POnTE
Pest Organisms Threatening Europe

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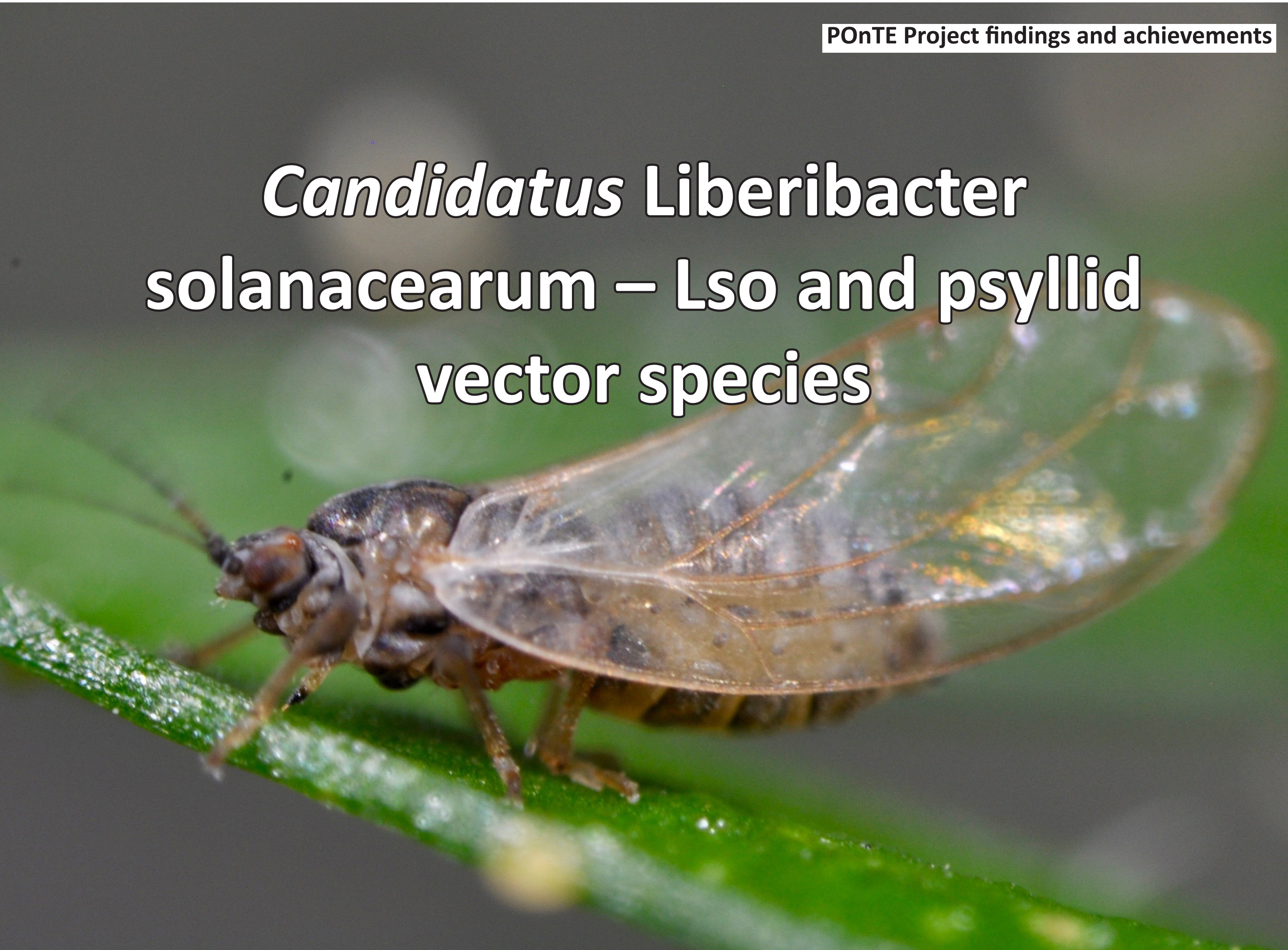
POnTE Project findings and achievements



Candidatus Liberibacter solanacearum – Lso and psyllid vector species

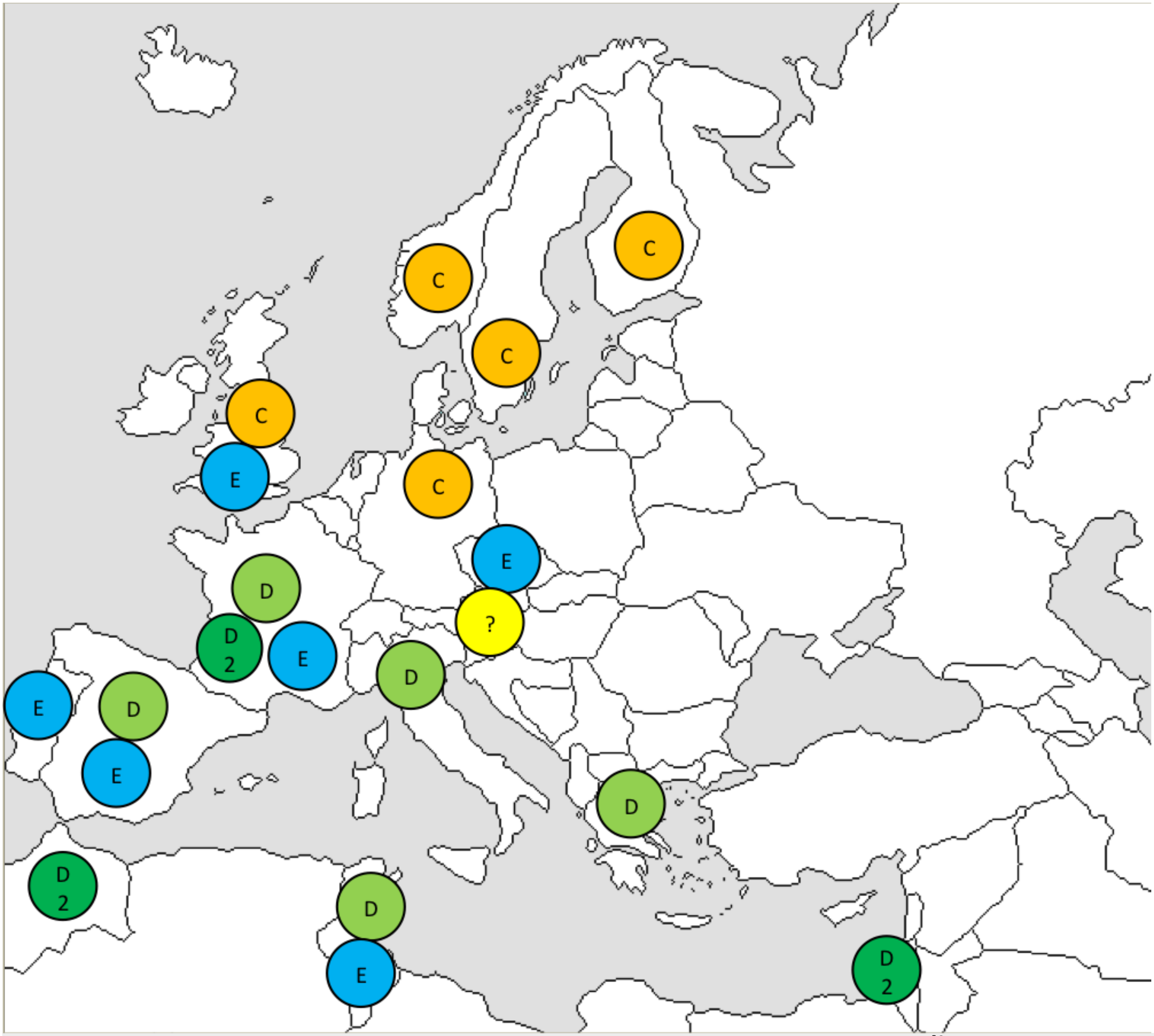
Candidatus Liberibacter is a genus of gram-negative bacteria belonging to the order of *Rhizobiales*. Among these bacteria, there are damaging and well-known plant pathogens such as the agents of the citrus greening and potato zebra chip diseases. In fact, the *Candidatus* Liberibacter spp. causal agent of Huanglongbing disease of citrus, and *Bactericera cockerelli,* the highly polyphagous vector of the *Candidatus* Liberibacter solanacearum causing potato chip disease, are included in the list of the EU priority pests, which means they deserve special surveillance from the European Union Member States authorities.

The 2015–2019 EU Horizon2020 POnTE Project focused on *Candidatus* Liberibacter solanacearum, also known as Lso. Scientists around the world described many different haplotypes of the bacterium and quite different interactions of those with the vectors (psyllids), susceptible hosts and environment. The emergence of new Lso haplotypes in carrots and celery in Europe has raised serious concerns about the risk that they could pose to potato and other solanaceous crops across the EU. The POnTE Project was the occasion to take stock of the previous research on the bacterium and to carry out a program of extensive sampling at the EU level.

Haplotypes and vectors

Lso haplotypes A and B, that can cause disease in solanaceous plants, are present in the US and New Zealand and their spread is related to the psyllid *Bactericera cockerelli*. Neither these haplotypes nor the correlated psyllids have been detected in Europe. Lso haplotypes C, D and E, instead, have been found in several EU countries and in the Mediterranean basin in plants of the *Apiaceae* family. More specifically, haplotype C is present in Northern Europe (Austria, Estonia, Finland, Germany, Norway, Sweden, and the United Kingdom) and is transmitted by the psyllid *Trioza apicalis.* Haplotypes D and E have been detected in Southern Europe (Belgium, France, Greece, Italy, Portugal, and Spain including the Canary Islands) and non-EU countries of the Mediterranean basin, and the main vector is *Bactericera trigonica*. These haplotypes cause vegetative disorders in carrot and celery. In Spain and Finland especially, the Lso-associated symptoms make carrot and celery affected impossible to be marketed, with economic losses for growers. According to estimates of the POnTE Project, the current impact is around 24 million euros for the whole carrot production in Finland alone.

Within the POnTE Project, in 2018, a team of scientists described haplotype U in *Urtica dioica* and the associated psyllid species *Trioza urticae* in Finland.



Credits: INRA

Map of geographical distribution of the *Candidatus* Liberibacter solanacearum haplotypes in Europe

How does it spread?

Lso is phloem limited. It can be transmitted by propagative plant material as shown in an experimental setup, but psyllids are the main vector of the transmission of the pathogen. When a psyllid feeds on the phloem sap of a Lso-infected plant, the bacteria can be ingested by the psyllid and then be transmitted with salivary secretions into a new host plant during psyllid feeding. As of today, there is no treatment for Lso and research to contrast its spread focuses on the management of psyllid population.

Symptoms in *Apiaceae*:

- Leaf curling
- Yellowish, bronze and purplish discoloration of leaves
- Stunting of the carrot shoots and roots
- Proliferation of shoots and secondary roots
- Disruption of the fruit set
- Production of small and poor-quality fruits

Credits: Alberto Fereres

Symptoms of Lso on carrot

Findings of the POnTE Project

Lso in Europe

Along with former research results, the work carried out in the POnTE Project helped developing new scientific knowledge on genetic diversity of Lso, enabling researchers to map the geographical distribution of the pathogenic haplotypes in Europe. Although the complete genome sequence of the European haplotypes of Lso is not complete yet, the geographical distribution studies gave a more precise view of the impact of Lso and of the possible strategies to contain it in different areas in Europe.

Finland

Trioza apicalis is the main vector of Lso in Finland and in other Center-Northern European countries. On top of that, scientists in the POnTE Project detected *Trioza anthrisci* to be associated with the infection of new wild plant host *Antriscus sylvestris*, and *Urtica dioica* and the corresponding psyllid species *Trioza urticae* are associated with the new haplotype U. Scientists tested population control methods, finding that kaolin treatments significantly reduced the number of *T. apicalis* eggs and nymphs on the plants compared to the untreated controls. Chemical control programs proved to be highly dependent on environmental conditions. However, the insect net proved to be more effective than the other control measures to prevent both the *T. apicalis* feeding damage and Lso transmission into carrots.

Credits: Anne Nissinen

Kaolin application and insect nets to control *T. apicalis* psyllid population in Finland

France

There is no visible impact of the presence of Lso in *Apiaceae* crops in France. Lso was not detected in potato crops in France. *Bactericera trigonica*, recently introduced in the country, seems to be the principal vector of Lso. So far, seed marketing has been most affected.

Spain

The psyllid *Bactericera trigonica* was present in all the carrot field plots sampled at high population densities. The high abundance of this vector is consistent with the high incidence of Lso in Spain. *Bactericera nigricornis* is also a vector of Lso and is associated with the carrot and the potato plots. It is the only psyllid species able to colonize and reproduce in both potato and carrot crops. *Bactericera trigonica* was found to be a highly efficient vector of Lso in carrots and celery, but a weak vector in potato. Regarding the control of the psyllids, in Spain, scientists tested an insect-proof mesh that effectively prevented the feeding by *Bactericera trigonica* as well as transmission of Lso. The IPM control programs consisting of products such as the maltodextrin, natural pyrethrin, *Beauveria bassiana*, and acetamiprid were shown to be more effective than paraffin oil applications alone. Drip irrigations instead of irrigation by sprinklers improved the effect of all treatments avoiding the washing of the applied products.

Israel

The fairly rapid change in carrot yellows disease etiology (from phytoplasma to liberibacter) may be a result of vector population shift (leafhoppers to psyllids), rather than a recent introduction of a new pathogen.

Being prepared: the risk for other crops and regions and new detection tools

The sole concrete risk for the potato industry in Europe is the possible introduction of *Bactericera cockerelli* via trade. Within the POnTE Project, scientists monitored through suction trap networks across Europe and field-scale sampling. In an extensive monitoring campaign carried out in Germany, Sweden, Spain and the UK, the only psyllid found to be infected from suction trap samples from Germany was *Trioza urticae* carrying Lso haplotype U. Haplotype C was found in *Trioza anthrisci* in Sweden and the UK, and in plants within the UK along with novel Lso haplotypes from psyllids not previously reported to harbour Lso. Comparisons of suction trap screening and in-field sampling suggest that in-field sampling is the best way to monitor Lso and field-scale psyllid diversity. To identify psyllid species, a DNA barcoding database was built using ITS2 and CO1 gene regions of 60 psyllid species from 14 different countries. The psyllid DNA database was used to survey psyllid diversity and ecology and as a basis to design qPCR diagnostic assays to rapidly identify important psyllid vectors of Lso such as *Bactericera cockerelli*, *B. nigricornis*, *B. trigonica*, and *Trioza apicalis*. These will be **important tools in the prevention and detection of introductions of psyllids such as *Bactericera cockerelli***.

Importantly, scientists in the POnTE Project found that settling, oviposition and feeding preferences of *B. trigonica* suggest that this psyllid is not able to colonize potato crops. Consequently, **the risk of Lso transmission mediated by *B. trigonica* to potato is very low**. Furthermore, *B. trigonica* was unable to reach the phloem of potato, so Lso transmission would be very unlikely.

Credits: Alberto Fereres

Scientists at work on the field

Also, scientists tested the effect of temperature and inoculum load on Lso disease symptoms and concentration in carrot plants. According to the results of experiments in Israel, disease symptoms developed more rapidly and Lso haplotype D reproduced faster *in planta* under 18° than under 30° growing temperature. The north European haplotype C, on the contrary, enhanced its development at the higher temperatures (20–25°C).

In general, more research is needed to define the host range of the European Lso haplotypes and their vectors as well as the environmental requirements for their multiplication and reproduction in order to understand their epidemiology and to be able to predict if they could pose a risk to other cultivated crops.

Scientists improved protocols for Lso detection and tested new ones. As far as DNA extraction procedures are concerned, direct extraction from symptomatic plant materials is efficient when used for real-time PCR detection and not for conventional PCR. Regarding detection in plant material, CTAB, NucleoSpinFood and NucleoMag (Macherey-Nagel) are the most universal methods for DNA extraction to detect Lso. As for insect vectors, CTAB, TNES and Quick-Pick (Bio-Nobile) allow the detection of Lso and the identification of the psyllid species. Importantly, the TNES method is non-destructive and allows the preservation of the specimen.

Identification of natural hosts associated with Lso in Europe

Thanks to extensive field surveys in Finland, France, Israel and Spain, in collaboration with Croatia and Tunisia, in areas of solanaceous and apiaceous plant production, and the subsequent use of DNA extraction techniques in the POnTE Project, **for the first time the researchers found Lso on new hosts**. These were cultivated *Apiaceae* species, such as parsley, fennel, chervil, and parsnip, and wild plants. Most of the wild samples were also from the *Apiaceae* family. That was the case in Finland. As anticipated, Lso was found in cow parsley (*Antriscus sylvestris*), a perennial wild plant closely related to the carrot and very common in the country. Similarly, in Israel, a symptomatic wild carrot and asymptomatic wild fennel were found to be positive for Lso.

Credits: Anne Nissinen

Symptomatic* leaves of *A. sylvestris

Also, the tests enabled scientists to detect Lso in wild plants which are not from the *Apiaceae* or *Solanaceae* families. The samples originated from the edge of fields infected with the bacterium. However, the findings on other wild plants seem to represent new haplotypes and, therefore, scientists hypothesize that they do not play an epidemiological role on crop plants.

Some results of the research suggest that wild *Apiaceae* plants might be Lso ‘natural reservoirs’ in the margins of highly contaminated fields. The wild hosts could provide a permanent supply of infected sap for the psyllids, even when the crops are not cultivated. The interaction among these newly discovered hosts, the pest and the vectors should be better investigated. It is important to find out if the Lso haplotypes detected in wild plants are the same as those identified in cultivated plants. Moreover, plants belonging to different families may have different psyllid species feeding on them.

Digital tools for Lso early detection and psyllid surveillance

Within the POnTE Project, agricultural engineers tested a digital platform equipped with proximal sensing equipment combined with aerial remote sensing for Lso monitoring and a system of automatic stations for the surveillance of psyllids. They mounted a sequence of high-resolution hyperspectral, multispectral and thermal cameras on a robot moving on the fields and drones flying over them, created high-resolution field maps and analysed hyperspectral and vegetative indices. The robot was used to test combinations of digital tools that can be part of future 'conventional' machinery, such as tractors. The robot was equipped with:

- Three DSLR (Digital Single Lens Reflex) cameras, two of them within the near-infrared (NIR) range from 700 to 1000 nm
- Blue NDVI (normalized difference vegetation index)
- A thermal camera
- A multispectral camera capable of capturing eight monochromatic images in 558, 589, 623, 656, 699, 732, 769 and 801 nm.

Aerial, terrestrial and laboratory tests using remote sensing, however, so far have not been completely successful in the asymptomatic detection of Lso.

In addition, engineers developed a prototype of **automated trap** as a permanent monitoring and surveillance system, catching and sending images of the vectors captured in the field to a remote server. A special Irwin trap equipped with a camera (on top of a water trap with a green tile having reflectance similar to vegetation) captures images at programmable intervals and sends the images to entomologists via a specific server. The energy supply is ensured by a long-life battery thanks to solar panels. This system is potentially patentable.

A way to 'cultivate' Lso

Scientists presented the first results of the attempts to work in a genome annotation-based in silico approach for the **design of a culture medium for this bacterium**. According to preliminary results, microaerophilic and anoxic conditions, not aerobic, might induce Lso culture in the laboratory. Carrot root phloem extract might contain specific chemical compounds that permit culturing Lso *in vitro* and comparative genome studies suggest the design of a complex culture medium.

Credits: Alberto Fereres

Prototype of automated trap for Lso vectors monitoring

Text: Angelo Di Mambro
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