoplasma may be seed transmitted 1000 seeds from two other varieties that had tested positive for '*Ca* P asteris' growing in field trial plots at SASA were planted in a glasshouse and

observed for symptoms. Some yellowing leaves were observed but these plants tested negative by real-time PCR for phytoplasma (also negative for Lso).

PHYLIB: Where to next

Mariano Cambra described the proposals for the Pest Organisms Threatening Europe (POnTE) project submitted in June 2014 under the Horizon 2020 - EU Research and Innovation Framework Programme with nearly €80 billion of funding available over 7 years (2014 to 2020). POnTE, which comprises 25 partners, including some from the PHYLIB project, was submitted by its coordinator, Donato Boscia (Institute for Sustainable Plant Protection, Italy). The project was for work on the following pests: *Xylella fastidiosa, Candidatus* Liberibacter solanacearum, *Hymenoscyphus pseudoalbidus (Chalara fraxinea)* and *Phytophthora* spp. (Phy)'. For Lso the work packages were: 1) Isolation of the bacterium and genotyping (including *in vitro* culturing and biological significance of haplotypes); 2) Implementation of diagnostic procedures for monitoring and surveillance programs (including use of automatic and aerial devices, thermal imagery); 3) vector monitoring and epidemiology (including use of electrical penetration graphics, molecular identification of psyllids, forecasting of disease epidemics, assessment of risk to Europe, 4) Managing and mitigation strategies (including carrot seed treatments, use of biopesticides, carrot seed production under cover). The project proposal had passed the first round of vetting and a final decision was expected in November 2015.

David Kenyon (Scottish Government coordinator for EUPHRESCO) provided some background to the PHYLIB project and why phytoplasmas and Lso were combined into one project (they are nonculturable bacteria and the aims of the project were similar). For the next round of EUPHRESCO his preference would be to continue the work started in PHYLIB but as two separate projects.

Since this end of project meeting, the POnTE project, despite a very high score of 13.5/15 failed to get funding. Proposals have been submitted to the EUPHRESCO board for a continuation of work on phytoplasmas and Lso as separate projects.

PHYLIB project publications

Bertolini E, Teresani GR, Loiseau M, Tanaka FAO, Barbé S, Martínez C, Gentit P, López MM, Cambra M (2014) Transmission of '*Candidatus* Liberibacter solanacearum' in carrot seeds. *Plant Pathology.* DOI: 10.1111/ppa.12245

Nisbet C., Ross S, Monger WA, Highet F, Jeffries C (2014) First report of '*Candidatus* Phytoplasma asteris' in commercial carrots in the United Kingdom. *New Disease Reports* 30, 16.

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Pearson K, Jeffries C, Kenyon DM (2014) Potato phytoplasmas and *Candidatus* Liberibacter solanacearum: the EUPHRESCO PHYLIB project. *Crop Protection in Northern Britain* pp. 209-214.

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Teresani GR, Bertolini E, Alfaro-Fernández A, Martínez C, Tanaka FAO, Kitajima EW, Roselló M, Sanjuán S, Ferrándiz JC López MM, Cambra M, Font MI (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.

Teresani G, Hernández E, Bertolini E, Siverio F, Marroquín C, Molina J, de Mendoza AH and Cambra M (2015) Search for potential vectors of '*Candidatus* Liberibacter solanacearum': population dynamics in host crops . *Spanish Journal of Agricultural Research*. 13, e10-002, 11 pages (2015) eISSN: 2171-9292 http://dx.doi.org/10.5424/sjar/2015131-6551

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