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The European research project "POnTE - Pest Organisms Threatening Europe"

| info@ponteproject.eu | | /ponteproject.eu | | @ponteproject.eu |

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POnTE is a multiactor research consortium integrating different expertise in plant pathology, risk management and economics, with the objective to advance the knowledge on several pathogens threatening relevant species in both agriculture and forestry. This 4-year project, started in November 2015, targets *Xylella fastidiosa (Xf)*, *Candidatus* Liberibacter solanacearum (*Ca*Lsol), *Hymenoscyphus fraxineus* and emerging species of *Phytophthora*. Studies include research experiments on the genetics and biology of the target pathogens, elucidation of the mode of transmission and ecology of the insect-vectors, early detection and prevention, modelling and risk assessment tools, sustainable approaches for controlling the spread and the impact of the diseases caused by the target pathogens.

Main research results achieved so far

Genetics and genomics: The complete genome of the *Xf* strain associated with the olive epidemic in southern Italy ('De Donno' strain), one of the most severe EU strains, was successfully obtained (Fig. 1). This represents the first EU reference sequenced strain, boosting future genomics studies relevant to answer important epidemiological questions (drivers of the epidemic spread and evolution of the bacterial populations). Indeed, several laboratories have developed skills and expertise for determining the subspecies and sequence type (ST) associated with the infections detected in the countries currently facing this quarantine pathogen.

The subspecies and the ST of the strains, along with the list of the susceptible hosts, are the key information supporting the continuous update of the EU phytosanitary measures.

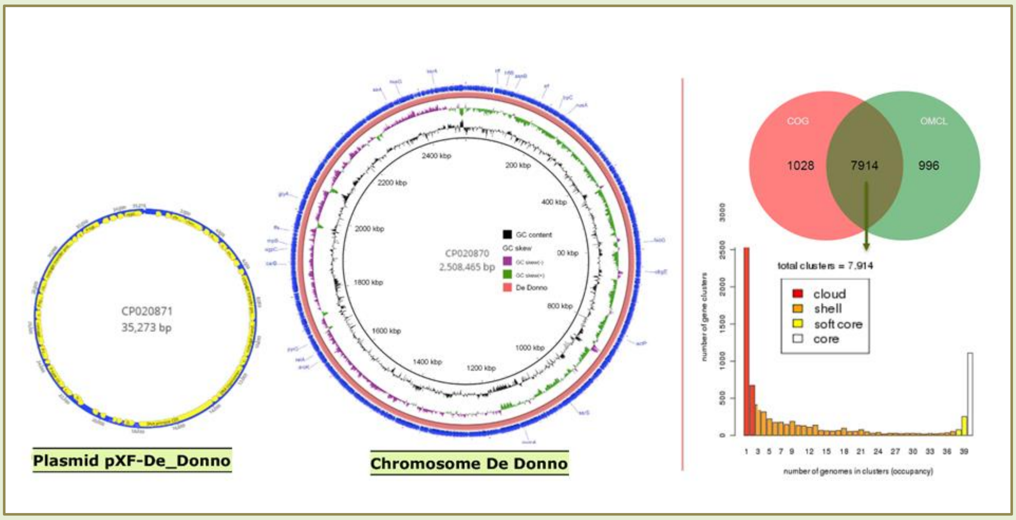

Fig 1. Complete genome diagram of *Xf* 'De Donno' strain.

Similarly, characterization of the haplotypes of different *Ca*Lsol positive samples from Europe and other Mediterranean countries allowed to identify the haplotypes occurring in Europe and to draw a map of the geographical distribution of *Ca*Lsol haplotypes. The full genomic sequence of *Ca*Lsol haplotypes C and D has been obtained and the genome of the haplotype D has been annotated.

The identification and characterization of new species of *Phytophthora* isolates have been carried comprising different hosts and environments (soil, water, decaying trees) and different countries (Austria, Germany, Portugal, the UK, Serbia, Vietnam). Interestingly, several new species/taxa have been detected, including potential hybrids: 23 new *Phytophthora* taxa, a diverse array of known and new taxa of *Phytopythium, Pythium and Elongisporangium* and *Nothophytophthora*.

Biology, pathogenicity and host interactions: The collection of available *Xf* isolates has been enriched with isolation from different hosts in Italy, Spain, France and Costa Rica.

Pathogenicity tests conducted in Italy clearly showed the pathogenic role of *Xf* pauca 'De Donno' strain in the emerging Olive quick decline syndrome (OQDS). Screening of olive germplasm for *Xf* resistance, under field and greenhouse conditions, proved that olive cultivars Leccino and FS-17® hosted lower bacterial populations and expressed less severe OQDS symptoms (Fig. 2). The discovery of these traits of resistance allowed the Italian authorities, in accordance with the Commission Implementing Decision (EU) 2017/2352, to abolish the prohibition of planting host plants in the infected areas.

Surveys and testing for *Ca*Lsol in crops, weeds and seed lots revealed a wider host range than expected, and the presence of around 3–5% of contaminated seeds of carrot and parsnip.

Fig. 2. Field trials of screening for resistance traits in different olive cultivars.

More than 1,200 *Phytophthora* isolates have been isolated in different countries and identified by morphological and molecular methods. A possible *Phytophthora* hybrid on beech (*Fagus sylvatica*) was discovered in England. A total of 38 *Phytophthora* non-quarantine species were detected in British soils. *Phytophthora* was found to be widespread on alder in Norway. *P. europaea*, *P. gallica* and *P. gregata* were reported for the first time in Serbia and 217 self-sterile *Phytophthora* isolates have been identified in from Vietnam, Portugal and Taiwan.

About 100 isolates of *H. fraxineus* have been obtained and identified at the species level in different *Fraxinus* hosts in Serbia, the UK and Austria (Fig. 3). *H. fraxineus* was detected for the first time in Serbia on both *Fraxinus excelsior* and *F. angustifolia*. In addition, it was detected for the first time in Montenegro and in the Isle of Man.

Fig. 3. A. Declining ash trees; B. Leaf necroses; C. Ascocarps of *Hymenoscyphus fraxineus*; D. Ascocarps in the litter; E. Culture of *H. fraxinues*.

Insect vectors: Major efforts have been devoted to study the biology, phenology, host-shifting, active dispersal movement and the capability to acquire and transmit the bacterium by *Philaenus spumarius*, the first ascertained *Xf* vector. Results of the mark-release-recapture experiments provided the first measurement of the active movement of the spittlebugs, which could be recaptured after 10-15 days within a 100-m radius from the release points.

Two new vector species were identified in southern Italy: *Neophilaenus campestris* and *Philaenus italosignus* (Fig. 4). Surveys in the Apulian epidemic area and comparative transmission tests have shown that the *N. campestris* is less abundant in olive groves and its transmission efficiency is lower than *P. spumarius*. On the other hand, the role of *P. italosignus* can be negligible in the current Apulian epidemic, since its presence has never been detected in the infected area.

Fig. 4. Vectors of *Xf* identified in southern Italy: *Philaenus spumarius* (left), *Neophilaenus campestris* (middle) and *Philaenus italosignus* (right).

Surveys on different crops to determine the occurrence and distribution of psyllids have shown that *Bactericera trigonica* is the most abundant species. The feeding behaviour of *Bactericera trigonica* has been characterized by means of the electrical penetration graph (EPG) technique and correlated with the *Ca*Lsol transmission (Fig. 5). *B. trigonica* was found to be a highly efficient vector of *Ca*Lsol in Apiaceae but a very poor vector in potato. *B. tremblayi* was proved able to acquire the bacterium but unable to transmit to carrots.

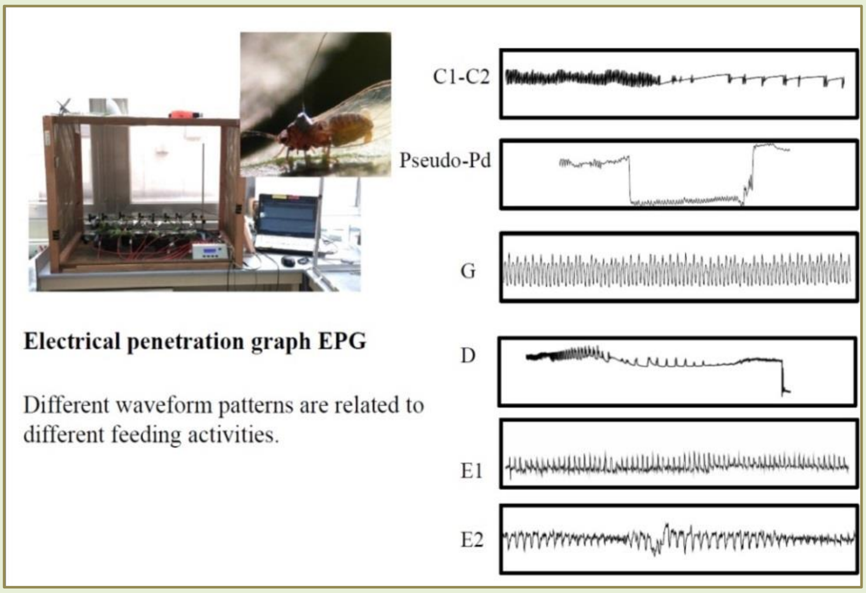

Fig. 5. Electrical penetration graph (EPG) technique used for studying feeding behaviour of *Bactericera trigonica*.

Disease and vector control: Different strategies have been explored for controlling *Xf*-induced diseases. The potential therapeutic effect of molecules (i.e. N-acetyl cysteine) has provided initial encouraging results by slightly lessening OQDS symptoms in olives. Preliminary information on the microbiome characterizing olive xylem tissues has been developed by next-generation sequencing, and bacterial/fungal endophytes have been isolated from infected olives and their potential use as *Xf* bio-control agents is under study. The beneficial bacterium *Paraburkholderia phytofirmans* PsJN was shown to be able to colonize olive, therefore its potential *Xf* bio-control is under testing (Fig. 6).

Fig. 6. Testing bacterial and fungal endophytes as *Xf* bio-control agents.

Intense research experiments are devoted to control *Xf* vectors: (i) natural enemies, i.e. *Zelus renardii* (Hemiptera: Reduviidae) (Fig. 7); (ii) use of cover crops (i.e. *Lolium* and *Hordeum*) proved to be efficacious to reduce the abundance of the juvenile populations; (iii) testing natural and synthetic insecticides confirmed the highest efficacy of neonicotinoids and pyrethroids for controlling adults of spittlebugs on olives.

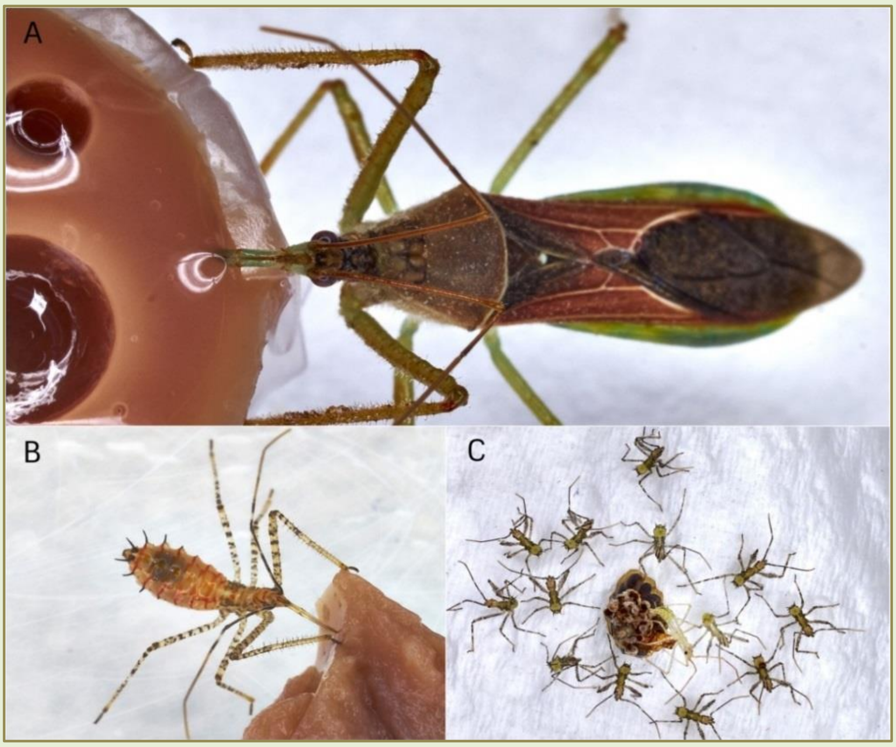

Fig. 7. A and C. *Zelus renardii* artificial diet; B. *Zelus* newborn.

Application of kaolin was tested for reducing the number of eggs and nymphs of *Trioza apicalis* in the field (Fig. 8). However, growing plants under net provided better results than the tested applications of maltodextrin, natural pyrethrin, *Beauveria bassiana*, paraffin oil and acetamiprid.

Fig 8. Field trials of *Trioza apicalis* psyllid control strategies.

Hot water treatment for ash seeds has been applied for controlling *Hymenoscyphus fraxineus*. Tolerant ash seedlings have been identified and will be vegetatively propagated onto disease-free *F. excelsior* rootstocks to produce approximately 2,500 grafted plants, to form a clone bank of tolerant trees, which could provide material for future breeding.

An accreditation scheme for the production of *Phytophthora*-free plants in nurseries is under development in the UK. The guidelines and requirements to avoid the introduction and spread of *Phytophthora* species into production fields/forest nurseries are being implemented in Austria and plant growth media are being screened in Norway.

Diagnostic tools for early detection: Numerous protocols for detection of the target pathogens were evaluated and validated, contributing to revise and update the official EPPO diagnostic protocols. Their application in monitoring and surveying led to the detection of new outbreaks (of *Xylella* in Italy, France, Spain; *Ca*lsol in Tunisia, Israel, Hf in Serbia and Phy in Great Britain).

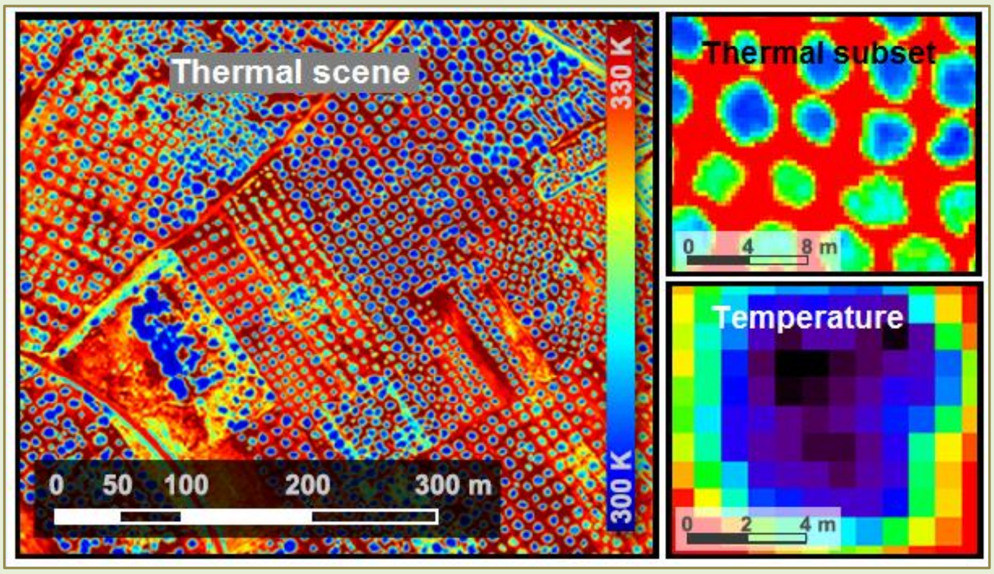

Fig. 9. Airborne hyperspectral and thermal imagery for *Xf* early detection.

Successful pre-visual identification of olive trees infected by *Xf* has been achieved using high-resolution airborne hyperspectral and thermal imagery acquisition with aerial vehicles (Fig. 9). Indicators for early and/or asymptomatic detection of *Xf* infection were developed to achieve a method for the automatic classification of *Xf* infection and severity at a large scale.

A specific robotic platform equipped with proximal sensing equipment has been developed to make fast and precise surveys of *Ca*Lsol experimental fields and serve as a future surveillance platform (Fig. 10). An aerial remote sensing system and the robot developed were used to survey carrot fields in Spain, and acquire high-resolution hyperspectral, multispectral and thermal imagery. In addition, automated traps have been developed that acquire and send images of the vectors captured in the field to a remote server.

Fig. 10. Robotic platform for *Ca*lsol surveys.

Disease impacts, modelling and pest risk assessment: The potential economic impact of *Xf* within European olives was assessed by estimating the foregone profits from declining yields and the lost investments from the premature death of orchards over a period of 50 years. The results indicate that the expected economic damages due to Xf run into the tens of billions of euros, and imply the needs to strengthen the ongoing resistance breeding campaigns and the regulatory support for farmers as well as to ensure the rejuvenation of the European olive crops.

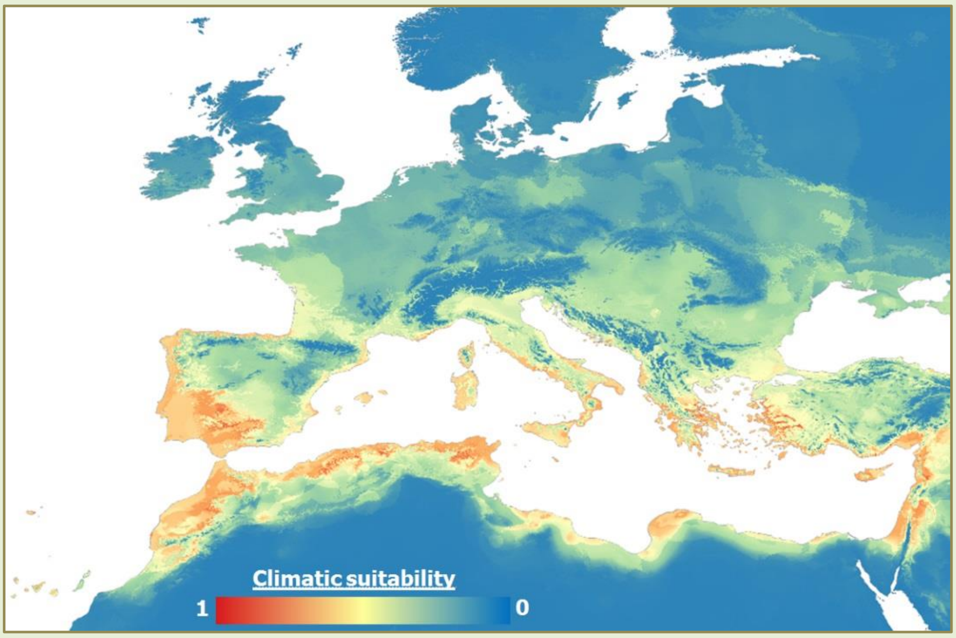

Fig. 11. Climatic suitability map for *Xf*: MaxEnt.

A climatic suitability map (Maximum Entropy) provided the area of the potential establishment (Fig. 11). The spread was simulated by means of a radial range expansion as well as a logistic growth models. The spatial pattern of olive trees affected by the OQDS in Apulia at the field scale has been characterized and proved to be aggregated.

Dissemination and communication: The outcomes of the POnTE project were made publicly available throughout different communication channels targeting different groups. The interactive project website was developed from the beginning of the project providing the communication within the project community as well as with the wider auditorium. The general public was provided with scientific information through different media, organization of meetings targeting the growers, agricultural specialists and stakeholders. The scientific results were exchanged by free access scientific papers, conference organizations and presentations, development of laboratory protocols and organization of trainings.

The developed knowledge and expertise were made available to policy makers through periodic reports as well as by participation of the project experts in *ad hoc* committees, working groups and bodies (Fig. 12).

Fig. 12. Participants of the Joint Annual meeting POnTE | XF-ACTORS, Palma de Mallorca, 2017.

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The POnTE Consortium

1. CNR, ITALIAN NATIONAL RESEARCH COUNCIL, Italy
2. UNIBA, UNIVERSITY OF BARI ALDO MORO, Italy
3. INRA, FRENCH NATIONAL INSTITUTE FOR AGRICULTURAL RESEARCH, France
4. ANSES, FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH AND SAFETY, France
5. IVIA, VALENCIAN INSTITUTE FOR AGRICULTURAL RESEARCH, Spain
6. CSIC, SPANISH NATIONAL RESEARCH COUNCIL, Spain
7. SG SASA, SCOTTISH GOVERNMENT–SCIENCE AND ADVICE FOR SCOTTISH AGRICULTURE, United Kingdom
8. FORESTRY COMMISSION RESEARCH AGENCY, United Kingdom
9. BFW, FEDERAL RESEARCH AND TRAINING CENTRE FOR FORESTS, NATURAL HAZARDS AND LANDSCAPE, Austria
10. LUKE, NATURAL RESOURCES INSTITUTE FINLAND, Finland
11. WU, WAGENINGEN UNIVERSITY, The Netherlands
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13. UCR, UNIVERSITY OF COSTA RICA, Costa Rica
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22. AGRITEST SRL, Italy
23. CITOLIVA FOUNDATION, INNOVATION AND TECHNOLOGY CENTER FOR OLIVE FARMING AND OLIVE OIL, Spain
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