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Xylella fastidiosa & the Olive Quick Decline Syndrome (OQDS)

A serious worldwide challenge for the safeguard of olive trees

Edited by:
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The Meadow Froghopper philaenus spumarius,
photo courtesy of Dr. Vincenzo Cavalieri, IPSP-CNR, Italy

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Foreword

Olive trees (*Olea europaea*) have been landmarks of the Mediterranean region for thousands of years. Unfortunately, this precious resource is now severely threatened by the bacterium *X. fastidiosa*, a devastating xylem-restricted quarantine organism, which affects more than 350 plants worldwide, belonging to crop, ornamental, forestry and natural vegetation species. This pathogen is vector-transmitted by several xylem-feeding insects and is originated from the American continent. The outbreak of *X. fastidiosa* in the South of Italy in 2013, as first report of this pathogen in the European and Mediterranean regions, poses the great risk of its spread in the whole area primarily on olive trees. Nevertheless, several host plants may stay symptomless or show mild symptoms. The strain of *X. fastidiosa* found in Italy (named "CoDiRO") belongs to the subspecies *pauca*, infects at least 28 host species and is vectored by the spittlebug *Philaenus spumarius*. After the finding in Italy, several interceptions of the infection occurred in Europe, mainly on coffee plants originated from Costa Rica and Honduras. Several emergency measures were taken, strengthened and updated with the aim to preventing the further spread of the bacterium within the EU (EU implementing Decision 2015/789). Outbreaks of different *Xylella* subspecies were also detected in France (2015), Germany (2015) and Spain (2016). There is no record of successful eradication of *X. fastidiosa* once established outdoors due to its broad range of host plants and vectors. Control efforts imply preventing the introduction and managing infected areas to reduce the spread of the pathogen.

Following the outbreak of *X. fastidiosa* in Italy associated to the olive quick decline, the National Plant Protection Organizations (NPPOs) of the North-African and Near-East countries (NEPPO) and the Euro-Mediterranean countries (EPPO) have strongly expressed their concern and the need to get information and training for setting up efficient measures for preventing the introduction and spread of this pathogen and its vectors. Therefore, the Food and Agriculture Organization (FAO), the International Plant Protection Convention (IPPC) and the CIHEAM, with the support of NEPPO and EPPO, have jointly organized a dedicated international workshop to share updated information on the infection and to train NPPOs on the recent developed methods and technical protocols for early surveillance, diagnosis and control of *Xylella fastidiosa* and its vectors. The workshop, held at CIHEAM Bari (19 - 22 April, 2016), was attended by 140 participants from 40 different countries, mainly from Europe and the Mediterranean region. The first day of the workshop was also open to plant protection inspectors from Italian regions and delivered in streaming. In the workshop, a great scientific contribution was provided by the Puglia research institutions deeply involved in the research on the pathogen and its vectors and by researchers from the EUPHRESCO network.

Following the success of the FAO-IPPC-CIHEAM initiative, a second international workshop was jointly organized by the International Olive Council (IOC) and the CIHEAM at the end of 2016 (CIHEAM Bari, 28-30 November, 2016). It was aimed at developing capacity and raising awareness on the threat of *X. fastidiosa* for the safeguard of olive trees worldwide in order to propose recommendations to prevent, identify, and combat this pathogen. The workshop was also held and attended by representatives of IOC members from 14 countries (Albania, Algeria, Argentina, Egypt, the EU, Iran, Israel, Jordan, Lebanon, Libya, Morocco, Tunisia, Turkey and Uruguay). Updated information on the advances of research and EU phytosanitary measures on *X. fastidiosa* was presented, primarily focusing on the Puglia strain causing the Olive Quick Decline. The workshop was concluded with a round table coordinated by CIHEAM and animated by IOC, FAO-IPPC, EPPO and all the other research institutions involved. It provided IOC members, as stakeholders of the olive sector, explanations and suggestions on actions to be taken in their own countries for preventing or early detecting *X. fastidiosa*.

The information acquired during the workshops and the technical visits to the outbreak area of Puglia showed the destructive impact of *X. fastidiosa* infections primarily on olive trees. The main outcomes from the 2 workshops are hereafter summarized:

- *Xylella fastidiosa* is a phytosanitary priority for Europe, the Mediterranean region and olive-producing countries worldwide.
- *Philaenus spumarius* is the only assessed vector of the CoDiRO strain of *Xylella* affecting olive trees in Puglia; all xylem-feeding insects may be potential vectors of the pathogen.
- The number of recognized host plant species worldwide is about 380, 28 of which are presently assessed as hosts of the CoDiRO strain; however, this number is going to increase as the infection spreads in new areas.
- Running research projects are providing knowledge on the pathogen, its vectors and control measures.
- Several tools for pathogen surveillance and early detection are now available.
- No treatment solutions are currently applicable for pathogen elimination in the open field; however, specific treatments for the control or mitigation of the pathogen/disease in olive trees and the evaluation of tolerance/resistance of olive cultivars are under investigation.
- The certification of plant propagating material of olive and ornamental host plants should be mandatory for *X. fastidiosa*.
- An efficient communication plan for stakeholders and civil society should be prepared in advance by the NPPO for raising awareness and building capacity in the surveillance of the infection and in the application of phytosanitary measures. The plan will supply media & press with a correct information.
- Scientific, technical and political collaboration among countries should be strengthened through the establishment of a dedicated forum and through the technical support of quarantine laboratories.
- International organisations and institutions which are dealing with this emergency and with the olive sectors should strengthen the cooperation for combating *X. fastidiosa* worldwide.

This publication represents a valuable source of information on the state of the art of *X. fastidiosa* infection in the EU and in the Mediterranean region. The book is structured in three parts, with an introduction by the promoting International Organizations of CIHEAM, FAO, IPPC, IOC and EPPO. In part one, a general overview on the infection, pathogen surveillance and diagnostic methods is given; in part two, the focus is on control strategies, legislative aspects, capacity development and communication. In part three, a consistent scientific bibliographic review is provided. Contributions of the experts from the 2 workshops are included as short notes.

Cosimo Lacirignola
Secretary General of CIHEAM

Introduction

CIHEAM actions to combat *X. fastidiosa* in the Mediterranean region

Cosimo Lacirignola[1], Maurizio Raeli[2]

[1] CIHEAM - Paris
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For its strategic role in the Mediterranean agricultural area, CIHEAM, an intergovernmental organization gathering 13 member countries, could not turn a blind eye to the serious phytosanitary emergency due to the introduction of *Xylella fastidiosa* in Puglia (southern Italy), a region which hosts one of its four Institutes, the CIHEAM Mediterranean Agronomic Institute of Bari (MAIB).

CIHEAM Bari, which was founded in 1962, has hosted more than 40 000 trainees, technicians, experts, researchers and public officers; the activities that it has been carrying out for more than 50 years have involved 3 000 people, 650 scientific institutions of 50 different countries; at present, it plays an active role in 90 research and cooperation projects.

The phytosanitary status of Mediterranean crops, and, in particular, that relating to the "Certification of the plant propagating material" and to "Quarantine", has always been strategic for the Mediterranean agriculture. To this end, in 1985, jointly with the FAO, CIHEAM Bari launched the Master of Science course on "Sanitation and Certification of the Plant Propagating Material" which is now a course on the "Integrated Pest Management of Mediterranean Crops".

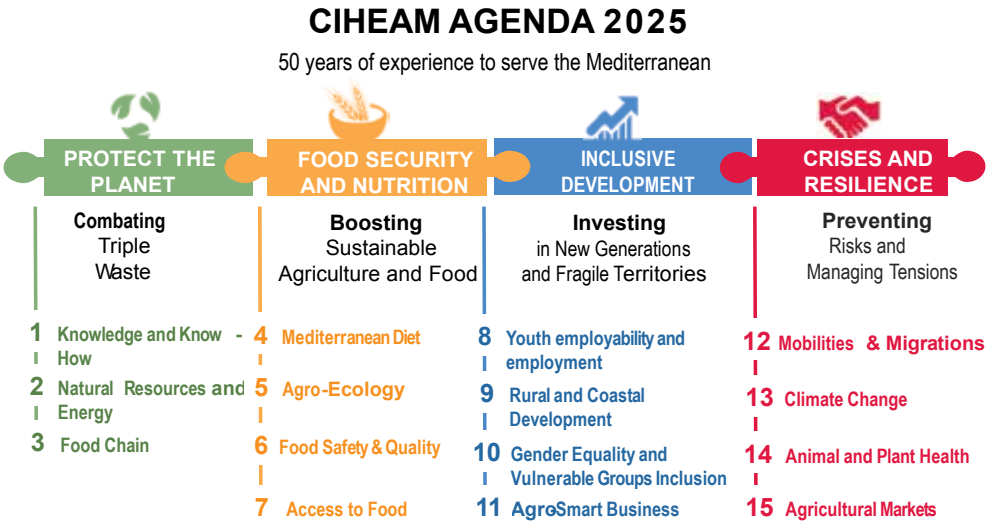
Over the years, awareness-raising and training actions on these topics have been backed up by the promotion of meetings, research networks with several Mediterranean scientific institutions, and of bilateral research and cooperation projects (with Albania, Algeria, Egypt, Lebanon, Kosovo, Malta, Serbia, Syria, Tunisia, etc.) in which CIHEAM Bari has instilled its long experience gained in the regional territory. In fact, it has been involved, together with institutions and the phytosanitary service, in activities of Voluntary Certification of the plant propagation material and of monitoring and eradication of some major quarantine diseases (sharka of stone fruits, citrus tristeza, grapevine golden flavescence, fireblight of pome fruits, etc.).

Thus, CIHEAM Bari has also contributed to finding solutions to the serious threat posed by *Xylella fastidiosa* to the Puglia olive industry. This is witnessed by the scientific publications on this topic, 13 Master of Science or PhD theses in collaboration with other Italian scientific institutions, the training of over 300 local and foreign technicians on investigation and diagnostic techniques, about 45 000 diagnostic tests on plants and/or insects, participation in some major national (e.g. Mix-CoDiRO) and international (e.g. Horizon 2020, *Xf*-Actors) research projects. A ceaseless activity which has yielded fruitful results mainly in the framework of plant pathology diagnostics (technical protocols of DTBIA and real-time LAMP techniques, which are reliable, highly sensitive, ready to use) and of IT tools for large-scale pest surveillance (XylApp and XylWeb).

The correct scientific investigation, the tight collaboration with scientific and institutional operators from different countries and timely measures are crucial for tackling such emergencies. This is why CIHEAM has promoted the organization of two international workshops on *Xylella fastidiosa* and the Olive Quick Decline Syndrome (OQDS) which have triggered so much interest: the first in collaboration with IPPC-FAO (International Plant Protection Convention) in the framework of the CIHEAM-FAO Memorandum of Understanding signed in 2015, with the involvement of NEPPO and EPPO (Near East and Euro-Mediterranean Plant Protection Organizations), addressed to officers, technicians and experts of Mediterranean and Middle-East National Plant Protection Organizations (NPPOs); the second with the International Olive Council (IOC) with the purpose

to provide updated information on the current situation of the infection in order to propose recommendations to prevent, identify, and combat the OQDS in IOC member countries worldwide.

“Plant Health” is one of the 15 pillars in the CIHEAM Strategic Agenda 2025 which will enable CIHEAM to serve the Mediterranean area through its 50 years of experience in the field of education and training, research and cooperation in agriculture. An ambitious programme which aims at: (i) the protection of the planet struggling against triple waste (not only waste of food and natural resources but also of knowledge); (ii) food security and correct nutrition with the use of sustainable agriculture and promotion of the Mediterranean diet; (iii) inclusive development (investing in new generations and women) and development of marginal areas; and (iv) understanding the root causes of crises (migrations, climate change, emerging diseases) through risk prevention and management of international tensions.



FAO preventive actions to the introduction and spread of the Olive Quick Decline Syndrome OQDS - associated to *Xylella fastidiosa* in NENA Region

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The occurrence of the Olive Quick Decline Syndrome (OQDS) caused by *Xylella fastidiosa* in Puglia region (Italy) with the strain Co.Di.R.O affecting olive trees poses an enormous threat to olive production in all the Mediterranean countries. *X. fastidiosa* is a regulated pest in many countries in the world. Around 95% of olive trees are cultivated in the Mediterranean region, where the Near East and North Africa (NENA) countries rank second in terms of global production of olives, after Southern European countries (Spain, Italy and Greece). In spite of their economic relevance, olive trees have important historical and cultural roots in the NENA countries, where Syria is known as the first olive-producing land in the ancient world and the area of origin of olive growing. The olive production and olive oil sector is one of the important sources of income of thousands of families and of food security in the region. The first report of *X. fastidiosa* on olive trees in Southern Italy and consequent estimated cost of olive trees losses in this region (€250 millions) raised an alert on the emerging threat to the entire Mediterranean Basin due to favourable climatic conditions for the epidemic spread of the infection. The host complexity of *X. fastidiosa* and diversity of the ways of its spread increase the risk of its introduction into the countries of the NENA region through the movement and trade with potentially infected host plants. These facts impose the necessity for reviewing and strengthening the phytosanitary measures applied in the region and putting in place a harmonized surveillance programme in the NENA countries. However, the NENA countries lack technical capacities/expertise to deal with this new emerging disease; therefore they approached the FAO for technical assistance. Hence, the FAO Regional Office for the Near East and North Africa (FAO-RNE) embarked on preparing technical support programmes to help countries raise the awareness about this disease, strengthen their capacities for the enforcement of appropriate phytosanitary regulations/measures, and put in place effective surveillance and monitoring programmes. The FAO-RNE has communicated the risk of the OQDS disease to all National Plant Protection Organizations (NPPOs) and other stakeholders through email and other media. Special side sessions on the Olive Quick Decline Syndrome and *X. fastidiosa* were organized by the FAO and IPPC in the framework of the Regional IPPC Workshop held in Amman, Jordan, in September 2015 and the 11th Session of the Commission for Phytosanitary Measures held in Rome, Italy, in April 2016.

An FAO Regional Technical Cooperation Programme (TCP) will be launched in the mid of 2016 to support the Near East and North Africa Countries in their efforts to enforce preventive measures for the introduction and spread of *Xylella fastidiosa* – Olive Quick Decline Syndrome in their territories.

TCP will support the governments' efforts to reduce the risk of introduction and spread of *X. fastidiosa*, and its harmful effects. TCP is expected to support participating countries in developing a contingency and surveillance plan to prevent the introduction of the disease; it will build institutional capacity and the skills of technical staff and farmers on early detection, diagnosis, surveillance and phytosanitary measures. In addition, TCP will raise the awareness of

all stakeholders on the risk of the disease and ensure their active involvement in the promotion of preventive measures. The regional coordination and information and knowledge sharing will be established and maintained by TCP between the countries in the region and with the international experts and partner institutions involved in the project.

The actions envisaged by the project aim to provide a legislative basis for dealing with the emergency caused by *X. fastidiosa*. At the same time, activities for upgrading and professionally training phytosanitary inspectors and technical experts involved in the phytosanitary diagnosis are planned, in order to increase the professionalism of those who are involved in the emergency. Similar training will be targeted at public and private technicians engaged in agricultural extension services. All this can be the basis for future interventions supported by the countries or donors, which may include the supply of instruments and equipment necessary for monitoring and diagnostic tests, as well as training and awareness raising activities.

Improved technical capacity will enable countries to take proper action in case of emergency, and raise strong public awareness on the seriousness of the disease and its associated risk; it will facilitate the implementation of the measures to be taken to reduce the risk of introduction and spread of *X. fastidiosa* in their territories.

The expected impact of the project is “Prevention of the risk of the introduction and spread of *X. fastidiosa* – Olive Quick Decline Syndrome in the NENA region, and saving the livelihood and income of olive growers and national economies”. Hence, it is envisaged that the project will positively contribute to saving more than 40 million hectares of olive trees and the income and livelihood of more than 500,000 farmers in all participating countries.

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The International Plant Protection Convention (IPPC) and its standards on pest surveillance and diagnostics to prevent the entry of *Xylella fastidiosa*

Adriana Gonçalves Moreira[1], Jingyuan Xia[2], Sarah Brunel[1]

[1] International Plant Protection Convention (IPPC)
[2] Food and Agriculture Organization of the United Nations (FAO)

1. International travel and trade are greater than ever before – and as people and commodities move around the world, organisms that present risks to plants travel with them. Pest introductions and outbreaks cost governments, farmers, consumers and the environment billions every year. The global community has responded to these challenges through international agreements and other mechanisms.

2. The International Plant Protection Convention (IPPC) established in 1952[1] is an international plant health agreement that aims to protect cultivated, wild plants and plant products by preventing the introduction and spread of pests. As of March 2017, the IPPC has grown into a body that encompasses a worldwide membership of 183 contracting parties.

3. The implementation of the Convention involves collaboration by the IPPC contracting parties, which have the obligation to set up a national plant protection organization (NPPO), the official services established by governments to discharge the functions specified by the IPPC, and regional plant protection organizations (RPPOs), which can act as coordinating bodies at a regional level to achieve the objectives of the IPPC. Suggestions for international standards can be made by national or regional plant protection organizations. They are developed by expert drafting groups and reviewed by technical committees, NPPOs and RPPOs and adopted by the CPM. The Secretariat of the IPPC is provided by the Food and Agriculture Organization of the United Nations.

4. In agreeing to the rights and obligations of the Convention, contracting parties shall make provision, to the best of their abilities, for an official NPPO with the following responsibilities: (a) the issuance of Phytosanitary certificates; (b) the surveillance of growing and wild plants and of plant products; (c) the inspection of consignments of plants and plant products moving in international traffic; (d) the disinfestation or disinfection of consignments of plants, plant products and other regulated articles moving in international traffic, etc.

5. To implement these responsibilities, the IPPC provides an international framework for plant protection that includes developing International Standards for Phytosanitary Measures (ISPMs) for safeguarding plant resources.

6. International Standards for Phytosanitary Measures (ISPMs) are internationally agreed upon phytosanitary measures that have been adopted by the Commission on Phytosanitary Measures (CPM), which is the governing body of the IPPC. ISPMs cover invasive pests of plants, including invasive alien species that cause damage to plants both directly and indirectly. In order to help manage these pests, they must be regulated within the territory of the importing contracting party, and these are defined as “regulated pests”. ISPMs extend beyond the protection of cultivated plants to the protection of wild flora. The standards cover the movement of traded goods such as plants and plant products and also apply to vehicles, ships, aircraft, containers, storage places, soil, wood packaging and other objects

that could harbour plant pests. ISPMs facilitate safe trade by providing general and specific guidance on procedures, regulations and treatments that can be used to manage pest risks associated with the international movement of goods and conveyances.

7. The IPPC is the only standard setting organization for plant health recognized by the members of the World Trade Organization under the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement).

8. There are 80 ISPMs[2] as of March 2017, including 22 diagnostic protocols for regulated pests as Annexes to ISPM 27 (*Diagnostic protocols for regulated pests*) and 21 phytosanitary treatments as Annexes to ISPM 28 (*Phytosanitary treatments for regulated pests*). All ISPMs, including those in draft and consultation stages, are freely available through the IPPC Web site (www.ippc.int) or from the IPPC Secretariat.

9. The ISPM 6 (*Guidelines for surveillance*) was adopted in 1997 and describes the components of survey and monitoring systems for the purpose of pest detection and the supply of information for use in pest risk analyses, the establishment of pest free areas and, where appropriate, the preparation of pest lists. Surveillance is an obligation of NPPO and underpins other obligations and phytosanitary decision-making. It is a critical part of the national phytosanitary system. Plant pest surveillance thus plays a key role in the overall mandate of the NPPO.

10. A national plant pest surveillance programme should be conducted in such a way that its results are accurate, credible and contribute to national goals and priorities. Management support is critical to a strong, sustainable programme. Surveillance activities can be expensive. However, as activities that support national phytosanitary policy, the benefits will invariably outweigh the costs. This guidance on surveillance are of uttermost relevance for the early detection of *Xylella fastidiosa*.

11. Diagnostic protocols are crucial for an accurate pest diagnosis, as pest diagnosis is the basis of an effective pest surveillance. Thus, diagnostic services are fundamental to the success of a national plant pest surveillance system. The ISPM 27 (*Diagnostic protocols for regulated pests*[3]) and its annexes contain the minimum requirements for reliable diagnosis of a specified regulated pest, and provide flexibility to developing and developed countries to ensure that methods are appropriate for use in the full range of circumstances for which a diagnostic protocol may be used (e.g. from detection of a pest in a consignment, to surveillance, to routine diagnosis). The methods included in diagnostic protocols are selected on the basis of their sensitivity, specificity, and reproducibility. Only methods of relevance for diagnostics are given in the protocol. Information on record keeping and contact points are provided to help laboratories and NPPOs in the implementation of the methods described in the protocols. The IPPC has in its work programme the development of a diagnostic protocol for *Xylella fastidiosa*, which is expected to be submitted for adoption in 2018.

12. The IPPC provides a framework and forum for international cooperation, harmonization and technical exchange between contracting parties, which are crucial for the prevention of *Xylella fastidiosa*. Organizations can share and consult contributed resources on this pest, as well as other phytosanitary material freely at http://www.phytosanitary.info.

Notes

[1] The IPPC convention text was adopted by the 6th Conference of the Food and Agriculture Organization of the United Nations (FAO), superseding previous international plant protection agreements.

[2] Adopted ISPMs: https://www.ippc.int/core-activities/standards-setting/ispms

[3] ISPM 27. Diagnostic protocols for regulated pests: https://www.ippc.int/publications/diagnostic-protocols-regulated-pests

Overview of the activities and projects on *Xylella fastidiosa* of the International Olive Council (IOC)

Abdellatif Ghedira, Francesco Serafini

International Olive Council (IOC), Madrid - Spain

The International Olive Council is the world's only international intergovernmental organization in the field of olive oil and table olives. It was set up in 1959 under the auspices of the United Nations. The Council is a decisive player in contributing to the sustainable and responsible development of olive growing and serves as a world forum for discussing policymaking issues and tackling present and future challenges.

For this reason, it is the ideal framework for driving effective multilateral cooperation among all its member countries to help find joint solutions to any problems and to encourage collaborative research and development activities on topics of general interest to Members.
These activities help to convert the objectives and aspirations of individual countries into collective efforts. Communication, cooperation and coordination to achieve a common objective and information sharing are just some of the benefits of collective action channeled through joint projects.

In October 2015, the new International Agreement on Olive Oil and Table Olives, 2015, was negotiated in Geneva under the umbrella of UNCTAD. This new Agreement will enter into force on 1 January 2017 and lays down the terms of reference for the IOC to carry out activities for the benefit of its countries. The new Agreement has many objectives in different spheres ranging from chemistry and standardization to technical cooperation and promotion. In the specific case of technical cooperation, its aims are:

- to promote technical cooperation and research and development in the olive sector by encouraging the cooperation of public or private bodies and/or entities, whether national or international;
- to conduct activities for the identification, preservation and utilization of the gene sources of olive trees;
- to study the interaction between olive growing and the environment, particularly with a view to promoting environmental conservation and sustainable production, and to ensure the integrated and sustainable development of the sector;
- to foster the transfer of technology through training activities in the fields connected with the olive sector by organizing international, regional and national activities;
- to encourage the exchange of information and experience on olive growing in the phytosanitary field.

Beside these key objectives assigned in the Agreement, the member countries have tasked the IOC to work on issues relating to *Xylella fastidiosa* for which it is a reference marker.
Xylella is a problem of close concern to all the member countries of the IOC, which produce almost 98 per cent of the world's olive oil.

In 2015, to try to grapple with this problem, the IOC brought together representatives from international organizations such as EPPO, EFSA and the EU as well as from the Ministries of Agriculture of France, Italy and Spain to fix the terms of reference of an international IOC workshop on the subject of *X. fastidiosa*.

In the meantime, the IOC has given its support for projects submitted under the EU Horizon 2020 programme to find solutions to this problem.

In recent years, the IOC has implemented various R & D projects as part of its technical cooperation programme. One in particular – the RESGEN project – has brought together 22 producer member countries with the common aim of conserving, collecting and using their genetic olive resources. Each country has morphologically characterized its autochthonous varieties and created a reference collection housing most of the genetic variability in the *Olea europaea* species present in their country. More than 1300 accessions have been recovered.

In addition to the 22 national reference collections created under the project, the IOC has also set up and recognized three world collections to which all the plant material recovered and conserved by the individual countries is being shipped. These three world collections are located in Cordoba, Marrakech and Izmir. Clearly, this is just a first step. A new project will shortly get off the ground using molecular markers (microsatellites) and a joint methodology to characterize the plant material held in the reference national and world collections in order to shed light on the ever-persistent problem of homonymy and synonymy.

The IOC is willing to make all this plant material available to the scientific community to allow testing for varietal resistance/tolerance of the *Xylella* bacterium. This can be done either through agreements with the IOC and with the countries that ship the material or by implementing an *ad hoc* project. This wealth of varietal material could be the starting point for projects and training activities for member countries which will thus have the opportunity to see the problem at first hand and learn how to handle it.

At the end of 2016 (28-30 November), IOC and CIHEAM organized, at the Mediterranean Agronomic Institute of Bari (IAMB), the international workshop on "*Xylella fastidiosa: a serious worldwide challenge for the safeguard of olive trees".* The workshop included sessions by international specialists, a field technical visit to the demarcated area of *Xylella* in the South of Puglia and a round table to identify common measures.

The purpose of the meeting was to provide an update on the current situation and the development of the bacterium in order to propose recommendations to prevent, identify, and combat this infection. To this end, the organizers had invited specialists from some national and international institutions, namely the Italian Research Council (CNR), the Food and Agriculture Organization of the United Nations (FAO), the European and Mediterranean Plant Protection Organization (EPPO), the French Agency for Food, Environment and Occupational Health & Safety (ANSES), the Council for Agricultural Research and Agricultural Economic Analysis (CREA), the Centre for Agronomic Research, Experimentation and Training (CRSFA), the International Plant Protection Convention (IPPC-FAO), the Directorate General for Health (European Commission), the University of Basilicata, the University of Foggia and the University of Bari.

Following the opening speech by the Secretary General of CIHEAM, Cosimo Lacirignola, the subjects addressed during the first day, chaired by Francesco Serafini, Head of the IOC Department of Research & Development and the Environment, and Françoise Petter of the EPPO, concerned the monitoring tests carried out on the bacterium and its vector, strategies and agricultural measures to combat the rapid decline syndrome in olive trees, plant certification, research, current legislation and the instruments available to monitor the bacteria and detect it in its early stages.

The participants, including the Vice Chairperson of the IOC Advisory Committee and the representatives of some IOC member countries (Albania, Algeria, Argentina, Egypt, the EU, Iran, Israel, Jordan, Lebanon, Libya, Morocco, Tunisia, Turkey and Uruguay), could observe the impact of *Xylella fastidiosa* on the ground as well as the symptoms of infestation, familiarize with the

sampling techniques used on plant material and vector insects and examine the results of the tolerance/resistance tests carried out on different olive varieties.

During the round table session, coordinated by CIHEAM-IAMB, the participants shared their concerns regarding the disease and highlighted the need to strengthen scientific, technical and political collaboration among countries, to create a forum for the exchange of information and to support the quarantine laboratories in member countries to prevent the proliferation of the bacterium through the international trade of plants and plant material by adopting a certification procedure. They also recommended some measures to strengthen cooperation between the IOC, and international organizations currently working on this disease, namely the CIHEAM, the FAO (IPPC), EPPO and the CNR, which invited the IOC to contribute to the work of an *ad hoc* committee in the EU Horizon 2020 research project initiative on *X. fastidiosa* (*XF*-Actors).

At the end of the workshop, the Executive Director of the IOC, Abdellatif Ghedira, thanked the specialists from IOC member countries, which account for 94% of olive growing worldwide, the Vice Chairperson of the Advisory Committee and the international experts. He indicated that *Xylella fastidiosa* is one of the priorities of the Executive Secretariat and recalled that, in addition to the IOC's role as an international centre for documentation and information on the sector, the International Agreement on Olive Oil and Table Olives (2015) gave particular importance to international cooperation and to scientific and technical exchanges on olive growing among member countries.

From science to policy, the contributions of EPPO and Euphresco to the *Xylella fastidiosa* emergency

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Given the very serious threat to the agriculture and environment, *Xylella fastidiosa* was added to the EPPO A1 list of pests recommended for regulation as quarantine pests in 1981. Following the recent detection of the bacterium in Italy and France, the EPPO (European and Mediterranean Plant Protection Organization) member countries have agreed to start several activities under the coordination of the EPPO Secretariat.

In October 2013, the EPPO Secretariat prepared a specific webpage on *Xylella fastidiosa* http://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa/Xylella_fastidiosa.htm which includes a brief description of the pathogen and its known vectors, the symptoms in the main host plants, its geographical distribution (with details on the most recent outbreaks in Italy and France) as well as an easy access to specific EPPO data e.g. EPPO Datasheet, EPPO Diagnostic protocols, EPPO Standards on phytosanitary procedures and other useful resources e.g. EFSA database on host plants of *Xylella fastidiosa*, EU Commission webpage on *Xylella fastidiosa*.

Besides supporting knowledge exchange in the region, the EPPO Secretariat ensures a number of other activities in plant quarantine: identification and evaluation of potential risks and development of pest risk analyses, recommendations on pests which should be regulated as quarantine pests (EPPO A1 and A2 lists), preparation of regional Standards (e.g. official control, diagnostic protocol, inspection procedures). In view of the high profile of the outbreak of *X. fastidiosa* in Europe, the EPPO Working Party on Phytosanitary Regulations agreed that the EPPO Diagnostic protocol on *X. fastidiosa* should be revised (previous version dated from 2004) and two Inspection Standards on *X. fastidiosa* should be prepared. The three Standards were prepared and sent to members for approval through an official EPPO country consultation. The National Plant Protection Organisations will provide their feedbacks on the documents whose content will be amended accordingly.

The Standards on 'Phytosanitary procedures for inspection of places of production' and on 'Phytosanitary procedures for inspection of consignments' have been prepared under the leadership of the EPPO Panel on Phytosanitary Inspections. The first document describes the procedures for inspection of places of production of plants for planting which are susceptible to *X. fastidiosa* for export or for internal country movements. The second one describes the procedures for inspection of consignments for detection of *X. fastidiosa* on host plants and insect vectors. The main content of these Standards is presented below.

Descriptions of symptoms in the main host plants are presented to support visual inspection and selection of plant material. Recommendations on how to sample are also provided. These recommendations are as follows. In the case of symptomatic plants, the sample should consist of branches/cuttings representative of the symptoms seen on the plant and containing at least 10 to 25 leaves (depending on leaf size). The Standard recommends that symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected from several plants showing similar symptoms. For asymptomatic plants, the sample should be representative of the entire aerial part of the plant. Foliage, branchlets, leaves and all accessible container surfaces, including floor or walls, should be examined to look for live insect vectors. The size of the unit of inspection (minimum number of individuals to be examined) to be selected for inspection at a specified level of infection in a specified lot size, is given according

to ISPM no. 31 'Methodologies for sampling of consignments'. To maximize the likelihood of detection, inspections and sampling during the period of active growth and after warm periods is recommended. For outdoor plants in Europe this period is usually between late spring up to autumn. For tropical plant species grown indoors such as coffee plants, sampling all year round is considered appropriate. Sampling after warm periods (e.g. late summer-early autumn) increases the probability for an accurate bacterial detection.

An Expert Working Group was formed for the revision of the EPPO Diagnostic protocol on *Xylella fastidiosa* (PM 7/24). In this Standard, recommendations for the preparation of the sample in the laboratory are provided, based on the type of sample (individual plants, composite samples, dormant plants and cuttings) and on the host plants and type of tissue (petioles, midribs, leaves, etc.). The screening tests described in the Standard are either serological (immunofluorescence, direct tissue blot immunoassay -DTBIA-, enzyme-linked immunosorbent assay -ELISA-) or molecular (conventional PCR, real-time PCR, loop mediated isothermal amplification -LAMP-). Testing for asymptomatic plants in an outbreak area or a buffer zone around an outbreak often implies that a high number of tests need to be performed. In such a situation and given that the concentration of the bacterium is expected to be higher than in an area thought to be pest free, a single test including serological tests (e.g. ELISA) may be performed. Unlike other EPPO protocols for bacteria, isolation is not recommended as a screening test as the bacterium is very difficult to isolate. Subspecies determination by molecular tests (PCR for multi locus sequence typing, conventional PCRs, multiplex PCR) and/or sequencing analysis should then be performed. Validation data for most of the tests included in the EPPO Diagnostic protocol are available from the EPPO Diagnostic Expertise Database http://dc.eppo.int/validationlist.php.

The EPPO Country Consultation will close on April 30, 2016. The EPPO Panel on Diagnostic in Bacteriology will meet in Paris to discuss the comments received and finalise the Standards taking into account the remarks received.

Since April 2014 the EPPO Secretariat has been hosting the Euphresco network Secretariat, thus coordinating phytosanitary research in the region. Euphresco members have funded so far ca. 60 research projects for a total budget of 12 M€, and 15 projects were funded in 2015 among which the project 'Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors (PROMODE)'. Coordinated by the CNR of Bari (Italy), the consortium is composed of 18 research institutes directly funded through Euphresco and 5 organizations that are participating with their own funds. PROMODE will provide further validation of sampling methods to effectively determine the presence of *X. fastidiosa* in symptomatic and asymptomatic plant materials and insect vectors to be used for the development of guidelines for sampling. Improved protocols for bacterial isolation from difficult matrices will be developed, and work undertaken to adapt tests (digital PCR, Next Generation based MLST) for a sensitive detection of *X. fastidiosa*. A test performance study and training workshops will be organised to support knowledge exchange and the use of best practices.

In the framework of Euphresco, an inventory of the national research projects focussing on *X. fastidiosa* was performed in 2015. Such information will allow to identify competences scattered throughout Europe, to efficiently exploit the scientific knowledge produced in order to avoid programme duplication and to ensure that valuable results are readily disseminated to the plant health community to support further research and provide scientific evidence for the policy makers.

Regular meetings are organised with other international organisations sharing an interest in *X. fastidiosa*, such as the International Plant Protection Convention Secretariat, the Near-East Plant Protection Organization, the European Commission, the European Food Safety Agency and the International Olive Council, to avoid duplication of efforts.

Part One

General overview, surveillance & diagnostic methods

The world threat of *Xylella fastidiosa*

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Xylella fastidiosa is a xylem-limited, fastidious bacterium, considered as quarantine organism in many countries. It was mainly present in America but it has been recently identified in Italy in 2013 (Saponari *et.al.*, 2013) and in France in 2015 (EPPO, 2015). Its economic importance is due to several characteristics of this pathogen and to the disease it causes (Janse, J.D., Obradovic, A. 2010): 1) the large number of hosts (359 potential hosts according to EFSA, 2015a, and new plant hosts are discovered every year), the severe symptoms induced (mainly leaf scorch, wilting, dieback and decline); 2) the different diseases it causes in economically important plants (Pierce's disease on grapevine, citrus variegated chlorosis, plum leaf scald, almond leaf scorch, olive quick decline syndrome, etc.); 3) the long list of xylem sap-sucking vectors (among the local *Homoptera*, *Cicadellidae* and *Cercopidae* present in the different countries), the absence of latent period in the transmission, meaning that bacteria are persistently transmitted once the adult vector has acquired the pathogen, and 4) the very difficult and expensive chemical or integrated control (of the vectors and the disease). In addition, resistant varieties of commercial interest are not yet available for the most important hosts. Consequently, exclusion, eradication or contention are the main management options when the bacterium is identified in a new area (EFSA, 2015b).

X. fastidiosa has six subspecies that have been reported as causing symptoms in a more or less high number of hosts but its epidemiology is not well known in most of them, with the exception of Pierce's disease and olive quick decline syndrome. The bacterium can adapt to different climatic conditions although low winter temperatures (below -8ºC annual minimum temperature) limits its dissemination. Symptoms are mainly shown in late spring, summer and early autumn and they are favoured by high temperatures (25-28ºC) and stressed conditions for the crop. The bacterium is present in roots and the aerial parts and forms biofilms in the plant vessels, making water and nutrient transport difficult and favouring symptoms appearance.

The unexpected discovery of *X. fastidiosa* in Italy in 2013 and in France in 2015 has made more evident the risk that this pathogen represents not only for European commercial crops, landscape trees and ornamentals, but also for agriculture in other continents. The available information suggests that the CoDiRO strain detected in olive in Italy could have been introduced there with ornamental plants imported from Costa Rica. In addition, there have been many interceptions since 2014 in the inspections performed in several borders of the European Union in coffee plants but also in other ornamentals imported from Costa Rica, Honduras, Ecuador and Mexico by Austria, France, Italy, Germany, Slovakia and The Netherlands.

This demonstrates, once more, that the uncontrolled global market could lead to a global dissemination of some quarantine organisms. The Italian outbreak is the paradigm of how the plant pathogenic bacteria have been able to overcome the European Union legislation that protects the international trade without taking into account the phytosanitary risks.

However, the European Union has reacted fast against this pathogen, published several Commission Decisions in 2014, 2015 and 2016 and drastic controls have been implemented to avoid the introduction of contaminated plants in its territory. However, the European Union (and probably many other countries) have imported in the last ten years a large number of plants of *X. fastidiosa* hosts, from areas where the bacterium was present and the plants were not specifically analyzed for this pathogen. As an example, there were more than 35000 t of potted

plants imported every year since 2010 to 2014 from Costa Rica, Guatemala, Honduras and other countries where this pathogen is present that were not analyzed. Unfortunately, in the majority of the countries, the phytosanitary certificates for export are provided without any analysis against *X. fastidiosa*, just after visual inspection of the plants. It is now clear that the detection in asymptomatic plants is essential, because this bacterium can have a long period of latency in the host, or even does not show symptoms in some contaminated plants. In this context, the European and Mediterranean Plant Protection Organization (EPPO) has published very useful inspection and diagnostic protocols (EPPO, 2016).

Bacteria and vectors are not limited by borders and common actions and collaborative practical work are necessary. As *X. fastidiosa* in Italy is a threatening phytosanitary emergency (Martelli *et al.*, 2015) and the bacterium has demonstrated to be a world threat, each country should prepare its own risk assessment for this pathogen, design a contingency plan in different scenarios and be ready for a rapid eradication of an eventual introduction. The governments, Plant Protection services, phytosanitary inspectors, laboratories of diagnostic, nurserymen, growers, mass media and the public in general should know the *Xylella* issues, identify the regional and local risks and take complementary preventive actions against this pathogen because unfortunately, new outbreaks could be detected and all the countries should be well prepared for dealing with this serious problem.

Note. In 2016-2017 *X. fastidiosa* has also been reported in a greenhouse in Germany and in three Balearic islands in Spain.

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The olive quick decline syndrome

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The quick decline syndrome of olive (OQDS) is a disease that appeared suddenly some years ago in a *Olea europaea* grove near the city of Gallipoli, on the Ionian coast of the Salento peninsula (south-east Italy), and began spreading fast in lower Salento. OQDS has been the object of reviews (Martelli *et al*., 2015; Martelli, 2016) which the readers are referred to for a more exhaustive information. The disease is characterized by the apperance of scattered desiccation of twigs and small branches. Leaves are the first to be affected. Scorching starts at their tip and progresses towards the petiole, extending to the whole blade. Dead leaves remain attached throughout summer to the twigs, which are also desiccating, and are shed in the rainy season. Symptoms are first localized in the upper part of the crown, then they extend to the rest of the canopy. Trees of susceptible cultivars, i.e. 'Cellina di Nardò' and 'Ogliarola salentina', that account for nearly the totality of the Salentinean olive industry, decline and die within a few years from the appearance of symptoms. These trees, especially the centuries-old ones, are often pruned heavily, forcing them to push new growth which, eventually, will wither and desiccate. The investigations carried out at Bari by the local University, a phytopathological outfit of the National Reseach Council of Italy (CNR), the CIHEAM Mediterranean Agronomic Institute of Bari (MAIB) and the Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia at Locorotondo (Bari), disclosed that in addition to other putative disease agents, i.e. wood-inhabiting fungi of the genera *Phaeoacremonium, Pseudophaemoniella* gen. nov., *Pleumostomophora* and *Neofusicoccum,* OQDS-affected trees consistently hosted *Xylella fastidiosa* (*Xf*), a most feared quarantinable pathogen, which had never been detected in any of the European Union (EU) countries. *Xf* is a Gram-negative bacterium of the family Xanthomonadaceae, that enters the xylem vessels of the hosts and is ranferred from plant to plant by xylem fluid feeding insects of the family Cicadellidae. Colonization of the xylem vessels results in their clogging by the bacterial biofilm which impairs water uptake. This has a major pathogenic effect. However, a putative lipase/esterase (LesA) secreted by bacterial cells has recently been identified as a virulence factor that initiates marginal scorching of grapevine leaves (Nascimiento *et al*., 2016). Because of its biology, which does not conform to that of bacterial plant pathogens, *Xf* has long been thought to be a virus, until its isolation in axenic culture (Wells *et al.,* 1987). *Xf* is subdivided in subspecies, four of which, *Xf fastidiosa, Xf multiplex, Xf pauca* and *Xf sandyi*, are currently retained as taxonomically valid. These subspecies have a different geographic distribution in the American continent, the site of origin of *Xf*, and an extremely wide host range: 75 botanical families, 204 genera and 359 plant species (EFSA, 2016a), 22 of which proved to be hosts of the bacterial strain present in Salento, called CoDiRO. When *Xf* enters a new environment with favourable climatic conditions, it becomes entrenched because of its polyphagy and is no longer eradicable. This seems to be the case of Salento, as suggesed by the outcome of the studies underway at Bari, whose major results are listed hereafter.

(i) First identification of *Xylella fastidiosa* in different plants (olive, almond, oleander) showing lef scorch symptoms in the Salento peninsula (Saponari *et al*., 2013).

(ii) Different fungal species colonize the wood of declining olive trees of the Salento peninsula (Nigro *et al*., 2013; Crous *et al*., 2015).

(iii) Finalization of serological (ELISA, DTBIA, immunofluorescence) and molecular (PCR, Real time PCR, LAMP) procedures for the reliable identification of *Xf* in host plants and vector (Loconsole *et al*., 2014; Djelouah *et al*., 2014; Yaseen *et al.,* 2014; Cariddi *et al*., 2014).

(iv) Isolation in axenic culture of strain CoDiRO from olive and other naturally infected plant species (Cariddi *et al*., 2014; Elbeaino *et al.,* 2014).

(v) Identification of CoDiRO as a strain of *Xf pauca*. Molecular evidence of its identity with a bacterial isolate (ST53) of the same subspecies present in Costa Rica, a country from which it may have landed in Salento with an unidentified ornamental plant (Loconsole *et al.,* 2014; Giampetruzzi *et al.,* 2015).

(vi) Complete sequence of the genome of strain CoDiRO, a DNA molecule of 2.46 MB (Giampetruzzi *et al.,* 2015a).

(vii) Identification of the spittlebug *Philaenus spumarius* (family Aphrophoridae) as the main if not the only vector of strain CoDiRO, and determination of its biological cycle (Saponari *et al*., 2014; Cornara *et al*., 2016).

(viii) Elecron microscopic detection and identification by gold immunolabelling of the bacterium in xylem vessels of infected plants and in the foregut of the spittlebug vector (Cariddi *et al*., 2014; Cornara *et al.,* 2016).

(ix) Identification of 22 alternative hosts of strain CoDiRO in the province of Lecce out of more than 600 trees, shrubs and weeds analysed, including grapevines and citrus (Potere *et al.,* 2015; P. La Notte, unpublished information).

(x) Experimental evidence that upon mechanical inoculation with bacterial cultures, strain CoDiRO does not infect grapevines (cv. Cabernet sauvignon) and citrus (orange Madame Vinous and Navelina, mandarin, grapefruit Duncan, citranges Carrizo, Troyer and C35), whereas it multiplies readily in olive seedlings and in rooted cuttings of cv. Cellina di Nardò and other olive cultivars (Coratina, Frantoio, Leccino), and oleander (Saponari *et al.* 2014, 2016; EFSA, 2015).

(xi) Complete sequence of the genome of CO33, a coffee-infecting isolate of *Xylella fastidiosa* intercepted in northern Italy, a DNA molecule of 2.68 MB (Giampetruzzi *et al.,* 2015b).

(xii) Host plants exposed to infective *Philaenus spumarius* in the field are infected at different rates. *Xf* was detected by laboratory assays in still symptomless olive plants as soon as six months after caging with infective vectors (Saponari *et al.,* 2016).

(xiii) Bait plants. Of the young trees of olive, oleander, citrus, grapevine and almond planted in diseased olive orchards for exposure to infective vectors, only olives and oleanders became infected within 12 months and started to show symptoms 16-18 months after planting (Saponari *et al.,* 2016).

(xiv) Fulfilment of Koch's postulates upon mechanical inoculation of different hosts (olive, *Polygala myrtifolia,* oleander) with pure cultures of strain CoDiRO (Saponari *et al.,* 2016, as certified by EFSA, 2016b). The Salentinian strain of *Xf pauca* is a primary pathogen causing desiccation and necrosis of inoculated susceptible hosts.

(xv) A comparative analysis of the transcriptome of infected and healthy plants of cvs Leccino and Ogliarola salentina showed that genes coding for receptor-like kinases (RLK) and receptor-like proteins (RLP) involved in plant defence responses are differentially expressed in the two cultivars. Partial resistance of cv. Leccino to strain CoDiRO seems to be expressed essentially through a remarkable reduction of the bacterial population, i.e. 130.000 CFU/ml of tissue extract in cv. Leccino vs 2,094,000 CFU/ml in cv. Ogliarola salentina (Giampetruzzi *et al.,* 2016).

Being a quarantine pathogen, *Xf* is regulated by EU Directive 2000/29/CE, which must be enforced in all member States, Italy included. This Directive dictates the protective measures to be implemented against the introduction and spreading of such pathogens in the EU territory. Eradication is mandatory or, should this be no longer possibile, measures must be adopted for restraining pathogen dissemination. Based on the knowledge acquired with the above-listed investigations, a plan was envisaged by the Italian Ministry of Agriculture and Forestry for confining the contagion within the province of Lecce, its current boundaries, through the control of *P. spumarius,* the OQDS vector: (i) mechanical weeding against the larval stages; (ii) chemical treatments against the adults; (iii) uprooting alternative hosts and infected olive trees in newly identified foci. Stumbling blocks have prevented the enforcement of this plan, thus the disease is moving north, and has reached the neighbouring provinces of Brindisi and Taranto.

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Xylella fastidiosa and its role in the Olive Quick Decline Syndrome

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The Olive Quick Decline Syndrome (OQDS) appeared suddenly some years ago in the municipality of Gallipoli, Puglia region (southeast Italy) and its spread was very fast through the heavily olive-grown countryside. Search for the causal agent(s) of this new olive disease disclosed the consistent presence of *Xylella fastidiosa* (*Xf*) in the diseased olive trees, as well as in other hosts exhibiting leaf scorching symptoms (Saponari *et al*., 2013). *X. fastidiosa* is a xylem-restricted pathogenic bacterium native to the Americas, where it has been confined for long time. Following the report of its finding in Italy, monitoring and surveys have been intensified throughout the European Union and the Mediterranean Countries. Currently, confirmed records of its presence come from Iran, Italy, France, Germany and Spain, with epidemic field outbreaks occurring only in Iran (Amanifar *et al*., 2014), southern Italy (Salento peninsula, southern Puglia) (Martelli *et al*., 2016) and in insular (Corsica) and continental (French Riviera) France (Anonymous, 2016). Thus, *Xf* has no longer a geographical distribution limited to the Americas and these novel records demonstrate that the bacterium continues to conquer new areas (Almeida and Nunney, 2015). *Xf* infections to olive were first reported by Krugner *et al.* (2014) in trees exhibiting leaf scorch and dieback symptoms in California (USA). In the Salentinian olive groves, *Xf* outbreaks were consistently associated to the olive groves affected by OQDS, consisting in leaf scorching and scattered desiccation of twigs and small branches, which, in the early stages of the infection, prevail on the upper part of the canopy. Over time, symptoms become increasingly severe and extend to the rest of the crown, which acquires a blighted appearance. Desiccated leaves and mummified drupes remain attached to the shoots. The most severely and impressively affected olives are the centuries-old trees of the locally grown highly susceptible cultivars Cellina di Nardò and Ogliarola salentina, which the growers unsuccessfully try to save through a drastic rejuvenation pruning to stimulate new growth. In fact, the new vegetation pushed by these skeletal-looking trees will soon wither and desiccate. These trees show also a variously extended browning of the sapwood of branches and trunks associated with the presence of fungal species of the genera *Phaeoacremonium*, *Phaemoniella*, *Pleumostomophora* and *Neofusicoccum* whose penetration is favored by the leopard moth galleries and which are thought to act as disease aggravators (Nigro *et al*., 2013). The overall aspect of OQDS and the modality of its spreading recalled very much the outcome of *Xf* infections to grapes, fruit and shade trees as described in the North American literature.

Interestingly, olive diseases strikingly resembling the Puglia OQDS have been described from Argentina (Haelterman *et al*., 2015) and Brazil (Coletta-Filho *et al.,* 2016). In both cases, symptomatic plants are infected by *Xf* strains closely genetically related to the subspecies *pauca*. Although belonging to the same subspecies occurring in Puglia, the Argentinean and Brazilian *Xf* strains differ from the Salentinian isolate, known as CoDiRO, whose genome, a DNA molecule of *ca.* 2,500,000 bp in size, has been sequenced (Giampetruzzi *et al*., 2015) and found to be molecularly identical to a bacterial isolate from Costa Rica, recently reported in an outbreak in France (Menton) along with the isolates of the subsp. *multiplex* commonly identified in the French outbreaks.

In early 2016, symptoms resembling those observed in the infected olive groves were recovered under experimental conditions in two independent experiments of mechanical inoculation (needle

inoculation) of potted olive plants with a bacterial suspension of the CoDiRO strains (Saponari *et al*., 2016 and unpublished). Twig dieback starting from the apexes and progressing with the desiccation of the entire branches were observed 8-14 months after the inoculation. Indeed, similarly to the field conditions, sprouts from the rootstocks (seedlings) of the inoculated grafted plants remained symptomless for a longer period, with few starting to become symptomatic 2 years after the inoculations. This study provided also evidence that the bacterium is capable to move downstream and to invade and colonize efficiently the olive roots.

In addition to mechanical inoculations, successful vector-mediated infection of the olive trees was achieved using field-collected adults of *Philaenus spumarius.* The experiments conducted using the vectors demonstrated that the natural infectivity rates of *P. spumarius* collected from olive orchards in Puglia were reasonably high (at least 25%), and that these individuals transmit the bacterium to host plants from May to October (Cornara *et al*., 2016).

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Main insect vectors of *Xylella fastidiosa* in Italy and worldwide

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Xylella fastidiosa (Wells *et al*., 1987) is a xylem-limited pathogenic bacterium, originating in America, that xylem sap-sucking insect vectors transmit. The bacterium infects more than 300 plant species belonging to 63 plant families (EFSA, 2015) causing different plant diseases, the most important being Pierce's disease of grapevine and citrus variegated chlorosis (Chang *et al*., 1993). All *X. fastidiosa* insect vectors belong to Auchenorrhyncha (Hemiptera) and are distributed within the superfamilies Cercopoidea (spittlebugs and froghoppers), Cicadoidea (cicadas) and Membracoidea (Cicadellidae: Cicadellini). The insect vector acquires the bacterium as it feeds on infected plants and transmits it while feeding on another host plant. Transmission does not show a latent period, and it is neither transovarial nor transstadial. Once the bacterium is acquired, adult vectors can transmit it during their lifetime since *X. fastidiosa* can multiply and persist into their foregut (Almeida *et al*., 2005). In Nearctic and Neotropical regions, there are many plant diseases caused by different *X. fastidiosa* genotypes. In the same areas, there are abundant xylem-sap feeding leafhoppers belonging to the subfamily Cicadellinae, which includes the main known vectors of *X. fastidiosa*. These insects are commonly named sharpshooters. In total, 39 species of Cicadellinae and 5 spittlebugs (Aphrophoridae) are vectors of *X. fastidiosa* in American region (Redak *et al*., 2004). The transmission of *X. fastidiosa* is not specific, and all xylem sap-sucking insects are considered potential vectors. Furthermore, transmission efficiency varies depending on insect vector species, *X. fastidiosa* genotype and host plant (Redak *et al*., 2004). The most important sharpshooter vectors of the bacterium in North America are *Graphocephala atropunctata* (Signoret) and *Homalodisca vitripennis* (Germar). They are responsible for the spread of Pierce's disease of grapevines. Whereas in South America the main vectors are: *Bucephalogonia xanthophis* (Berg), *Dilobopterus costalimai* Young, *Acrogonia citrina* (Marucci and Cavichioli) and *Oncomeopia facialis* (Signoret), *Macugonalia leucomelas* (Walker), all known vectors of citrus variegated chlorosis on citrus (Almeida *et al*., 2005).

Recently, Saponari *et al*. (2013) and Cariddi *et al*. (2014) detected, isolated and confirmed the presence of *X. fastidiosa* in olive trees, oleander and almond in south-eastern Italy as the first record in the European Union. Pathogenetic tests confirmed the bacterium responsibility for a new disease: the olive quick decline syndrome (OQDS or CoDiRO) (Saponari *et al*., 2016). The syndrome begins with severe leaf scorch and scattered twigs desiccation of the upper part of the canopy. Later, the symptoms expand on the plant until the host death (Martelli *et al*., 2016). The disease is lethal, and knowledge of the candidate and actual *X. fastidiosa* vectors are crucial for a correct risk assessment of this threat. The xylem sap-sucking insect species present in Europe are seven sharpshooters (Cicadellidae, Cicadellinae), twenty-six spittlebugs (Aphrophoridae), seven Cercopidae, fifty-four cicadas (Cicadidae and Tibicinidae) (EFSA, 2013; Bosco, 2014). Of these, only two species were considered potential vectors of the bacterium in Europe, *Philaenus spumarius* (L.) and *Cicadella viridis* (L.) (Janse & Obradovic, 2010). Surveys over the infected or diseased olive groves found three homopteran species positive to *X. fastidiosa*. Namely, two species belonging to Aphrophoridae: *P. spumarius* and *Neophilaenus campestris* Fallén and one species of Cicadellidae: *Euscelis lineolatus* Brullé (Elbeaino *et al*., 2014; Ben Moussa *et al*., 2016). Transmission tests with insects collected in infected olive groves showed the ability of *P. spumarius* to acquire and transmit the bacterium among olive trees and other host plants

(Saponari *et al*., 2014; Cornara *et al*., 2016; Cornara *et al*., 2017). By the time, *P. spumarius* is the only vector of *X. fastidiosa* in Europe. On-going studies will help understand the role of other candidate xylem-sap feeders vector species in the spread of the pathogenic bacterium.

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Xylella fastidiosa: the status of the infection and control measures in France

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The xylem-limited bacterium *Xylella fastidiosa* is the causal agent of diseases on a large host range of plants. It has been observed in Americas since the XIXth century causing notably Pierce's disease of grape (*Vitis vinifera*) in North America, citrus variegated chlorosis on orange tree (*Citrus sinensis*) in South America and diseases on fruit trees (*Prunus dulcis*, *P. domestica*, *P. persica*, etc.), ornamentals (*Coffea* spp., *Nerium oleander*, *Platanus occidentalis*, etc.) and forest trees (*Quercus* spp., *Ulmus* spp., etc.). *X. fastidiosa* is a quarantine pest for the European Union and as such is listed on the directive 2000/29/EC and the introduction of many plants into the European Union is regulated. A risk of introduction is still pending due to the high number of non-regulated plant hosts which are imported from contaminated areas and to the presence of asymptomatic contaminated plants.

In 2012, 2014 and 2015 several interceptions of coffee plants (*Coffea arabica* and *C. canephora*) contaminated with *X. fastidiosa* occurred in France, generally on asymptomatic plants. Different subspecies and sequence-types were identified, demonstrating a great diversity: *X. fastidiosa* subsp. *fastidiosa* (ST75), *X. fastidiosa* subsp. *sandyi* (ST72 and ST76) and *X. fastidiosa* subsp. *pauca* (ST53 and ST74) (Jacques *et al.*, 2016; Denancé et al., 2017). In the same time, *Coffea* sp. were intercepted on different occasions in other EU Member States (The Netherlands, Italy, Germany, Switzerland…).

In 2013, the causal agent of the syndrome of quick decline of olive in Puglia was identified as *X. fastidiosa* subsp. *pauca* (Elbeaino *et al.*, 2014).

In France, the first outbreak of a disease caused by *X. fastidiosa* was observed in July, the 22nd 2015 in the island of Corsica on *Polygala myrtifolia*, a popular ornamental shrub which has been widely planted. Since then, *X. fastidiosa* has been detected on twentyseven plant species (21 genera) in Corsica, including ornamentals and native flora. At the beginning of November 2016, the total number of outbreaks reaches 289 in Corsica. Two strains have been identified belonging to the subspecies *X. fastidiosa* subsp. *multiplex* (ST6 and ST7).

On October, the 22th 2015, the first outbreak was discovered in mainland along the south-East Mediterranean coast of France on *Polygala myrtifolia*. Fifteen outbreaks were notified in Provence - French Riviera area at the beginning of November 2016 and two new host plants have been identified (*Spartium junceum* and *Lavandula angustifolia*) infected by. *X. fastidiosa* subsp. *multiplex* (ST6 and ST7). These three hosts are in common with Corsican ones. Recently, in the same area, in Menton, *X. fastidiosa* subsp. *pauca* (ST53) was identified on *Polygala myrtifolia* in

a single outbreak which is now eradicated. In France including Corsica, *X. fastidiosa* has never been detected on olive tree, citrus species, grapevine and oleander.

Since 2012, ANSES has carried out methodology work based on Real-Time PCR and DNA extraction kits on various plant matrices such as coffee (*Coffea* spp.), grapevine (*Vitis vinifera*), peach (*Prunus persica*), orange (*Citrus sinensis*) and other host plants in order to provide efficient tools for early detection and to validate the more performant detection method. After officialisation by the French Ministry of agriculture, a reference method was published in October 2015 by ANSES (MA039 version 1 - https://www.anses.fr/fr/system/files/ANSES_MA039_Xylellafastidiosa_final.pdf). The method is based on Real-Time PCR (Harper *et al*., 2010) after QuickPick™ Plant DNA kit (Bio-Nobile) DNA extraction. In order to assure high throughput DNA extractions, KingFisher™ (Thermo Fisher Scientific) robots are used allowing 15 or 96 samples serial extractions. Each sample is composed of 0.5 to 1 g of petioles, from 5 to 100 petioles according to plant species (e.g. oleander vs olive), or green twigs for very small plants or plants with leaves without petiole (e.g. *Rosmarinus* sp.). The limit of detection (LOD) of this method reaches 10^2 bact./mL on some matrices (i.e. *Citrus sinensis*) but on others, due to the presence of PCR inhibitors as polyphenolic compounds, the LOD aims about 10^5 bact./mL, for example on oaks (*Quercus* spp.) and olive tree (*Olea europaea*). But this protocol remains more sensitive than others such as ELISA or PCR.

Since November 2015, official analyses have been transferred to a network of five laboratories approved by the Ministry of agriculture after training and successful proficiency's assessment.

The Plant Health Laboratory – ANSES performed confirmation analyses on positive samples when concerning new outbreaks or new host plants in the "contaminated" area. The characterization of isolates directly on plant extract or on pure strain isolated from plant material is performed since May 2016 according to a multilocus sequence analysis/typing (MLSA/MLST) (http://pubmlst.org/xfastidiosa/) using partial sequences of seven housekeeping genes (cysG, gltT, holC, leuA, malF, nuoL and petC) following EPPO 2016, Diagnostic PM 7/24 (2) *Xylella fastidiosa*. INRA IRHS – EmerSys team (Beaucouzé - France) is implementing modifications in the amplification protocol (Denancé *et al.*, 2017).

For additional confirmation purpose, isolation is performed on modified PWG medium. Plant Health Laboratory-ANSES set up a collection of 20 strains isolated from 10 different host plants sampled in Corsica, 4 strains from PACA area and 8 strains from intercepted *Coffea* spp.. These strains are transferred to the Biological Resource Center CIRM-Plant Associated Bacteria (CIRM-CFBP) (Beaucouzé-France).

Xylem sap-feeding insects belonging to Hemiptera order and *Auchenorrhynca* sub-order are known to be the main way for *X. fastidiosa* spreading. In consequence, forty seven species belonging to Aphrophoridae, Cercopidae, Cicadellidae and Cicadidae families are potential vectors and present in mainland France and twelve are present in Corsica island.

The Aphrophoridae *Philaenus spumarius*, known as vector in Puglia (Saponari *et al.,* 2014, Cornara *et al*., 2016), is widespread in mainland France and Corsica as in all Europe. More than three hundred *P. spumarius* specimens collected from October to December 2015 on eight Corsican outbreaks were tested individually for the detection of *X. fastidiosa* by Real-Time PCR (Harper *et al*., 2010) in duplex with internal controls 18S (Ioos *et al.*, 2009) after DNA extraction using a commercial kit usable with a robot. The observed contamination rate of individual insect varied, according to the outbreak locations, from 4% to 25% with an average of 8.8% (non-official results – method being in development and validation).

Controls and surveys have been implemented by the French Ministry of agriculture to investigate dispersion of the disease and to eradicate the outbreaks according to the Commission Implementing Decision (EU) 2015/789 of May 18th 2015. The main regulatory measures consist in the delimitation of a demarcated zone around each outbreak comprising an infested zone

with a radius of 100 m and a buffer zone with a radius of 10 km around the outbreak in order to confine the bacteria and to avoid its spreading. Eradication measures are maintained for 5 years after the discovery of the last infected plant of an outbreak. Into the infected zone, insect control/ eradication and removal of host plants and symptomatic plants are processed and specified plants are sampled for analysis. Planting of host plants is prohibited into the infected zone. Visual inspection and sampling of symptomatic plants are performed into the buffer zone and the movement of specified plants is forbidden outside the buffer zone. Outside the demarcated zones, a monitoring plan has been applied since 2015 consisting in inspections of the territory, production sites, retailers and controls at the EU Entry points (ship and port facilities, airports, etc.). From January 1, 2015 to November 07, 2016, more than 15000 samples have been tested. 11090 samples from Corsica were processed, among which 6.91% of positive samples, 2385 samples from Provence-French Riviera area, among which 2.25% of positive and 1630 samples among which 1.08% of positives for other French areas. For the latter case, positives are related to intercepted coffee plants imported from third countries.

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State of the art of the research on *X. fastidiosa* in Puglia

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The recent emergence of *Xylella fastidiosa* in Europe raised several concerns for the risks and the severe impacts that this plant pathogenic bacterium can determine on the EU agriculture/forestry sectors and natural environment. Effective preventive and containment measures rely on the knowledge of the complex interactions (bacterium-host plant- vector- environment) determining the epidemic spread of this bacterium. Major consequences related to the introduction of this invasive pathogen in the EU territory take into account that: (i) several known hosts of *X. fastidiosa* include many cultivated and wild species growing in Europe and in the Mediterranean Basin; (ii) a wide range of European wild plant species have never been exposed to the bacterium, thus no information is available on their susceptibility; (iii) all xylem fluid-feeding insects in Europe may represent potential vectors; (iv) one of the most susceptible crop species in the EU outbreaks is olive (Martelli *et al*., 2016), a major crop and a relevant species for the Mediterranean landscapes. In response to the first *X. fastidiosa* outbreaks (Saponari *et al*., 2013) in the Puglia region (southern Italy), research programs have been activated (at regional and international level) to develop critical information on the biology and genetics of the Puglia isolate, and on the epidemiology of the infections. International collaborations, with research groups with long-term experience in studying host-pathogen-vector interactions, have been important to capitalise the existing knowledge and to promote the research capability building of the local Institutions. The outcomes of these research programs have significantly contributed to unravel information on the genetic relatedness of the Puglia strain and other intercepted strains, the natural host range, the susceptibility of major crop species, the vector-transmission and the epidemiology of the infections, the impact of the infections. One of the major scientific contributions of the ongoing research investigations refers to the evidence on the pathogenicity and on the role of *X. fastidiosa* strain CoDiRO in the severe olive disease associated to the *Xylella*-outbreak in Puglia. Taken together, the overall experimental results lead toward the implementation of the future scientific knowledge and the reduction of the uncertainties regarding the risk assessment and the development of effective surveillance and management strategies.

Taxonomic position of the Puglia strain

Multilocus sequence typing (MLST) for *X. fastidiosa*, first introduced by Scally *et al.* (2005) and then refined by Yuan *et al.* (2010), has been successfully used to study *X. fastidiosa* diversity at the species/subspecies level, and to infer the phylogenetic placement of newly identified isolates. The MLST data have resulted in a robust taxonomy for the species and for the classification of isolates into sequence types (STs) (unique genotypes based on the 7 loci used in MLST) (Alemida and Nunney, 2015).

MLST analysis of the *X. fastidiosa* isolates recovered from different infected olive trees found in the first outbreak (Gallipoli district) proved that all isolates collected belonged to ST53 (Elbeaino *et al*., 2014; Loconsole *et al*., 2014). Up to now, isolates with the same ST are known to occur only in Costa Rica, associated to *X. fastidiosa* infections on oleander and coffee plants (Nunney *et al*., 2014). The phylogenetic network derived from all MLST data publicly available indicated that ST53 clusters with *X. fastidiosa* subspecies *pauca*. Further molecular investigations assessed that this ST is the only allelic profile associated to the isolates causing infections in olive trees

located in geographically distant foci in Puglia and in different naturally infected hosts found in the Puglia infected area (Saponari *et al*., 2014; Loconsole *et al*., 2016).

Host range and pathogenicity tests

Preliminary data on the susceptibility of ornamentals (i.e. oleander and *Polygala myrtifolia*) important tree species (i.e. olive, stone fruit, citrus, grape and holm oak) in the EU to the Puglia strain of *X. fastidiosa* were obtained by conducting small-scale experiments under controlled and field conditions. Needle inoculations with the *X. fastidiosa* strain CoDiRO and exposure of plants to natural infective vector populations have provided critical information that substantiated the field observations made in the last 2 years after the discovery of the first Puglia outbreak. In summary, (i) olives appear to be highly susceptible to infections caused by isolates of the subspecies *pauca*, and in particular to the strain CoDiRO; (ii) olive cultivars display a differential response to *X. fastidiosa* infection, multiplication and movement; (iii) upon systemic infections, symptoms similar to those observed in the affected field (desiccation and dieback) were observed on inoculated olive plants; (iv) amongst the cultivars tested, Cellina di Nardò clearly resulted as the most susceptible to the CoDiRO strain (Saponari *et al.,* 2016).

The field experiments with the exposure of young plants to natural inoculum and to naturally infective specimens of *P. spumarius* resulted in successful infection of the known host plants (olive, oleander and *P. myrtifolia*); conversely no transmission occurred on grapevines, citrus and holm oak. So far no symptoms have been observed on the infected plants under field conditions.

Major research outcomes

- Sequence analyses on the *X. fastidiosa* isolates recovered from different hosts and foci in Puglia showed that all infected samples harbored a single ST, denoted as ST53, with phylogenetic relationships with the subspecies *pauca*. The finding that isolates with the same ST53 have been so far identified only in Costa Rica, provided evidence toward the better understanding of the introduction pathways in the EU, and supports the single introduction hypothesis associated to the Puglia outbreak.
- Artificial inoculations confirmed that olives, oleander and myrtle-leaf milkwort support systemic infections of *X. fastidiosa* strain CoDiRO, and develop symptoms (Fig. 1) resembling those observed in the outbreak area. These results contributed to disclosing the etiology of the olive quick decline syndrome and the role of *X. fastidiosa* strain CoDiRO in this novel olive disease.
- Upon artificial inoculations and the field exposure of citrus, grapevines and holm oak, no successful systemic infections were detected on these plant species, which so far appear to be not susceptible to *X. fastidiosa* strain CoDiRO.

Figure 1. Symptoms recovered on the mechanical inoculated plants with *Xylella fastidiosa* strain CoDiRO. A) Desiccated olive twig; B) Shoot tip dieback on olive inoculated shoot; C) Chlorotic leaf pattern and initial necrosis observed on the mature leaves of oleander; D) Desiccated shoot on *Polygala myrtifolia*.

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Current EU research initiatives on *Xylella fastidiosa*

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The large outbreak of *Xylella fastidiosa* (*Xf*) affecting olive groves in the Salento peninsula of the Puglia region (southern Italy) and the numerous *Xf* outbreaks reported by the French Authorities in the French island of Corsica, and later on the mainland, pose major risks to the EU agriculture and plant biosecurity. Because of the complexity of the *Xf*-associated diseases, the management and the control of the infections rely on deep knowledge of the susceptible hosts, of the biology and genetics of the isolate(s), and on their interactions with the insect vector population(s), the climate conditions and the agriculture practices. As such, the EU Commission mobilized resources within the EU framework programme for research and innovation Horizon 2020. The first research program started at the end of 2015 with the project "Pest Organisms Threatening Europe" (POnTE) dealing with *X. fastidiosa* and other relevant emerging pathogens. Indeed in 2016, an H2020 project "*Xylella Fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy" (*XF*-ACTORS) targeting exclusively *Xf* has been funded with the aim of fulfilling the research gaps on *Xf* and of developing tools and strategies for prevention and containment of the impact of the disease spread under different agriculture management regimes. To strengthen the capability of the national plant health services to conduct robust surveys in EU and Mediterranean countries, a EUPHRESCO network has been set up to promote validation and interlaboratory performance tests and provide harmonized diagnostic approaches. These actions involve very large Consortia with ambitious work-plans covering basic and applied research on prevention, detection, surveillance and innovative control strategies for *Xf* and its vector(s). The multi-actor approach ensured by these large Consortia will facilitate interactions among research groups, share previous experiences, establish new and strengthen current collaborations among European and non-European research organizations, and increase awareness about scientific work previously done. Moreover, the European Food Safety Authority (EFSA) promoted in 2014 a "Pilot project on *Xylella fastidiosa* to reduce risk assessment uncertainties" and more recently a specific action to collect literature information and experimental data on the vectors "Collection of data and information on biology and control of vectors of *Xylella fastidiosa*". Best practices to manage the EU resources are put in place in order to maximize the efforts while avoiding research duplications.

Major expected research advances and outcomes from the ongoing projects:

POnTE "Pest Orga POnTE "Pest Organisms Threatening Europe" (H2020 cod. 635646)

- Discovery of biomolecules that can be applied to prevent or reduce host colonization by *Xf*;
- Selection of tolerant or resistant varieties;
- Discovery of endophytic bacteria that can cross protect against *Xf*;
- Development of early detection of the pathogens that can be applied for inspection at port of entry to interdict the exotic pathogens and pests;
- Discovery of an optimal biological control agent for vectors of *Xf*;
- Development of pest management regimes to mitigate the impact and the further spread of emerging diseases and alien pests.

XF-ACTORS "*Xylella Fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy" (H2020 cod. 727987)

- Extensive knowledge on the biology, genetics and pathogen-host/environment interactions;
- Extend the knowledge on the vector biology, ecology and role in disease epidemiology;
- Integrated research data on *X. fastidiosa* and implementation of regional pest risk assessment (PRA);
- Strengthen preventive measures by the promotion of integrated measures for rapid and efficient response against emerging outbreaks of *Xf;*
- Screening of a larger number of olive selections for their susceptibility to *Xf;*
- Develop sustainable management strategies aimed at reducing the economic, environmental and social impacts.

PROMODE "Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors" (EUPHRESCO2015-F-146)

- A common protocol for sampling and processing of plant/bacteria extract, with special emphasis on isolation of viable cells of *X. fastidiosa*;
- Harmonized and validated detection methods to determine prevalence of *X. fastidiosa* in plants and insects;
- Evaluate the risks of *X. fastidiosa* transmission from infected oversea ornamentals to native host plants.

CIHEAM/IAMB innovative tools for early surveillance and detection of *Xylella fastidiosa*

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Xylella fastidiosa is a xylem-limited, gram-negative bacterium, infecting about 380 plant species worldwide, in most of the cases without causing symptoms. Typical symptoms are leaf scorching and leaf wilting, eventually followed by the death of the plant. Any xylem sap-feeding insect is a potential vector for this pathogen. Four subspecies are officially recognized: *fastidiosa*, *pauca*, *multiplex* and *sandij*. *X. fastidiosa* appeared in the southern part of Italy in 2013, as first report in the EPPO region, causing the death of million olive trees, with severe economic, environmental and social consequences (Saponari *et al*., 2013). The strain CoDiRO (**Co**mplesso del **Di**sseccamento **R**apido dell'**O**livo) found in olive trees and several other host plant species induces the Olive Quick Decline (OQD) and is genetically identical to a strain from oleander in Costa Rica (Elbeaino *et al*., 2014). The only assessed vector is the Aphrophoridae *Philaenus spumarius* (Saponari *et al*., 2014). There is no record of successful eradication of *X. fastidiosa* once established outdoors due to its broad range of plant hosts and vectors. However, the only control means are the prevention of pathogen introduction in free areas (e.g. use of healthy propagating material) and the containment of the outbreak where the pathogen is not well established yet (e.g. eradication of infected plants and vector control).

The severe threat posed by *X. fastidiosa* in Italy prompted the Italian Ministry of Agriculture to declare a state of emergency for *X. fastidiosa*. To this aim, a special Commissioner was soon appointed and a national scientific committee was established for advising technical decisions. An action plan was established for implementing measures as for the: - removal of host plants located near roads, canals, green areas; - control of young stages of the vectors on ground vegetation; - phytosanitary treatments for the control of adult vectors; - removal of infected plants; - destruction of host species in nurseries; and other horizontal activities.

Based on the infection status, a demarcated area was established, which includes the infected zone and buffer zone (10km surrounding the infected zone). In the infected zone, where the pathogen is considered established, measures concern planting prohibition of EU host plants, while intensive monitoring and the removal of infected plants are restricted to a 20km-wide strip at the border with the buffer zone. In the buffer zone, where the pathogen is not present, measures concern the intensified monitoring of specified plants, vector control, movement restrictions out of the buffer zone. In all the above zones, the infected plants have been mapped and the management of the monitoring data has been fully computerized. The graphical representation of the areas monitored and their results are available on the official website of the Puglia Region (www.emergenzaxylella.it).

The early surveillance and detection of *X. fastidiosa* is so difficult that it is necessary to develop an efficient and sustainable management of the infection. It should be based on a thorough knowledge of the: territory (e.g. cartography, land cover), time and space evolution of the infection since its first outbreak, priority risky sites to be monitored, diagnostic protocols to be applied, etc.. The surveillance flow of both qualitative and quantitative data should be managed properly in order to provide accurate indications to the National Plant Protection Body for the application of control measures. Relatively new approaches as the remote sensing coupled with the availability of large-scale datasets, the rapid development of computer technology and biotechnology, are leading to considerable improvements in strategic and tactical decision making on plant disease

surveillance and management. To this aim, the system developed by CIHEAM Bari for the official surveillance of *X. fastidiosa* in South of Italy is aimed to early detect the pathogen integrating innovative tools for: territorial analyses (e.g. photointerpretation of aerial images), accurate on-site data acquisition (XylApp), rapid on-site pathogen detection (DTBIA, real-time LAMP) in plant material and 'spy insects' (D'Onghia *et al*., 2014; Lacirignola *et al*., 2015).

This surveillance system, which is multidisciplinary, multifunctional and multi-actors, allows the traceability of different types of data which converge in a central server (XylWeb) for their rapid storage and analysis. The main components of this system are hereafter briefly described.

The assisted photo interpretation of high resolution aerial images was developed for the rapid recognition of olive trees showing OQD-like symptoms on a large scale, being this species the primary host of the CodiRO strain in Puglia region (Gualano *et al*., 2014). This approach allows the implementation of precision intervention at local and territorial levels.

All suspected infected sites were investigated by visual observations for confirming OQD symptoms and assessing the presence of the pathogen. This method can also provide indications on: the presence of symptoms in olive trees which have been pruned before visual inspections; the application of some measures included in the action plan (e.g. soil tillage in spring to significantly reduce vector juveniles' populations), etc.

XylApp is an application for "android systems", designed and developed with the aim of facilitating, optimizing and rationalising collection, geolocalisation and storage of data related to plant (e.g. OQDS photointerpreted trees) and/or insect samples (vectors and spy insects) collected in the field during the monitoring phase (Santoro *et al*., 2014). The application consists of five independent modules for on-site data acquisition by inspectors: *Sampling, Explore & Sampling, Find, Archive* and *Vademecum.*

The spy insects approach is based on the monitoring of insect vectors or potential vectors of *X. fastidiosa*, which have been assessed to harbour the pathogen as *P. spumarius*, the only assessed vector, *Neophilaenus campestris* and *Euscelis lineolatus* (Elbeaino *et al*., 2014; Ben Moussa *et al*., 2015). The detection of the pathogen in these insect species can early reveal the presence of the infection before symptom development in the buffer zone and in the pathogen-free area. Due to the different dynamics of seasonal population of the spy insects in Puglia region, their monitoring can be carried out during the whole year.

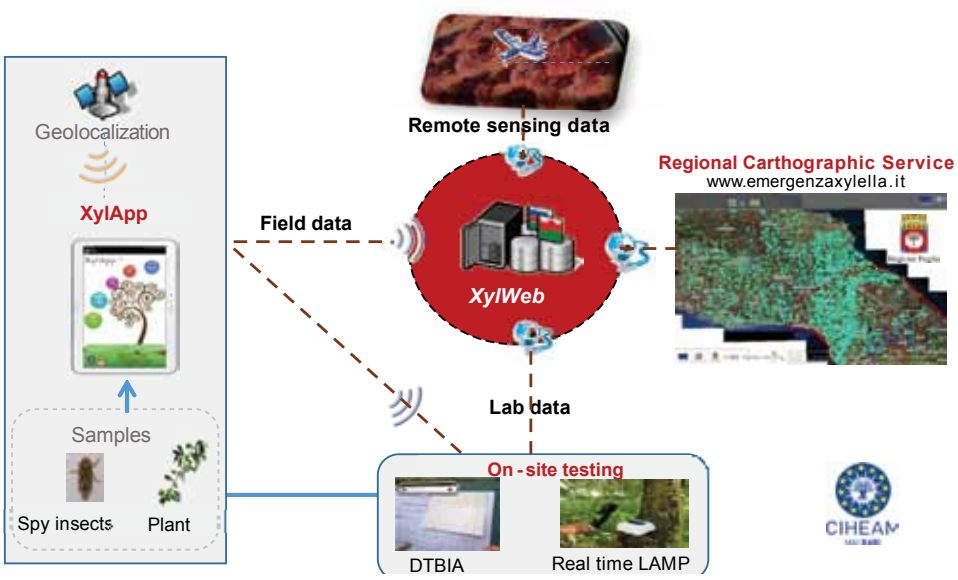
On-site rapid detection of *X. fastidiosa* has been developed using the real time LAMP (loop-mediated isothermal amplification) and DTBIA (Direct Tissue Blot ImmunoAssay). The real time LAMP can be totally performed on site in plants and 'spy insects' using a field device (Yaseen *et al*., 2015) while, in the case of DTBIA, membranes can be printed in the field with plant material and processed in laboratory (Djelouah *et al*., 2014). However, in both cases the movement of infected plant material in Xylella-free areas for pathogen testing can be avoided.

XylWeb is a web-based software for the collection, storage and management of surveillance data for *X. fastidiosa* (Gualano *et al*., 2014). This software represents the core of the surveillance system in which all data converge, e.g. daily data acquired by XylApp are transmitted in real time to XylWeb. XylWeb allows data traceability and real time analyses for producing reports and other elaborates. The application consists of the following independent modules: *Sample; Processing; Browse; Management; Downloads;* and *Links.* Its implementation with the regional cartography provides a clear map on the distribution of the samples, infected plants, etc..

In the framework of this surveillance system in South of Italy, more than 100 000 diagnostic tests have been conducted (in the buffer zone and the 20km-wide strip of the infected zone surrounding the buffer zone) with the aim of determining the presence and spread of the infection, thus applying eradication/containment measures as indicated in the Commission Implementing Decision EU 2015/789.

Thanks to the ongoing research at national and EU levels (e.g. Horizon 2020 *Xf*-Actors), the surveillance system for *X. fastidiosa* will be enhanced exploiting the potential of hyperspectral and thermal data for early pathogen detection (using manned or unmanned vehicles), smart applications (e.g. dedicated Apps for end users) and prediction models of ecophysiological stress responses of olive trees, of spatial and temporal spread of the infection, etc..

The innovative system for early surveillance of *Xylella fastidiosa*



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IT platform based on smart device and web-application for the survey of *Xylella fastidiosa*

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The identification of the first outbreak of *Xylella fastidiosa* in Puglia, South of Italy (Saponari *et al*., 2013), poses a serious threat to the agriculture and landscape in Europe and in the Mediterranean region. The assessment of the bacterium in the host plants, primarily on olive trees which show the Olive Quick Decline Syndrome (OQDS), and the control of its spread throughout Puglia have raised attention on the importance of early identification of the infection in the surveillance programme on large scale. Therefore, the timely collection of information on the host plant species, their geographical position, presence of symptoms, presence of the vector(s), characteristics of the surrounding environment and pathogen detection is the first step for the survey and statistical forecast of the infection.

At the beginning, the survey on *X. fastidiosa* in Puglia was managed through the traditional approach based on field data collection with the use of GPS devices, maps, etc. This method brought up wrong data in time and space, inconsistent statistics mainly due to the huge mass of geographic and alphanumeric information acquired and their processing by operators.

A new approach was set up on the use of Information and Communication Technology (ICT) for the management and processing of survey data for *X. fastidiosa* with a focus on the sample collection, coding, transmission, storage, phytosanitary analysis, management and visualization on a map (Santoro *et al.*, 2014; D'Onghia *et al.*, 2014). The IT architecture is made up of two specific types of software, XylApp and XylWeb, developed for Android mobile clients and for the web-based data collection unit (server), respectively, as reported in the Figure 1.

Figure 1. Client server architecture: XylApp - XylWeb.

XylApp, installed on tablet/smartphone, combines the acquisition of field data with the GPS-GLONASS satellite positioning; it is thus possible to overlap vectorial maps or rasters, grids having different cartographic scale or official demarcation areas, which are available in the mobile terminals both off-line and on-line (2G/3G/4G/WiFi). Once data are acquired, the application generates an encryption, stores it and transfers it to the server in real time through functions, which make the app user-friendly, robust and accurate.

In particular, XylApp includes five modules: (i) “Sample”, which allows to acquire data and geolocalize the samples taken from the field without any cartographic support; (ii) “Sail and Sample” to acquire data from the samples taken from the field with cartographic support; (iii) “Find” which allows to find one or more samples knowing the geographic coordinates; (iv) “Archive”, to store and transfer data to XylWeb; (v) “Vademecum” to provide guidelines on the most important notions on the monitoring activities (equipments, host species, symptoms, vectors, spy insects, etc.). The software is run on-line; a registered user (Phytosanitary Observatory, inspector, lab manager/technician, etc.), can remotely access the server to get information and make relevant operations on data (data entry, modification, processing, display, export, etc.) through a system of differentiated access keys. Data generated during the survey converge into XylWeb through XylApp (position of plants and/or caught insects, field technicians, survey date); XylWeb collects data from the application of remote sensing techniques, results from accredited laboratories for analyses, the manual entry of external samples which are not codified by the App. This enables to facilitate, harmonize, standardize and keep track of the flow of data, which are associated to the sampling in the surveyed sites. XylWeb, is made up of five independent modules (“Sample”, “Processing”, “Management”, “Downloads” and “Links”), which allow to archive, manage and process large amounts of information. In particular, the web-based application generates statistical elaborations and reports, makes available an updated version of information from monitoring activities as both figures and geographical representation of data on maps or graphs. The IT architecture, developed for the whole Puglia area, was tested starting from the official survey of *X. fastidiosa* in 2015. The phytosanitary inspectors and the technicians involved could identify olive plants and other host species, with or without symptoms, in a simple, accurate, effective and real-time way. Data acquired with XylApp were then transferred to the regional cartographic service, which has been in charge of the on-line publication, updating of all the sampling steps since the first outbreak of this disease in Puglia.

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Sampling procedures of plant material for the survey of *Xylella fastidiosa* in Puglia Region, Italy

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After the identification of the bacterium *Xylella fastidiosa* in the region of Puglia, the first outbreak in Europe and in the Mediterranean basin (Saponari *et al*., 2013), and of *Philaenus spumarius* as its vector (Saponari *et al*., 2014), the National and the Regional (Puglia) Phytosanitary Service developed a surveillance plan with the scientific support of research institutions which deal with quarantine plant diseases.

The Regional Phytosanitary Service-Puglia (RPS) promptly worked out and implemented all over the region a strategic survey programme to demarcate *X. fastidiosa*-infected area, check the spread of the bacterium and set up suitable *ad hoc* measures for the control of the pathogen in compliance with the European, national and regional legislation. The work plan was elaborated on the basis of the epidemiology of the bacterium known in countries (United States and Brazil) where it was already reported on different species (grapevine, citrus) and the experience gained by scientific institutions based in Puglia in the framework of mandatory programmes to control other quarantine pathogens (e.g. *Citrus tristeza virus*, *Erwinia amylovora*). The monitoring system, type of sampling, diagnosis protocols and/or diagnostic techniques have been modified or adjusted following the scientific information acquired on the bacterium, hosts and vectors or potential vectors since its first outbreak (Loconsole *et al*., 2014; Djelouah *et al*., 2014; Elbeaino *et al*., 2014, 2014a; Saponari *et al*., 2014; Yaseen *et al*., 2015; EPPO standard PM 7/24 2). Furthermore, CIHEAM Bari experience on the use of information technology supporting monitoring enabled the development of the application XylApp (Santoro *et al*., 2014), to acquire field data (e.g. geolocalization) on the plant sample and insect as potential vector and to send data to the laboratory for analysis, and XylWeb that collect all the monitoring data (D'Onghia *et al*., 2014; Gualano *et al*., 2014).

The sampling procedures have been defined at national (Italian Ministerial Decree of 26 September) and European level (EU Implementing Decision 2015/789; Guidelines for the survey of *X. fastidiosa* in the Union territory; EPPO standard PM 7/24 based on the experience gained in Puglia to tackle the phytosanitary emergency (Regional Council Deliberation n. 1824, 5 September 2014). Since October 2013, several phytosanitary measures have been issued for the prevention and containment of the infection. They are continuously complemented, amended and updated along with the evolution of the epidemic in Italy, its outbreak in France, Germany and Spain and the new knowledge acquired by research works.

After the first infection outbreak, a wide-mesh survey was carried out all over the region using the Regional Cartographic Reticulum whose rectangular meshes, each with a surface of ca. 1,000 ha, were visually inspected and sampled if symptoms ascribable to *X. fastidiosa* were found. After the evaluation of the north limit of the infection, both the infected and buffer zone were demarcated; however, their demarcation is constantly modified after new outbreaks are reported.

Referring to the current infection status in the Italian outbreak area, a demarcated area has been defined in the EU implementing Decision 2015/789, which includes the infected and buffer zone (10km surrounding the infected zone). Surveys are conducted for early detection in the pathogen-free area. As for the demarcated area, intensive survey is conducted in the whole buffer zone and in the infected zone surrounding the buffer zone (a 20km wide strip) through visual checks and sampling, preferably from symptomatic plants. As for the rest of the area, which is considered

pathogen-free, the survey is carried out in the selected risky sites (e.g. main roads,airports, nurseries, parks, tourist areas, etc.) and in selected submeshes (about 1 Ha) intensively olive grown; the latter because olive is identified as the priority plant species for Puglia region.

In each area/zone an appropriate number of visual checks and samples are considered in relation to the risk factors which are listed in the national survey plan. In Puglia, visual checks and sampling are carried out by phytosanitary inspectors and/or agents belonging to the RPS, Forestry police or other institutions. However, personnel in charge of this activity is trained through courses provided by the RPS with the technical support of Puglia scientific Institutions actively involved in the research on this pathogen (CIHEAM Bari, CNR-Bari, University of Bari, CRSFA-Locorotondo). The field technical team is composed of at least 2 units well equipped with materials and tools for sampling (pruning shears, telescopic pruners, plastic bags, adhesive tags, cool box, etc.) and with a tablet for the use of XylApp.

Each team is tasked with the inspection and sampling of a portion of territory per day. In the buffer and containment zones, all the plants hosting *X. fastidiosa* strain CoDiRO are checked giving priority to olive, oleander and *Polygala*. The sample is taken from plants with suspect symptoms (e.g. leaf scorch, desiccated twigs/branches, etc.) or, if symptoms are absent, from a host plant selected at random in the inspected submesh.

A unit identifies the plant to be sampled (ID) and labeled the plant reporting all the data in the XylApp (georeferencing, species, varieties, symptoms, etc.), another takes the sample.

The sampling procedure on the plant, the type of sample and the sampling period depend on the host species and on the presence or absence of suspect symptoms.

- The sampling period shall coincide with that of visual checks since samples shall be taken from suspect plants. It is usually carried out from late spring to early autumn and in summer for deciduous plant species when the bacterium concentration is high. Only for olive and oleander, sampling can be made throughout the year since summer symptoms persist and the bacterium is always detectable. If symptoms are absent, it is advisable to sample in late summer early autumn when an accurate bacterial detection is higher.
- As to symptomatic plants, the plant material shall be taken from the areas close to the symptoms but not from necrotic tissues. In the event of asymptomatic plants (trees or shrubs), the sample shall be taken from the four cardinal points at different levels; it shall include non-herbaceous twig parts, and/or mature leaves with petioles from woody twigs. As regards the olive, the upper part of the canopy shall be sampled where the infection is often localized with the exclusion of young shoots, suckers or young leaves. For annual herbaceous plants, portions of stems with basal leaves shall be collected; wherever possible the whole plant with its root system.
- Each sample includes at least 8 cuttings/tree of 15-20 cm, or 10-12 mature leaves with petioles from woody twigs. It is closed in a bag and tagged with a daily identification code (ID), geographical coordinates, code of samplers, date, and presence or absence of symptoms (as generated by XylApp).
- Shake the sample before bagging it not to spread potential vectors of *X. fastidiosa.*
- Disinfect all pruning tools (sodium hypochlorite) before taking sample from a new plant.
- Complete the operations on one site, bags with individual samples shall be transferred into a larger bag; report sampling date, site and team on the tag.
- Place the samples in a cool box for transport; deliver the sample to 1st level official laboratories not later than one day after sampling, otherwise, keep the samples in a refrigerator at 4°C.

Laboratories that carry out official diagnostic assays for *X. fastidiosa* are authorized in compliance with the Italian Ministerial Decree of 14 April 1997. Before the arrival of samples, laboratories receive a list with information for each sample so as to organize assays. Upon delivery, samples are checked in an *ad hoc* premise for conformity with the indications produced by XylApp, and for the presence of anomalies (e.g. open bag, damaged sample). Information is reported in an entry register; any mistake is timely reported to SFR-Puglia. Samples eligible for testing are kept for at least 12 hours at 4°C before opening the bags to reduce the viability of potential insect vectors.

The plant tissue may be taken only and exclusively in the laboratory for the analyses of quarantine pests. The remaining plant material shall be placed in a special container for "Plant residues to be autoclaved". It is mandatory to disinfect (e.g. sodium hypochlorite solution) pruning tools for the preparation of the sample.

At the end of the above-cited operations, the plant material is closed in a bag and kept in a refrigerator for quarantine purposes till completion of diagnostic analyses. The plant material will be then autoclaved.

Figure 1. Type of sample from *X. fastidiosa* host plants: a) *Olea europaea* L. (twigs with mature leaves) and b) *Euphorbia terracina* L. (the whole plant).

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The "Spy Insect" approach for monitoring *Xylella fastidiosa* in absence of symptomatic plants

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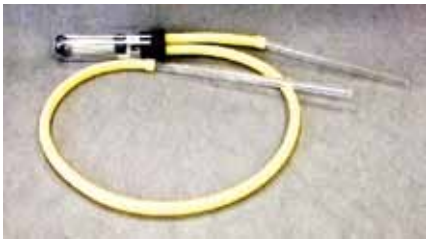
Xylella fastidiosa, a vector-borne bacterium, has recently been reported in southern Italy infecting olive trees and more than 28 plant species. The pathogen induces typical leaf scorch and quick decline symptoms; however, many host plants may remain symptomless for years. Its spread by the adults of *Philaenus spumarius* (L.), the only assessed vector, seems very fast due to the poor agronomical practices in the olive groves (e.g. no tillage, no insect control) and to the warm climatic conditions which favour population density and extend the life of infected adults through the whole year. In addition to *P. spumarius*, other 2 insects were reported by Elbeaino *et al.* (2014) as potential vectors because able to harbour *X. fastidiosa*, *Neophilaenus campestris* (Fallén) and *Euscelis lineolatus* (Brullé). The monitoring of the infection in absence of symptomatic hosts in the buffer zone and pathogen-free areas is difficult and requires a randomised sampling for pathogen detection. Due to the quick dissemination of *X. fastidiosa* in Puglia, an effective approach was therefore developed for the early detection of the bacterium in the symptomless areas (D'Onghia *et al.*, 2014). The three Auchenorrhyncha specimens *P. spumarius*, *N. campestris* and *E. lineolatus* are used as 'spy insects', i.e. as indicators of the presence of *X. fastidiosa* in apparently uncontaminated areas (Ben Moussa *et al.*, 2016). They have a different seasonal population density which allows the possibility to monitor the pathogen through the whole year. From spring to early autumn, *P. spumarius* followed by *N. campestris* are the most numerous for sampling, while *E. lineolatus* is more frequent in autumn and winter months. A site/submesh is identified and georeferenced, selecting areas with high presence of pathogen host plants. Insects are mainly collected from the ground vegetation or from the host plants using about 10 sweeps with the sweeping net (Fig. 1). However, a D-Vac or yellow sticky traps may be also used but are less efficient. Adults of spy insects are carefully collected by aspiration directly *in loco*, put in small tubes (Fig. 1) containing 70% ethanol, codified and brought to the laboratory for testing and, eventually, identification. If few specimens or no specimen are collected, it is preferred to change the site or combine the collection of 2 sites for a total amount of about 10 adults/site.

The list of the samples and relative code numbers is sent as excel file through XylApp, the application used for field data acquisition (Fig. 1), to the laboratory for analyses and to the central web server, XylWeb (D'Onghia *et al*., 2014).



a)

b)

c)

Figure 1. a) Aspirator; b) XylApp; c) Sweeping net

The bacterium is successfully detected in insects by molecular assays (real time PCR and real time LAMP). Nonetheless, real time LAMP is the preferred method because it is fast and accurate; moreover, the use of the field device allows the on-site detection of *X. fastidiosa* in insects and plant material (Yaseen *et al.*, 2015). After results of testing, only the positive insects are identified using the keys of Holzinger *et al.* (2003) classification. Once a positive insect is found, the monitoring of the infection is carried out in a more capillary way in a 100mt radius from the positive sampled site, either collecting plant material from all plant hosts either or capturing other insect's specimens.

The presence of infected insects has two possible explanations: the first one is that the insects have acquired the bacterium from symptomless infected host plants present in the apparently "*Xf*-free" area; the second one is that the insects could have acquired the bacterium in the outbreak area and moved to the pathogen-free area also through indirect transport.

This approach is effective for the early detection of the pathogen in the buffer zone and in the pathogen-free areas. Sampled site for insect captures should be located in the risky points of introduction (e.g. existing trade patterns, traffic ways, nurseries and sites where plants originating in risky areas are grown or kept).

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Use of conventional DNA- and protein-based techniques for the detection and characterization of *Xylella fastidiosa* in Italy

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The recent finding of *Xylella fastidiosa* (*Xf*) in southern Italy and its fast expansion in that region prompted the necessity to apply techniques that guarantee a fast, simple and efficient detection tool to be applied at large spectrum in different host-plant species, environments and insects, *i.e*. *ca.* 30 different plant species for *Xf* (EFSA, 2015) and 3 insect species, for which only *Spumarius philaenus* (L.) (Hemiptera: Aphrophoridae) was experimentally ascertained to be a vector of *Xf* in Italy (Elbeaino *et al*., 2014; Saponari *et al*., 2014). The Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assays (ELISA) were among the first techniques, afterward combined with the Immuno-fluorescence microscopy and isolation, to shed light on the bacteriological nature and the etiology of the severe disease of olive in Italy, named as "Olive quick decline syndrome" (OQDS; Cariddi *et al*., 2014).

Numerous serological techniques and genotyping approaches have been used for the detection, diagnosis and characterization of *Xf* worldwide (Holt, 1994). However, ELISA assay remains the first choice detection tool for this pathogen, being mostly adapted to screen high number of samples in a short lapse of time without a laborious work and high costs. In fact, based on an inter-laboratory validation study that was completed in November 2013 (Loconsole *et al*., 2014), its application to detect *Xf* in infected olive plants was found suitable, and therefore it was included in the EFSA and EPPO protocols as a conventional method to screen thousands of suspected olive samples collected from areas adjacent to already claimed infected zones in Salento region (Italy). At the same time, results of screening olive samples using this serological test were in harmony with those obtained by PCR assays. Two commercial antisera, provided by Agritest s.r.l (*Xylella fastidiosa* Cat. No. K-27B, Italy) and Loewe Biochemica GmbH (*Xylella fastidiosa* Cat. No. 07119S, Germany) are essentially used to investigate the presence of this bacterium in infected samples through a DAS-ELISA test. The validation of both antisera to detect *Xf* in other susceptible host plants (oleander, almond, cherry, ornamentals, etc.) is in due course of evaluation through an inter-laboratory test in the frame of many projects at the European level, *i.e*. Euphresco, POnTe, *Xf*-actors, etc.

For the PCR assay, the bacterial genomic DNA is extracted using a CTAB buffer (Hexadecyl trimethyl-ammonium bromide) and\or a commercial kit designed for pathogens with DNA genomes.

A portion of the RNA polymerase sigma-70 factor gene is amplified using a conventional set of primers (RST31\33) generating an amplicon of 733 bp in size (Minesavage *et al*., 1994), previously adopted in quarantine programs (EPPO, 2004). Two additional couples of primer pairs targeting a hypothetical protein HL (Francis *et al*., 2006) and the 16S rDNA genes (Firrao and Bazzi, 1994) are also recommended since they are more suitable for accurate detection of a wider number of genetically diverse strains of *Xf* (Harper *et al*., 2010). However, a multiplex PCR for detection of all *Xf* strains in both plant tissue and insects, using primers against *Xf*-gyrase b gene and 16S rDNA genes are also reported in the literature (Rodrigues *et al*., 2003).

An additional conventional DNA-based technique, i.e. the Multilocus sequence typing system (MLST; Maiden *et al*., 1998), was also used and helped to acquire more genetic data on the type of *Xf* strain affecting the Salentinian olive groves (Elbeaino *et al*., 2014) that was found to belong

to the subspecies pauca with a sequence type 53 (ST53). The application of this technique is relatively simple, since it is based on a preliminary amplification operation of seven housekeeping genes of *Xf* (*leuA, petC, lacF, cysG, holC, nuoL and gltT*), conventionally used as key factors for strains characterization, followed by gene sequence concatenation (4161 nucleotides), profile and phylogenetic analyses. However, the application of this technique is conditioned by the success of isolating *Xf* in a culture medium, besides to being applied on genomic DNA extracted from living cells of a single bacterial colony. It is noteworthy mentioning that in this era of Next-Generation Sequencing (NGS), many conventional DNA- and protein-based techniques were left archaically behind since they cannot overtake the huge and complete information generated by this new technology to unveil or characterize the identity of many plant pathogens.

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PCR assays for the detection of *Xylella fastidiosa*
Review and comparison of published protocols

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Xylella fastidiosa (Wells *et al.*) is a widespread pathogenic bacterium, causing severe plant diseases with enormous economic impact on agriculture. *X. fastidiosa (Xf)* is regulated in the EU and other countries as quarantine organism. The official surveillance of *Xf* requires valid and appropriate detection tests. Due to the wide host range and the different subspecies and strains, the selection of an appropriate assay is of major concern.

When drafting an international diagnostic protocol for this pathogen (IPPC diagnostic protocol), an extensive literature search (ELS) was performed. One part of the study was to gather information on tests, appropriate for the detection of *Xf* at species level. The detection at species level requires a broad-spectrum detection of different subspecies and strains and the suitability for a wide range of host matrices (plants and insect vectors).

Different literature sources and the EPPO validation database were used to gain data on analytical and diagnostic sensitivity and specificity of currently available PCR assays. Based on the tested strains and non-targets the test performance criteria according to Hughes et al. (2006) were calculated and compared.

Endpoint PCR assays with generic primer for *Xf*

Different endpoint PCR assays with generic primer for *Xf* are available in the literature (1, 2, 3, 4). The primer set Rst31/33 developed by Minsavage et al. (1994) is widely used for surveillance activities. The test results from different sources (1, 8, 10) were compiled (in total 45 different *Xf* strains including subsp. *pauca*, *multiplex*, *sandyi & fastidiosa* and the European strain *Xf* subsp. *pauca* CoDiRO strain and 30 closely related or host related non targets). The compilation of these data revealed that this assay was tested mainly with strains from grapes, citrus and almonds, and that it failed to detect some strains from oaks [*Xf* red oak, US (C. Chang); *Xf* red oak, US (OAK0023 and OAK0024)] as well as from grapes [*Xf V. rotundifolia*, US (C. Chang); *Xf* grapes, US (CR. Almedeida); *Xf* grapes, US (PD0001]. A summary of the test performance criteria for the different endpoint PCR assays are reported in table 1.

Realtime PCR assays with generic primer for Xf

Five different realtime assays for the detection of *Xf* are currently available (5, 6, 7, 8, 9).

The primer set HL5p6 (Francis et al., 2006) was comprehensively tested in different studies (7, 8, 9, 10). The assay failed to detect 5 different *Xf* strains (fig. 1). The primer set *Xf*-fpr (Harper *et al.*, 2010) was tested on 94 different strains, mainly on CVC, PD, OLS and ALS strains and on a broad-spectrum of non targets (fig.2). It showed a high diagnostic sensitivity and selectivity.

Table 1. Compiled data of test performance criteria for endpoint PCR assay.

Reference	Primer	Diagnostic sensitivity	Diagnostic specifity	Relative accuracy	Analytical sensitivity (primary lit. source)	Number of tested *Xf* strains/host combination	Number of tested non-targets
Minsavage *et al.*, 1994 (validated by Harper *et al.*, 2010)	Rst 31/33	100/**63.64**	100/100	100/**76.47**	1 x 10^2cfu/ml	93/19	31
Firraro *et al.*, 1994	XF 1/6	100	100	100	7.6 x 10^2cfu/ml	5/5	7
Pooler & Hartung 1995 (validated by Huang, 2009)	271-1-int/272-2-int	100/100	100/100	100/100	-	57/13	8
Rodrigues *et al.*, 2003	Set A, B, C Gyr	100	100	100	1 x 10^2cfu/ml multiplex: 10 cells	30/10	36

The test performance criteria for the different realtime PCR assays are summarized in table 2.

Table 2

Reference	Primer	Diagnostic sensitivity	Diagnostic specifity	Relative accuracy	Analytical sensitivity (primary lit. source)	Number of tested *Xf* strains/host combination	Number of tested non-targets
Schaad et al., 2002 (validated by Li *et al.*, 2013)	XfF1P1R1	100/100	100/**35.7**	100/**90.1**	1 x 10^3 cfu/ml	93/18	31
Francis et al., 2006 (validated by Harper *et al.*, 2010 and Li *et al.*, 2013)	HLP5p6	100/ **90.5**/ **96.1**	100/ **100**/ 100	100/ **94.1**/ **96.7**	10 copies per reaction	108/21	38
Harper et al., 2010 (validated by Li *et al.*, 2013)	XF-fpr (Rim PCR)	100/100	100/100	100/100	10 copies per reaction	95/20	26
Li et al., 2013	XF16Sfpr	100	100	100	2-3 copies per reaction	77/15	14
Ouyang et al., 2013	Xf.Csp6	100	100	100	3 copies per reaction	27/5	15

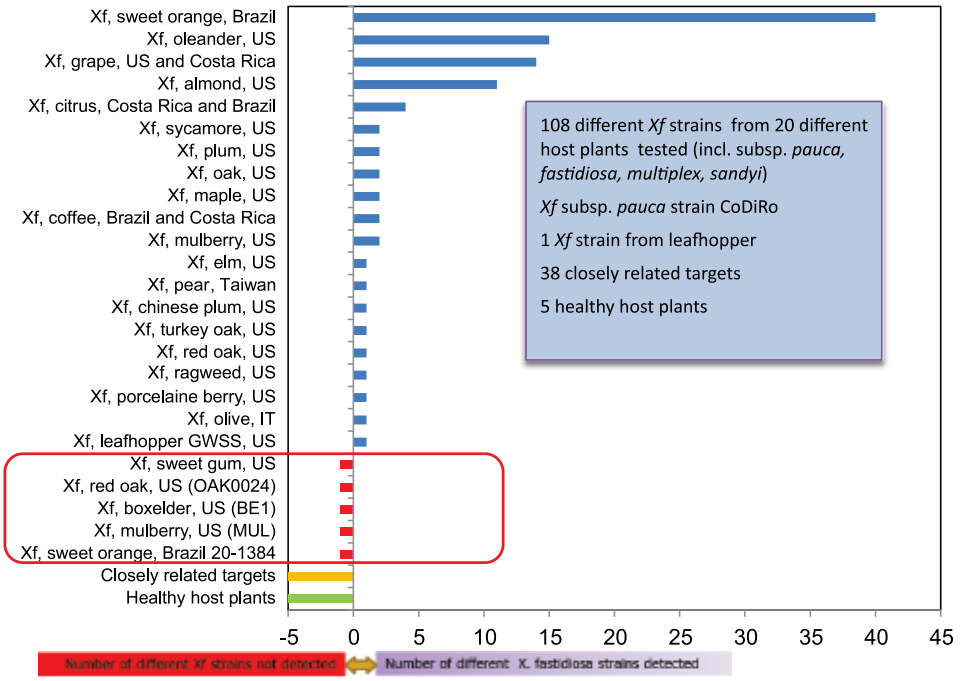

Figure 1. Compiled data from different sources (1, 8, 10) with test results using HL5p6 primer (Francis *et al.*, 2006).

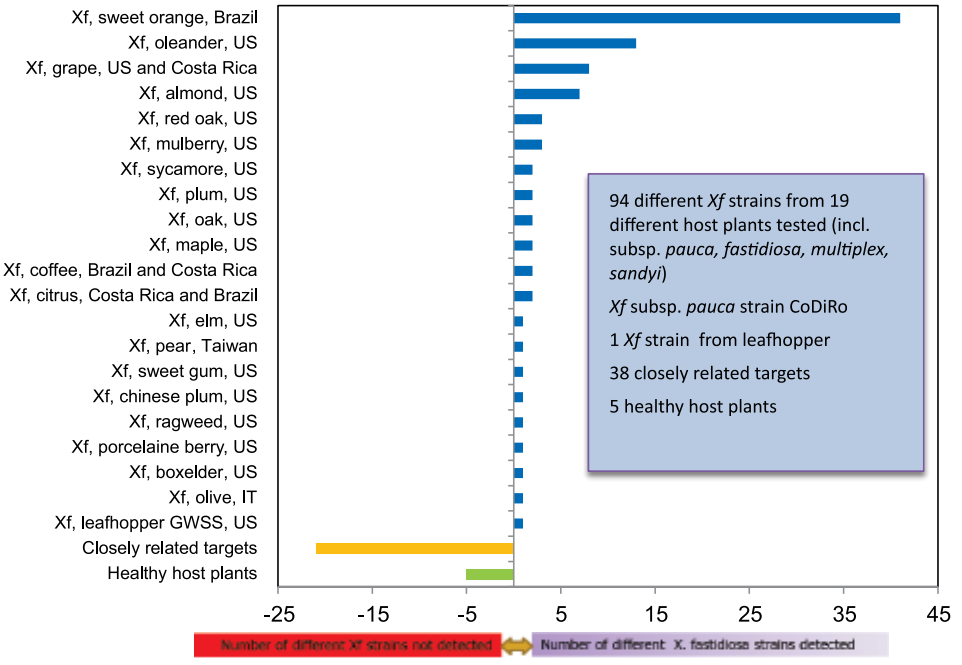

Figure 2. Compiled data from different sources (1, 8, 10) with test results using *Xf*-fpr primer (Harper *et al.*, 2010).

The compiled data from different sources can serve as a decision basis for selecting appropriate PCR assays for the specific requirements of surveillance or research activities. In particular for the detection of a pathogen like *Xf* with different subspecies and strains the limits of an assay can provide important information for further verification and validation studies.

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Direct Tissue ImmunoBlot Assay (DTBIA), an efficient tool for the mass detection of *Xylella fastidiosa* in infected olive trees

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Following the first identification of *Xylella fastidiosa* (*Xf*) under field conditions from the province of Lecce (Puglia region, southeastern Italy), in association with a devastating disease of olive known as "olive quick decline syndrome" (OQDS) (Saponari *et al*., 2013), the pathogen was characterized as a strain of the subspecies Pauca (Cariddi *et al*., 2014) and found to be transmitted primarily by a xylem fluid-feeding insect vector *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae); moreover, apart from olive, other host species of the bacterium were identified, most of which are ornamentals or belong to the typical Mediterranean bush.

To face this threat, the local Plant Protection Services requested an immediate monitoring campaign in the region, in order to establish the exact distribution of the pathogen and to limit further spread of the bacterium. Before to conduct a large-scale monitoring program for *Xf* detection in Puglia, the validation of ELISA and PCR protocols was necessary. In this context the CIHEAM-Bari laboratory was part of the ring-test with other accredited laboratories and ELISA was recognized as the official diagnostic assay in large-scale monitoring; while PCR was identified as a method for the confirmation of ELISA assay.

Following these key studies, thousand samples of different plant species, primarily olive trees, were efficiently tested using ELISA for routine pathogen detection (Loconsole *et al*., 2014). However, despite all precautions that can be taken, the risks associated with the handling and safe movement of plant material to the laboratory which can often carry infected vectors, remained very high and were considered as critical issues for avoiding dissemination of this pathogen to "pathogen-free" areas, in which most of the laboratories are located.

In order to perform mass analyses of plant samples directly in the field, thus avoiding the potential pathogen spread through sample delivery, Direct Tissue Blot Immunoassay (DTBIA), an on-site rapid diagnostic assay that requires very little sample manipulation, was investigated in the CIHEAM Bari laboratories, as an alternative diagnostic tool to ELISA. This technique was already successfully applied in the mass detection of some citrus disease agents, mainly *Citrus tristeza virus* and *Citrus psorosis virus* (Garnsey *et al*., 1993; Cambra *et al*., 2000; D'Onghia *et al*., 2001; Djelouah and D'Onghia, 2001). Some attempts to use DTBIA applications were also reported for the detection of *X. fastidiosa* from citrus affected by variegated chlorosis (Garnier *et al*., 1993) and for other infectious agents (Lin *et al*., 1990).

The DTBIA technical protocol for the detection of *Xf* (Djelouah *et al*., 2014) was achieved by using plant tissues collected from different *Xf* infected olive and oleander trees from four orchards located in Gallipoli, Parabita and Taviano municipalities (Salento peninsula). In this framework, different explants, nitrocellulose membranes and reagents were analysed. The adopted DTBIA protocol by using Enbiotech kit was compared with ELISA and PCR tests through the use of specific antibodies to *X. fastidiosa* (Loewe Biochemica GmbH and Agritest) and the RST31/ RST33 set of primers targeting the 16S rDNA gene (Loconsole *et al*., 2014; Minsavage *et al*., 1994) respectively. In both procedures, the olive and oleander samples were correctly categorized as positive and negative.

The fresh mature twigs (2-5 mm in diameter), collected from the four quadrants of the tree, showed very distinct and homogeneous stained areas and proved to be the best explant for pathogen detection; moreover, the 0.45 µm nitrocellulose membranes evidenced better results in terms of blot colour reaction. Similarly, concerning the protein-binding sites, the use of 1% fat milk solution, coupled with a gentle stirring on a shaker for 1 h, performed better than BSA, which has been commonly used in previous research (Garnsey *et al*., 1993; Garnier *et al*., 1993; D'Onghia *et al*., 2001). Interestingly, no apparent difference was observed between fresh, 1 month stored blotted membranes as well as between membranes printed directly in the field, and those printed in the laboratory.

Results obtained using the DTBIA also highlighted the importance of adopting appropriate sampling for effective pathogen detection in large scale monitoring especially in the early stages of infection. Because the bacterium is unevenly distributed within the canopy, and because four twigs can be printed in each quadrant of the grid drawn on a membrane, two quadrants can be allocated to each tree to be tested, so that a number of representative twigs is therefore analysed. Whereas, young suckers should be avoided for sampling since they are probably infected at a later stage because of the xylem translocation of the bacterium.

The overall results of this protocol by using the Enbiotech commercial Kit proved that the technique is user-friendly, fast and does not require sophisticated equipment or highly skilled operators. DTBIA is an accurate and highly reliable serological test for processing a large number of samples. The efficiency comparable to ELISA and PCR, combined with the advantages of easier handling, speed and cost, makes DTBIA a valid alternative to ELISA in large-scale surveys for occurrence of *Xf*. Thus, it could be proposed as an effective alternative method for on-site detection of *Xf*. Moreover, the printing of membranes directly in the field prevents infections from spreading to *Xf*-free areas, through the movement of plant material with pathogen vectors for laboratory testing.

In table 1 is reported the DTBIA protocol for the detection of *Xylella fastidiosa*

Table 1. DTBIA PROTOCOL FOR THE DETECTION OF *XYLELLA FASTIDIOSA*

MEMBRANE PREPARATION & TISSUE BLOTS	
Distinct squares are delimited on nitrocellulose membranes 0,45 µm pore size with high affinity for binding proteins.	
Smooth cut sections are made from cross sections of mature twigs (2 mm in diameter), then gently pressed to the nitrocellulose membranes. Each twig is printed twice in grid. Printed membranes are left to dry for 20 - 30 min at room temperature.	
BLOCKING ASPECIFIC SITES	
The membranes are incubated in 1% fat milk solution for 1h at RT on an orbital shaker, for the saturation of protein-binding sites.	
The membranes are washed with PBS Buffer (0.05% Tween 20) on an orbital shaker 3 times x 3 min each.	
ADDITION OF *Xf* SPECIFIC ANTIBODIES & MEMBRANES STAINING	
The membranes are immersed for 2h in a solution containing *Xf* specific alkaline phosphatase-conjugated antibodies.	
The conjugated antibodies solution is discarded and the membranes are washed three times using the washing solution.	
The membranes are stained by immersion in a solution obtained by dissolving one tablet of Sigma FastTM BCIP-NBT in 10ml of distilled water, until a purple-violet colour appears in the positive samples (around 2-3 min). The reaction is stopped by washing the membranes with tap water.	
MEMBRANES READING	
The membranes are observed under a low power magnification lens (X10-X20). The presence of purple-violet coloration on stem section reveals the presence of *X. fastidiosa* infection.	

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Specific, Sensitive, and Rapid Diagnosis of *Xylella fastidiosa* from olive plant material by a new Loop-Mediated Isothermal Amplification (LAMP) system

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The gram-negative, insect-vectored, *Xylella fastidiosa* has recently been detected in Italian olive trees severely affected by Olive Quick Decline Syndrome (OQDS). The Italian isolate of this bacterium has been characterized and classified as "CoDiRO" strain of the subspecies *pauca* (Cariddi *et al.*, 2014; Elbeaino *et al.*, 2014; Loconsole *et al.*, 2016) and the spittlebug *Philaenus spumarius* L. (Aphrophoridae) was ascertained to be an effective vector in Italy (Saponari *et al.*, 2014). In Puglia region, a large-scale monitoring campaign was implemented by the Regional Plant Protection Service in order to demarcate the contaminated area boundaries and to implement adequate control measures. To this aim, Enzyme linked Immuno Sorbent Assays (ELISA) and Polymerase Chain Reaction (PCR) assays were largely used. Since the movement of large amounts of infectious materials to the laboratories for testing greatly exposes "*X. fastidiosa*-free areas" to the risk of contamination, the use of rapid and on-site detection methods was highly desirable. The suitability of a new on-site Loop-mediated isothermal amplification (LAMP) system (Enbiotech s.r.l., Italy), composed of a portable instrument (icgene mini) and a ready to use diagnostic kit denominated "Xylella Screen Glow", was therefore evaluated in this study for the detection of *X. fastidiosa* in host plants and insects. To this aim, its specificity and sensitivity were compared with those of PCR and real-time qPCR assays.

For PCR assay, *X. fastidiosa* RNA polymerase gene was amplified using RST31 and RST33 specific primers, which generate a PCR product of 733 bp (Minsavage *et al.*, 1994). The amplification reaction was conducted at 94°C for 5 min, 35 cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C for 45 sec, and a final elongation at 72°C for 7 min.

Quantitative Real-time PCR (qPCR) assay was performed to amplify the *X. fastidiosa* rimM gene using *XF*-F and *XF*-R primers together with a 6′FAM/BHQ-1 labelled probe *XF*-P (Harper *et al.* 2010). The assay was conducted in a Thermal Cycler (IQ™5, Bio-Rad Laboratories, Italy, at the following conditions: 50°C for 2 min and 94°C for 2 min, then 40 cycles of 94°C for 10 sec and 60°C for 40 sec. The threshold value was set automatically by the software (iQ™5 Optical System, V2.0). A cycle threshold (Ct) value below 35 was scored as a positive result.

Real-time LAMP assay was carried out using Enbiotech's LAMP system. The system envisages a rapid preliminary nucleic acid extraction from the sample, genetic amplification using LAMP technology, detection of the fluorescence emitted and automatic interpretation of results. The kit contains a strip with extraction buffer and another with dried primers and a LAMP mix stable at room temperature for the amplification and detection. Amplification conditions were set at 65°C for 25 min.

For the specificity tests through PCR, real-time qPCR and real-time LAMP methods, pure cultures of *X. fastidiosa* and a group of 19 non-target bacterial species and/or patovars were used. All bacterial species were grown on Nutrient Agar media while *X. fastidiosa* was grown on buffered cysteine-yeast extract (BCYE) agar medium (Wells *et al.* 1987) as a control. The bacterial DNAs were extracted using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich s.r.l., Italy) and quantified at the spectrophotometer at 260 nm (A260).

Identical results were obtained for all three techniques adopted, as only *X. fastidiosa* DNA was amplified and no aspecific amplification was observed. Moreover, using the real-time LAMP system, *X. fastidiosa* was detected after only 15 min, while qPCR and PCR required about 30 min and 1.5 h, respectively.

Sensitivity tests were performed on DNA obtained from *X. fastidiosa* pure cultures harvested from BCYE agar medium and from artificially inoculated olive plants. Serial decimal DNA dilutions from 10 ng/µl to 1 fg/µl were prepared and analysed through all three diagnostic techniques.

Healthy olive extract used for diluting the bacteria suspension was obtained following two different procedures. In the first one, excised petioles and midribs (0.3 - 0.5 g) were extracted in the presence of Cetyl Trimethyl Ammonium Bromide (CTAB) extraction buffer and homogenized. The serial diluted extracts were heated at 65°C for 30 min and centrifuged at 10.000 rpm for 5 min. Then the DNA was extracted from the supernatant by mixing in an equal volume of chloroform-isoamyl alcohol (24:1) and precipitated with isopropanol, after incubation at -20°C for 1h. In the second procedure, sap was extracted from olive cuttings by injecting with a syringe 100µl extraction buffer through the plant shoot vessels. The serial dilutions of *X. fastidiosa* were directly used for the real-time LAMP reaction, after incubation at 65°C for 10 min. The remaining part of the extract was purified through a QIA shredder mini spin column (DNeasy Plant Mini kit, Qiagen, Milan, Italy), for using in the Real-time PCR and PCR sensitivity assays.

X. fastidiosa DNA extracted from pure culture successfully amplified by PCR and qPCR until a DNA concentration of 0.1 pg/µl; only real-time LAMP was able to detect up to 10fg/µl concentration.

Also in this test, the real-time LAMP system allowed to detect the presence of *X. fastidiosa* DNA at a very low concentration within a shorter reaction time (20 min instead of 40 and 90 min required by qPCR and PCR assays, respectively), using the crude extract from the plant or from insect vector. Using the crude extract qPCR and PCR assays were not able to detect the DNA of the bacterium.

Using saps extracted from plant tissue, real-time LAMP method allowed the detection of *X. fastidiosa* DNA from a concentration of 105 up to 10 CFU/ml with both purified and non-purified sap extracts, similarly to qPCR analysis. Conversely, the PCR assay showed to be highly influenced by the extraction method adopted, since it was unable to detect *X. fastidiosa* DNA from olive sap extracts in the samples containing less than 104 CFU/ml.

Unlike the other two techniques, the sensitivity of Enbiotech's real-time LAMP system showed to be not affected by the grade of purity of DNA samples and required shorter amplification times. In addition, the Enbiotech's real-time LAMP system did not require laborious sample preparation and expensive equipment, thus being applied also by non-specialized personnel.

These results, together with the simplicity of the extraction procedure and the brief reaction time required, make the Enbiotech's real-time LAMP system highly suitable for *X. fastidiosa* detection directly in the field, thus minimizing the risk of carrying infectious plant material (and vectors) in pathogen-free areas.

The Protocol of Real time LAMP for *Xylella fastidiosa* is reported in Table 1.

Table 1. Protocol of Real time LAMP for *Xylella fastidiosa*

DNA extraction

This method of DNA extraction could be applied for insect vectors/potential vectors or for plant material.

Insect: stored insect at 80% ethanol should be removed from ethanol and dried on tissue paper for 5 min; the extraction could be done for a single insect or for a pool up to maximum 5 insects.

Plant material: a) olive leaves from different parts of the plant (4-5 peduncles with a diameter of 2-3 mm) could be used for DNA extraction; b) for other host plant species, 5µl of ELISA crude extract could be used; ELISA extract prepared using 0.5-1 gr of midveins and petioles excised + 1-2 ml of extraction buffer, homogenized using the semi-automated homogenizer or by using a hummer.

- Collect a number of tubes equal to the number of specimens that will be tested.
- Add 200 µl of extraction buffer (1% Triton x-100, 20 mM Tris-HCl, 20 mM EDTA) in 0,2 ml tubes.

Open the tubes and add the specimens for DNA extraction (4-5 peduncles 1-2 mm of peduncle with a diameter of 2-3 mm immediately after cutting or an intact insect), or 5µl of ELISA extract or animal specimens (integral insect up to 5 insects in one single tube).

Vortex.

Place the tubes directly into LAMP (device or any heating device) and start the DNA extraction program (65°C for 10min).

Use 2.5 µl of DNA extracted for each tube for amplification process in the next step.

Preparing the Real time LAMP reaction

Prepare 0.2ml safe-lock tube with the same numbers of extraction procedure; add 5ul of primer mix (1 µM of each internal primer (FIP and BIP), 0.1 µM of each external primer (F3 and B3), 0.5 µM of each loop primer (LF and LB). To simplify the assay Primer mix generally provided in ENBOTECH Xylella screen Glow dried in a separate strip as ready to use Primer Mix tubes.

For each tube, add 17.5 µl of LAMP master mix, 30 µl of mineral oil and 2.5 µl of extracted DNA to the Primer Mix tube (If you are using Xylella screen Glow you should add 22.5 µl of LAMP master mix).

It is recommended to use positive control and negative control for each run.

Place the tubes directly into LAMP device, associate a number of each sample in the tablet RT LAMP software.

Perform the amplification program and wait 25 min for the results.

Results will be automatically viewed in tablet screen at the end of amplification.

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Organization of ring tests on diagnostic methods among Italian laboratories

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The occurrence of the olive quick decline syndrome (OQDS) in Puglia region represents one of the most serious plant health emergencies of recent years in which Italy and the entire European Union (EU) have had to deal with. Particularly crucial was the recovery of the quarantine pathogen *Xylella fastidiosa* in association with the disease, because it was never established in the EU territory before (Saponari *et al.*, 2013).

The need of official diagnostic protocols is a very critical issue when severe epidemics as the OQDS occur. A wide comparison among the available diagnostic methods is needed to obtain validation data of each method to be taken into consideration for the revision of the official diagnostic protocols (i.e. European Plant Protection Organization protocol). With this purpose a national test performance study (TPS) was coordinated by CREA-PAV, in the frame of an agreement with the Italian Ministry of Agriculture, by involving sixteen Italian laboratories that include several Plant Protection Services (PPS), SELGE, University of Milano, University of Catania, Centro di Ricerca, Sperimentazione e Formazione in Agricoltura (CRSFA), International Center for Advanced Mediterranean Agronomic Studies (CIHEAM of Bari). A working group was constituted, to define this interlaboratory comparison that included the following Institutions: CREA-PAV, (CNR-IPSP), PPS Lombardy, PPS Tuscany, PPS Liguria, PPS Veneto, PPS Emilia-Romagna, PPS Trentino Alto Adige, UNIMI.

The activity was organized in two steps: a PRE-TEST and the final TPS. The objectives of the PRE-TEST were the establishment of the analytical sensitivity of each method to select the bacterial contamination of samples and the methods to be assessed for the final TPS; moreover the repeatability, the analytical specificity, the relative accuracy were calculated. All performance criteria were elaborated following PM 7/76 (3) and PM7/98 (2) EPPO standards (EPPO 2014a, EPPO 2014b). The final TPS involved 16 laboratories to detect the reproducibility of the selected methods. For the development of PRE-TEST, three laboratories (CREA-PAV, CNR-IPSP, PPS Lombardy) received 16 samples consisting of two series of olive extract spiked with a devitalized *Xylella fastidiosa* CoDiRO strain suspensions ten-fold diluted from 10^7 cfu/ml to 10 cfu/ml. These samples, prepared by SELGE, consisted in: i) crude extracts to be used for direct analyses of ELISA (Agritest s.r.l.; Loewe s.r.l.) and LAMP (Enbiotech s.r.l.) and ii) olive extract to be extracted by C-TAB based method (Loconsole *et al*., 2014) to obtain total DNA for PCR (Minsavage *et al*., 1994), real-time PCR (Francis *et al*., 2006; Harper *et al*., 2010; IpadLab-Hyris s.r.l.) and LAMP (IpadLab-Hyris s.r.l. and Enbiotech s.r.l.). The real time PCR of the *cox* gene was used as internal control (Li *et al.,* 2006) obtaining 100% for all performance criteria.

As expected, the analytical sensitivity was lower when the crude extracts were used as target if compared with the total DNA. In particular, the values ranged from about 10^5 cfu/ml for the two ELISA tests to 10^3-10^4 cfu/ml for the LAMP (Enbiotech s.r.l.).

By using total DNA samples, the higher values (10-10^2 cfu/ml) were obtained by the real-time PCR and by real time LAMP (Enbiotech s.r.l.) processed in the ic-gene dedicated instrument. LAMP (IpadLab-Hyris s.r.l.) gave 10^2-10^3 cfu/ml whereas conventional PCR (Minsavage *et al.,* 1994) had lower sensitivity (10^4 cfu/ml).

The relative accuracy that consisted in the closeness of agreement between a test result and the accepted reference value (or the expected response from reference material) confirmed the higher reliability of real time LAMP (73%) with respect the two ELISA tests (respectively 63% Agritest s.r.l., and 56% Loewe s.r.l.) by using crude extract samples. The use of DNA as target showed the highest performance (100%) of the real-time PCR Francis *et al.* (2006) and the real time LAMP Enbiotech (s.r.l.). The repeatability was detected as the level of agreement between 5 replicates of a sample under the same condition, and resulted in: 100% for ELISA (Loewe s.r.l.), real-time PCR (Harper *et al.,* 2010 and Francis *et al.,* 2006), real time LAMP (Enbiotech s.r.l.). Conventional PCR (Minsavage *et al.,* 1994) gave 80% repeatability with undiluted DNA extracts and improve to 100% with ten-fold dilution DNA (probably due to an inhibition of PCR reactions).

Finally, exclusivity was assessed by CREA-PAV testing 36 different bacterial strains of several species. Real-time of Francis *et al*. (2006) gave aspecific or abnormal peaks with respectively *X. arboricola* pv. *celebensis* (NCPPB 1832), *Brenneria populi* (NCPPB 4299T) and B*. quercina* (NCPPB 1852^T), *Pantoea agglomerans* (ISF 438), *Pseudomonas marginalis* (CREA-PAV 1229), *X. hortorum* pv. *pelargonii*. Real-time PCR of Harper *et al.* (2010) did not show false positive results. The activity of the final TPS for the evaluation of reproducibility is currently in progress.

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Part Two

Control strategies, legislative aspects, capacity development and communication

Transcriptome profiling of two olive cultivars infected by *Xylella fastidiosa*

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A devastating outbreak of a strain of *Xylella fastidiosa* is ravaging the olive trees of southern Puglia, Italy (Saponari *et al*., 2015). The bacterium, discovered in 2013, causes the Olive Quick Decline Syndrome (OQDS), a destructive disease characterized by apical and marginal leaf scorching, extensive branch and twig dieback, followed by death of the trees (Martelli *et al*., 2016).

Genome studies (Giampetruzzi *et al*., 2015; Loconsole *et al*., 2016;) have shown that the olive-infecting strain of *X. fastidiosa* belongs to the subspecies *pauca*, sequence type 53 (ST53), whereas epidemiological investigations and transmission tests proved that the xylem-feeding meadow froghopper *Philaenus spumarius* is the vector of the bacterium (Saponari *et al.,* 2014).

The impossibility of curing *X. fastidiosa*-infected plants calls for the implementation of management strategies aimed at preventing infection and containing bacterial spread in the field. The international experience acquired with some of the best known *Xylella*-induced disorders, such as Pierce's disease of the grapevine and Variegated chlorosis of citrus, indicates that disease containment relies on the mechanical and/or chemical control of the vector(s), and the elimination of inoculum sources, i.e. infected hosts. It is also common knowledge that these measures can hardly eradicate the pathogen, when it has entrenched itself in areas with favourable climatic and environmemtal conditions. In these cases, co-existence with the disease, e.g through the identification and use of resistant plants would represent a desirable option.

With this aim in mind, field surveys carried out for scouting resistant germplasm allowed the identification of a possible source of resistance in olives of cv. Leccino which, in areas with a very heavy inoculm pressure, showed limited signs of infection, contrary to the extensively grown local cvs Cellina di Nardò and Ogliarola Salentina.

Quantitative determination of the bacterial population in infected olive trees showed that cv. Leccino hosts a much lower concentration of bacterial cells than cv. Ogliarola salentina, suggesting that it may possess genetic constituents and/or regulatory elements counteracting *X. fastidiosa* infection.

A global transcriptome profiling comparing healthy and infected trees of the two above-mentioned cultivars, was performed for determining whether a tolerant/resistant condition of cv. Leccino exists. Statistical analysis of the differentially expressed genes (Figure 1) from plants of the cv. Leccino (LC) and Ogliarola salentina (OG) healthy (H) or infected by *Xylella fastidiosa* (X), showed a distinct clustering of healthy and infected plants of both cvs and locates the infected cv. Ogliarola salentina more distantly than the cv. Leccino from the corresponding healthy olives.

Quantitative analysis revealed that 659 and 447 genes were differentially regulated upon *Xylella fastidiosa* infection, in cvs Leccino and Ogliarola salentina, with respect to the healthy plants of the same cultivars (Figure 2). The differential gene expression of infected *vs* healthy plants demonstrates that olives of both cvs perceive the presence of *Xfp*. Moreover, in depth analysis

of main altered genes of the susceptible cv Ogliarola salentina indicated that they are related to water stress and to an intense activation of the defense response imposed by *Xylella fastidiosa* infections.

These findings suggest that cv. Leccino is endowed with an intrinsic tolerance to *Xylella fastidiosa*, which makes it eligible for further studies aimed at investigating molecular pathways underlying its different defense response.

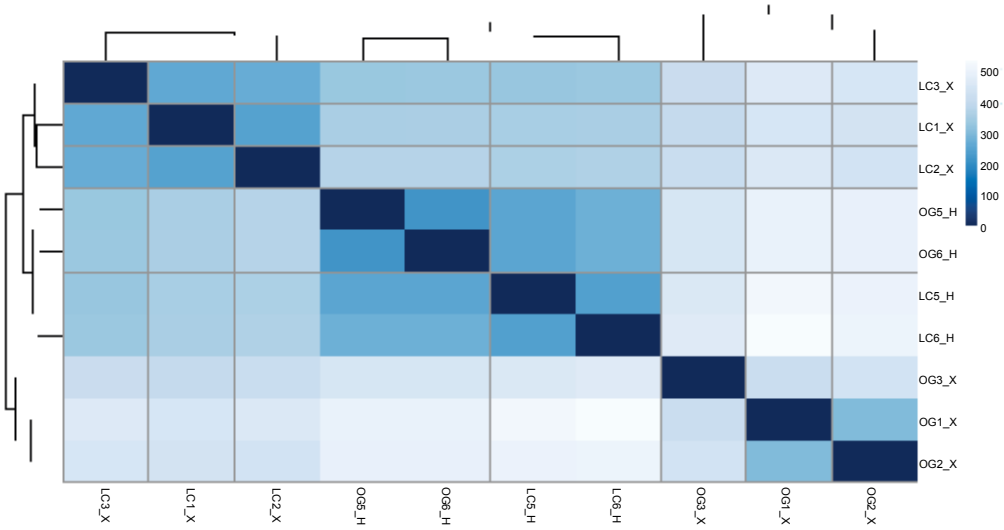

Figure 1. Statistical analysis of the transcripts libraries using rlog trasformation dato of gene expression. The heatmap shows the hierarchical clustering of biological replicates using sample-to-sample distances. Blue and white colors represent low and high values of distance, respectively. Healthy (LC5_H, LC6_H, OG5_H, OG6_H) and *Xylella fastidiosa*-infected plants (LC1_X, LC2_X, LC3_X, OG1_X, OG2_X, OG3_X) plants of cvs Leccino (LC) and Ogliarola salentina (OG) are showed.

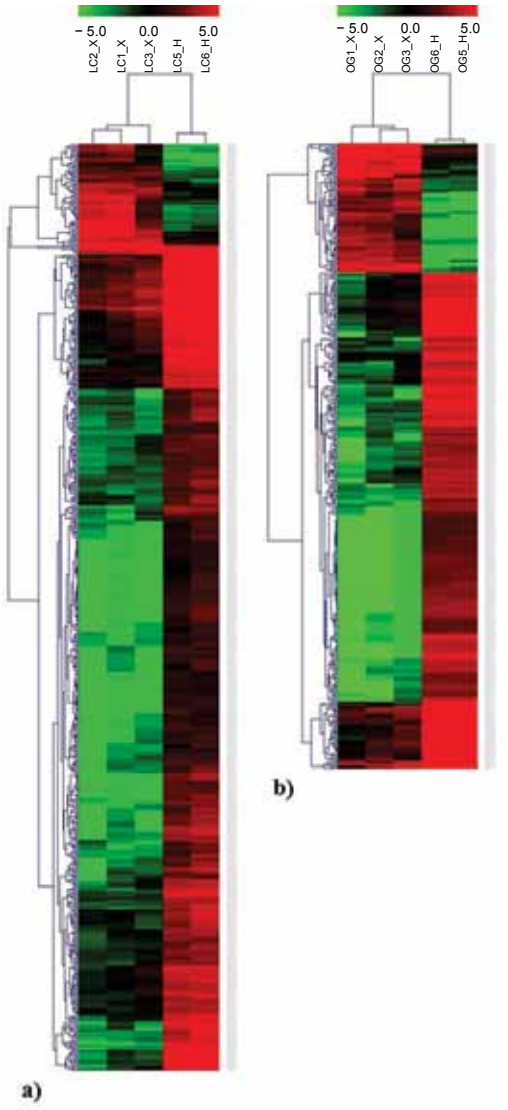

Figure 2. Heat maps of expression levels (log2) created with MeV v4.9 (http://www.tm4.org/mev) showing the 659 (a), 447 (b) differential expressed transcripts of the Xf-infected cv. Leccino (LC1_X; LC2_X; LC3_X) vs healthy (LC5_H; LC6_H), the Xf-infected cv. Ogliarola (OG1_X; OG2_X; OG3_X) vs healthy (OG5_H; OG6_H). Fold expression values are indicated by different colours.

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Preliminary results on field trials to control *Xylella fastidiosa* on olive trees in Puglia

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During early 2015, a total of 110 adult olive (*Olea europaea*) trees (cvs. Cellina di Nardò and Ogliarola salentina) grown in the province of Lecce (Puglia, Southern Italy) in three different counties, namely Galatina, Galatone and Veglie, were chosen to test the field efficacy of a CE fertilizer, patented in Israel and employable also in organic agriculture, containing zinc (4% w/w) and copper (2% w/w) complexed to citric acid, to possibly control *Xylella fastidiosa*, currently found associated with the "olive quick decline syndrome" in the Salento peninsula. The olive orchards were trained according to the common traditional way of Salento (i.e. not regular pruning, minimum soil tillage and pest control measures). The compound was preliminarily tested for verifying its capability to systemically move within the tree both upon trunk endotherapy and foliar spray application. Blocks of trees were chosen as experimental design. In the orchards, the presence of the pathogen was ascertained by means of real-time PCR (Harper *et al*., 2010), and a molecular test was preliminary set up to precisely point out which part of the tree and leaf should be taken to reliably detect the presence of *X. fastidiosa* for the additional quantitative assessment of the pathogen within the tree. Half of trees were not treated and served as control plants. A total of six spray treatments were applied to the canopy of the trees (i.e. 5 kg/ha) from early April to October. During summer (i.e. July and August), any treatment was applied. The same spray treatment scheme was carried out both in 2015 and 2016. For each tree, the total number of new shoots that wilted during the vegetative season was counted. The ANOVA and ARM programs were applied to the data recorded in the fields to test the statistical significance of the treatment. During 2015 and 2016, the treatments significantly reduced the occurrence of new wilting shoots during the season in the three olive orchards. The reduction of wilted twigs resulted higher during spring, early summer and autumn. By contrast, the non-treated trees showed an increasing incidence of the disease (i.e. presence of new wilting shoots and branches) that progressively appeared during the season. During summer, the highest number of wilted twigs both in the treated and untreated trees was recorded. None of the treated trees resulted dead. By contrast, at the end of summer 2015, in Galatone and Galatina orchards, some non-treated trees appeared dead. In these two orchards, during early spring 2016, a severe pruning (i.e. removal of a great part of some main branches) was performed to both treated and non-treated trees to verify if such a measure could reduce the symptoms severity during the following years. During 2016-2017, in the Veglie orchard, in addition to the further recording of the incidence of the disease, the quantitative real-time PCR technique of Harper *et al.* (2010) was also applied to determine the rate of reduction of *X. fastidiosa* within the canopy of some treated trees in comparison with non-treated ones. To this aim, some trees officially ascertained by the regional phytosanitary service for the presence of the pathogen before the starting of the trial were chosen. The monitoring and quantification of *X. fastidiosa* within the canopy of such olive trees is currently under way. These preliminary data would indicate that the treatments should not be suspended during summer.

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Preliminary results of comparative efficacy evalutation trials against *Philaenus spumarius* L., vector of *Xylella fastidiosa*

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The meadow spittlebug *Philaenus spumarius* L. is currently considered as one of the most common insects in the world; the species is ubiquitous and widely polyphagous. *P. spumarius* has never been associated to significant direct damage on agricultural and ornamental crops, thus no specific control measures targeting this insect species have been so far developed. However, within the xylem-feeders, this species has been considered as one of the potential vectors of *Xylella fastidiosa* in the European and Mediterranean countries; a hypothesis unfortunately confirmed upon the finding of *X. fastidiosa* outbreaks in southern Italy, associated to a novel severe disease of olive, the quick decline syndrome (OQDS). So far *P. spumarius* is the only ascertained EU vector of *X. fastidiosa*.

The fact that both *P. spumarius* and *X. fastidiosa* are associated with a large number of woody and herbaceous plants and that *P. spumarius* turned to be an efficient vector of *X. fastidiosa* and the most abundant species of *Auchenorrhyncha* are detected in the olive orchards in the infected area (Cornara *et al*., 2016; Ben Moussa *et al*., 2016) poses major risks related to the rapid spread of the *Xylella*-induced disease. An integrated pest management strategy aimed at reducing the juvenile and adult spittlebug populations is extremely urgent for suppressing the vector population. A good control of the nymphs of *P. spumarius* can be achieved when they still feed on weeds by mechanical interventions, which have limited environmental impact. At this stage the interventions are highly effective as the insects do not harbour the bacterium yet. Conversely, the control of the adult population requires the use of insecticides. No active compounds are currently registered in Italy for the control of *P. spumarius* and no data are available on the efficacy of any insecticide for the control of this species. In 2015 and 2016, a 120-day temporary registration was granted to a formulation based on a citrus oil extract. Therefore in 2015, four field trials were set up in the infected area of Salento to evaluate the effectiveness against *P. spumarius* of different organic and chemical insecticides. All trials were carried out in semi-field conditions, with a randomized block and 6 replicates per trial. Each replication was made of an olive branch caged with insect net, in which a pre-fixed number of insects was introduced. Ten adults of *P. spumarius* were introduced in cages before application 3 and 7 days for all trials, with the exception of one trial in which a single introduction was performed, before application, with 20 adults of a spittlebugs.

Insecticides were selected on the basis of previous experiences reported in the literature for the control of other *Auchenorrhyncha* (*Aphrophoridae* and *Cicadellidae*) (Akey *et al*., 2001, 2002; Bezerra-Silva *et al*., 2012; Grafton-Cardwell *et* al, 2003; Janse and Obradovic, 2010; Purterka, 2002, Redak and Bethke, 2003), while taking into account the guidelines drawn up by the Puglia Region in 2014 upon the emergence of *X. fastidiosa* epidemics. A total of twelve different formulations based on active compounds belonging to different chemical/ organic families and with different mechanisms of action and translocation were tested. The doses tested were defined based on maximum doses at which these formulations are currently used for the control of other sucking insects. The treatments were performed by spraying the entire canopy with motor pumps

that supplied the equivalent of 1,500 L/ha, with the exception of a formulation based on sweet citrus oil in trial D, for which a volume of 2,000 L/ha was used, to ensure a good distribution of the product on the olive canopy. In all trials, four inspections of the cages were carried out, 3 (DAT_3 (days after treatment)), 7 (DAT_7), 10 (DAT_{10}) and 15 (DAT_{15}) days after the application of the insecticide. During each inspection, living and dead insects were counted; living adults were left in the cages whereas dead adults were removed. The data recovered were used to calculate the percentage of mortality based on total individuals introduced for each trial in the cages and the effectiveness index calculated on the cumulative value of living insects.

Under our experimental conditions neonicotinoids (acetamiprid and imidacloprid) and pyrethroids (deltamethrin and *lambda*-cyhalothrin) showed a high mortality rate, followed by etofenprox which gave similar results but with slightly more gradual action. The formulations containing these active substances showed a persistence of about a week that rapidly decreased ten days after the application. The action of both formulations based on dimethoate was poor and slow in time. No toxicity effects against the spittlebugs were recorded using buprofezin and pymetrozine; unexpectedly for its mobility in the plants, also using spirotetramat. Among the organic compounds tested, extract of citrus oil showed very low mortality when applied at the volume of 1,500 L/ha, while a good insect mortality was observed when applied at the volume of 2,000 L/ha. In both trials natural pyrethrin showed very low mortality, while no toxicity effect was recorded using azadirachtin; indeed, no persistence was recorded for any of these compounds.

Altogether, these results provide preliminary evidence on the efficacy of different formulations for their potential use for the biological and integrated control of *P. spumarius* toward the implementation of the containment strategies for *X. fastidiosa* induced diseases.

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Sustainable strategies to contain the Olive Quick Decline Syndrome in southeast Italy

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The definition Olive Quick Decline Syndrome (OQDS) comes from the fact that the symptoms observed were initially confused and non-specific, since more abiotic and biotic factors were thought to be involved. Indeed, the Salento olive trees affected by OQDS were seen to be massively infested by *Zeuzera pyrina*, while the wood contained various vascular fungi such as *Phaeoacremonium parasiticum*, *Pm. rubrigenum*, *Pm. minimum* (= *Pm. aleophilum*), *Pm. alvesii*, *Phaeomoniella* spp. and fungal species belonging to the family Botryosphaeriaceae. The presence of *Z. pyrina*, whose larvae develop in tunnels bored into branches and twigs, is generally regarded as a factor predisposing the plant to attacks by secondary pests, including beetles, bark beetles, longhorn beetles, bacteria and fungi. Moreover, the scientific literature points to numerous fungal pathogens, such as *Verticillium dahliae* (Baidez *et al*., 2007), *Phoma incompta*, *Cytospora oleina*, *Eutypa lata*, *Pm. parasiticum*, *Pm. rubrigenum*, *Pm. minimum*, *Pm. alvesii*, *Pm. italicum*, *Phaeomoniella* spp., *Pleurostomophora richardisae* (Carlucci *et al*., 2013; 2015), and species belonging to Botryosphaeriaceae (Carlucci *et al*., 2013; 2015; Kaliterna *et al*., 2012; Romero *et al*., 2005; Taylor *et al*., 2001; Urbes-Torres *et al*., 2013) that in some cases are able to block the xylem vessels, while in others can cause cankers affecting large areas of the woody tissue of branches and the main trunk of olive trees. However, the presence of *Xylella fastidiosa* has been confirmed and considered as the primary causal agent of OQDS in Salento olives crops (Saponari *et al*., 2013).

In 2015, an experimental trial was conducted on olive groves located in the infected area of Salento (Gallipoli area), affected by rapid desiccation in order to assess the possibility of containing the symptoms by using products with different activities combined with good farming practices. For this purpose, products with very low environmental impact were chosen, such as agricultural chemicals, resistance-inducing biostimulants and fertilisers. Each trial setup included seven plants more than 70-80 years old showing clear symptoms of desiccation in leaves and branches. Eighteen tests were performed: one untreated control, 12 in which the olive trees were treated with only one of the 12 products available and five in which the olive trees were treated by combining two or three of those products. Root and leaf treatments (according to the instructions on the label of each product) were performed at intervals of 25-35 days apart, up to a maximum of six applications. Starting from 60 days after the first treatment, the effectiveness of sustainable products used was evaluated by: (i) assessing the vegetative development of the plants treated by determining the number of leaves that developed on the last twig produced after the first treatment starting from the point of insertion; (ii) assessing the chlorophyll index, i.e. the total chlorophyll content in plant tissues, which provides an indirect marker of the nutritional status of the plant; and (iii) assessing the leaf stomatal conductance, i.e. the plant water potential indicating the capacity for sap to travel from the roots to the leaves. The data collected were subjected to variance analysis (ANOVA) and the averages were compared using the Duncan test ($P < 0.05$). Moreover, laboratory research focused on assessing the direct or collateral inhibitory effects of certain products used in the 'open air trials' against fungal pathogens associated with common olive diseases. The fungi tested were *Colletotrichum acutatum* and *C. gleosporioides* (responsible for drupe anthracnose), *Phaeoacremonium minimum*, *Pm. parasiticum*, *Pm. italicum*, *Pm. scolyti*, *Phaeomoniella* spp., *Pleurostomophora richardsiae* (both responsible for vascular diseases and

cankers), *Lasiodiplodia citricola* (responsible for wood diseases), *Verticillium dahliae* (responsible for vascular and root disease) and *Rosellinia necatrix* (responsible for root rot).

The preliminary results obtained showed that the treatments performed on olive trees suffering from OQDS were all effective, regardless of the numeric value. In particular, treated plants displayed increased vegetative vigour (number of leaves), higher chlorophyll index and stomatal conductance values, and were thus statistically significant. The best results were obtained from plants tested using a combination of the products.

The results of the field tests highlighted the capacity of olive plants to react to pathogenic attack when standard agricultural practices (ploughing, milling, and pruning) and phytosanitary treatments were put in place. Moreover, the best results seem to have been achieved in plants treated with combined applications of two or more products with different characteristics. This would indicate the desirability of a strategic approach meeting all the plant requirements, ranging from nutrition to protection and defence, particularly in situations such as those prevalent in the Salento.

These preliminary and partial results of experimental activities of just one year need to be confirmed and validated by subsequent experiments in next years. Based on these experiments, it is possible to state that, at least in the 'infected' area considered in the Salento, it is possible for olive trees to coexist with the bacterium, and for the bacterium to coexist with the territory, since the olive plants' productivity was not compromised by the presence of the bacteria. This situation was also helped by performing good agricultural practices, i.e. proper ordinary agricultural and phystosanitary management. Furthermore, we believe that more time is needed for scientific verification and confirmation, and that treatment cycles should be repeated for at least another two years (in 2016 and 2017) to validate the encouraging results obtained scientifically.

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Good agricultural practices in the management of the Olive Quick Decline Syndrome

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Intensive agricultural practices have determined the loss of soil fertility and the depletion of soil organic carbon with negative effects on soil fertility, pollution, yield and fruit quality (Halvorson *et al*., 2002; Kabiri *et al*., 2016). Humus is the bond between living and non-living parts in soil and is part of the soil organic carbon that has severely declined mainly in the last 6 decades. Plants depend on beneficial soil organisms to protect them from pathogens, to help them to uptake nutrients from the soil, to increase water efficiency and to break down toxic compounds that could inhibit growth (Nannipieri *et al*., 2002; Acosta-Martinez *et al*., 2007).

Biotic and abiotic stresses are the main causes of productivity decrease and yield losses for crop species (Suzuki *et al*., 2014). In the case of Salento olive groves, the non-rational soil and canopy management could have facilitated the spread of the Olive Quick Decline Syndrome (Xiloyannis *et al*., 2015). Thus, soil management for sustainable olive production must involve the introduction of a number of practices with the general aim of improving orchard performance, bringing about greater autonomy for the grower, while at the same time increasing the stability of production (yield and quality), and reducing risk to the environment.

Increase of carbon in the soil

Soils represent the most diverse and important ecosystem on the planet. Soil has been recognized to play a double role in the entire agro-ecosystem: it is important for a good production as well as for a healthy environment (Noel *et al*., 2009; Turner *et al*., 2016). The sustainable management implies the increase of carbon in the soil. Research works showed that the recovery of carbon in the soil is a relatively slow process and takes 7-10 years before it can be revealed (Montanaro *et al*., 2012). This aspect emphasizes the urgency of promoting the actions for its recovery.

Soil and microbial activity

Most of the biodiversity of agroecosystems is found in the soil, and the functions performed by soil biota have considerable direct and indirect effects on crop growth and quality, nutrient cycle quality and sustainability of soil productivity (Barrios, 2007). The composition, complexity, genetic diversity and the use of nutrients of the soil microbial communities are positively influenced by a sustainable management system (Ghimire *et al*., 2014). This is the case of a study in which an olive grove managed for 12 years with sustainable practices showed a greater genetic, functional and metabolic diversity, and a greater amount of microbial species, compared to the conventional management (Sofo *et al*., 2010). Microbiological analyses revealed significant changes in the soil microbial communities in response to sustainable farming practices (Sofo *et al*., 2014). The management mode of the land has a significant effect on the number and biodiversity of fungal and bacterial populations of the soil. According to Sofo *et al*. (2014), the diversification of microbial communities was likely enhanced by the inputs to the soil of different quality organic material.

Increase of microbial-biodiversity in leaves and fruit

The interface between the aerial part of the plants and the atmosphere (phyllosphere for leaves and carposphere for fruits) constitutes a very specific habitat for epiphytic microorganisms and

is normally colonized by a variety of bacteria, yeasts and fungi. Both in the phyllosphere and carposphere, bacteria are by far the most numerous organisms. On this basis, the bacterial communities of phyllosphere and carposphere of olive plants subjected for several years to two different management systems (conventional and sustainable) were recently characterized (Pascazio *et al*., 2015). From this study, it emerged that sustainable soil management significantly modified the composition of the bacterial communities of phyllosphere and carposphere, increasing their biodiversity.

Endophytic bacteria

Endophytes are organisms, often fungi and bacteria, that live between plant cells within a plant and usually establish a symbiotic relationship with the plant (Hallmann *et al*., 1997). Endophytic bacteria may play a significant role in protection against plant pathogens and in the overall productivity of an agricultural ecosystem (Mercado-Blanco and Lugtenberg, 2014). Different studies revealed that *X. fastidiosa* interacts with endophytic bacteria present in the plant xylem, and that these interactions, particularly with *Methylobacteriurn mesophilicum* and *Curtobacterium flaccumfaciens*, may affect disease progress. In addition, high frequency of *C. flaccumfaciens* had been observed in asymptomatic citrus plants and this suggests a role for this organism in the resistance of plant to Citrus Variegated Chlorosis (Lacava *et al*., 2009).

Agricultural practices

Water and nutritional stresses are often determining factors in the development of symptoms once a plant has become infected with *X. fastidiosa (*Janse *et al*., 2010*)*. Field practices should therefore be directed towards healthy, well growing plants and adequate nutrition. Iron deprivation possibly provides a way to reduce disease severity by preventing biofilm formation in the xylem vessels (Toney and Koh, 2006).
A good canopy management with frequent pruning may play a key role in the *X. fastidiosa* infection management, since it facilitates air circulation and prevents the increase in relative humidity. In this way, it is possible to reduce the amount of inoculum and of the upstream migration of the pathogen in the plant. In the areas in which the presence of *Xylella* is detected, it is necessary to remove all the infected shoots by cutting at 5-10 cm below the symptoms and disinfect the tools used for pruning before moving on to the next crop plant. After the cut, it is possible to perform a treatment with copper-based products for preventive purposes. After the attack of *Xylella fastidiosa*, a plant in a good nutritional status reacts issuing new lateral shoots, thus rebuilding the vegetation quickly. This is important when the attacks occur both on the upper or outer side of the canopy. In this situation, the sectoral pruning could permit to save the plant without eradicating it (Xiloyannis *et al*., 2015). Studies of bacterial infections on different crops (e.g. *Erwinia amilovora* and *Pseudomonas syringae* in kiwifruit, *X. fastiodiosa* of the Pierce's Disease in grapevine and *X. fastidiosa* of the Citrus Variegated Chlorosis in citrus) revealed that it is possible to coexist with the infection through the adoption of cultural practices directed towards healthy, well growing plants aimed at reducing the spread of the disease.

The conventional, non-sustainable, agronomic practices should evolve in a more sustainable management addressed to ameliorate the ecological networks and nutrient cycling in which soil microorganisms are involved. The adoption of a sustainable management of olive groves can increase the soil fertility and biodiversity and its capability to generate benefits for the environment. It is important to consider the olive grove as a whole and improve its "health condition" by adopting sustainable agricultural practices in order to increase the ability of plants to overcome the biotic and abiotic stresses. In this way, the coexistence with the *X. fastidiosa* bacterium will be possible limiting the spread of the disease.

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Risk assessment of *Xylella fastidiosa* in the EU territory and other EFSA activities

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Since the first detection of *Xylella fastidiosa* in Europe (Lecce, Italy, October 2013), the European Food Safety Authority (EFSA) conducted several activities to provide scientific advice and assistance to the European decision makers facing this emergency. In a first scientific advice provided in November 2013 (EFSA, 2013), EFSA concluded that prevention of pathogen entry is the most effective option to reduce the risk of its establishment and spread, particularly when targeted to the pathway of plants for planting and to the xylem-sap feeding insects, whose European species should be all regarded as potential vectors. Later, the EFSA Scientific Panel on Plant Health (the PLH Panel) conducted a more comprehensive evaluation which included the pest risk assessment of the pathogen for the EU territory and the evaluation of the effectiveness of all available risk reduction options against *X. fastidiosa* entry, spread and establishment. The final document, published in January 2015, provides a detailed analysis of the risk components and the management options against both the pathogen and its vectors (EFSA PLH Panel, 2015a). In appendix, a first list of the reported host plants and a review of confirmed non-European and potential European vector species are provided. In the same document, the PLH Panel recommended to conduct further research on the biology, epidemiology and control of the Puglia strain of *X. fastidiosa*. To address this last point and to reduce the uncertainties highlighted in the PLH Panel opinion, EFSA funded a pilot project to collect preliminary data on the susceptibility of important crops to the Puglia strain of *X. fastidiosa*. The overall goals were: (i) to study the host range of *X. fastidiosa* subsp. *pauca* strain CoDiRO; (ii) to provide an accurate description of the symptoms in olive and other known susceptible hosts; (iii) to investigate if other economically relevant plant species, like grapevines, citrus and oak, which have not been found infected under natural conditions in the outbreak areas, were susceptible to strain CoDiRO; (iv) to provide research-based information with regards to the implementation of a program for the evaluation of the susceptibility of a wider panel of host species. The project, which lasted for 18 months, was conducted by the CNR of Bari in collaboration with the University of Bari and the CRSFA of Locorotondo, Bari. Its findings (Saponari *et al*., 2016) substantially contributed to increasing the knowledge on the current situation in Puglia and furthermore provided evidence that strain CoDiRO is the causative agent of the olive quick decline syndrome, also highlighting differential responses of various olive varieties to *X. fastidiosa* strain CoDiRO infections. On 12-13 November 2015 (Brussels), EFSA hosted a workshop titled "*Xylella fastidiosa*: knowledge gaps and research priorities for the EU", where more than 100 scientists reflected on current knowledge and research priorities for the European territory. After the plenary session, the participants separated in discussion groups on i) surveillance and detection; ii) vectors' identity, biology, epidemiology and control; iii) plants: host range, breeding, resistance and certification; and iv) pathogen: biology, genetics, and control.

In addition, in the period 2015/ 2016, EFSA produced scientific and technical advice providing further clarifications on specific aspects:

- responding to an NGO claim (EFSA, 2015): EFSA concluded that the hypothesis that tracheomycotic fungi are the primary causal agents of olive quick decline syndrome was not supported by scientific evidence.
- assessing the effectiveness of hot water treatment against *X. fastidiosa* in dormant grapevine planting material (EFSA PLH Panel, 2015b): the PLH Panel considered the conditions prescribed to sanitize grapevine planting material against flavescence dorée (50°C for 45 min) also effective against *X. fastidiosa* and its subspecies.
- critically reviewing some studies on grapevine susceptibility to CoDiRO strain (EFSA PLH Panel, 2015c): the short timeframe of observations and the intermediate aspect of the results available allowed only to conclude that the results presented were coherent and provided converging lines of evidence that grapevine (*Vitis vinifera*) is not a major susceptible host of *X. fastidiosa* strain CoDiRO. However, it was considered premature to exclude that systemic infection of *V. vinifera* and *Vitis* sp. could occur and that infections at limited foci could serve as a source of inoculum.
- updating the *X. fastidiosa* host plant database (EFSA, 2016): the current version of the database includes reports of hosts of *X. fastidiosa* published up to 20 November 2015, with a list of *X. fastidiosa* host plant species counting 359 plant species (including hybrids) from 204 genera and 75 different botanical families. Compared to the previous version of the database, 44 new species and 2 new hybrids, 15 new genera and 5 new families were introduced, the majority of which (70%) was identified for the first time in Puglia, Corsica and southern France outbreaks.
- responding to six queries statements regarding the EU control strategy against *X. fastidiosa* (EFSA PLH Panel 2016a, b, c) and in particular on:
  - *factors affecting symptom expression and spread of X. fastidiosa:* all interventions that support vigorous growth and development of the plant lead to improving its health status, its resilience and prolong its productive phase but do not cure the plant from bacterial infections;
  - *the etiology of the CoDiRO disease on olives:* it was confirmed by the EFSA funded pilot project (Saponari et al., 2016) that the pathogen *X. fastidiosa* subsp. *pauca* is the causal agent of the CoDiRO disease of olive plants;
  - *host plant removal as an option for containment or eradication:* this was considered in a system-based approach as an option to prevent further spread of the pathogen to new areas;
  - *secondary effects of pesticides on the interaction of X. fastidiosa with infected olive trees:* such effects were not substantiated as currently there is no evidence on negative effects of such treatments on the severity of symptoms and the outcome of the infection;
  - *efficacy of current treatment solutions to cure X. fastidiosa diseased plants, in particular those under evaluation by two research groups in Puglia olive orchards:* the Panel acknowledged the potentially positive effects of such treatments in prolonging the productive phase of olive trees and their putative relevance for the management of olive orchards, particularly in the containment area where eradication of the pathogen is considered no longer possible;
  - *assessing the diversity of the population of Xylella fastidiosa subsp. pauca in Puglia:* this evaluation is currently ongoing.

In the future EFSA aims to keep supporting the EU and its Member States in the prevention and control of *X. fastidiosa*, as well as other emerging plant health threats, by providing up to date

scientific advice and assistance to risk managers and developing and maintaining tools such as the databases and models for pest risk assessment.

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A Pest Risk Analysis on *Xylella fastidiosa* for the countries of the Near East Plant Protection Organization, focusing on the olive-infecting strain

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A strain of *Xylella fastidiosa* (*Xf*) subsp. *pauca* was first reported causing a disease of olive, commonly referred to as Olive Quick Decline Syndrome (OQDS), in Puglia, southern Italy, in 2013. This strain has subsequently spread, with devastating consequences, to other olive production areas within the province. In 2015, olive oil production in Italy was estimated to have fallen by around 50% as a combination of drought and *Xf*. Many cultural and environmental consequences associated with OQDS are also evident, if harder to quantify.

With respect to *Xf* pathogenic on olive, currently reported cases have all been attributed to *Xf* strains with closest genetic homology to *Xf* subsp. *pauca* and "are limited" to Italy, Brazil and Argentina. This withstanding, *Xf* is known to cause other serious diseases such as Pierce's Disease on grapevine. The scientific review of *Xf* suggests that much is not known about the causal agent, with substantial gaps in knowledge on host range and spread by insect vectors. To date approximately 226 natural and 190 experimental hosts of *Xf* have been recorded. Almost parallel to the discovery on olive, in October 2015 outbreaks of another *Xf* subsp., subsp. *multiplex*, were reported for Corsica and Provence-Alpes-Côte d'Azur in France on native *Polygala myrtifolia*. In response to the disease on olive, on *P. myrtifolia* and a potential wider threat of *Xf* on other crop and environmental plants, the European Union declared an emergency status in the Member States.

Countries of the Near East and North Africa region (Algeria, Egypt, Iraq, Jordan, Lebanon, Libya, Malta, Morocco, Palestine, Pakistan, Syria, Sudan, Tunisia and other neighbouring countries) have a heavy reliance on agriculture, amongst which olive is a critical crop, for both domestic and export markets. By example of Palestine and Tunisia, olive is the most cultivated crop, and for Palestine represents 2.15% of the national income. Other crops that have also been identified as potentially at risk from *Xf* also figure strongly in the region. Examples here include citrus, stone fruit crops and Quercus.

At the time of writing, within the NEPPO region and neighbouring countries *Xf* has only been reliably reported from Iran (2014). In the light of the events unfolding in Italy, a Pest Risk Analysis (PRA) has been undertaken for the NEPPO countries to document the prevailing status of the regions and its vulnerability to *Xf*, focusing on the olive-affecting strain. This PRA highlights gaps in the capacity of NPPOs to monitor for *Xf* in the environment and with imported traded commodities, alongside knowledge gaps on the presence and prevalence of known and possible vectors. The

ability of *Xf* to move in the plant material intended for planting, and to be asymptomatic, presents a particular challenge. It is noted that many countries of the Near East and North Africa region are with instability that makes them fragile to additional pressures. Accordingly, economic and social costs associated with the wide-scale spread of *Xf* on olive and/or other crops are seen as substantial.

Pest risk analysis on *Xylella fastidiosa* in Palestine

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Agriculture is considered as one of the main components of the national identity, history, heritage, society and economy of Palestine. About 11.5% of the workforce in Palestine is in agriculture. The total agricultural area is about 120 706 hectares, about 21% of the total area of the West Bank and Gaza strip. About 81% of the agricultural area is rain-fed, while the other 19% is irrigated. Palestine has a semi–arid Mediterranean climate and a wide topographical variation, which permit a high biodiversity and natural resources. The main crops are divided into 3 groups: field crops represent about 27%, vegetables 11% and fruit trees 62%. Olive orchards account for more than 80% of the total fruit tree area and about 50% of the cultivated area . Olive production relies on natural precipitation and constitutes 2.15% of the Palestinian national income.

Given the major importance of agriculture in Palestine and the threat posed by *Xylella fastidiosa* in the Mediterranean area after the first outbreak in 2013 in Italy on olive orchards, the National Plant Protection Organization (NPPO) is going to apply different actions to prevent entrance, establishment, spread and the destructive effect of this disease.

Pest Risk Analysis

Xylella fastidiosa is a destructive disease which attacks a wide range of plant species including economically important crops (citrus, stone fruits, grapevine and olive), in addition to wild forest trees, shrubs and landscape plants. From the literature, *Xf* is a gram-negative bacterium (family *Xanthomonadaceae*) that has evolved with plants to exist as a xylem-limited endophyte. It is known to have a remarkably broad host range, with 359 plant species, from 204 genera and 75 different botanical families recorded. *Xf* is transmitted by xylem-feeding insects, which are limited to the order Hemiptera, sub-order Auchenorrhyncha (Cicadellidae, Aphrophoridae and Cercopidae).

The geography of Palestine, in particular the broad range of climatic and environmental conditions and the wide range of host plants present, could favour the establishment and spread of *Xf*. More research and surveys need to be conducted to determine the presence of the many xylem-feeding insects from the previous mentioned order, which could be widely distributed. The lack of accurate information about the vectors and their associated hosts means that it is difficult to evaluate the likelihood of introduction, and requires more in-depth research and monitoring.

Pest Risk Assessment

Regulatory status. The Borders of Palestine (West Bank & Gaza Strip) are not under Palestinian control. All trade is indirect, through Israeli ports where checks occur and strict import regulations are applied. NPPO follows two legal mandates in addition to Israeli Regulations: the up-dated national law of Agriculture No. 2 of 2003 and its amendments No.11 of 2005; and 3 relevant bylaws - Agricultural Nurseries bylaw, Agricultural Quarantine bylaw and the Production of Seeds and Plant Propagation Material bylaw.

Current situation of the disease. To date, *Xf* has never been reported in Palestine. Suitable conditions and availability of host plants mean that it is of high importance to conduct a

comprehensive survey for the disease and for insect vector(s) detection. The former should be based mostly on symptom observation and testing of suspected plants. The possibility of entrance of *Xf* by trade of plant material is rated to be low. Entry via passengers carrying plant material and by insect vector is rated to be moderate to low.

Host plants and vectors. A wide range of plants, which are known as hosts of *Xf*, are present in Palestine, including cultivated fruit trees, weeds, ornamentals and forests species. In addition, a wide range of xylem-feeding insects are present and may be potential vectors of the pathogen.

A thorough survey is needed to be sure whether the pest is present (or absent). Laboratory analyses, infrastructures and financial support are needed. There are no abiotic factors that could limit *Xf* establishment. Study and survey programs to determine the status of the potential vectors of *Xf* in Palestine are essential, leading to consider more appropriate management options with the possible risk.

Pest entrance by plant material. Importing fruit tree planting material is prohibited according to the law. All types and quantities of imported commodities are subjected to Israeli authorities checks at the borders (Paris economic agreement). Agricultural commodities should have a phytosanitary certificate from the country of origin. Moving plants or plant seedlings between Palestine and Israel is possible with an internal arrangement. Therefore, the possibility of entrance by plant material appears to be unlikely. In the case of fruits, there is no evidence that fruit can transmit *Xf*.

Pest entrance by insect vectors. Insect vectors are unable to move from country to country by themselves. Movement of vehicles into Palestine is not possible, except trade vehicles which are obliged to remain on the borders or ports for a long time. It is moderately likely to unlikely that *Xf* can be introduced by insect vectors.

Pest entrance by human movement. Very few citizens from Palestine can travel to EU countries, North and South America and other *Xf* infected countries. However, many Israeli citizens are residents in all mentioned countries and travel back to Israel. These passengers may be moderately likely to transfer host plants for plantings or the vector to the country. It is important to establish bilateral discussions and professional meetings and establish efficient measures to raise the public awareness about the dangers of importing plant material.

Pest establishment. Under the current situation - availability of wide range of host plants, suitable weather conditions for the bacterium and insect vector - the possibility for *Xf* to become established in Palestine is considered to be very likely. The availability of the insect vectors would play a major role in the establishment and spread of the pathogen. However, further studies are needed to define the status of xylem-fluid feeding insects in Palestine. If these potential vectors are present then the establishment of the pathogen will be rated as very likely based on the wide host range of cultivated crops, in addition to the possible wild and asymptomatic host plants, the confidence will be high. The lack of efficient cultural practices to limit this pest or control measures also increases the probability of establishment.

Economic, environmental and social impact. The establishment and spread of *Xf* would have major negative impacts on the agriculture sector and national income. Trade in horticultural goods would slow and *Xf* would reduce the production quality and quantity and cause host deterioration, negatively affecting the international and national markets, food security and safety, in addition to farmers' incomes. Intervention against the pest or its vector/s will incur additional costs, taking into consideration the budget for eradication or treatment.

Olive and other fruit trees such as Citrus, grapevine and stone fruits would be negatively affected. More than 100 thousand Palestinian families, the majority living in rural areas (14% of total population), rely on the olive sector either directly or indirectly (labour, transportation, industry, nurseries and traders). The value of olive production at the farm gate consists of 3.5% of the total value of agricultural production. The olive sector comprises 13.2% of total agricultural exports.

Many plant species have a positive environmental impact, limiting soil erosion and runoff, desertification, carbon dioxide uptake and contributing to biodiversity, in addition to being beneficial to tourism. Control measures against *Xf,* mainly pesticide use against the vectors, may have side effects on beneficial insects, wild life, biodiversity and the environment.

Area endangered by the pest. Cultivated areas with olives, citrus, grapes, almond, ornamental plants, and forest are the main endangered areas in the country.

Pest Risk management

Prevention. Importation of agricultural commodities from countries where the pest is present should be prevented (plant material of pathogen host species). Each country should carry out surveys and establish if the pest and its possible vector/s are present. Other commodities should be checked to ensure that they do not harbour any insect vectors before shipping. Further inspection should be done at the borders before entrance either for passengers' baggage and/or commodities. For Palestine, import of planting material especially of fruit trees is prevented, and *Xf* is considered a quarantine pest.

Eradication. Once the bacterium has established or spread, eradication is very difficult and unlikely, because of the huge range of host plants (symptomatic and asymptomatic) and potential vectors. In addition, latent infections, which could act as an inoculum source, are hard to detect.

Public awareness on the disease, focusing on symptoms on different plant species, is required. Stakeholders should look for symptoms of *Xf* and report any suspected plant to the NPPO for assessing the presence of the bacterium through laboratory testing.

The implementation of the legislation provision to contain the spread of the disease will involve the following actions:

- premoval and burning of infected host plants taking into consideration the latent infections and the buffer zone; tools should be disinfected after use in infected orchards or when moving from one orchard to another; for nurseries, all plants should be destroyed if *Xf* is detected;
- isolation of the infected area, establishment of clear borders and prevention of movement of any host plant out of the infected area;
- control measures against the vector(s) within the contaminated area and the surrounding buffer zone using pesticides, trapping and/or any method to decrease vector population;
- survey in the orchards to identify infected plants (inspection and sampling for laboratory tests); eradication or containment measures improve with early detection within a region.

Exclusion. No treatments are efficient for eradicating the bacterium once it is introduced or established. Insufficient relevant information about the bacterium, its host plant range and insect vectors could increase uncertainty. Procedures are beaded to improve management for this disease and to reduce its negative effect. The followings should be considered:

- phytosanitary certificate, indicating that the seedlings, commodities and any agriculture products come from a pest-free area. Issue of strict regulation to prevent importation of any possible host plant/part of plant from infested area;
- information exchange between countries, organizations and stakeholders about the pest, host plants, vector/s and contaminated area;
- survey and inspection for the nurseries, mother plants, orchards and natural or wild areas, for the presence of symptoms, infected plants or insect vector/s;

– additional intervention such as treatment of imported consignments and packaging material (either at origin or at borders before entrance) to eliminate any possibility of any infestation by vectors;

– public awareness for farmers and citizens, using posters and local media, etc.

A contingency plan should be soon prepared by the NPPO with the support of all relevant organizations in order to identify and train the responsible for the different actions (e.g. surveillance and monitoring, surveys, sampling, laboratory analyses).

Work done and actions taken on *Xylella fastidiosa* in Lebanon

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The importance of the olive tree goes back a long way in human history. It was cultivated by many ancient civilizations that settled in the Mediterranean basin. Olive (*Olea europaea* L.) is cultivated in Lebanon on a surface of ca. 53 600 ha, accounting for 43% of the area given over to perennial crops (Anonymous, 2010).

In autumn 2013, an epidemic outbreak of *Xylella fastidiosa* was reported in the Mediterranean region, namely in Puglia (South-Eastern Italy), where this pathogen is the main cause of the so-called quick decline syndrome (QDS) of olive trees, leading to destructive dieback and wilting of trees. The pathogen also infected oleander and almond, which exhibited typical leaf scorch symptoms (Guario *et al*., 2013; Saponari *et al*., 2013) and presently, has extended to include sweet cherry, and several landscape plants (Catalano, 2015). There are concerns that this disease could also reach Lebanon and destroy many economically important crops as well as the culturally important and beautiful centennial olive trees.

In Lebanon, *X. fastidiosa* was recently reported as being associated with leaf scorch, chlorosis and stunting symptoms of oleander growing in the American University of Beirut (AUB) campus (Temsah *et al*., 2015); ELISA tests for this pest were positive as were in situ observations made with a scanning electron microscope (SEM). Although isolation and culturing on appropriate media are mandatory for a first occurrence of *X. fastidiosa* (EPPO, 2004), and molecular assays are crucial to confirm ELISA-positive samples (Amanifar *et al*., 2014; Loconsole *et al*., 2014), neither of these two techniques were performed on these oleander ELISA-positive samples from AUB to confirm the presence of the pathogen in Lebanon.

Since 2013, symptoms of leaf scorch, accompanied in a few cases with severe branch defoliation, have been commonly observed on olive in the main growing areas in Lebanon. Furthermore, despite the outbreak of *X. fastidiosa* in Puglia, Lebanon continued to import olive seedlings and ornamentals from Italy, until the decree 1/161 in 04 March 2015 (Amendment to the list of quarantine pests for Decree Nr. 783/1 dated 26/8/2011) (Annex 1"Arabic version") was issued by the Lebanese Ministry of Agriculture, to impose the import of plant propagation material only from *X. fastidiosa* pest-free areas. In addition, the Decree Nr. 1068/1(Annex 2 "English version") for the protection from *Xylella fastidiosa* when importing plant product was issued. Within this decree all consignments of fruit seedling and ornamental plants, including plants, plant parts such as leaves, branches, twigs, roots, flowers (except seeds), must be accompanied by an original phytosanitary certificate and mention in the additional declaration that consignment is free from the bacterium *Xylella fastidiosa* and must be accompanied by an additional declaration certificate as proof that consignment was produced in Pest Free Area Zone free from *Xylella fastidiosa* under the supervision of the NPPO of the country of origin specifying the name of the place of production.

Considering the risk this pathogen presents and the devastating disease it can cause in Lebanon, on both agricultural and landscaping plants, it was crucial to quickly monitor the area in which the occurrence of *X. fastidiosa* is suspected.

A survey on the spread of *X. fastidiosa* in Lebanon was organized by the Department of Plant Protection of the Lebanese Agricultural Research Institute (LARI)-Tal Amara, the Laboratory of Mycology, Department of Plant Protection LARI-Fanar, the Department of Olive and Olive Oil, LARI-Tal Amara and the Department of Plant Protection of the Lebanese Ministry of Agriculture (MoA) in May 2014, starting from the main olive growing areas in which symptoms similar to those described for QDS were observed. Samples (twigs and mature leaves) were collected during three vegetative seasons: spring 2014 and 2015, and autumn 2014. A total of 82 different olive trees from 24 groves were sampled at different heights of the canopy. In addition, 30 grapevine plants expressing symptoms similar to Pierce's disease were also sampled during fall 2014.

Following the report on the occurrence of *X. fastidiosa* on oleander in Lebanon (Temsah *et al*., 2015), and upon the request from the Phytosanitary service of the Lebanese Ministry of Agriculture, twigs samples were collected from oleander plants at AUB campus (Beirut) to confirm the previous ELISA results by molecular, serological and cultural methods. Four twigs per plant were taken from 15 oleander plants showing leaf scorch and stunting symptoms and located in the same landscape area where the bacterium was previously reported; in addition, 10 asymptomatic plants located in the surroundings were also sampled. Seven samples from symptomatic oleander were also collected from landscape areas in Byblos and Bekaa Valley.

In spring 2015, samples were gathered from nurseries that imported ornamentals and olive seedlings from Italy during 2014–2015. In total, samples were collected randomly from 26 three-year-old olive grafted seedlings and 48 ornamental plants, already reported as hosts of *X. fastidiosa* in Puglia.

All the samples showing symptoms similar to those described on olive, almond, oleander and other ornamentals in Southern Italy were used in attempts to isolate the bacterium on different media, according to the procedures described by Cariddi *et al*. (2014).

For serological detection on symptomatic and asymptomatic samples, tissue extracts obtained from leaf petioles and midveins excised from 8–10 mature leaves and macerated in plastic bags (Loconsole *et al*., 2014) were tested by Double Antibody Sandwich ELISA using specific antibodies to *X. fastidiosa* (Loewe Biochemica GmdH, Germany) following the manufacturer's instructions. In addition, Direct Tissue Blot Immunoassay technique for the detection of *X. fastidiosa* was carried out as described by Djelouah *et al*. (2014).

For molecular detection on symptomatic and asymptomatic samples, DNA was extracted using CTAB-based extraction procedure (Loconsole *et al*., 2014) with slight modification. Molecular detection of *X. fastidiosa* in the samples was carried out by PCR using three sets of primers: RST31/RST33, which is mandatory for the detection of quarantine pathogen, according to the EPPO protocol (EPPO, 2004); FXYgyr499/RXYgyr907 and HL5/HL6. Laboratory activities were undertaken at the Department of Plant Protection, Tal Amara at the Lebanese Agricultural Research Institute in collaboration with the Department of Soil Sciences, Plant and Food, University of Bari, Italy.

Results of all tests were negative and unequivocally demonstrated that all the collected samples were free from the pathogen. In addition both serological and molecular tests and attempts at isolating the pathogen demonstrated that oleander samples gathered from American University campus in Beirut, where *X. fastidiosa* was previously reported, were not infected. A scientific article entitled "*Xylella fastidiosa* Does Not Occur in Lebanon" was published in Journal of Phytopathology on the results of this survey (Habib *et al*., 2016) (Annex 3). Nevertheless, continuous and large monitoring and rigorous control measures of propagative materials are necessary to prevent the introduction of *Xylella fastidiosa* in Lebanon.

Numerous species of xylem fluid-feeding sharpshooters and spittlebugs are known to transmit the bacterium worldwide. The species ascertained as an effective vector for the CoDiRO Italian strain

is the meadow spittlebug, *Philaenus spumarius*, although potential vectoring roles of *Neophilaenus campestris* and the phloem feeder *Euscelis lineolatus* have been reported. *Philaenus spumarius* has not yet been reported in Lebanon, but any xylem fluid-feeding hemipteran should be regarded as a potential vector of the bacterium. To date, in Lebanon, no research has been carried out on insect vectors of *Xylella fastidiosa*. The knowledge of the insect vectors is crucial for well-timed and efficient control strategies, to avoid further spreading of the bacterium. The study of the entomofauna related to the crops of interest is not always easy and often different sampling techniques should be combined, due to the different life cycle of the insects. Moreover the search should not be restricted only to the crop(s), but it should be extended to the surrounding weeds. For this reason, to optimize the search for the vectors in Lebanon, a clear plan and in depth training for LARI and MoA staff is needed in order to choose the best sampling method, to identify the effective/potential vectors based on the transmission trials.

Among the awareness raising and training activities undertaken in the country, LARI research staff organized several meeting with MoA Agricultural Engineers in order to prepare a decree to impose the import of plant propagation material only from *X. fastidiosa* pest-free areas. During the field inspections, LARI staff explained to farmers about the serious damage that this quarantine pathogen could cause if it is spread in olive groves. Two seminars were carried out for farmers to make them aware them about the economic impact of this lethal disease and other olive diseases. Training was given to Dr. Elia Choueiri at IAM Bari during May 2015 where he had the opportunity to discuss and exchange scientific knowledge with Italian experts about the main characteristics of Olive Quick Decline Syndrome. This was part of a Training course on Early Warning System for Integrated Pest Management in the framework of the ClimaSouth Project. In addition, for the other Scientific staff from LARI; Dr. Wassim attended a Workshop "*Xylella fastidiosa* threat on food industry" at IAM-Bari, Italy (2014); Dr. E. Choueiri, Dr W. Habib and Eng. Elvis Gerges participated in the Round Table "*Xylella fastidiosa*: a serious menace to the Mediterranean fruit industry" during the 14th congress of Mediterranean Phytopathological Union, Istanbul, Turkey (2014) and lastly Eng. Farah Baroudy from LARI attended the International symposium on the European outbreak of *Xylella fastidiosa* in olive, Locorotondo, Italy (2014).

Recently, a Regional Technical Cooperation Programme (TCP) on *Xylella fastidiosa* for Lebanon expected to be funded by FAO is upcoming with the following objectives: (i) Building capacities of the technical team from MoA and LARI on monitoring and management of *Xylella fastidiosa*; (ii) Development of a national *Xylella fastidiosa* survey; (iii) Building capacities for the survey team; Awareness raising for farmers; (iv) Building capacities of phytosanitary officers on identification of *Xylella fastidiosa*; (v) Development of a manual/handbook for the identification of *Xylella fastidiosa* at border points; (vi) Development and implementation of regulations to limit the entry of *Xylella fastidiosa*.

Acknowledgments

Finally, I would like to thank the scientific staff of LARI (Dr. Wassim Habib, Eng. Elvis Gerges, Dr. Milad El Riachy, Eng. Fouad Jreijiri and Dr. Elia Choueiri), MoA personnel (Eng. Youssef Al Masri) and Bari University (Dr. Franco Nigro) for their effective participation in this field survey and laboratory activities.

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Regulations enforced against *Xylella fastidiosa* in Jordan

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The olive tree is one of the most important traditional cultivated trees in Jordan from economic and social perspectives. It is an essential part of the cultural heritage. The total area planted with olive in Jordan is about 120 000 hectares. This corresponds to approximately 73% of the total fruit trees and 36% of the total cultivated area. The estimated number of olive trees is 15-20 millions. The production of olive fruit was about 200 000 tonnes in 2015 compared to 155 640 tonnes in 2012. Olive oil production was about 29 611 tonnes in 2015, and 21 548 tonnes in 2012. Olive production is the main source of income for around 85 000 Jordanian families. The olive Unit Directorate in the Ministry of Agriculture plays an important role in supporting and improving the cultivated olive crop within the agricultural sector.

In addition to olive trees, other fruit trees are major cultivated crops in Jordan especially grape (production of 47 131 tonnes), almond (9 981 tonnes), peach (59 058 tonnes), citrus trees (120 087 tonnes). The total production of fruit trees was approximately 275 461 tonnes in 2014. Production areas of fruit trees are reported in Table 1. Therefore, destructive pests, such as the bacterium *Xylella fastidiosa*, can destroy these crops and would have a serious impact on the livelihood of the farmers in Jordan.

Table 1. Areas of commercial production of fruit trees in Jordan in 2014 (ha).

Provinces	Olive	Grape	Citrus	Fruit trees	Total
Amman	12546.8	1102.6	8.3	1307.8	14965.5
Madaba	6105.1	802.8	1.2	542	7451.1
Zarqa	15140.8	1244.6	12.9	708.3	17106.6
Irbid	25099.1	489.8	18.3	1325.7	26932.9
Jerash	6977.8	681.4	713.6	785.1	9157.9
Ajloun	11913.5	3715.1	64.8	343	16036,4
Al mafraq	23885	1418.1	1	5757.1	31061,2
Al balqa	3926.2	3230	86.5	3818.3	11061
Karak	2629.5	1418.1	19.6	289	4356.2
Tafila	2629.5	376	15.6	430.5	3451.6
Maan	1712	200.6	10	2613.8	4536.4
Aqaba	608.5	143.3	36.7	421	1209.5
Jordan valley	372.5	94.6	6946.6	2410.2	9823.9

Plant Quarantine is an important part of the Ministry of Agriculture. The Plant Protection & Phytosanitary Directorate is divided into three sections: Phytosanitary, Pest Risk Analysis, and Pesticide Registration. The Plant Health Laboratories Directorate are divided into three laboratories: Phytosanitary Laboratory, Pesticide Residue Laboratory, and Formulation Laboratory. There is a close cooperation of the Plant Protection & Phytosanitary Directorate with Jordan Universities the National Center for Agriculture Research & Extension (NCARE) on many topics concerning agriculture.

The Plant Quarantine issues are discussed by the Phytosanitary committee, which consists of: the Plant Protection & Phytosanitary Directorate, the Plant Health Laboratory Directorate, the Faculty

of agriculture/plant protection/University of Jordan, NCARE, Agriculture Marketing Directorate, private sector/Jordan Exporters and producers Association for fruit and vegetables (JEPA).

Since the bacterium *Xylella fastidiosa* was found to infect olive trees causing "Olive Quick Decline Syndrome" in the region of Puglia in Southern Italy, and EPPO declared the first report of *Xylella fastidiosa* in Europe in October 2013, Jordan implemented procedures to prevent the entrance of any host of *Xylella fastidiosa* to the country as reported in the regulation reported in Annex.

The scientific name and common name of imported plant material should be written on the phytosanitary certificate.

The infrastructure of the Phytosanitary & Bacteriology Laboratory in Plant Health Directorate should be upgraded for the detection of *Xylella fastidiosa* in imported plant material.

The current plans for *X. fastidiosa* are:

- survey on different host plants in Jordan;
- training;

  technical training for staff from Plant protection and Plant Health Directorate;

  diagnostic training for Border Staff and Extension Agents;

  specialist training for bacteriologists and entomologists for detection techniques and methodology;
- awareness campaign for Farmers and Extension agents in cooperation with NCARE through field days, publications and media;
- upgrade of infrastructures of the Phytosanitary Laboratory including Real-time PCR, PCR, Immunoflurescence microscope.

Ministry of Agriculture Regulations of the Hashemite Kingdom of Jordan

The status of regulations that have been issued in Jordan against the devastating pathogen *Xylella fastidiosa* is as follows.

According to the Agriculture law No. 13/2015 and plant quarantine regulations No. z-19/2016:

The import of seedlings from Italy, France and Iran, and from other countries in which this disease is listed as present, complies with the following conditions:

The import of seedlings from non-host plants of *Xylella fastidiosa* is allowed.

The import of seedlings from *Xylella fastidiosa* - free areas is allowed; this is mentioned in the additional declaration on the phytosanitary certificate.

The import of seedlings from host plants of *Xylella fastidiosa* from Italy, France and Iran, and from other countries in which this disease is listed as present is not allowed.

Eng. Kholoud Aranki, Director of Plant protection & phytosanitary Directorate, Ministry of Agriculture. Amman, Jordan

Regulatory status and phytosanitary measures implemented to face *Xylella fastidiosa* and its vectors in Egypt

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1. Regulatory status

- *Xylella fastidiosa* is a quarantine pest that is absent in Egypt.
- The most effective way to control this pest is to prevent its entry.

2. Phytosanitary measures

a. Emergency measures

Notification of Emergency Measures on WTO website ″G/SPS/N/EGY/75″:

«Temporary suspension of the importation of ornamental plants and seedlings of fruit trees from countries where *X. fastidiosa* is present».

b. Notification for the Egyptian ports and entry points

A publication was prepared based on the WTO notification; it contains all the measures which should be taken in the Egyptian ports and entry points.

c. Rapid Pest Risk Analysis (PRA)

The Rapid PRA considers the risk of economic harm due to the entry, establishment and spread of *X. fastidiosa* within the countries (Algeria, Egypt, Iraq, Jordan, Libya, Malta, Morocco, Pakistan, Syria, Sudan, Tunisia) and neighbouring countries (Lebanon, Palestine) of the Near East Plant Protection Organisation (NEPPO).

3. Actions Taken

- Cooperation with the Central Administration for Plant Quarantine (CAPQ) and the Central Administration for Pest Control to prevent the introduction of *X. fastidiosa*.
- Reviewing case studies of the infection in the countries where the pest is present.
- Reviewing up-to-date research on this topic.
- Applying the EPPO Inspection and diagnostic protocols for this pest.
- Informing research stations of the inspection measures and procedures.
- Raising awareness of farmers so that they may report suspicious symptoms and cooperate with inspections/surveys.
- Organizing field inspections.
- Informing farmers on field management practices for reducing the risk of infection.

4. Future Plans

- Cooperation with other global research bodies to conduct research on *X. fastidiosa* and its vector(s).
- Pest Risk Analysis (PRA) for *Xylella fastidiosa* in Egypt.
- Optimization of diagnosis protocol.
- Undertaking an annual Egyptian National Surveillance Program for *Xylella fastidiosa* according to ISPM#6 in cooperation with CAPQ.
- Training of inspectors, surveyors, diagnosticians, farmers and producers.

Xylella fastidiosa in the framework of the EU plant quarantine law

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Xylella fastidiosa is regulated in the EU as quarantine organism under Council Directive 2000/29/EC ("plant health directive") on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. As such, the introduction of this organism into, and spread within all Member States, shall be banned. The plant health directive provides Member States with the legal obligations to take, once the organism is known to be present and irrespective of the symptoms, all necessary measures to eradicate it, or if that is impossible, inhibit its further spread.

Following the first outbreak of *Xylella fastidiosa*, subspecies pauca, notified by the Italian Authorities in the region of Puglia, in October 2013, preliminary EU emergency measures were taken in February 2014, detailed in July 2014 and further strengthened in May 2015 with the aim to prevent the further spread of the bacterium within the EU. Current emergency measures have been updated in November 2015 and April 2016 to ensure that they are well targeted and based on latest scientific evidences. Most particularly, the latest revision enlarged the demarcated area in Puglia taking into account latest spread of the bacterium outside the province of Lecce and the pest free area declared by the Italian Authorities.

Four audits were carried so far by the Commission's Food and Veterinary Office in Puglia, confirming the limited implementation of the eradication/containment measures (e.g. removal of infected plants) and the further spreading of the bacterium out of the province of Lecce. No movement of specified plants is so far authorised to be moved within and out of the demarcated areas established in Puglia.

In July 2015, French Authorities notified the first outbreak of *Xylella fastidiosa*, subspecies multiplex, in Corsica. Numerous outbreaks have been reported since then in the area, including some outbreaks reported in the PACA region (France mainland). *Polygala myrtifolia* is the main host plant, although other numerous host plants have been also confirmed to be infected (e.g. *Prunus cerasifera, Quercus suber*). No positive cases have been reported so far on *Olea europaea*. Trace-back activities are ongoing to confirm the source of infection. EU emergency measures are being taken. A Commission's audit was carried out in February 2016 and the official report will be published soon.

Lastly, EU co-financed survey activities were carried out across Member States during the 2015 growing season and no further findings were reported. EU guidelines have been made also available with the aim to harmonise survey activities across Member States.

EU Legislation on *Xylella fastidiosa*

Pasquale Di Rubbo

DG SANTE, European Commission

Xylella fastidiosa (Xf) is a vector-borne bacterium, regulated in the EU as quarantine organism under the *Council Directive 2000/29/EC (*"plant health directive*") on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community*. As such, the introduction of this organism into, and spread within all Member States, shall be banned. The plant health directive provides Member States with the legal obligations to take, once the organism is known to be present and irrespective of the symptoms, all necessary measures to eradicate it, or if that is impossible, inhibit its further spread.

Following the first outbreak of *Xylella fastidiosa*, subspecies *pauca*, notified by the Italian Authorities in the region of Puglia, in October 2013, preliminary EU emergency measures were taken in February 2014, detailed in July 2014 and further strengthened in May 2015 (Decision (EU) 2015/789) with the aim to prevent the further spread of the bacterium within the EU. Emergency measures have been updated in several occasions, taking into account new scientific and technical evidence to ensure they are well targeted and based on latest scientific evidences.

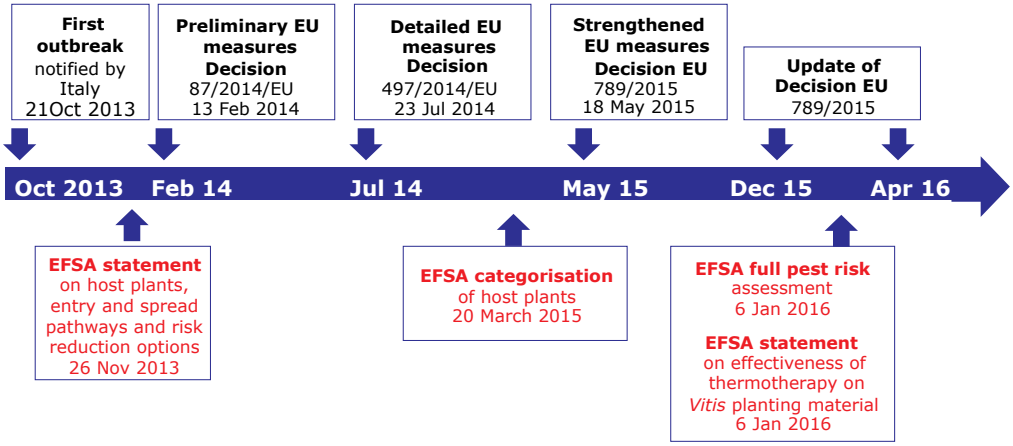

Figure 1. EU Regulatory framework of *X. fastidiosa* from its first outbreak in EU (2013), following scientific developments.

Emergency measures laid down under Decision (EU) 2015/789 are applicable to all Member States and are applicable to any subspecies of *Xylella fastidiosa.* Among others, general obligations are in place for all Member States to implement regular surveys activities (Art. 3); awareness raising campaigns (Art. 13a); mandatory Contingency plans (Art. 3a) and intensified controls on import in case of specified plants imported from infected non-EU Member States (Art. 18). Regulated plant species are divided into two categories: (1) "host plants", indicating those plant species found infected in the Union territory, and (2) "specified plants", indicating those plant species, including the host plants, found infected worldwide.

When the presence of *Xylella fastidiosa* is confirmed, Member States shall immediately proceed with the establishment of a demarcated area (DA) (Art. 4), consisting of an infected zone and a 10 km surrounding buffer zone. In this latest zone, intensive monitoring of specified plants is applied, vector control practices implemented and movement restrictions in place for all specified plants. In the infected zone, instead, eradication measure consist in the removal of all host plants located within a 100 m radius around the infected plants, plus removal of any symptomatic plants, intensive monitoring, phytosanitary treatments and implementation of agricultural practices against the vector population. Containment measures (Art. 7) are applied only in the infected zone of Puglia, Italy, and consist in intensive survey and felling activities of at least infected plants located in proximity of: (1) nurseries and growing sites; (2) plants with cultural, social or scientific value; (3) within the upper 20km adjacent to the buffer zone. Movement of specified plants within the infected zone is possible without any restriction.

Strict conditions are applied for movement of specified plants originating in a demarcated area/ infected non-EU Member States. Specified plants shall indeed be grown under insect proof conditions, in a site surrounded by a 200 m buffer zone free from *Xf*, subject to sampling, testing and phytosanitary treatment prior to movement, with traceability requirements. Specific derogations are in place for dormant *Vitis* planting material which have undergone hot water treatment, or in case of *in-vitro* planting material. On a precautionary approach, plant passport is also mandatory for all host plants grown outside the demarcated areas and moved within the Union territory. On import side, a declaration of the *Xf* status is mandatory by non-EU Countries wishing to export to the EU, informing the Commission whether import will take place from a Pest Free Country, a Pest Free Area, or Pest Free Production Site, established in accordance with Decision (EU) 789/2015. Import of Coffee plants from Costa Rica and Honduras is banned due to their level of risk.

As regards the state of play of *Xf* in the Union territory, at the present time, based on official survey activities carried out by all EU Member States, the bacterium is only present in some limited parts of the Union territory. More particularly, it is considered to be established in South of Puglia (Italy) where containment measures are currently applied, while it is under eradication in Corsica and PACA region (France) as well as in Germany, where an isolated case was detected in June 2016 on 4 ornamental potted plants. Lastly, on 10 November, 2016, an outbreak of *Xf* was detected in Mallorca (Spain) in a garden centre where strict eradication measures in place. Based on current knowledge, different subspecies have been detected so far in the different demarcated areas of the Union: subsp. *pauca* in Italy, and in one outbreak in France (Menton); subsp. *multiplex* in all remaining outbreaks of France; subsp. *fastidiosa* in Germany and Spain. Trace-back activities are still ongoing to confirm the source of the infection, while movement of all specified plants remains prohibited. A list of demarcated areas established in the Union territory is available at the following http://ec.europa.eu/food/sites/food/files/plant/docs/ph_biosec_pwn_demarcated-areas.pdf

Finally, EU co-financing possibilities exist for the implementation of general survey acitivities and emergency measures (e.g. felling and removal of plants, monitoring, sampling and testing, etc.), as well as the compensation to operators for the value of the destroyed plant material (from January 2017). This is complementaty to funding activities of DG AGRI as part of the Rural Development Programmes (e.g. advisory service, restoration of the agricultural production potential damaged by *Xylella*). Moreover, dedicated funding for research on *Xylella* has been also available in the framework of the HORIZON 2020 EU Programme (POnTE, *Xf*-Actors).

Further information is available at the following link: http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa_en

International legal framework for phytosanitary protection: obligations and responsibilities under the IPPC

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The purpose of this presentation is to introduce the international regulatory framework for plant protection, taking into consideration the Agreement on Sanitary and Phytosanitary Measures approved in the framework of the World Trade Organization (hereinafter the SPS Agreement), as well as the International Plant Protection Convention (hereinafter the IPPC). To conclude, we will briefly summarize how countries implement the IPPC in their national plant protection legislation. In the interest of time, this presentation will not provide an exhaustive explanation of all the elements included in the IPPC and its international standards for phytosanitary measures (ISPMs), but just a quick revision of key principles that countries should take into consideration to draft their national phytosanitary legislation. The presentation does not enter into some aspects of the IPPC such as the establishment of regional plant protection organizations that are exhaustively covered by other speakers.

International regulatory framework for phytosanitary protection

The main international obligations for states with regard to the protection of plants and the natural environment from the negative effects of pests derive from the WTO SPS Agreement and the IPPC.

The SPS Agreement aims to facilitate trade by preventing the use of sanitary and phytosanitary measures as disguised barriers to international trade. WTO members can apply phytosanitary measures to achieve their desired level of phytosanitary protection as long as these measures are applied only to the extent necessary to protect plant and environmental health, and comply with a number of principles, which will be explained in detail. The SPS Agreement refers to the standards approved in the framework of the IPPC as international reference standards for plant protection.

Together with the SPS Agreement and the IPPC, the Convention on Biological Diversity (CBD) is also relevant for the trade of plants and plant products. The CBD imposes obligations on contracting parties with regard to invasive alien species. Article 8(h) of the CBD requires each contracting party to “prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species.” Since most invasive species can be categorized as plant pests, the CBD reinforces governments’ responsibility to address these threats under phytosanitary legislation.

The SPS Agreement

The SPS Agreement recognizes that countries have the right to define their desired level of sanitary protection and to adopt sanitary and phytosanitary measures to protect such status, as long as a number of principles are met.

Sanitary and phytosanitary measures must be based on a science-based risk analysis and be technically justified, proportional to the risk and not stricter than necessary to achieve their desired impact (principles of **technical justification, proportionality and necessity**). Countries

might be requested to demonstrate that these conditions are met. Article 5.7 recognizes the right of countries, in cases where relevant scientific evidence is insufficient, to "*provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information" (…).* In this case, members should "*seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time"*.

To facilitate this scientific justification and to foster global **harmonization** on sanitary and phytosanitary measures, the agreement recognizes the standards of three international standard setting organizations as reference for SPS matters (article 3 and Annex A). The measures included in the standards approved by these three organizations (the Codex Alimentarius, the OIE and the International Standards for Phytosanitary Measures (ISPM)) are considered science-based and not stricter than necessary to maintain the desired level of sanitary protection. Countries may decide to apply measures stricter than the international reference standards, as long as they can demonstrate that these measures are science-based, necessary to achieve their desired level of protection and applied in a proportionate and **non-discriminatory** manner.

For the implementation of the SPS Agreement, importing countries are obliged to accept the measures of other member states if the exporting country demonstrates that its measures achieve the importing country's desired level of protection (principle of **equivalence**). Because this desired level of phytosanitary protection can often be achieved in several manners, and if those alternatives are economically feasible and provide the same level of phytosanitary protection, governments should select those which are not more trade restrictive than necessary to meet their desired level of phytosanitary protection (principle of **minimal impact**).

The SPS is aimed at improving transparency in the implementation of sanitary and phytosanitary measures. Accordingly, SPS measures must be approved on the basis of an appropriate assessment of the actual risks involved, and, if requested, countries should make known what factors they took into consideration, the assessment procedures they used, and the level of risk they determined to be acceptable. The SPS Agreement further introduces obligations of notification and information sharing to other member countries and to the SPS secretariat, including notification of phytosanitary requirements, which may affect trade.

Finally, WTO members are admonished, where they apply phytosanitary measures as a condition for import of plants and plant products, not to arbitrarily or unjustifiably discriminate between countries with identical or similar phytosanitary status (principle of **non-discrimination**).

The IPPC

The IPPC was adopted in 1951 and revised twice, in 1979 and in 1997. The 1997 text (the "New Revised Text") came into force in October 2005. The IPPC is a multilateral treaty whose main purpose is to secure "common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control". The New Revised Text reflects the role of the IPPC as recognized by the SPS Agreement, which, as noted above, identifies the IPPC as the organization responsible for international phytosanitary standard-setting and promotes the harmonization of phytosanitary measures to facilitate trade.

The IPPC introduces a number of principles, some of which must be reflected in national legislation, such as the principle of state **sovereignty**, which recognizes the right of countries to use phytosanitary measures, including emergency measures to protect plant health and the environment from risk associated to plant pests. This principle is tempered by other principles such as the principle of **necessity**, which requires countries to adopt restrictive measures only when they are necessary for phytosanitary protection. The principles of **proportionality** and **minimum impact** require restrictive measures to have the least possible impact on international trade. Other important principles are **cooperation** among countries to prevent the introduction

and spread of pests, and **non-discrimination** between countries with the same phytosanitary status and, in the case of regulated pests within a country, between domestic and imported consignments.

As a multilateral treaty, the IPPC becomes binding for its contracting parties once it is adopted and ratified according to their constitutional systems. By virtue of its first article, contracting parties are also requested to adopt national legislation for the implementation of the IPPC in their territories. This legislation should apply not only to plant and plant products, but also to all materials, objects and organisms capable of harbouring or spreading plant pests (regulated articles).

Among the obligations included in the IPPC, by virtue of Article IV, contracting parties are requested to designate a national entity as a National Plant Protection Organization (NPPO), and to give this organization the legal mandate necessary to undertake a number of functions, including surveillance on growing plants, plant products and regulated articles. Countries must regulate the issuance of phytosanitary certificates and assurance of the phytosanitary security of consignments after certification, the approval of import requirements and conduction of pest risk analysis, inspection, and when necessary, disinfestation of consignments in international trade. Finally, countries are called to share of phytosanitary information with the IPPC and with other contracting parties.

The NPPO should verify that all exported plant, plant products and regulated articles comply with the import requirements of the country of destination and, to this purpose, they must issue a phytosanitary certificate following the model approved by the IPPC. Phytosanitary certificates are official documents and must be signed by a public officer.

Article VI on regulated pests recognizes the right of contracting parties to require phytosanitary measures for regulated pests (quarantine and non-quarantine pests), as long as these measures are not more stringent than the measures applied to the same pests if present within the territory of the importing contracting party (principle of non-discrimination); they are limited to what is necessary to protect plant health and/or safeguard the intended use, and can be technically justified by the contracting party concerned (principles of non-discrimination and technical justification).

Import requirements are regulated in Article VII, which recognizes the sovereign right of contracting parties to regulate the entry of plant, plant products and regulated items, including prohibiting restrictions of regulated pests and biological control agents. These measures must be based on phytosanitary considerations, pest risk analysis, sufficient technical justification and be notified.

The IPPC further recognizes the duty of contracting parties to cooperate by exchanging information on phytosanitary threats and by providing technical and biological support and participating in campaigns to combat pests that need action at the international level.

Contracting parties should also cooperate in the development and implementation of International Standards for Phytosanitary Measures (ISPMs) to be approved by the Commission on Phytosanitary Measures (Article X). As we mentioned, the IPSMs are recognized by the SPS agreement as the international reference standards for plant protection. Additionally, article X of the IPPC recognizes the duty of contracting parties to participate in the adoption and to implement ISPMs.

The 36 existing ISPMs are of very different nature, from general reference standards (such as ISPM 1 or 5) to very specific and technical standards prescribing concrete surveillance methods and treatments, such as ISPM 27. Depending on their nature, the ISPMs would have different effects and be implemented in a different manner in national legislation.

ISPM 1 recognizes and develops the content of the general principles embraced by the IPPC. These include (i) the principle of ***sovereignty*** of contracting parties to prescribe and adopt

phytosanitary measures; (ii) the principle of ***necessity***: measures should be adopted only where necessary to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests; (iii) the principle of ***managed risk***, according to which contracting parties "shall institute only phytosanitary measures that are ... consistent with the pest risk involved ..." (Article VII.2(g)); (iv) the principle of ***minimal impact***: contracting parties shall institute only phytosanitary measures that ... represent the least restrictive measures available, and result in the minimum impediment to the international movement of people, commodities and conveyances" (Article VII.2(g)); and (v) the principle of ***technical justification*** of phytosanitary measures "on the basis of conclusions reached by using an appropriate pest risk (...)" (Article II.1) parties shall not, under their phytosanitary legislation, take any of the measures specified in paragraph 1 of this Article [VII] unless such measures ... are technically justified." (Article VII.2(a), Article VI.1(b). IPSM 1 also addresses the principles of transparency, harmonization, non-discrimination, cooperation and equivalence, among others.

Other relevant ISPMs for the purposes of this meeting are ISPM 6, on surveillance, ISPM 9, on pest eradication; ISPM 11 on pest risk analysis of quarantine pests; ISPM 14 on integrated measures for pest risk management and ISPM 29 on the recognition of pest-free areas and areas of low pest prevalence.

Implementation of the IPPC in national legislation

To conclude, we will provide a snapshot of how countries implement the IPPC and the ISPM in their national legislation. As we mentioned, contracting parties are obliged to implement the obligations in the IPPC and their ISPMs in their national legislation, and this implies an obligation to designate an NPPO with the functions included in Article IV of the IPPC. The NPPO should further receive the legal mandate (and capacity) to undertake risk assessment (through PRA) and risk management decisions, and could also contribute to risk communication. The NPPO should be legally responsible to implement the legislation, and should also find in legislation the inspection and enforcement powers necessary for this purpose. Countries may also want to take into consideration the impact of the obligations included in legislation through a regulatory impact assessment, including the socio-economic impact of specific phytosanitary measures.

Legislative aspects for the mandatory control of *Xylella fastidiosa* in Puglia and in Italy

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Puglia is the main olive-growing region in Italy (32% of the national olive-growing area) and olive trees cover 29% of the Puglia agricultural surface. In summer 2013, numerous cases of olive trees, mainly the ancient ones, showed Olive Quick Decline symptoms (OQDS) in the Southern part of Puglia (Lecce province). In October of the same year the National Research Council of Bari (CNR) reported for the first time the presence of the quarantine bacterium *Xylella fastidiosa*, subsp. pauca, strain CoDiRO as the main cause associated to the OQDS. Following the indications of the EU Directive 2000/29 (08.05.2000), the Regional Plant Protection Service of Puglia (RPPS) immediately communicated the finding to the Ministry of Agriculture, Food and Forestry Policies (MiPAAF) and to the European Commission; a series of measures and actions against *X. fastidiosa* were soon taken (Table 1). A large scale monitoring of the pathogen was conducted soon after the finding in the entire Region by analysing over 16,000 host plant samples (primarily olive trees) and delimiting the infected and buffer zones. The movement of plants from the infected area was blocked and strict measures were adopted for nurseries and producers.

The dramatic nature of the emergency and the increasing extent of the infection prompted MiPAAF in 2014 to adopt urgent measures for the containment of the bacterium across the whole Puglia. In February 2015, the Italian Council of Ministers declared a state of emergency and appointed a Commissioner (head of the Civil Protection department) and a national scientific committee for advising technical decisions. The Commissioner adopted all available means in order to prevent the pathogen from further spreading, thereby endangering olive cultivation in Puglia, in Italy, in Europe and the whole Mediterranean region. Several emergency measures were then implemented, with regard to territorial management and in response to the measures of the European Commission. An Action Plan for the rapid implementation of the mandatory control measures against Xylella, as indicated in the Ministerial Decree (MD) no. 2777 (26.09.2014) was applied throughout 2015. The main measures were: the elimination of infected plants, in order to reduce possible pathogen inoculum, and the containment of the insect vector population, *Philaenus spumarius* , also known as "meadow spittlebug", which is the only confirmed vector in Puglia.

Philaenus spumarius could have an important epidemiological role in spreading the infection, because it is a polyphagous insect showing a high population density in the South of Puglia due to the favourable climatic conditions and poor agronomical practices in most of the olive groves. This insect is believed to have only one generation per year, developing mainly from spring to autumn, with overwintering eggs. Its biological cycle starts in April when nymphs hatch from the eggs. The nymphs live on the stems of the herbaceous vegetation, and cover themselves in a liquid foam to maintain their correct moisture level and protect from their natural enemies. Once they reach the adult stage, they fly onto the aerial parts of trees and feed on xylem with their stylets. Objective of the Action Plan was to reduce the number of nymphs as much as possible through the application of specific agronomical practices (e.g. tillage) against wild herbaceous plants in spring or else by burning, using string trimmers or applying insecticides registered for use against phytophagous insects, which are effective against the juvenile stages. The control was also against the adult stage, which is the most dangerous for the dissemination of the infection, through phytosanitary treatments applied in autumn on olive trees and other fruit trees in the affected areas. Awareness

campaigns were developed in the Action Plan such as: dedicated website www.emergenzaxylella.it, Ministry press, meetings with farmers, nurserymen and stakeholders, posters, leaflets, video on media, etc.

Following the MD issued on 19.06.2015, official investigations were conducted on host plants and insect vectors in all Italian regions, primarily in areas considered at higher risk of introduction of *Xylella* (nurseries, garden centres and production sites). A total of 17186 sites were inspected and 13766 analyses were performed without any finding of the infection. In the region of Puglia a similar number of sites were also inspected (17124 sites) but with a higher number of analyzed samples (50.488 samples).

In 2016, the survey programme was co-financed by the European Commission and results showed that infection had spread across the whole province of Lecce, which was the original infected area, and reached the provinces of Brindisi and Taranto. Financial compensation was planned for economic loss due to *X. fastidiosa* and the cost of removal of olive trees.

Following the EU Commission Implementing Decision 2015/789, phytosanitary measures were issued based on the new demarcated area, which includes the infected zone and buffer zone (10km surrounding the infected zone). Intensive monitoring, eradication and containment measures, vector control, movement restrictions of plants, planting prohibition of host plants are the main actions conducted in the buffer zone and in the infected zone surrounding the buffer zone (a 20km-wide strip). As for the buffer zone, in addition to the infected plants, all pathogen host species are removed in a radius of 100mt around the infected plant/s, regardless of their health status.

Since its first discovery in 2013, more than 200.000 plants have been tested mainly in the buffer and containment areas with the aim of determining the presence and spread of the infection for the application of eradication/containment measures. The spread of the infection covers approximately 180000 ha, i.e. 16% of the national olive-growing area. Sampled and infected plants in the demarcated area have been mapped and the management of the monitoring data has been fully computerized. The graphical representation of the areas monitored and their results are available on the official website of the Puglia Region (www.emergenzaxylella.it). A series of different activities took place in order to raise awareness such as dedicated website, meeting with farmers, distribution of 16.000 informative leaflets and others. Plant Protection Service, Forestry and Municipality police (about 500 units) have been employed in the application of the phytosanitary measures as indicated in the contingency plan against *X. fastidiosa*.

The work conducted by the RPPS of Puglia was very hard due to the limitation in the removal of the infected trees caused by the civil protest and by the appeals of the Regional Administrative Court.

Table 1. Synthesis of the main actions taken after the first finding of *Xylella fastidiosa* in EU (2013-2016) by Regione Puglia, Ministero Italiano delle Politiche Agricole, Alimentari e Forestali (MiPAAF) and European Commission.

2013	
October	OFFICIAL COMMUNICATION OF XYLELLA FASTIDIOSA FINDING IN PUGLIA REGION
	Measures for the movement of host plants
	Temporal Blocking of plants movement from the Province of Lecce (Apulia)
	Provisions on the implementation measures for *X. fastidiosa* in Puglia region
November	1st Monitoring of *X. fastidiosa* for the definition of the delimited areas
2014	
February	COMMISSION IMPLEMENTING DECISION (EU) 2014/87 as regards measures to prevent the introduction into and the spread within the Union of *X. fastidiosa*
March	Removal of infected trees in Apulia region
April	Definition of the outbreak area of Apulia region
July	COMMISSION IMPLEMENTING DECISION (EU) 2014/497 as regards measures to prevent the introduction into and the spread within the Union of *X. fastidiosa*
July	Definition of the infected and buffer zones
September	Regional Council Deliberation of Puglia n. 1824 - Declaration of the extraordinary phytosanitary emergency for *X. fastidiosa*
	Ministerial Decree - Establishment of the national Technical Scientific Committee on *X. fastidiosa*
	Ministerial Decree - Emergency measures for the prevention, control and eradication of *X. fastidiosa* in the Italian territory
	Guidelines for the containment of the spread of *X. fastidiosa* sub. pauca, strain CoDiRO in Puglia region
October	2nd Monitoring of *X. fastidiosa* for the definition of the delimited areas in Puglia region
2015	
February	Appointment of a special Commissioner for the emergency of *X. fastidiosa*
March	Action plan for implementing measures for *X. fastidiosa* in Puglia region
	Definition of the delimited areas for *X. fastidiosa* in Puglia region
March	COMMISSION IMPLEMENTING DECISION (EU) 2015/789 as regards measures to prevent the introduction into and the spread within the Union of *X. fastidiosa*
June	Ministerial Decree - National surveys and updating of the demarcated area in Apulia
September	New Action Plan for *X. fastidiosa* in Puglia region
	Removal of spontaneous/host plants
	Control of vectors
	Pruning of olive trees removing symptomatic parts
	Strengthening of checks on the movement of specified plants
	Surveys activities
	Removal of infected plants and host plants in 100mt radius
December	Definition of the delimited areas for *X. fastidiosa* in Puglia region
	Ministerial Decree - Extension of financial contributions to the farmers
December	COMMISSION IMPLEMENTING DECISION (EU) 2015/2417 amending Implementing Decision (EU) 2015/789 as regards measures to prevent the introduction into and the spread within the Union of *X. fastidiosa*
2016	
February	Ministerial Decree - Official recognition of pest-free areas in all Italian regions, with the exception of the demarcated area in Apulia
May	COMMISSION IMPLEMENTING DECISION (EU) 2016/764 amending Implementing Decision (EU) 2015/789 as regards measures to prevent the introduction into and the spread within the Union of *X. fastidiosa*
September	3rd Monitoring of *X. fastidiosa* for updating the demarcated areas

April 2014 **July 2014** **March 2015**

June 2015 **May 2016**

Figure 1. Definition of the demarcated areas in Puglia for *Xylella fastidiosa* following results of the monitoring activities in the period 2013 – 2016.

The elements of successful capacity development for *Xylella fastidiosa*

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The International Plant Protection Convention (IPPC) has made significant strides to support its contracting parties to implement the Convention and its Standards through phytosanitary capacity development activities. Phytosanitary capacity development is defined as "the ability of individuals, organizations and systems of a country to perform functions effectively and sustainably in order to protect plants and plant products from pests and to facilitate trade, in accordance with the IPPC" (IPPC, 2012).

Capacity development activities are crucial at various regulatory intervention points, which correspond to the 3 regulatory stages: prevention, detection and rapid response, as shown in table 1. Contingency plans should encompass all this range of activities.

Table 1. Capacity development activities needed for different regulatory intervention points.

Regulatory stages	Regulatory intervention points for which capacity development is needed
Prevention	Pest Risk Assessment Pest Risk Management (incl. legislation, certification) Surveillance
Detection	Pest Diagnostic Import verification Inspection
Rapid response	Monitoring Eradication Containment

For each one of these intervention points, relevant stakeholders should be engaged to conduct the different activities as best as possible. A stakeholder is defined as "a person, group or organization that has interest in the phytosanitary activities of an NPPO" (IPPC, 2015). A proposal of stakeholders to be involved, as well as the material needed and available for these activities on *Xylella fastidiosa* is presented in table 2.

The IPPC Secretariat has made available the website www.phytosanitary.info to provide resources to its contracting parties in support of the development of their phytosanitary capacities. The website provides hundreds of relevant resources developed by the IPPC Secretariat and others. The IPPC Secretariat currently runs a pilot project on surveillance and available capacity development resources were aggregated for *Xylella fastidiosa*. These resources and others are regularly posted on the www.phytosanitary.info website.

Table 2. Capacity development and other needs and available resources from this workshop for different Capacity development intervention points for which stakeholders to be engaged have been identified.

Capacity development intervention points	Stakeholders (non exhaustive list)	Capacity development and other needs	Available resources from this workshop
Pest Risk Assessment (PRA)	National Plant Protection Organization (NPPO), research, experts and other professional resources	Expertise in PRA, taxonomy and other required areas	Data sharing on biology (hosts, vectors), impacts, all scientific publications, existing PRAs
Pest Risk Management (PRM) (incl. legislation, certification)	NPPO, legal experts, policy makers	Expertise in PRM, specialized skills to develop and assess appropriate risk management options (including integrated options), certified propagating material	Existing PRM, existing legislations
Pest Diagnostic	NPPO, research and other diagnostic entities	Training in diagnostic methods, well equipped laboratory, infrastructure and resources, access to expertise, technical protocols and Standard Operating Procedures (SOPs)	EPPO standards and related trainings, innovative on site diagnostic methods, laboratory accreditation
Import verification Inspection	NPPO, customs, immigration	Trainings in documentary checks, trainings for collaborative agencies such as customs, immigration, postal services	Updated list of host plants to be inspected at entry points
Inspection	NPPO, diagnostic entity	Trainings for inspectors in sampling and diagnostic methodologies, and documented procedures, SOPs	Symptoms on hosts, vectors identification, on site detection tools
Surveillance and monitoring	NPPO, authorities, extensions, media, researchers, plant owners (nurserymen, producers, etc.), importers/exporters, police, military, citizens	Legislation, technical protocols, trainings for inspectors and various stakeholders, communication strategy and advocacy, infrastructures, human and other resources	General surveillance: global pest distribution, remote sensing applications, webserver and GIS development Monitoring: apps, detection tools and technical protocols
Eradication and containment	NPPO, authorities, extensions, media, researchers, plant owners (nurseryman, producers, etc.), police, military		Buffer zone, delimiting survey, uprooting of infected plants, vector control

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The importance of communication on phytosanitary issues

The case of *Xylella fastidiosa*

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The bacterium *Xylella fastidiosa* (Xanthomonadaceae) is a quarantine pest known for its negative social and economic impact on agriculture, the environment and trade. Various strains of this bacterium can infest more than 350 host plants, including important crops such as grape, *Citrus* spp. and olive trees. The outbreak of this bacterium in the Puglia region in southern Italy has led to a rarely seen social and media crisis. Local associations and individual citizens opposed emergency measures such as the up-rooting of trees, amassed strong opposition in the media through social networks, and ultimately sued the authorities and scientists in court. Local authorities and scientists were accused of having voluntarily introduced the bacterium.

To face the tremendous challenge of tackling pests that increasingly cross borders through trade of plants and plant products, National Plant Protection Organizations (NPPO) are the official institutions in charge of preventing the introduction and spread of pests in their territories, as defined by the International Plant Protection Convention (IPPC). NPPOs have the authority and mandate to take measures to prevent, control or manage *X. fastidiosa* within their country. However, the burden of responsibilities of an NPPO are too often at odds with the little financial and human resources NPPOs have at hand to operate in a comprehensive and efficient way to face the challenges of a pest incursion.

Communication is an essential, but too often forgotten, strategic component in responding to a phytosanitary outbreak. A communication plan would strive to unite all stakeholders by informing them on how they are impacted by the outbreak both separately and together. A communication plan would also engage stakeholders to conduct activities that will facilitate the implementation of the contingency plan. Communication aspects have seldom been explored in the realm of plant protection. The outbreak of *X. fastidiosa* in the Puglia region represents a rich opportunity to gain knowledge on how to set an efficient communication plan on this pest. These lessons could be applied to any other quarantine pest in the future.

Learning from the experience in the Puglia region, and inspired by other experiences, proposals for the setting of a communication plan for contingency planning for *X. fastidiosa* could include:

- Outlining the roles and responsibilities in a communication plan: who should be involved, what is the line of command, what are the activities to be undertaken? In terms of governance, it is important that the message be centralized and issued by the NPPO.
- Defining messages to be communicated on *Xylella fastidiosa*: defining clear, straightforward, succinct messages is fundamental for the success of a contingency plan. Messages should be adapted to the national or local context and should communicate clearly to everyone the status of the pest in the country. These messages are most appropriate to situations in which pest is absent or of limited distribution in the country.
- Identification of the activities within the communication plan, including meetings with the media, press conferences, organizing training workshops to visit production places and international workshops to exchange information, developing communication material such as webpages, social media accounts, fliers, videos, etc.

Part Three
Bibliographic review

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Other links

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EPPO (European Plant Protection Organization) https://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa

FAO-IPPC (International Plant Protection Convention) https://www.ippc.int/en/search/?q=Xylella&type=

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Annex

Food and Agriculture Organization of the United Nations

International Plant Protection Convention
Protecting the world's plant resources from pests

CIHEAM
IAM BARI

FAO-IPPC-CIHEAM International Workshop on

Xylella fastidiosa & the Olive Quick Decline Syndrome (OQDS)

19-22 April, 2016
CIHEAM/Istituto Agronomico Mediterraneo of Bari
Via Ceglie, 9 70010 Valenzano (BA) ITALY

This international workshop is organized by FAO, IPPC & CIHEAM with the support of the Near East Plant Protection Organization (NEPPO) and the European Plant Protection Organization (EPPO)

NEPPO
منظمة وقاية النباتات للشرق الأدنى
Near East Plant Protection Organization

***Options Méditerranéennes**, A No.121, 2017 - Xylella fastidiosa & the Olive Quick Decline Syndrome (OQDS)*
A serious worldwide challenge for the safeguard of olive trees

Agenda

FAO-IPPC-CIHEAM International Workshop on
Xylella fastidiosa and the Olive Quick Decline Syndrome (OQDS)

19 April (streaming)

08.30	Registration
09.00 - 09.40	Opening remarks by the Secretary General of CIHEAM, ***C. Lacirignola***, the Representative of the Italian Ministry of Agriculture, Food and Forestry Policies, ***M. Barba***, the Representative of Puglia Region, ***G. Nardone*** and the IPPC Secretary, ***J. Xia***.
Session 1 *Chairperson*	*Xylella fastidiosa* and its impact ***B. Giovani****, European and Mediterranean Plant Protection Organization (EPPO)*
09.40 - 10.00	The world threat of *X. fastidiosa* ***M. M. Lopez****, IVIA-Spain*
10.00 - 10.20	*X. fastidiosa* and the etiology of Olive Quick Decline Syndrome in Italy ***G. P. Martelli & F. Nigro****, UNIBA-Italy*
10.20 - 10.40	Main insect vectors worldwide & in Italy ***F. Porcelli****, UNIBA-Italy*
	Coffee break
11.00 - 11.20	State of the art of research on *X. fastidiosa* in Puglia, Italy ***M. Saponari****, CNR, Bari-Italy*
11.20 - 11.40	Breaking news of *X. fastidiosa*, an emerging plant pathogen in France ***M. A. Jacques****, INRA-France*
11.40 - 11.55	Preliminary results on field trials performed in Puglia (Southern Italy) to control *X. fastidiosa* in olive trees ***M. Scortichini****, CREA, Caserta-Italy*
11.55 - 12.10	Sustainable strategies to contain the Olive Quick Decline Syndrome in South-East Italy ***A. Carlucci****, UNIFG-Italy*
12.10 - 12.30	Discussion
12.30 - 13.00	Poster presentation
	Lunch
Session 2 *Chairperson*	International collaboration and projects ***S. Aldobai****, FAO Near East*
14.30 - 14.50	From science to policy: the contributions of EPPO and Euphresco ***B. Giovani****, EPPO*
14.50 - 15.10	The EU project 'Pest Organisms Threatening Europe' (POnTE) ***D. Boscia****, CNR, Bari-Italy*
15.10 - 15.30	The International Plant Health Standards (ISPMs), their relevance to combat emerging pest situations: the case of *Xylella fastidiosa*? ***J. Xia****, IPPC Secretariat*

15.30 - 16.00	Risk assessment of *X. fastidiosa* in the EU territory and other EFSA activities ***S. Winter**, Leibniz Institute DMSZ - Germany*
	Coffee break
16.20 - 16.40	A Pest Risk Analysis on *X. fastidiosa* for the countries of the NEPPO, focusing on the strain infecting olive ***M. Chouibani**, Near East Plant Protection Organization (NEPPO)*
16.40 - 17.00	Presentation of the FAO Near East project ***S. Al-Dobai**, FAO - RNE*
17.00 - 17.10	Overview of the activities and projects of the International Olive Council ***A. Ghedira**, International Olive Council (IOOC)*
17.10 - 17.30	Discussion

20 April (streaming)

Session 3 *Chairperson*	Legislative aspects ***C. Bullon**, FAO Development Law Service*
09.00 - 09.20	Obligations and responsibilities under the IPPC ***C. Bullon**, FAO Development Law Service*
09.20 - 09.40	*X. fastidiosa* in the framework of EU plant quarantine law ***H. Arijs**, DG SANTE, European Commission*
09.40 - 09.55	Italian Decree for the mandatory control of *X. fastidiosa* ***B. Faraglia**, MiPAAF-Italy*
09.55 - 10.10	Application of the Italian Decree in Puglia ***S. Schito**, Puglia Region-Italy*
10.10 - 10.30	Discussion
	Coffee break
10.30 - 10.45	New regulations on *X. fastidiosa* in Australia ***L. Paglia**, Department of Agriculture and Water Resources, Australia*
10.45 - 12.15	Presentations on legislations in Mediterranean countries and implications for trade by Mediterranean experts
12.15 - 13.00	Discussion
	Lunch
Session 4 *Chairperson*	Surveillance and diagnostic methods I ***M. Chouibani**, Near East Plant Protection Organization (NEPPO)*
14.30 - 14.40	International Standard on Pest Surveillance *A. Moreira, IPPC Secretariat*
14.40 - 14.55	EPPO Standards related to *X. fastidiosa* *B. Giovani, EPPO*
14.55 - 15.10	Symptoms description and host species *D. Boscia, CNR, Bari*-Italy
15.10 - 15.25	The innovative surveillance system for *X. fastidiosa* in Puglia *A.M. D'Onghia, CIHEAM/IAMB-Italy*
15.25 - 15.40	The remote sensing approach for early surveillance *S. Gualano, CIHEAM/IAMB-Italy*

15.40 - 16.00	Discussion
	Coffee break
16.20 - 16.35	The Android device XylApp for field data acquisition *F. Santoro, CIHEAM/IAMB-Italy*
16.35 - 17.00	Sampling procedures *F. Valentini, CIHEAM/IAMB-Italy; F. Porcelli, UNIBA-Italy*
17.00 - 17.15	Organization of ring tests on diagnostic methods at Puglia and national level *S. Loreti, CREA, Rome-Italy; G. Loconsole, UNIBA-Italy*
17.15 - 17.30	Discussion

21 April

Session 5 *Chairperson*	Surveillance and diagnostic methods II ***M. Digiaro**, CIHEAM/IAMB-Italy*
07.30 - 12.00	Demonstration of field data acquisition
	Lunch
15.00 - 17.00	Demonstration of field data acquisition *Technical support by CIHEAM/IAMB, CNR-Bari, UNIBA, CRSFA*

22 April

Session 6 *Chairperson*	Surveillance and diagnostic methods III ***A. M. D'Onghia**, CIHEAM/IAMB-Italy*
09.00 - 09.30	Innovative diagnostic methods for *X. fastidiosa*: real time LAMP & DTBIA ***T. Yaseen & K. Djelouah** CIHEAM/IAMB-Italy*
09.30 - 13.00	Demonstration of DTBIA and real time LAMP *Technical support by CIHEAM/IAMB, CNR-Bari, UNIBA, CRSFA*
	Lunch
Session 7 *Chairperson*	From knowledge to implementation, the need for capacity development ***S. Brunel**, IPPC Secretariat*
15.00 - 15.45	Experiences of awareness raising and capacity development initiatives in the Mediterranean basin by Mediterranean experts
15.45 - 16.00	The elements of successful capacity development for *X. fastidiosa* ***S. Brunel**, IPPC Secretariat*
16.00 - 16.40	Discussion: the way forward
16.30 - 17.00	Closing remarks by ***S. Brunel**,* IPPC Secretariat *&* ***A.M. D'Onghia*** *CIHEAM/IAMB*

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International Workshop
Xylella fastidiosa:
a serious worldwide challenge for the safeguard of olive trees

28-30 November, 2016
CIHEAM/Istituto Agronomico Mediterraneo of Bari
Via Ceglie, 9 70010 Valenzano (BA), ITALY

28 November

09.00 - 09.45 **Opening**

C. Lacirignola *Secretary General of CIHEAM*

Morning Session
Chairperson F. Serafini, IOC

09.45 - 10.00 *X. fastidiosa:* a serious world threat
G. P. Martelli *UNIBA-Italy*

10.00 - 10.15 *X. fastidiosa* and the Olive Quick Decline Syndrome in Italy
M. Saponari *CNR, Bari-Italy*

10.15 - 10.30 *X. fastidiosa*: the status of the infection and relative control in France
B. Legendre *ANSES-France*

10.30 - 10.45 ***Coffee break***

10.45 - 11.00 Preliminary results on field trials to control *X. fastidiosa* in olive trees in Puglia
M. Scortichini *CREA, Caserta-Italy*

11.00 - 11.15 Discussion

11.15 – 11.30 Preliminary results in the control of the vector of *X. fastidiosa* using different formulates
E. Dongiovanni *CRSFA-Italy* & ***V. Verrastro*** *CIHEAM-Bari*

11.30 - 11.45 Sustainable strategies to contain the Olive Quick Decline Syndrome in South-East Italy
A. Carlucci *UNIFG-Italy*

11.45 - 12.00 Good agricultural practices in the management of the Olive Quick Decline Syndrome
C. Xiloyannis *UNIBAS-Italy*

12.00 - 12.15	The mandatory certification of plant propagating material of olive and other host species of *X. fastidiosa* ***M. Barba*** *CREA-Italy*
12.15 – 13.00	Discussion
13.00 - 14.30	***Lunch***

Afternoon Session
Chairperson F. Petter, EPPO

14.30 – 14.45	Main insect vectors of *Xylella fastidiosa* worldwide & in Italy ***F. Porcelli****, UNIBA-Italy*
14.45 – 15.00	Current EU research initiatives on *X. fastidiosa* ***D. Boscia*** *CNR, Bari-Italy*
15.15 – 15.30	IPPC activities on *Xylella fastidiosa* (skype call presentation) ***S. Brunel*** *IPPC – FAO*
15.30 – 15.45	EPPO contributions against *X. fastidiosa* ***F. Petter****, EPPO*
15.45 – 16.00	The EU legislation for *X. fastidiosa* (skype call presentation) ***P. Di Rubbo*** *DG SANTE, European Commission*
16.00 – 16.30	***Coffee break***
16.30 – 16.45	Italian Decree for the mandatory control of *X. fastidiosa* ***S. Schito*** *Regione Puglia-Italy*
16.45 – 17.00	*CIHEAM/IAMB* innovative tools for early surveillance and detection of *X. fastidiosa* ***A.M. D'Onghia*** *CIHEAM-BARI*
17.00 – 18.00	Discussion

29 November

10.30 - 11.45	Field visit in the demarcated area (Lecce province) ***F. Valentini, D. Boscia, M. Digiaro, V. Verrastro, F. Porcelli*** • Symptom identification in olive and other host species • Demonstration of sampling methods (plant material & insect vectors)
12.30 – 13.00	Preliminary results on tolerance/resistance of olive cvs in Puglia Auditorium at the Oil Mill in Racale, Lecce ***P. Saldarelli*** *CNR, Bari-Italy*
13.00 – 15.00	***Lunch***
15.00 - 16.00	Visit to the experimental field for the evaluation of resistance/tolerance to Xylella infections of different olive cultivars ***D. Boscia*** *CNR, Bari-Italy*
20.30	Social dinner in Bari

30 November

Morning Session

Chairperson A.M. D'Onghia, CIHEAM-Bari

9.00 – 9.30	The information technology applied in pathogen surveillance: XylApp & XylWeb ***F. Santoro, S. Gualano*** *CIHEAM-Bari*
9.30 – 10.00	Conventional & innovative early detection tools ***K. Djelouah, T. Yaseen*****,** ***T. El Beaino*** *CIHEAM-Bari*
10.00 – 10.30	Demonstration of XylApp & innovative diagnostic methods ***F. Santoro, K. Djelouah, T. Yaseen*** *CIHEAM-Bari*
10.30 – 11.00	***Coffee break***
11.00 - 12.30	Round table : proposal of measures for the prevention and potential containment of *X. fastidiosa* in olive growing areas *Representatives of IOC, CIHEAM, IPPC-FAO, EU, EPPO, EFSA, CNR, UniBA, UniFG, UniBas, CREA, CRSFA, ANSES*
12.30 – 13.00	Concluding remarks ***A. Ghedira*** *Director of the International Olive Council (IOC)*

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