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POnTE Project findings and achievements

Candidatus Liberibacter solanacearum – Lso and psyllid vector species

What is *Candidatus* Liberibacter solanacearum?

Lso is an abbreviation of the acronym *Ca*Lsol, that stands for *Candidatus* Liberibacter solanacearum. In North America and New Zealand, this plant bacterium infects tomatoes and potatoes (Solanaceae), causing the so-called zebra chip disease, inflicting significant economic damages. In Europe, the infection is associated with vegetative disorders in plants of the *Apiaceae* family only, especially carrot and celery, disrupting these crop yields in Finland and Spain, and seed market in France and Spain.

A global threat

The zebra chip name stems from the fact that affected potatoes develop black stripes, like the zebra ones, that become more visible when the tubers are fried. This makes the potatoes affected impossible to be sold on the market, impacting farmers' income. The pathogen belongs to the same family of bacteria bringing about the citrus greening, the most threatening citrus fruit disease, spread in Asian and African countries and some areas of Mexico and USA.

As far as the name of the bacterium is concerned, *Candidatus* is the tag for bacteria that cannot be maintained in culture. *Solanacearum* refers to the plant family of tomatoes, potatoes and peppers, that are the hosts from which the bacterium was isolated for the first time. In Europe, Lso affects the crops of the *Apiaceae* family, especially carrot and celery. The damage on those plants consists of vegetative disorders that can complicate lifting of the roots, reduce yield or make selling the products very difficult. Also, the bacterium has an impact on seed commercialization.

Following the vector

The extension and type of Lso infection are strongly related to its vector, the psyllids, or jumping plant lice. These insects are 2–3 millimetres long, and they feed much like aphids do, i.e. by sucking the sap from plant tissues. This feeding habit enables them to transmit Lso bacteria into the plants. The bug has a primary role in spreading the disease. As of today, the management of the psyllids' population is the most effective tool to prevent the spread of the Lso associated symptoms. This is why remarkable efforts in the POnTE Project were devoted to study biology, the mating habits and other characteristics of the vectors.

Credits: **Aberto Fereres**

Bactericera trigonica

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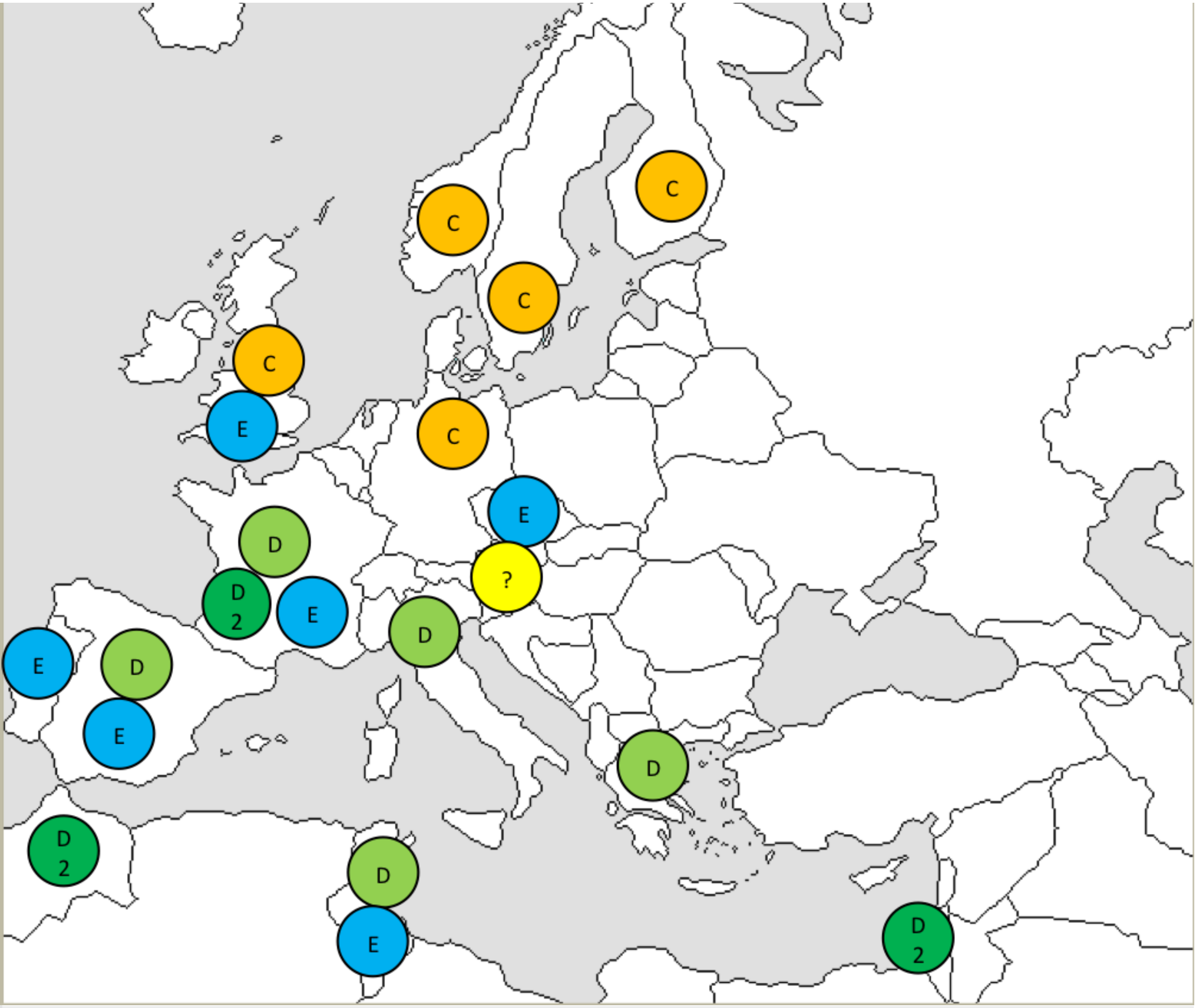
In Europe

- **Distribution and haplotypes**

Lso is present in all the continents, with substantial differences in regions. In Europe, Lso has restricted distribution in many countries across the continent. In order to investigate the origin of the organism and to understand the pathways of its distribution around the world, scientists started studying the geographic distribution of the Lso haplotypes.

In genetics, **the haplotype is a combination of variants in the DNA sequence which tend to always occur together**. So far, eight Lso haplotypes have been described, tagging them with the first letters of the alphabet, from ‘A’ to ‘G’, and one exception ‘U’. In Europe, haplotype ‘C’ has been found in Finland, Sweden, Austria, Estonia, Germany and Norway. Haplotypes ‘D’ and ‘E’ have been found in the Mediterranean region. Those haplotypes are different from the ones found in other parts of the world and this suggests the separation of populations of the bacterium in Europe has been long-lasting. This is confirmed by the difference in both psyllid species and psyllid plant hosts in Europe and North and Central America. In other words, the vector of the zebra chip disease, *Bactericera cockerelli*, is abundant in the USA, Mexico and in New Zealand where the disease impacts the solanaceus crops most. *Bactericera trigonica* is the psyllid associated with haplotypes D and E in the Mediterranean, and *Trioza apicalis*, also known as the carrot psyllid, is the psyllid associated with haplotype C in Northern Europe. Haplotype C is associated with different symptoms.



Credits: INRA

Distribution of *Candidatus* Liberibacter solanacearum haplotypes in EU and Mediterranean

- **The crops affected**

In Europe, Lso is associated with vegetative disorders on crops of the *Apiaceae* family, mainly carrot and celery.
Lso haplotype C is associated with a disease in carrots in northern Europe (Austria, Estonia, Finland, Germany, Norway, Sweden, and the United Kingdom). Haplotypes D and E are found in Belgium, France, Greece, Italy, Portugal, and Spain including the Canary Islands. Interestingly, concerning a survey of Lso in historical seeds from collections of carrot and related *Apiaceae* species (carrots, celeries), the bacterium was detected in seeds originating from countries which had not previously been reported to have it. This suggests that the distribution of Lso could be vaster than that already described.

- **Symptoms on the *Apiaceae* crops**

Leaf curling and yellowish, bronze and purplish discoloration of leaves, stunting of the carrot shots and roots, the proliferation of shoots (typical of haplotypes D and E) and secondary roots, disruption of the fruit set, and the consequent production of small and poor-quality fruits.

- **The impact**

In Spain and Finland, the Lso associated symptoms can make the marketing of carrot and celery impossible, with economic losses for growers. Current impact is estimated to be around 24 million euros for the whole carrot production in Finland alone.

The POnTE Project

The 2015–2019 POnTE Project is the first integrated response of European research to the emerging threat of invasive plant pests. POnTE stands for Pest Organisms Threatening Europe. Once considered to be a 'Northern world' problem originated by trade, today non-native plant pests are a global threat. According to some estimates, they cost global economies up to $1.4 trillion dollars. Global threats require a global response, and scientists and governments are expanding research networks all over the world, as well as the scientific disciplines involved, to be prepared to grapple with crop and forestry destructive pathogens.

Financed by the European Union Horizon2020 program, the POnTE Project relies on the 'know thy enemy' principle, bringing together 25 partners from Europe and Latin America with expertise in plant science, entomology, agro-engineering and economics in order to develop a comprehensive and multidisciplinary approach to three plant 'pathosystems'. *Candidatus* Liberibacter solanacearum is among them.

The goal is the development of early detection and surveillance tools, state-of-the-art knowledge and practical solutions against the spread of Lso and other emerging plant pests. The POnTE Project supported the intensification of research to find the most effective prevention, control, mitigation and management measures, covering phytosanitary as well as socio-economic dimensions.

In the case of Lso, scientists addressed epidemiological surveys in different countries in Europe, to find out which Lso haplotypes and vectors were present. Within the project, scientists also focused on psyllid species and their role as the main transmitter of the pathogen.

Credits: **ANSES**

Lso symptoms on celeriac. The symptoms make the crops impossible to be sold causing losses to growers

POnTE and *Candidatus* Liberibacter solanacearum: main findings and results

1. The geographical distribution of Lso in Europe

Along with former research results, the work carried out within the framework of the POnTE Project helped developing new scientific knowledge on the occurrence and genetic diversity of Lso, enabling researchers to map the geographical distribution of the pathogen haplotypes in Europe. In particular, scientists have confirmed that haplotypes A and B are not present in Europe. Both these Lso variants affect mainly potatoes and tomatoes in the US and New Zealand, causing serious damage to these crops. Moreover, *Bactericera cockerelli*, the psyllid transmitting Lso haplotypes A and B to healthy plants was found to be absent in Europe. The geographical distribution studies also gave a more precise view of the impact of Lso in different areas in Europe.

Finland

Trioza apicalis is the main vector of Lso in Finland, where the bacterium has been found in several plant and psyllid species. *Trioza anthrisci* was found to be associated with the infection of the new plant host *Antriscus sylvestris*, and the insect-plant host pair *Trioza urticae* – common nettle is associated with the new haplotype U.

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* was associated with the carrot but also with the potato plots. It is the only psyllid species known to be 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.

Credits: Alberto Fereres

***Bactericera nigricornis*: adult, nymphs and eggs**

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* seems to be the principal vector of Lso.

Why is this important?

The highest risk for the potato industry in Europe is probably the introduction of *Bactericera cockerelli* via trade. *Bactericera cockerelli* and *Candidatus* Liberibacter species causing the citrus greening are included on the European Union list of priority pests, which means that Member States are obliged to carry out special surveillance to prevent their arrival on the continent. Thanks to the POnTE Project, scientists are now better prepared for this possibility. The geographical distribution studies carried out within the project contributed to the findings of former and new research and to set up proper control strategies.

Challenges

The results have shown that Lso is more widespread in Europe and the Mediterranean region than it was previously known. 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 reproduction and multiplication in order to understand their epidemiology and allow predicting if they could pose a risk to other cultivated crops.

2. Identification of natural hosts associated with the Lso bacterium in Europe

Thanks to extensive surveys and molecular biology methods adopted in the POnTE Project, for the first time, the researchers found Lso on new hosts, meaning plants that were not known could be infected. These were cultivated *Apiaceae* species, such as parsley, fennel, chervil, and parsnip as well as wild plants. Most of the wild samples were also from the *Apiaceae* family. That was the case in Finland, where 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 an asymptomatic wild fennel were found to be positive for Lso.

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.

Why is this important?

Some results of the research suggest that wild *Apiaceae* plants might be 'natural reservoirs' of Lso at the margins of highly contaminated fields. The wild hosts could provide a permanent supply of infected sap for psyllids, even when the crops are not cultivated. The interaction among these newly discovered hosts, the vectors occurring as pests on carrots and the possible new vectors in Europe and the Mediterranean region should be investigated further.

Challenges

The scientific advances made within the POnTE Project can help manage and prevent the disease in the future. However, additional research is needed to better understand the biological and epidemiological significance of the new hosts. As far as non-*Apiaceae* and *Solenaceae* wild plants are concerned, more research on Lso haplotypes is needed. It is important to find out if the strains of bacteria 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.

Credits: Anne Nissinen

Symptomatic leaves of *Antriscus sylvestris*. In the POnTE Project, scientists detected Lso on new host plants

3. Digital tools for early detection and surveillance

Within the POnTE Project, scientists developed an Official diagnostic protocol. Moreover, **agricultural engineers tested** a system digital platform equipped with proximal sensing equipment and aerial remote sensing to make fast and precise surveys of Lso experimental fields and serve as a future surveillance platform. A robot on the ground and drones were used to survey carrot fields in Spain, to acquire high-resolution hyperspectral, multispectral and thermal imagery. In addition, automated traps have been developed as a permanent, automated monitoring and surveillance system, capturing and sending images of the vectors caught in the field to a remote server.

Why is this important?

Digital tools are more and more considered as instrumental to obtain accurate predictions of potential disease spread and simulation models. To this end, the collection and analysis of high-resolution remote sensing imagery through thermal and spectral cameras as part of early detection systems are a promising area of applied research on plant pests. Improving the promptness in the identification of diseased plants enables managing authorities to speed up the eradication strategies.

Challenges

The POnTe Project engineers developed state-of-the-art tools for early detection in the laboratory and tested them on the fields. One of the main problems with Lso is that a lot of plants can be infected showing no visible symptoms and in some circumstances digital cameras could help. However, now, all this should be put on the market so that cameras and other tools might be part of ‘conventional’ machinery such as tractors.

Credits: José Blasco, IVIA

The POnTE Project robot in Spain. The robot has been a tool for testing the possibility that high-resolution hyperspectral, multispectral and thermal imagery could help in Lso early detection

4. Lso management and control

Within the context of the POnTE Project, scientists tested various ways for the management and control of the population of two specific psyllids, *Bactericera trigonica* and *Trioza apicalis,* as principal vectors of Lso in Spain and France, and Finland, respectively. As already stated, the control of vector population is the best tool to prevent the spread of the infection. For both the insects species, an integrated approach (integrated pest management) leveraging mechanical tools such as nets, insecticide products – both synthetic and 'natural' such as kaolin,technologies such as drip irrigation proved to be more effective than single approach treatments.

As far as *Trioza apicalis* is concerned, growing plants under net provided better results than the chemical control programs tested. Kaolin treatment significantly reduced the number of psyllid eggs and nymphs, while the efficacy of chemical control programs was highly dependent on environmental conditions.

Similarly, an insect-proof mesh effectively prevented the feeding by *Bactericera trigonica* as well as transmission of Lso. Integrated control programs consisting of applications of products like maltodextrin, natural pyrethrin, *Beauveria bassiana*, and acetamiprid, which targeted different life stages of the psyllids, were more effective than paraffin oil applications alone.

The net use impacts positively the potential economic loss scenario. For a scenario in which all farmers use netting, the impact is estimated at around 1.76 million euros (instead of 24 million euros) in Finland alone.

Why is this important?

Setting the right control and management measures is important not only for farmers but for the social acceptability of eradication efforts. Recently, some invasive pests' epidemics in Europe, such as for instance *Xylella fastidiosa* in Apulia or the *Hymenoscyphus fraxineus* - ash dieback in the UK, became of public interest, triggering a debate on the most appropriate management protocols that went beyond the circle of the experts. In these protocols, the use of chemical products or the eradication of healthy plants susceptible of infection proved to be particularly sensitive, provoking heated controversy.

Challenges

One of the main challenges for scientists dealing with invasive plant pests is the dissemination and explanation of the research to laypeople. The POnTE Project was one of the first EU plant pest focused research endeavor including a working package to communicate the most relevant results to laypeople. This is a small but significant step to shorten the gap between science and society and to reinforce the trust between scientists and society, that appears to be one of the most sensitive issues of our time.

Credits: **Anne Nissinen**

Kaolin treatment and insect net to prevent the spread of Lso in a carrot field in Finland

Text: Angelo Di Mambro
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Pest Organisms Threatening Europe

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