PОnTE
Pest Organisms Threatening Europe

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PОnTE Project findings and achievements

HORIZON 2020 PROJECT PONTE Final Conference Ajaccio (France), October 2019

Report

From 2015 to 2019, the EU Horizon 2020-funded POnTE Project involved more than 120 researchers from 25 organizations in 10 European and three non-EU countries to investigate a group of invasive plant pests threatening EU agriculture and forestry.

New plant pests can cause hundreds billion euros of damage to Europe economy, landscape and biodiversity, as reported by the EU Commission Joint Research Center (JRC). Against this background, the EU Horizon 2020 POnTE (that stands for Pest Organisms Threatening Europe) Project is one of the largest and most comprehensive EU research endeavors to address the knowledge and strategies gaps existing about this kind of diseases.

Scientists presented the results and findings of POnTE at the final conference of the Project, with the participation of EU officials and stakeholders, in Ajaccio on 28 October 2019. The event was followed by the European Food Safety Authority (EFSA) 2^{nd} European Conference on *Xylella fastidiosa*, which was held on 29 and 30 October 2019.

The special focus dedicated to this bacterium is due to the fact that it is one of the most harmful plant pathogens in the world, at the top of the EU priority pest list, meaning that the Union's Member States are obliged to apply a higher level of surveillance to avoid its introduction and contrast its spread in Europe. Plus, the pathogen has already impacted olive groves tremendously in Apulia. According to JRC, *Xylella fastidiosa* alone has an economic potential impact of over 6 billion euros, accounting for the loss in yields and trade. The possible damage from all of the pests on the priority list related to European crops and forests are hundreds of billion euros worth.

Credits: **IPSP-CNR Bari**

The largest part of financing and research of the POnTE Project was dedicated to the emergency related to the *Xylella fastidiosa* epidemics in Apulia.

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 per year in biodiversity and economy losses. Global threats require a global response, and public research and governments are expanding research networks all over the world, as well as the number of disciplines involved, to grapple with crop and forestry destructive pathogens invading new territories.

The EU is a front-runner in this approach and POnTE Project brought together 25 partners from Europe and Latin America with expertise in plant sciences, entomology, agro-engineering and economics acting with a comprehensive and multidisciplinary approach. This perspective made the POnTE Project deliver also applied research suggesting up-to-date diagnostic standards, strategies to prevent the introduction and manage the epidemics of a group of plant 'pathosystems': *Xylella fastidiosa*, forest tree pathogens such as *Hymenoscyphus fraxineus* and *Phytophthora* species, and *Candidatus* Liberibacter solanacearum (also known by the abbreviation Lso), a bacterium causing serious damages on specific crops in various parts of Europe.

Credits: Forest Research, UK

Larches affected by *Phytophthora ramorum*

Major results and challenges of the EU H2020 POnTE Project on the control of *Xylella fastidiosa*

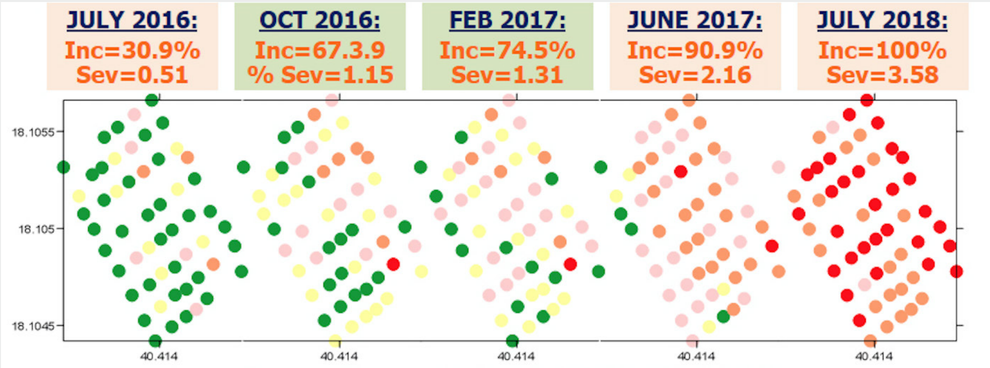
In Europe, the first detection of *Xylella fastidiosa* occurred in Apulia – Southern Italy – in 2013. Since then, this bacterium has been a top priority for plant scientists, pest managing authorities and made headlines news also in mainstream media. *Xylella fastidiosa* can colonize more than 550 plant species and it is pathogenic on a wide range of them, including grapevine, citrus, almond, oleander, peach, coffee, avocado, olive tree, and oak. Between 2015 and 2018, new *Xylella fastidiosa* outbreaks were detected in France, Spain, Germany, Portugal and, recently, in Tuscany.

Against this background, the POnTE Project was the first at the EU level to form an international research consortium to fill the scientific knowledge gap on the biology, ecology and epidemiology of the bacterium and its vectors in the EU territory. Consequently, the largest chunk of financing and research of the POnTE Project was dedicated to the emergency related to the *Xylella fastidiosa* epidemics. Here are the most important achievements:

The scientists within the POnTE Project:
Demonstrated that the large outbreak of *Xylella fastidiosa* in Apulia is linked to a novel severe olive disease, as **the bacterium is the principal cause of the olive desiccation epidemics (Olive Quick Decline Syndrome) in Southern Italy**.

- Isolated the strain of the bacterium present in Apulia and identified it as the most virulent that has been discovered in Europe so far. The POnTE network **detected diverse sequence types (ST) across distinct geographical regions**: ST6, ST7 and ST79 in Corsica and the Provence-Alpes-Côte d'Azur region (France), ST7 and ST81 in the Balearic Islands (Spain), ST6 in the province of Alicante and Madrid (mainland Spain), ST7 in the Douro Littoral region (Portugal), and ST87 in the region of Tuscany (Northern Italy). Given that current European regulation of *Xylella fastidiosa* is based on the subspecies present in each outbreak, these results combined with further pathogenicity tests on the main crops may help to establish management and regulation policy standards for the affected areas in Europe.

- Identified a pattern of the disease spreading in olive trees. Laboratory and field experiments have demonstrated that **the incubation of infection in olive trees is quite prolonged. It can take up to 12 months before the symptoms become visible**. Also, older plants may develop the symptoms faster than younger ones, especially under field conditions. Newly diseased trees often appeared close to the initial disease foci, suggesting a short distance secondary spread by insect vectors. Indeed, evidence was collected showing that over four years a dramatic increase of the disease incidence (reaching in most cases 100% of diseased trees) and symptom severity occurred in the affected olive groves.



Credits:
J.A. Navas (IAS-CSIC, Spain)

Graphic representation of the progress of incidence (Inc) and severity (Sev) of *Xylella fastidiosa* symptoms in a olive orchard in Apulia from July 2016 to July 2018

The results in experimental plots planted in the infected areas helped to identify a timeframe for infection. Firstly, infected trees could be identified as soon as one year after planting healthy trees in highly infected areas. Secondly, the bacterium could be readily detected even if the olive plants were still asymptomatic. Once the infection takes hold in a plot it spreads rapidly, and the number of infected plants can double in a year.

- Gathered and shared **essential information on vectors**, as well as a wide range of strategies for the control of their population on large areas and the scientists have found that the main ascertained vector of the epidemics in Apulia is *Philaenus spumarius*, a spittlebug species. In the infected area in Apulia, two other spittlebugs, *Neophilaenus campestris* and *Philaenus italosignus*, were found to be able to acquire and transmit the bacterium, even if populations of both species are less abundant than those of *P. spumarius*. Studies on the phenology and population dynamics of *Philaenus spumarius* remarked the need to focus on the control of the juvenile populations to accomplish a sustainable and effective reduction of the populations.
- To this end, the following practical solutions have been investigated:
- Soil tillage performed at the right time of the development of the nymphs remains the most effective strategy to suppress juveniles and significantly reduce the emergence of adults;
- Other tested means such as applications of herbicides, burning weeds, sowing gramineous plants and mulching were either less effective or less sustainable for the environment;
- For *N. campestris* soil tillage in late winter proved to be effective, probably because of causing a disruption of the egg masses;

Regarding control strategies for the adults:
- Testing different chemical formulations allowed to select those with the highest efficacy, however, the majority of the products had low persistence compared to the bugs' lifespan (i.e. from late spring to autumn).
- Use of inert compound (i.e. kaolin) as a repellent against adults did not reduce the spread of the infection in the long-term period. Similar results were obtained with chemical applications, confirming that short inoculation periods result in successful transmission events, posing major challenges for the containment of the infections.

Credits: IPSP-CNR Bari

The spittlebug *Philaenus spumarius*, the main vector of *Xylella fastidiosa* in Apulia

- **Provided tools to screen the susceptibility of different olive cultivated varieties to infection**, profiling those that might be tolerant of *Xylella fastidiosa*. Building on previous research carried out in other parts of the world, scientists investigating the *Xylella fastidiosa* epidemic in Apulia used RNA-sequence analysis to explore the interaction between olive trees and the bacterium, learning to recognize susceptible and resistant traits in the plants. The collected data confirmed findings from field observation indicating that the Leccino cultivar is less harmed by the bacterium if compared to the highly susceptible Ogliarola salentina cultivar. This finding enabled the repeal of the prohibition of planting new olive trees in the infected area in Southern Italy, giving new hope to olive growers living in the lands where the bacterium is impossible to be eradicated. More recently, other cultivars either in greenhouses or in field testing produced results similar to those recorded for the cultivar Leccino, used as a resistant control; indeed cultivars harboring high bacterial titers but not showing symptoms (i.e. with traits of tolerance) have also been identified. Although preliminary, these studies provide information on the spectrum of tolerance/resistance of additional olive cultivars and call for long term evaluation to gather robust evidence on the response of these promising cultivars.
- Regarding **early surveillance and detection of the pathogen**, the challenge of the visual inspections is one of the most relevant, because of the long latency of the symptoms that are non-specific and might be confused with other biotic or abiotic factors, and the practical difficulties of monitoring large areas. Under the POnTE Project, upon a 2-year campaign in Apulia using high resolution (sub-meter) images of nearly 200,000 olive trees, markers (plant indices) associated with the presence of the bacterium at the early stage of the infections were selected and proved to be useful to identify infected plants (symptomatic and asymptomatic) with 80% accuracy. This approach can be now used to screen large olive growing areas to seek for suspicious foci to be investigated and inspected.

Credits: IPSP-CNR Bari

Scientists and engineers at work on the system for the early detection of *Xylella fastidiosa*

- **Implementation and harmonization of the diagnostic tools at the EU level**. The large research network set up in the Project allowed to perform intense comparative and interlaboratory validations of laboratory diagnostic tests. Data on the performance and sensitivity of the different approaches were produced and used to prepare new and updated guidelines for testing plant tissues and insects. The availability and use of common approaches at the EU level for detecting *Xylella fastidiosa* is a prerequisite for ensuring common phytosanitary standards at the EU level.

- Models developed within the framework of the POnTE Project indicated which areas in Europe are under greater threat from *Xylella fastidiosa*, based on which an estimation of **the economic losses for growers and consumers and the geographical distribution** of the bacterium under current and future climate change scenarios were made. As part of the POnTE Project, economists developed a model to quantify the magnitude of potential economic impacts associated with the possible future spread of the bacterium in Italy, Greece and Spain, which together account for nearly 95% of European olive production. According to this model, growers could suffer production losses from 2.38 to 7.49 billion euros over 50 years if replanting with resistant varieties is not feasible. If replanting is feasible, the impact could range from 0.80 to 2.93 billion euros. Significantly, while the industry has some adjustment room, the economic impact of *Xylella fastidiosa* would be more significant.

Credits: **IPSP-CNR Bari**

Field trials of olive varieties resistant to the infection

Prof. Dr. Nenad Keča

Among the scientists involved in the POnTE Project, there was Prof. Dr. Nenad Keča, an eminent expert in Forest Pathology, especially poplar pathogenic fungi, from the University of Belgrade. Within the POnTE Project, he detected *Hymenoscyphus fraxineus* in Serbia for the first time. He passed away in August 2019. At Ajaccio, colleagues and friends of the POnTE Project wrote an obituary devoted to him.

Major results and challenges of the EU H2020 POnTE Project on forestry pathogens

A specific part of the POnTE Project was dedicated to emerging pathogens affecting woodland trees. Among these plant pests, the focus was on *Hymenoscyphus fraxineus*, a fungus known as the primary cause of the ash-dieback, and species of *Phytophthora* threatening tree health

The infection of *Hymenoscyphus fraxineus* starts on ash leaves and can cause the dieback of the tree

Credits: Forest Research, UK

Overview of *Hymenoscyphus fraxineus* within the POnTE Project

Hymenoscyphus fraxineus is a fungus, spreading through spores. The pathogen was detected in Europe (Poland) for the first time and affects mainly common ash (*Fraxinus excelsior*) and the narrow-leafed ash (*F. angustifolia*). The disease is usually fatal and nowadays is reported in most continental European countries as a very serious threat to ash populations. The first detection of this fungus in the UK in 2012 made the headlines news because it threatened a very common tree familiar in the British landscape. Since 2015, the disease has been monitored in England, Scotland and Wales. For plant health reasons, authorities prohibited all imports of ash seeds, plants and trees into Great Britain, and all inland movements within Britain of the same material. Northern Ireland has a similar measure in force. Within the POnTE Project, the fungus was detected on ash fruits and this was investigated further to determine whether the fungus was only present externally due to the airborne nature of the spores or it was also present internally, i.e. to determine if the infection was present on ash pericarps, seeds or embryos.

Within the POnTE Project, scientists:

- Detected the fungus on the European ash and narrow-leafed ash **in Serbia for the first time**
- For the first time found the disease on different non-ash species, in particular, *Phillyrea* species and *Chionanthus*
- Built on former research to investigate the role of ash fruits as a potential source for the spread of the disease, to determine if the infection was present on ash pericarps, seeds or embryos
- Tested a **hot-water treatment** as a control measure for *Hymenoscyphus fraxineus* eradication in seeds, to reduce biosecurity risks and facilitate the movement of seeds between diseased and non-diseased areas
- Identified **asymptomatic ash seedlings and trees**, an essential step in the research of tolerant trees.

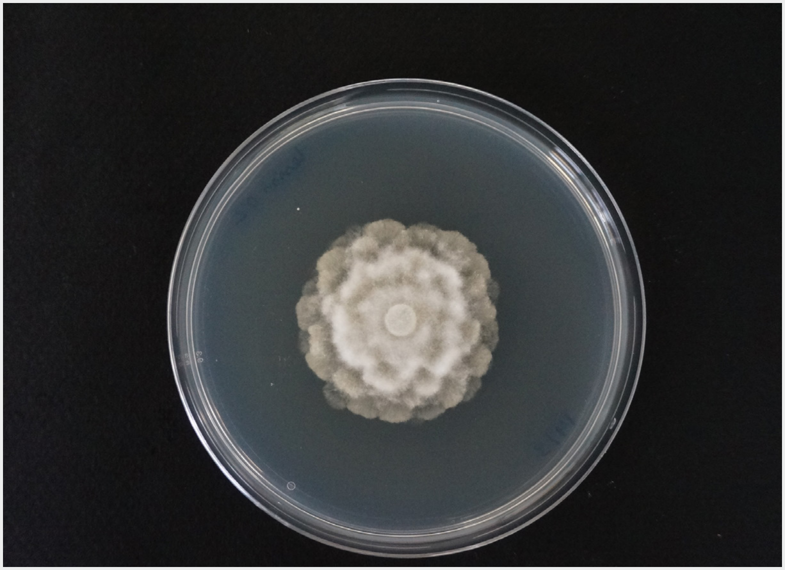
Credits: Forest Research, UK

Tests on hot water treatment for *Hymenoscyphus fraxineus* eradication in seeds

Insights into the biogeography and global diversity of *Phytophthora*

Within the POnTE Project, researchers monitored emerging *Phytophthora* species to understand why these organisms may be harmless in some areas and devastating in others. Firstly, also thanks to previous research, scientists could confirm the existence of a number of unknown species of *Phytophthora*. Secondly, a particular focus was dedicated to determining which *Phytophthora* species were present both in 'disturbed' sites (with frequent introduction of plants, soil movement and frequently visited by the public) and sites with very little disturbance, i.e. 'natural' ecosystems in Britain. In Serbia and Austria, scientists carried out *Phytophthora* surveys on specific declining woodland species.

Some of the species investigated proved to be highly adaptable. One *Phytophthora* specimen was found in the soil of a site where the disease had been eradicated nine years before.



Credits: Forest Research, UK

Phytophthora culture

Between 2013 and 2019, within the frame of several projects including POnTE, aiming at unravelling global diversity and biogeography of the genus *Phytophthora*, surveys were performed in natural ecosystems of Japan, Taiwan, Vietnam, Indonesia (Borneo, Java, Sulavesi and Sumatra), Chile, Nicaragua, Panama, Curacao, Egypt and eight countries in Europe. In total, 320 forest sites, 410 forest streams, 9 mangrove forests, 6 lagoons and 5 other marine sites were sampled. Baiting assays and direct plating of necrotic plant tissues were used for isolating *Phytophthora* species from forest streams, forest soils and woody plants. Isolates were identified using both classical identification and sequence analysis of ITS, cox1 and, if necessary, further gene regions. Overall, **13242 isolates were obtained which could be assigned to 65 known and 101 previously unknown species of *Phytophthora*** belonging to 11 of the 12 phylogenetic clades.

The POnTE Project helped to highlight some characteristics of this organism. In particular:

- Different *Phytophthora* species have been identified associated with specific tree declines in Europe.
- The combination of various state-of-the-art testing methodologies allowed the detection of up to 34 *Phytophthora* species in soil samples from different ecosystems.
- The abundance and high diversity of both known and new *Phytophthora* species discovered thanks to the POnTE Project are an alarm ring for European forests, agriculture and horticulture.
- Furthermore, it has been shown by these studies that **Southeast Asia is the center of origin of highly invasive wide-host-range *Phytophthora* species** like *P. cinnamomi* and *P. ramorum* which cause currently devastating forest epidemics in Europe and North America. This suggests that extensive host-range testing among European forest tree and horticultural crop species is urgently required to assess the **potential threat posed by the import of living plants from Southeast Asia**.
- Both natural and 'disturbed' ecosystems in Europe are likely to unravel the presence of previously unknown *Phytophthora* species. Thus, additional research is needed to better understand the biological and epidemiological significance of these findings and the potential impact of the *Phytophthora* subspecies on European forests.

Credits: Forest Research, UK

Trunk bleeding caused by *Phytophthora*

Candidatus Liberibacter solanacearum (Lso) and psyllid vectors

The gram-negative, phloem limited bacterium *Candidatus* Liberibacter solanacearum (also known by the abbreviation Lso) and its insect vectors are a major threat to the carrot and potato growing industry in Europe. So far, the most damaging *Candidatus* Liberibacter solanacearum haplotypes have affected potato and tomato crops in the US and New Zealand, where they are the cause of the so-called potato zebra chip disease severely damaging the crops. *Bactericera cockerelli*, the psyllid vector associated with this disease and the Lso species causing the citrus greening (Huanglongbing disease of citrus) are included on the European Union list of priority pests, which means that the Member States are obliged to carry out special surveillance to prevent their arrival on the continent. Thanks to extensive surveys carried out within the POnTE Project, scientists could exclude the presence of these two pests in the EU territory and prepare for the risk of their possible introduction via plant trade.

Credits:
Aberto Fereres

Lso symptoms on carrot leaves

The current main concern from Lso for European crops is the impact on the *Apiaceace*, such as carrot and celery. 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*. Haplotype 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.*

Collecting the results of the POnTE Project and former research, at Ajaccio, the European Food Safety Agency presented its survey card on Lso including information about the pest's biology and ecology, its detection and identification and the key elements for survey design, and the data requirements for performing a statistically sound sample size calculation.

Regarding Lso, at the POnTE Project final conference in Ajaccio, scientists presented:

- **An up-to-date map with the geographical distribution of the Lso variants in Europe**, giving a more precise view of the impact of Lso in different areas in Europe. Surveys during the Project led to the first detection of the haplotypes in the United Kingdom, Portugal, Israel and Tunisia. 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 estimated to be around 24 million euros for the whole carrot production in Finland alone.

- The results of the monitoring of the bacterium and its associated psyllid and plant hosts carried out to further understand the potential risk of Lso outbreak all across Europe. 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***.

Credits: **Aberto Fereres**

***Bactericera trigonica*, also known as the carrot psyllid**

- The evidence, for the very first time, of the presence of Lso on new hosts, both cultivated plants that were not known to have been infected previously, such as parsley, fennel, chervil, and parsnip, and wild plants.
- Developed a remote-controlled robot to inspect the presence of Lso in horticultural crops by remote sensing and automated traps such as a permanent monitoring and surveillance system

Trioza apicalis is the main vector of Lso in **Finland** and other Center-Northern European countries. On top of that, scientists within 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. The 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 the most effective compared to the other control measures to prevent both the *T. apicalis* feeding damage and Lso transmission into carrots.

In **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.

In **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 the transmission of Lso. The IPM control programs consisting of products such as maltodextrin, natural pyrethrin, *Beauveria bassiana*, and acetamiprid were shown to be more effective than paraffin oil applications alone. Drip irrigation instead of irrigation by sprinklers improved the effect of all treatments avoiding the washing of the applied products.

Credits:ANSES

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

In **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.

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, was enhanced at the higher temperatures (20–25°C).

Finally, as a *Candidatus* bacterium, Lso cannot be maintained in culture. At the Ajaccio conference, 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. In addition, 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:
Anne Nissinen

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

Beyond POnTE

The Ajaccio POnTE conference was also the occasion of taking stock of the information collected on Lso in recent years. Scientists presented results from tests – not included in the POnTE Project – on **vibrational communication and mating behavior of the psyllid *Bactericera cockerelli***. The findings support the hypothesis that the mating behavior of this species is likely to be vulnerable to manipulation by means of vibrations. More research will be conducted to improve the attractiveness of the stimulus, in order to develop mechanical monitoring and/or control technique.

Scientists discussed the **transmission of Lso via seeds**, that is a major problem for trade. This finding of seed transmission in a previous paper, has turned out to be difficult to replicate. For example, in the paper presented at the conference, the detection tests on seeds and plants from healthy lots were always negative. During the 6 months of the trial, no plants from the contaminated seed lots tested positive for the bacterium or showed any infection symptoms. However, the issue is still under discussion.

Credits:
Anne Nissinen

***Trioza apicalis*, the main Lso vector in Finland and other Center-Northern European countries**

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