POnTE
PestOrganisms
ThreateningEurope

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635646

POnTE Project findings and achievements

Xylella fastidiosa

Xylella fastidiosa (Wells et al.) is a quarantine phytopathogenic gram-negative bacterium belonging to the *Xanthomonadaceae* family. The bacterium is highly polyphagous and can live on a wide range of host plants: over 550 fruit trees and vegetable crops, ornamental plants, forest essences, weeds and wild species. Once confined to America, *Xylella fastidiosa* was first detected in Europe in 2013, on olive trees in Apulia, Southern Italy. In the EU, the bacterium is considered the most threatening in the priority plant pest list and action to contrast its introduction and spread is mandatory. Recently, the Joint Research Center of the European Commission and EFSA have estimated that *Xylella fastidiosa* full spread could ultimately cost the EU over €5.5 billion per year due to loss of production, with potential export losses of €0.7 billion per year.

How does it act?

Xylella fastidiosa establishes in the xylematic vessels, blocking the normal nutrition of the plant. The infection can bring the host plant to death of, depending on many factors, including the virulence of the *Xylella fastidiosa* subspecies and the susceptibility of the host plant. As of today, at least four subspecies of the bacterium are known: *fastidiosa*, *multiplex*, *sandyi* and *pauca*. Some experts indicate there are six subspecies, but *fastidiosa* and *pauca* have been the most known to plant pathologists so far, as they are agents of Pierce's disease of grapevine, which caused extensive damage to the California vineyards, and variegated citrus fruit chlorosis that devastated the citrus orchards in Brazil.

Symptoms

The symptoms caused by the pathogen vary depending on the host plant. In general, the most frequent are leaf scorching, with necrosis of dries in the apical and/or marginal part of the lamina (a symptom similar to that induced by excess salinity of the soil, hot winds, drought or other factors), reduced growth and drying up of shoots and branches.

Credits: POnTE Project

***Xylella fastidiosa* symptoms on olive tree**

The situation in Europe

After the first detection of the bacterium on olive trees in Southern Italy in 2013, since 2015 the EU has taken emergency measures to prevent further introduction and spread of the bacterium within the EU (Implementing Decision 2015/789/EU). The measures are amended regularly, based on experience, knowledge and new scientific evidence. The next update is expected in 2020.

Since the first detection in Apulia, the presence of the bacterium in the open field has been reported in France (Corsica and the Provence Alpes Cotes d'Azur region), Spain (Balearic Islands, Madrid and Comunitat Valenciana-province of Alicante), Italy (Tuscany) and Portugal (Porto). The pathogen has been also detected in two nurseries in Germany and Belgium, but in these cases, the infections were rapidly eradicated.

The strain established in Apulia is different from all the others found in Europe and has been one of the most virulent so far described worldwide, able to infect at least 34 different species of plants including olive, cherry, almond, laurel, as well as numerous shrub plants typical of the Mediterranean (myrtle, *Cistus*, rosemary, broom, *Phillyrea*) or ornamental (oleander, *Polygala*, geranium, lavender) and some herbaceous weed species (*Chenopodium*, *Conyza*). Managing the disease in the field is notoriously difficult due to the complexity of interactions among the harmful organism, the possible hosts, the vectors and the environment in which the infection occurs.

1. The challenge of visual inspections

Visual inspections and examination of the symptoms are quite challenging for detecting *Xylella fastidiosa* infection. Latent infections, long incubation period and non-specific symptoms are the main challenges. The European Food Safety Authority (EFSA) summarizes the visual symptoms as follows:

- Leaf scorching
- Wilting of foliage
- Stunting and dieback of shoots and twigs
- (Premature) defoliation
- Chlorosis or bronzing along the leaf margin
- Formation of new malformed (asymmetric) leaves
- Dwarfing.

However, the duration of the asymptomatic period varies depending on the host plant. Some infected plants can be asymptomatic for all the lifetime, whereas in others, there are no symptoms shown because infections do not become systemic. Plus, since *Xylella fastidiosa* has a wide host range, the latency period may vary significantly. This is also influenced by the subspecies and the strains causing the infections.

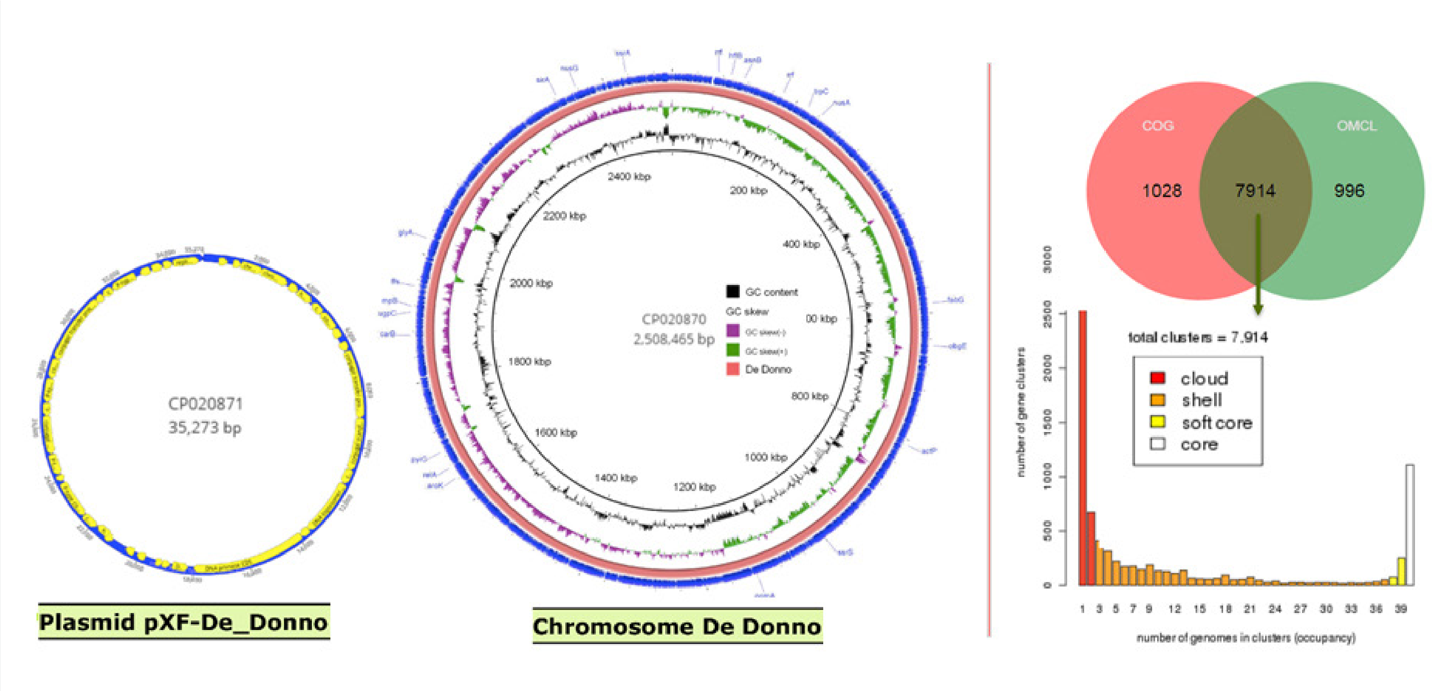
Credits: POnTE Project

***Xylella fastidiosa* symptoms on olive tree**

Thanks to the research carried out within the POnTE Project, we know more about the symptoms, the time of latency and the spread patterns in olive trees in Apulia. The etiological role of this *Xylella fastidiosa* strain (‘De Donno’) in the development of Olive Quick Decline Syndrome (OQDS) has been demonstrated through pathogenicity tests. This key achievement paved the way to investigate host–pathogen interactions and to screen cultivar susceptibility. Laboratory and field experiments have demonstrated that the incubation of infection is quite prolonged, and it can take more than 12 months before the symptoms become visible. Also, older plants may develop the symptoms faster than younger ones, especially under field conditions

The results in experimental plots planted in the infected areas helped to identify a timeframe for infection. Firstly, newly 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.

Symptoms can be non-specific and confused with other biotic or abiotic factors, pests or other factors which cause wilt and leaf dehydration, such as environmental stresses, water deficiencies, salinity, air pollutants, nutritional problems, sunburn, etc. This is the case of leaf scorch, for example.



Credits: POnTE Project

Genome sequencing of *Xylella fastidiosa* 'De Donno' strain

2. The epidemiology and the transmission

The transmission of *Xylella fastidiosa* occurs mainly through insect vectors belonging to the families *Cicadellidae* (sub-family *Cicadellinae*) and *Aphrophoridae*, which can acquire the bacterium through the mouthparts feeding on the xylem vessels of infected plants and pass it on to healthy ones. These insects are widespread in Europe and characterized by high polyphagia. This can contribute significantly to the rapid spread of the bacterium once introduced into a favorable environment with susceptible hosts. The most relevant vectors in the EU have been identified, as well as their preferred host plants. These findings have been followed by the characterization of their phenology and the achievement of important data of their potential natural spread through 'Mark-Release-Recapture' experiments. In the EU, these findings allow now the implementation of targeted surveys for the main vectors, field trials for the control of their populations, and determination of experimental-based parameters to develop models for the short-range spread.

POnTE research has proved that in the case of the Apulian outbreak the main source of inoculum is represented by infected olives and the bacterium spreads in affected groves with the aggregate pattern. New disease foci often appear close to the initial disease foci suggesting that a short distance secondary spread by insect vectors is the main pathway of the bacterium in the Apulian olive orchards.

Credits: POnTE Project

Philaenus spumarius

a. **The main ascertained vector of the epidemics in Apulia is *Philaenus spumarius* (L.), a spittlebug known as 'meadow spittlebug'**

The bacterium is retained in the foregut of the vector and adults need to feed on infected plants in order to acquire and transmit the pathogen. Vectors are not present during winter and have only one generation per year. According to the observations made by POnTE Project researchers on the field in Apulia, the insect overwinters at the egg stage laid in mass on plant residues or directly on the ground; the eggs hatch in a scalar manner, from the end of February to the end of March, freeing the first instar of 5 successive nymphal stages. The nymphs live, also in gregarious form, on many spontaneous and cultivated herbaceous plants sucking xylem sap and producing the typical foam (from which the name spittle and spittlebug) which protect the specimens during this stage; the nymphs do not fly, they are delicate, not very mobile and remain usually on the weeds. Adults (winged) appear in late spring/early summer according to the climatic conditions, and from the ground vegetation progressively move onto the tree canopies, like olives or other crops or perennial species. Adults must feed almost continuously and thus they are continuously searching for suitable succulent shoots to feed on. This feeding behavior implies that the adults can acquire the bacterium soon after their emergence from the canopies of the infected plants (most frequently from olive trees) and transmit it for the rest of their life (late autumn/early winter).
The mechanism of transmission is quite atypical compared to other vector-borne pathogens. The bacterium is retained and multiplies on the cuticle in the foregut. The anterior part of the mouthpart is the only tissue colonized by the bacterium, which does not invade the body (*hemocoel*) of the insect nor reach the eggs. As such, the transmission only occurs during insect feeding.

Credits: POnTE Project

Philaenus spumarius nymphs live on many spontaneous and cultivated herbaceous plants sucking xylem sap and producing the typical foam

The delicate juvenile forms are the most vulnerable stage to be targeted by the control. Within the POnTE Project, scientists assessed the possibility that other insect species can transmit the pathogen. Experiments on a cicada (*Cicada orni* L.) and a planthopper *Latilica tunetana* (Matsumura) gave negative results, indicating that these insects do not play a role in the transmission of *Xylella fastidiosa* to olives. On the contrary, scientists found that two other spittlebugs, *Neophilaenus campestris* (Fallen) and *Philaenus italosignus* Drosopoulos & Remane, are able to acquire and transmit the bacterium.

Scientists of the POnTE Project could assess the dispersal capacity of *P. spumarius* in managed olive orchards and not managed meadow areas. The results are included in the proceedings of the 2nd EFSA conference on *Xylella fastidiosa* (see the links at the end of this document).

b. **The spread through plant movement and trade**

The spread of the bacterium over long distances occurs mainly through the movement of infected plants (plants and plant parts, cuttings, buds). The most accredited hypothesis on the introduction of *Xylella fastidiosa* in Apulia is that it came with a plant from Central America. This has been confirmed by the studies conducted, within the POnTE Project, on genomic features of the strain that is affecting olive trees in the Apulia region. Furthermore, genetic studies outlined the great genetic variability among the isolates detected in Europe. The latter circumstance suggests that several independent introductions have occurred in the EU and confirms the high genetic variability of this pathogen. Phytosanitary import restrictions are in place at the EU level to protect the territory from the further introduction or movement from the current EU infected areas of infected materials to pathogen-free areas, and inspections are now mandatory in the Member States. As such, the uses of common approaches for inspection, sampling and testing need to be adopted. The POnTE Project significantly contributed to this challenge by promoting the update and harmonization of the diagnostic procedures.

3. Strategies to contrast the spread of the bacterium developed within the POnTE Project

Managing the bacterium related diseases in the field is very difficult due to its complex interaction with hosts and vectors. The most effective actions to be taken against the pathogen are: a) vector population control, b) prevention and/or containment measures such as the use of healthy propagating materials, c) surveillance and early detection of the pathogen and d) removal of the sources of inoculum by the destruction of infected plants. Specific challenges emerge for each of the actions mentioned and POnTE scientists proposed and tested practical solutions and/or indicated promising directions for the research.

a. **Prevention and implementation of surveillance strategies**

- The **validation and harmonization of diagnostic protocols** for *Xylella fastidiosa* carried out within the POnTE Project, together with the achievements related to the optimization of remote sensing (see early surveillance, below), will give a major contribution to the improvement of the surveillance plans at the EU level. Within this framework, a major contribution has been given to the three revisions of the EPPO standard diagnostic protocols for *Xylella fastidiosa*.

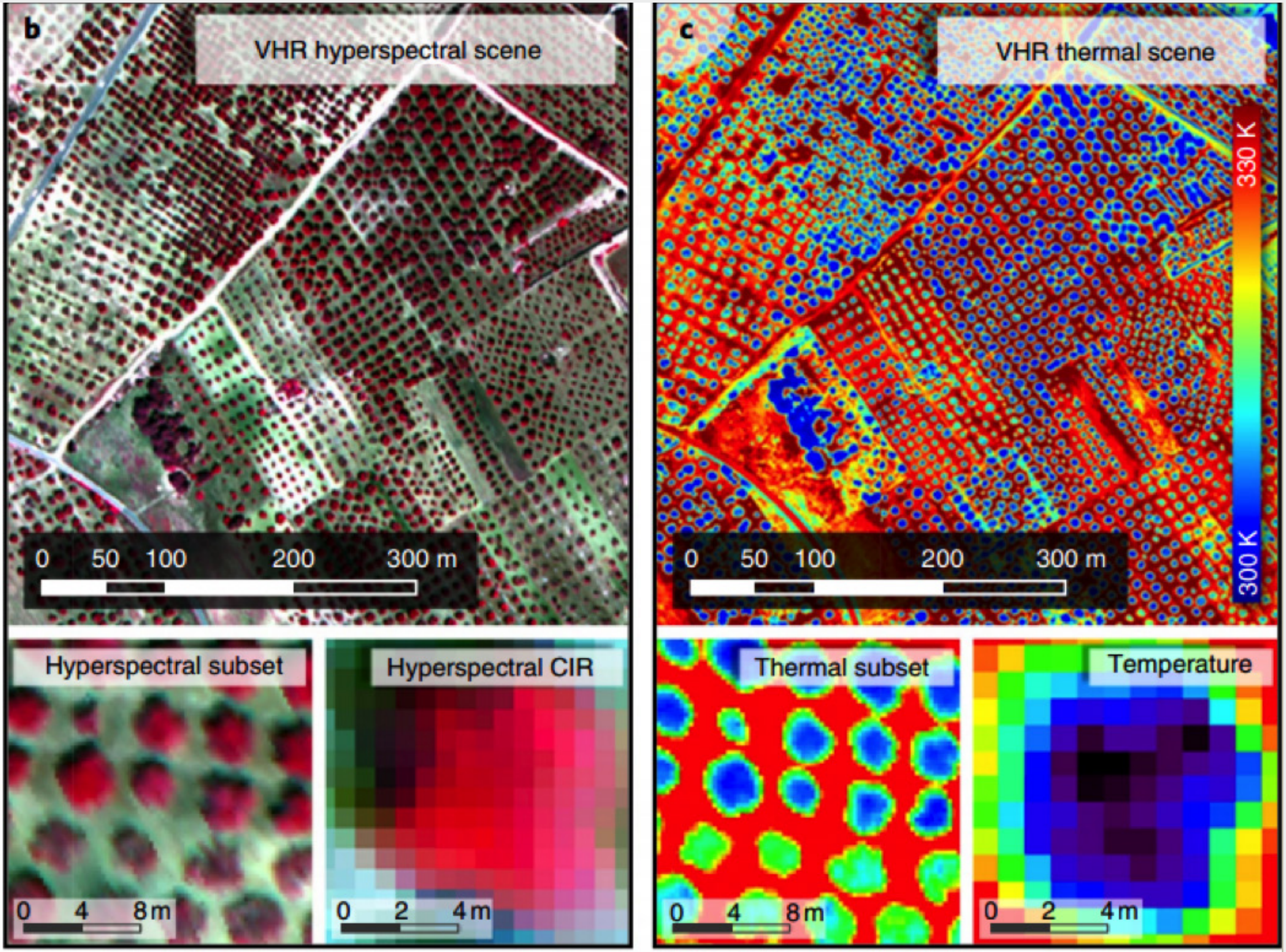
- Regarding **early surveillance**, the challenge of the visual inspections is one of the most relevant, because of the long latency of the symptoms, that are non-specific and might be confused with other biotic or abiotic factors, and the practical difficulties of monitoring large areas. Under the POnTE Project, **successful pre-visual identification of olive trees infected by *Xylella fastidiosa* has been achieved using high-resolution airborne hyperspectral and thermal imagery acquisition**. Indicators for early and/or asymptomatic detection of *Xylella fastidiosa* infection were developed to facilitate the automatic classification of OQDS incidence and severity at a large scale in order to further support the efficacy of the monitoring programs.

b. Search for tools to counteract the bacterial infection in the host plants

- Within the POnTE Project, a significant effort has been done to search sources of resistance to *Xylella fastidiosa* in the olive germplasm; it has been confirmed that phenotypic differences in olive cultivars are supported by different transcriptomic and ionomic profiles and that biological mechanisms involved are consistent with those previously identified in other hosts; preliminary encouraging results on the search of resistant/tolerant olive cultivars have been achieved with the identification of interesting traits of resistance in the cultivars Leccino and FS-17.

- On top of this remarkable achievement, the POnTE Project started various experiments to search for treatments of the disease in the host plants, such as:

- The experiments carried out to test the effectiveness of the N-acetyl cysteine (NAC) yielded slight attenuation of the severity of the symptoms at the initial stage of the disease, but afterwards, no differences could be recorded between the treated and non-treated plants. This result suggests that the damage and the interactions that *Xylella fastidiosa* establish in citrus (for which NAC treatments proved to be effective) are different from those in olives, which provides additional evidence on the great complexity of this pathogen requiring case by case specific experimental testing and control actions.



Credits: POnTE Project

Airborne hyperspectral and thermal imageries can lead to successful pre-visual identification of olive trees infected by *Xylella fastidiosa*

Credits: POnTE Project

Tests on the field of the Leccino (resistant) and Ogliarola salentina (susceptible) cultivars

- Exploring the endophytic bacterial community (microbiome) inhabiting olive xylem tissues. The description of the microbial communities associated with *Xylella fastidiosa* infection in olive will allow further investigation on the search for biological control agents. A similar approach gave encouraging results on grapevines infected by *Xylella fastidiosa* subsp. *fastidiosa* in the USA, but it has to be tested on olive trees in Apulia, that are subjected to the infection of a different subspecies

c. As far as the **vector control** is concerned, the POnTE Project scientists carried out tests on the field in the infected area in Apulia, concluding that an integrated pest management strategy based on control of nymphs and adults is needed to suppress populations of *Philaenus spumarius*, with strong evidence supporting the need to focus on the control of the juvenile populations.
Field trials, aimed to improve the control of the vectors, gave relevant information on the possibility to achieve significant results through appropriate vegetation management; i.e.: soil tillage in certain periods and/or cultivation of negatively selected plant species can reduce the populations of vectors.

Credits: POnTE Project

The results of POnTE Project highlighted the need to focus on the control of *Philaenus spumarius* juvenile populations

More specifically, results have been obtained on the following practical solution:

Control of the juvenile stages:

- 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 it causes a disruption of the egg masses;

Control strategies for 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.

To know more:
The POnTE Project website:
www.ponteproject.eu
XF-ACTORS Project website:
www.xfactorsproject.eu/
Gathering the data on the *Philaenus Spumarius*, check the tutorial developed by the EFSA and the POnTE project: https://www.youtube.com/watch?v=Rjh7FFQCtg8&t
The 2nd EFSA European Conference on *Xylella fastidiosa*:
https://www.efsa.europa.eu/en/events/event/conference-xylella-fastidiosa-29-30-october-2019
EPPO Standard describes a diagnostic protocol for *Xylella fastidiosa:*
https://onlinelibrary.wiley.com/doi/full/10.1111/epp.12469
EFSA, Update of the Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory:
https://www.efsa.europa.eu/en/efsajournal/pub/5665

Text: Angelo Di Mambro
Layout: Surrender Media Solutions

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