Food and Agriculture Organization of the United Nations

TCP/RAB/3601 project

"Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries"

Training Package

Course details

Dates: 30 January -03 February 2017

Location: Tunis, Tunisia

Training coordinator: **AlDobai, Shoki** Shoki.AlDobai@fao.org

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Food and Agriculture Organization of the United Nations

Agenda of training of the "Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries"
TCP/RAB/3601 project
Tunisia 30 January -03 February 2017

Date/Timing	Agenda Item		Notes
Day 1	***Monday 30 January 2017***		
8:30 -9:00	Registration		
9:00 - 09:30	Opening session - FAO Representative welcoming speech - MoA opening speech - FAO-RNE - Introduction of the project		Local & FAO Representatives
09:30 – 10:00	**Coffee break**		
10:00 - 10.30	Introduction to the training & Self-assessment test		All participants
10:30 - 11:30	- *Xylella fastidiosa (X.f.)* – General Overview		FAO Expert
11:30 - 12:30	- Main insect vectors of *X.fastidiosa worldwide* & in Italy		FAO Expert
12:30 - 14:00	- **Lunch break**		
14:00 - 15:00	- History and current situation of XF in the EU-Mediterranean area		FAO Expert
15-00 - 15:30	- *X. fastidiosa* in the framework of quarantine law.		FAO Expert
15:30 - 16.00	- **Coffee break**		
16.00 - 17:00	- Eradication & control methods as foreseen in the EU Decision 2015/789 & discussion		FAO Expert
Day 2	***Tuesday31 30 January 2017***		
8:30 - 9:30	- EU Surveillance methods (guidelines, survey plan and diagnostic)		FAO Expert
9:30 - 10:30	- Surveillance and Sampling procedures adopted in Apulia region (on olive, and other hosts)		FAO Expert
10:30 - 11:00	**Coffee break**		
11:00 - 12:00	- Innovative surveillance system for *X. fastidiosa* in Apulia region, Italy		FAO Expert
12:00 - 13:00	- Laboratory diagnostic methods for *Xylella fastidiosa* (EPPO standards)		FAO Expert
13:00 - 14:30	**Lunch break**		

14:30 – 15:30	- Case and management of the XF Outbreak management in Apulia region		FAO Expert
15:30 – 16:00	**Break**		
16:00 - 17:00	- Discussion Critical points for inspection and sampling		FAO Expert

Day 3	***Wednesday 01 February 2017, Field activity***	
8:00 – 17:00	*X. fastidiosa* survey organization	FAO Experts
	- Practical aspects of sampling methods	
	Field visit to farms/nurseries/places of production	
	- Insect sampling and conservation	
Day 4	***Thursday 02 February 2017, Laboratory activity***	
8:30 - 10:00	- Visit to diagnostic laboratory and illustration of the routine detection method - Organization of the sample acceptance	FAO Experts
10:00 - 10:30	**Coffee break**	
10:30 - 11:00	- Overview on the routine detection methods of *X. fastidiosa*	FAO Experts
11:00 - 12:30	- Training on DTIBA and Real time LAMP for the detection of XF in insect vectors and plant samples.	FAO Experts
12.30 - 14:00	**Lunch break**	
14:00 - 15:30	- Training on DTIBA and Real time LAMP for the detection of XF in insect vectors and plant samples.	FAO Experts
15:30 - 16:00	**Coffee Break**	
16:00 - 17:30	- Results interpretation	FAO Experts
Day 5	***Friday 03 February 2017***	
8:30 - 10:00	- Capacity development and awareness raising on *X. fastidiosa* – stakeholder involvement and media control	FAO Experts
10:00 - 10.30	**Coffee break**	
10:30 - 12:30	- Simulation exercises: **Exercise 1.** Planning activity following a detection of an infected plant and plant movement controls. **Exercise 2.** Import controls to detect *X. fastidiosa*	FAO Experts
12.30 - 14:00	**Lunch break**	
14.00 - 15.30	- Self-assessment test & discussion	All participants
15.30 - 16.00	Coffee break	
16.00 - 17.00	- Conclusions & End of the training session	Local & FAO Representatives

Nome & Surname: **Thaer YASEEN**
Email: y.thaer@iamb.it
Country: Italia
Role: Tutor
Present position:

- Consultant Researcher in Plant Pathology at CIHEAM - Istituto Agronomico Mediterraneo di Bari (IAMB);
- From 2015 is the scientific director of agriculture essay centre for pesticide efficacy in integrated control at CIHEAM / IAMB.
- Agronomist registered with the Provincial Agronomy and Forestry of Bari (Order No. 1422).

1997 Degree in **Agricultural Engineering** from the University of Damascus, Syria
2004 **PhD** from Bari University in "Plant protection" "MOLECULAR DIAGNOSIS AND BIOLOGICAL CONTROL OF PHYTOPHTHORA CITRUS ROOT ROT".

Background
20 years of experience in education/training, applied research and international cooperation in: pest surveillance, monitoring, characterization, epidemiology and control. **Since 2003** signor researcher in Plant Pathology at CIHEAM, 2005-present lecturer at MSc IPM course in IAMB. Scientific supervisor of about 53 MSc theses, Co-tutored about 13 PhD theses in collaboration with Italian and foreign Universities. Co- organized and/or lectured in several national & international training workshops at CIHEAM-MAIB or in other countries, e.g.:

- TAIEX Workshop (AGR 58587) **May 6th 2015** Tirana, Albania, on phytosanitary measures against ***Xylella fastidiosa***.
- IPPC-FAO-CIHEAM International workshop on ***Xylella fastidiosa*** and the olive quick decline syndrome — OQDS' (CIHEAM/MAIB ITALY- **April 2016**);
- FAO TCP/RAB/3601 Workshop 'Development of o contingency plan for ***Xylella fastidiosa*** in NENA countries' (Tunis, Tunisia – **28 August- 3 September 2016**)
- TAIEX Workshop (AGR 61696) **30 May to 01 June 2016** Ankara, Turkey on Managing the ***Xylella fastidiosa***.
- TAIEX Workshop (AGR 61694) **21 - 22 July 2016**. Podgorica, Montenegro, on Phytosanitary Measures against ***Xylella fastidiosa***.
- Tutor of TAIEX Study visit (AGR IND/STUD 61691) **24-28 October 2016** (CIHEAM/MAIB - Italy) on phytosanitary measures against ***Xylella fastidiosa***.

He has participated, as a keynote speaker in several Workshops and events in the last four years, primarily presenting the case of *X. fastidiosa* in Italy.

Field of expertise

- Development and validation of innovative methods of plant pathogens diagnosis.
- Epidemiology, morphological, and molecular characterization of new species and / or isolated from plant pathogens.
- Integrated pest control of fungal diseases of fruit trees and vegetables.
- Innovative eco-friendly methods to control plant pathogens in pre and post-harvest.

Nome & Surname: **Franco VALENTINI**
Email: valentini@iamb.it
Country: Italia
Role: Tutor
Present position:

- Consultant Researcher in Plant Pathology at CIHEAM - Mediterranean Agronomic Institute of Bari (IAMB);
- Responsible of bacteriology at the Department of Integrated Pest Management of Mediterranean of Fruit and Vegetable crops
- Agronomist registered with the Provincial Agronomy and Forestry of Bari (Order No. 1069).

2001 Degree in **Agricultural Science** from the University of Bari, Italy
2008 **PhD** from Perugia University in "Plant protection and Arboriculture" "Genetic and phenotypic diversity of Mediterranean populations of the olive knot pathogen, *Pseudomonas savastanoi* pv. *savastanoi*".

Background
15 years of experience in education/training, applied research and international cooperation in: pest surveillance, monitoring, characterization, epidemiology and control. **Since 2003** Researcher in Plant Pathology at CIHEAM, 2004-present lecturer at MSc IPM course in IAMB. Scientific supervisor of about 40 MSc theses, Co-tutored about 8 PhD theses in collaboration with Italian and foreign Universities. International consultant in the ANSES France) evaluation committee. Co- organized and/or lectured in several national & international training workshops at CIHEAM-MAIB or in other countries, e.g.:

- IPPC-FAO-CIHEAM International workshop on ***Xylella fastidiosa*** and the olive quick decline syndrome — OQDS' (CIHEAM/MAIB ITALY- **April 2016**);
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- Tutor of TAIEX Study visit (AGR IND/STUD 61691) **24-28 October 2016** (CIHEAM/MAIB - Italy) on phytosanitary measures against ***Xylella fastidiosa***.
- Tutor of BSTF workshop **7-9 November 2016** (CIHEAM/MAIB - Italy) on monitoring of ***Xylella fastidiosa***
- Tutor of COI workshop **28-30 November 2016** (CIHEAM/MAIB - Italy) on field Symptom description and identification in olive and other host species against ***Xylella fastidiosa***

He has participated, as a speaker in several Workshops and events in the last four years, primarily presenting the case of *X. fastidiosa* in Italy.

Field of expertise

- Epidemiology, morphological, and molecular characterization of new species and / or isolated from plant pathogens.
- Integrated pest control of bacterial diseases of fruit trees and vegetables.
- Scientific and technical assistance to the Apulian programme for the Monitoring of quarantine pests

"Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries"

TCP/RAB/3601 project

Xylella fastidiosa - *General Overview*

(Identity, hosts, geographical distribution, biology, symptoms etc.)

Franco Valentini, Thaer Yaseen, Khaled Djelouah
CIHEAM - Mediterranean Agronomic Institute of Bari, Italy

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Xylella fastidiosa overview

- Introduction
- The bacterium-Growth-Taxonomy
- Geographical distribution
- Host range
- Diseases / Lyfe cycle

2

Xylella fastidiosa

gram-negative

X. f. is one of the most harmful plant pathogen, affecting a number of commercial crops, landscape trees and ornamentals in North and South America

Pierce's disease

It is the causal agent of many diseases such as

- *Pierce's disease of grapes*
- *Citrus variegated chlorosis*
- *Almond leaf scorch*
- *Plum leaf scald*
- *Pear leaf scorch*
- *Oleander leaf scorch*
- *and many more……..*

CVC

X. f. is an EPPO quarantine pathogen of the A1 list.

OQDS (CoDiRO)

Xylella fastidiosa: the history

- **Pierce disease was firstly described in 1887**

  - in 1880s a severe disorder of the grapevine denoted Anaheim (CA) disease appeared in southern California

  - destroyed more than 14.000 grapevines ha in California, 50 vineries have to close in Los Angeles

  - The disease decribed by N. Pierce who called it "California vine disease"



- **Hewitt (1930s). Pierce's disease and postulated a "virus" as the causal agent**

Xylella fastidiosa: the history

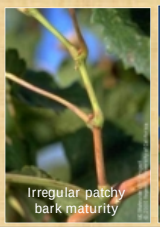
Not much attention was paid to Pierce's disease after the first outbreak, until it reappered dramatically in northern California in the 1920s

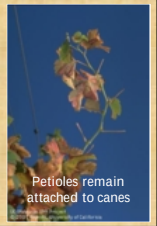
- **Demonstrated as transmitted by vectors in 1940's and Alfalfa dwarf was considered to be caused by same "virus"**
- **1970-80s Bacterial cells were discovered in the xylem vessels of PD-affected vines and Koch's postulated proved that the causal agent is a bacterium *X. fastidiosa*.**
- **Described in USA, Costa Rica and Mexico.**

Pierce's Disease

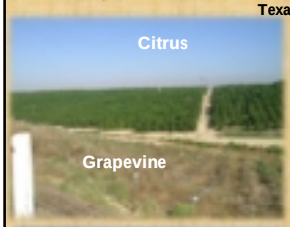

Sudden drying part of leaf


Foliar discoloring and distortion



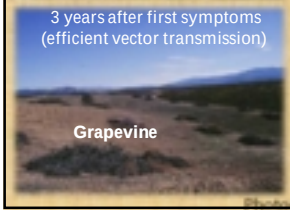


After 125 years is still the most severe disease of vineyards in USA (California, Texas, Florida)



Annual losses in California evaluated in more than 30 million US$ per year, increased since the end of the 1990's because of the presence of the very efficient vector "glassy-winged sharpshooter" (*Homalodisca vitripennis*).

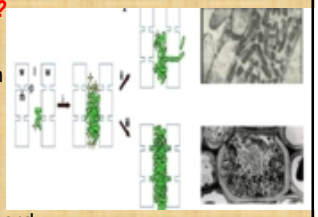
Temecula Valley
(South-California)



Grapevine

Photos by Mark Sisterson

What is Xylella fastidiosa?



- It's a xylem limited bacterium
- vector-transmitted
- It moves upward and downward
- It infects roots
- It formes biofilm in the vessels blocking the sap flow ……inducing the death of the branch or the tree

What is Xylella fastidiosa?

- *X. fastidiosa* is a polyphagous pathogen with large list of hosts and with new hosts discovered every year (more than 359 - EFSA, 2016)*
- The bacterium can have a long period of latency in the host, or even do not show symptoms.
- Long list of vectors (xylem-feeding insects) reported: variable in different countries, amond local Cicadellidae, Cercopidae and Aphrophoridae species
- Can adapt to different climatic conditions although low temperatures can limit its dissemination.
- Difficult control of plants and vectors.

*Among them, 44 new species, 15 new genera and 5 new families have been reported in the Italian and French outbreaks

What is Xylella fastidiosa?

- Oxidase negative and catalase positive
- Metabolism: aerobic (non fermentative), no halofilic
- Find mainly within plant xylem and transmited by xylem-feeding insect vectors (dual life style)
- Colonies: (cream to white), very small (0.2-0.35 mm after 21 days), visible after 7 to 21 days (medium / strain)
- Termophilic bacterium: Optimum growth at 25-28º

  Sensitive to low temperature (< 15°C);

  T< 10°C> 34°C limit its growth and survival

Fastidious growth:

- Very specific growth requirements (subspecies / strain-dependent)
- Easy to get contamination from other endophytic bacteria even with strong tissue desinfestation

egg. BCYE, PD3, PWG and other (Schaad 2001, Almeida *et al.*, 2004; Janse *et al* 2011; *etc*) .....

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What is Xylella fastidiosa?

- **Named and first described in 1987 (Wells *et al*.)**
- **Name: *Xy.lella.* Gr. n. *xylon* wood; M.L. dim. ending *-ella*; M.L. fem. n. *Xylella* small wood. *fastidiosa* 'fastidious growth'**
- **Gram negative** with cell wall**, lacking flagella (~~nonmotile~~)**
- **Taxonomy**

Filum:	***Proteobacteria***
Class:	***Gamma Proteobacteria***
Order:	***Xanthomonadales***
Family:	***Xanthomonadaceae***
Genus:	***Xylella***
Species:	***X. fastidiosa***
	X. taiwanensis*

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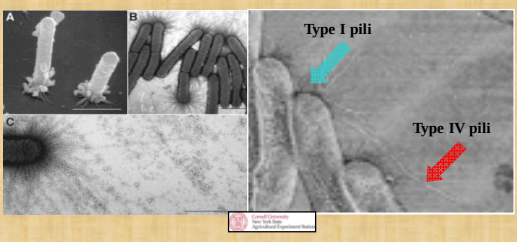
What is Xylella fastidiosa?



- **Single, straight rods (0,25-0,35 x 0,9-3,5 µm), can form long filamentous strands**
- **Has 'pili' type IV (vertical motility within plant xylem) and pili type I & II (biofilm formation, cell adhesion)**

Source: Wells et al. 1987 IJSB; Meng et al. 2005 J. Bacteriol.; Saddler & Bradbury, 2015. Bergey's

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Xylella fastidiosa

Two *Xylella* species are known: ***Xylella fastidiosa*** **and** the newly proposed *Xylella taiwanensis* (Pear, Nashi) are under scrutiny.

Strains of *X. fastidiosa* differ in host range and may be classified into subspecies, this bacterium has been divided in six subspecies, but only five subspecies are commonly accepted,

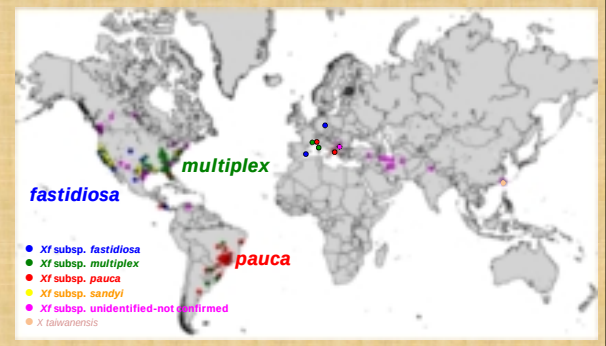
Demonstrated homologous recombination among subspecies

Xylella fastidiosa

Strains of *X. fastidiosa* differ in host range and may be classified into subspecies, this bacterium has been divided in six subspecies, but only five subspecies are commonly accepted,

Subspecies	Some host species	Diffusion	Reference
multiplex	*Prunus dulcis* (almond), *P. armeniaca* (apricot), *P. persica* (peach) *Quercus sp.* (Oak) *Ulmus americana* (Elm)	North America Europe (France)	Nunney *et al.*, 2013
	***Olea europaea* (Olive)**		
fastidiosa	*Vitis vinifera* (Grapevine) *Prunus dulcis* (Almond) *Medicago sativa* (Alfalfa)	North America (egg. United States) Europe (Spain & German)	Janse and Obradovic, 2010
sandyi	*Nerium oleander*		
pauca	*Citrus* spp., *Coffea arabica* ***Olea europeae* (Olive)**	South America Europe (Italy)	Schaad *et al.*, 2004 Cariddi *et al.*, 2014
morus	*Morus spp.* (Mulberries) and ornamental *Nandina domestica*	North America (United States)	Nunney *et al.*, 2014 Kostka SJ *et al.*,1986
taskhe	*Chitalpa tashkentensis*		

Xylella fastidiosa: subspecies distribution



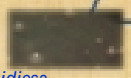
Adapted from EFSA. 2015. EFSA Journal 13 (1): 3989

X. fastidiosa diffusion factors

Vector:

xylem-feeding insects

- Environment (ex. Temperature)
- Ecology of the vector
- pathogen ecology
- Ecology of host plants
- Interactions between the various elements
- disease management



Pathogen:

Xylella fastidiosa

Host plants: >359….

…new emerging diseases

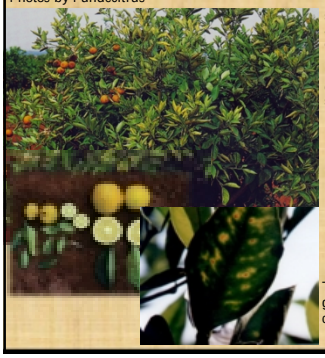
- Pierce's Disease (PD) in grapes in California (USA) – (N.B. Pierce, 1891*)
- Peach, plum in Southeastern USA (Cochran et al., 1951)
- Citrus Variegated Chlorosis (CVC) in South America (Brazil, Argentina) 1980s (Rossetti et al., 1990)
- Pear in Taiwan (Le and Su, 1993)

Old problem…

Citrus Variegated Chlorosis in Brasil

Leaf chlorosis like zinc deficiency

Photos by Fundecitrus



➢This new citrus disease, caused by *X. fastidiosa* subspecies *pauca* was first reported in Brazil in 1987 and then described in Argentina and few other countries.

➢ Widespread in several states in Brazil and economic losses especially important in Sao Paulo state.

➢ Responsible of eradication of more than 120 millions of trees in the 1990's before the detection of HLB.

➢ New vectors described

➢ Difficult control of vectors and disease

The name of citrus variegated chlorosis (CVC) was given because of the obvious bright interveinal chlorosis resembling zinc deficiency.

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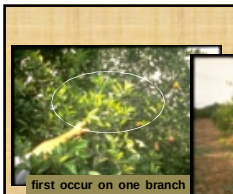


Symptoms more pronouced in older trees

Responsible of eradication of more than 120 millions of trees in the 1990's

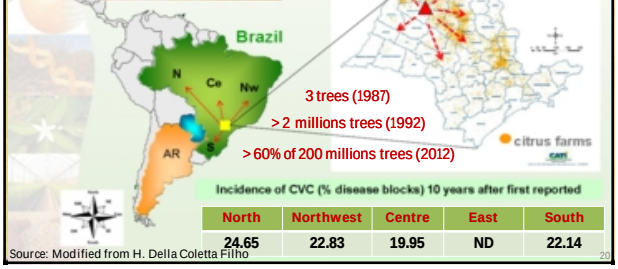
Difficult control of vectors and disease

- New vectors described

CVC management costs about US$120 millions/year (Bové and Ayres, 2007)

Citrus Variegated Chlorosis in Brasil

São Paulo Map and Geographic distribution of citrus farms



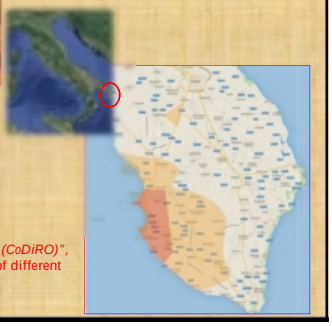
Incidence of CVC (% disease blocks) 10 years after first reported

North	Northwest	Centre	East	South
24.65	22.83	19.95	ND	22.14

Source: Modified from H. Della Coletta Filho

The Olive Quick Decline Syndrome (OQDS)

is a syndrome of olive which appeared for the first time in the South of Apulia (Gallipoli district)



The disease, denoted "*Complesso del disseccamento rapido dell 'olivo (CoDiRO)*", may be caused by the concomitant action of different factors

INITIAL , SOME OF THE EARLY HYPOTHESES

OQDS was associated to:

- ***Zeuzera pyrina***

- ***Severe attacks of olive antracnuose (Colletotrichum spp.)***

- **Tracheomycothic fungi**

Verticillium, Phaeoacremonium, Phaeomoniella spp.

***Journal of Plant Pathology* (2013), 95 (3), 659-668**
Disease Note
IDENTIFICATION OF DNA SEQUENCES RELATED TO *XYLELLA FASTIDIOSA IN* OLEANDER, ALMOND AND OLIVE TREES EXHIBITING LEAF SCORCH SYMPTOMS IN APULIA (SOUTHERN ITALY)
M. Saponari, D. Boscia, F. Nigro and G.P. Martelli

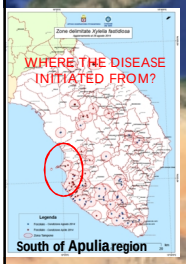
1st report on *X. fastidiosa* in Europe & Mediterranean region

X. fastidiosa was the main cause associated to OQDS trees

X. fastidiosa has been identified in coffee tree plants without symptoms for ornamental or industrial use, imported by Germany, Slovakia, France, Holland and Italy.

2013
First finding of X. fastidiosa in EU & Med region

Apulia region



Apulia is the largest olive producer region in Italy: 41% of the total olive production (ISTAT 2013)

X. fastidiosa: interceptions of infected plants in EU

BEFORE 2013

X. fastidiosa has been identified in coffee tree plants without symptoms for ornamental use in France 2011 (EPPO)

X. fastidiosa has identified in grape plants in Kosovo 1997 this data was publish but not confirmed

AFTER 2013

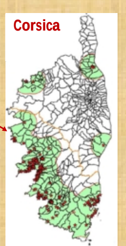
X. fastidiosa has been identified in coffee tree plants without symptoms for ornamental or industrial use, imported by Germany, Slovakia, France, Holland and Italy

	EU interception number	Plant species	Exporting country	Point of entry	Destination country	Class of commodity	Date of interception
1.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	SWITZERLAND	Plants for planting	06.02.2015
2.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	SWITZERLAND	Plants for planting	27.10.2014
3.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	SWITZERLAND	Plants for planting	16.10.2014
4.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	GERMANY	Plants for planting	21.10.2014
5.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	GERMANY	Plants for planting	17.10.2014
6.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	GERMANY	Plants for planting	29.10.2014
7.	[illegible]	Coffea arabica	COSTA RICA	GERMANY	GERMANY	Plants for planting	21.10.2014
8.	[illegible]	Coffea arabica	HONDURAS	NETHERLANDS	NETHERLANDS	Plants for planting	23.10.2014
9.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	GERMANY	Plants for planting	17.10.2014
10.	[illegible]	Coffea arabica	COSTA RICA	ITALY	ITALY	Plants for planting	29.10.2014
11.	[illegible]	Coffea arabica	COSTA RICA	NETHERLANDS	GERMANY	Plants for planting	17.10.2014

and……………….

2015
X. fastidiosa was reported in France

Situation in France March 2016



Provence, Alpes, Côte d'azur

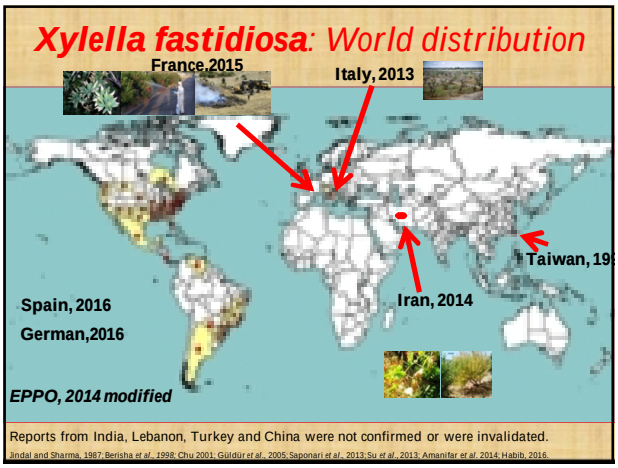
Isolated from 2 plant species
- *Polygala myrtifolia*
- *Spartium junceum*

★ foci
◯ buffer area

Isolated from 21 plant species

http://www.corse-du-sud.gouv.fr/xylella-fastidiosa-une-menace-qui-demande-une-a1409.html
http://www.alpes-maritimes.gouv.fr/Politiques-publiques/Securite-et-protection-de-la-population/Xylella-Fastidiosa/Communiques

Xylella fastidiosa: World distribution



Reports from India, Lebanon, Turkey and China were not confirmed or were invalidated.

Jindal and Sharma, 1987; Berisha *et al.*, 1998; Chu 2001; Güldür *et al.*, 2005; Saponari *et al.*, 2013; Su *et al.*, 2013; Amanifar *et al.* 2014; Habib, 2016.

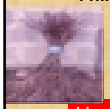
Hypothesis:
Possible pathogen introduction in Italy through infected material

The globalisation of plants trade has facilitated the import from exotic countries without evaluation of phytosanitary risks

The European Union (and 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 that did not analyse the plants for export

In the majority of the countries, the phytosanitary certificates for exports are provided without any analysis, just after visual inspection of the plants

Primarily by MAN



How can *X. fastidiosa* be transmitted?

HUMAN-MEDIATED INTRODUCTIONS
XF-infected plant material is a major pathway for long-distance dispersal

It does not spread through:

- Splash of water (rain and / or irrigation)
- -spores
- wind
- contact
- wounds

Once introduced, the pathogen is transmitted by insect vectors (mainly leafhoppers) with lack of latent period and trans-stadial or trans-ovarial transmission

Most important in USA

Homalodisca vitripennis

Graphocephala atropunctata

Persistence in adult insects and multiplication in the foregut.
Few bacterial cells required for transmission (Hill & Purcell, 1995)

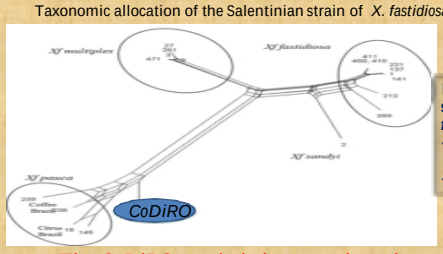
Philaenus spumarius
The assessed vector in Europe

Xylella fastidiosa in Apulia

ISOLATION

Bacterial colonies of *Xylella fastidiosa* isolated on BYCE / PWG medium, ca 20 days after plating from infected olive tissue and other.

Taxonomic allocation of the Salentinian strain of *X. fastidiosa*



MST; the Salentinian strain of *X. fastidiosa*
- ✓ genetically homogeneous
- ✓ highly identical

The *CoDiRO* strain belongs to the subspecies *pauca*

This variant is identical to that one infecting oleander in CostaRica

Cariddi *et al.*, 2014; Elbeaino *et al.*, 2014

Xylella fastidiosa: origin of the *CoDiRO* strain ?

Host plant found to be susceptible to *Xylella fastidiosa* subsp. *pauca* in the Union territory

Acacia saligna (Labill.) Wendl.
Asparagus acutifolius L.
Catharanthus
Cistus creticus L.
Dodonaea viscosa Jacq.
Eremophila maculata F. Muell.
Euphorbia terracina L.
Grevillea juniperina L.
Laurus nobilis L.
Lavandula angustifolia Mill.
Myoporum insulare R.Br.
Myrtus communis L.
Nerium oleander L.
***Olea europea* L.**
Phillyrea latifolia L.
Polygala myrtifolia L.
Prunus avium (L.) L.
Prunus dulcis (Mill) D.A. Webb
Rhamnus alaternus L.
Rosmarinus officinalis L.
Spartium junceum L.
Vinca
Westringia fruticosa (Willd.) Druce
Westringia glabra L.

.....(30)

X. fastidiosa: the vector in Apulia

Philaenus spumarius is the only assessed vector of *Xf*.
It is a polyphagous insect, showing an high density population in the *Xf*-infected area of Apulia (Saponari *et al*., 2014)


Potential vectors of *X. fastidiosa* in Apulia (Elbeaino *et al*., 2014)


Neophilaenus campestris Fallen


Euscelis lineolatus Brullé

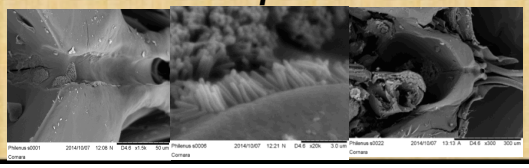
- ✓ Do not require a latent period
- ✓ Persistent transmission by adults
- ✓ No transstadial or transovarial (nymph vectors lose infectivity with moulting)

The vector

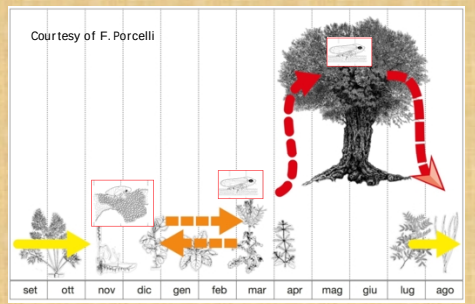
The spittlebug *Philaenus spumarius* is the main vector of *X. fastidiosa* CoDiRO. Hundreds of adults can be found on a single olive tree. A very high percentage of them (up to 70-80%) may contain the bacterium

Xf in *Philaenus spumarius*

Courtesy of F. Porcelli

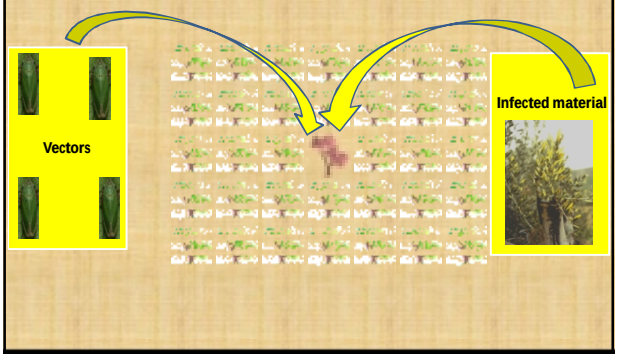


Biological cycle of *P. spumarius*



Olive is the favourite host of the spittlebug and the major source of inoculum for plant-to-plant spreading of *Xylella*

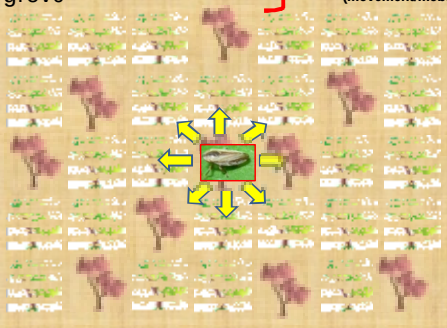
Primary spread:
the infection starts from a plant (0) in the grove



X. fastidiosa is transmitted naturally from plant to plant with the help of vectors

Secondary spread:
The infection spreads inside the grove

- **Vector transmission efficiency**
- **Vector population density**
- **Number of plants visited (movement/mobility)**



Acknowledgment

The research team

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SPECIAL THANKS


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D. Boscia

Case and Management of the XF Outbreak in Apulia

Thaer YASEEN

CIHEAM
Mediterranean Agronomic Institute of Bari (MAIB)
Division of Integrated Pest Management

Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries
TCP/RAB/3601 project

The first thing to do is

Trust the science

Practical aspect

Case of outbreak of *Xylella fastidiosa*

- EXTENSIVE SURVEYS OF SUSCEPTIBLE HOSTS
- Identify the contaminated area and determine the most appropriate management strategies
- Identify *X. fastidiosa* diffusion factors
- Define human assisted spread and vectors
- Awareness raising campaigns
- The use of validation of diagnostic tools
- Eradication of the infected plants

X. fastidiosa in the framework of quarantine law

Thaer YASEEN, Franco Valentini & Khaled Djelouah

CIHEAM
Mediterranean Agronomic Institute of Bari (MAIB)
Division of Integrated Pest Management

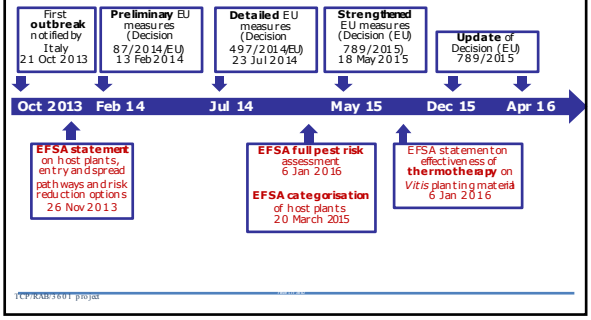
X. fastidiosa in the framework of the EU plant quarantine law

- Dir. 2000/29 and Decision (EU) 2015/789 -

Regulatory framework

- Regulated as **Quarantine Organism**: listed in Annex I, Part A, Section I of Dir. 2000/29/EC as organism not know to occur in the EU;
  - Introduction into and spread within the EU is banned;
  - Member States shall immediately notify any presence in their territories;
  - Take all necessary measures to eradicate, or if that is impossible, inhibit its spread;
- Regulatory status is currently being updated: proposed listing in Annex I, Part A, Section II as organism known to occur in the EU; NO CHANGES TO LEGAL OBLIGATIONS!

Regulatory framework hand in hand with scientific developments

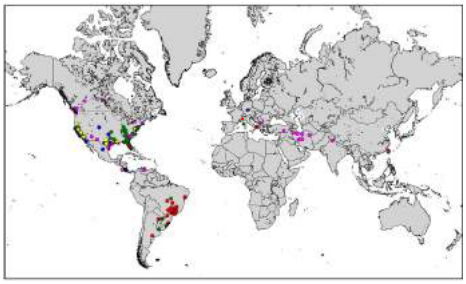


Scientific background (EFSA)

- Pathways of spread:
  - Plants for planting (no risk for seeds or fruit) - over 350 plants species affected;
  - Insect vectors;
- Different Xf subspecies: *pauca, multiplex, fastidiosa, sandyi* …
- Symptoms often with latency;
- No treatment solutions (scientifically validated) are currently available to treat diseased plants in open field;
- Establishment and spread in the EU is very likely;

Plant bacterium with huge impact worldwide

Global distribution of *Xylella fastidiosa*



blue = subsp. *fastidiosa*; green = subsp. *multiplex*; red = subsp. *pauca*;
yellow = subsp. *sandyi*; fuchsia = subsp. unidentified)

EFSA Pest Risk Assessment, January 2015 (readapted by DG SANTE)

Commission Implementing Decision (EU) 2015/789 of 18 May 2015 on *Xylella fastidiosa* (Wells et al.)

Consolidated version available at the following link
http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02015D0789-20160514

General rules applicable to all EU Member States

- Applicable to any Xf subspecies;
- **Surveys activities** on a yearly basis (Art. 3);
- **Awareness raising campaigns** (Art. 13a);
- **Contingency plans** by 31 Dec 2016 (Art. 3a)

Regulated plants species

- **Host plants** (found infected in the EU):
  - Listed under the Commission Database of Host Plants;

    http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en
  - ❖ Eradication/containment measures;
- **Specified plants** (found infected worldwide):
  - Listed in Annex I of Decision (EU) 789/2015
  - Host plants included;
  - ❖ Movement provisions;

Demarcated area (Art. 4)

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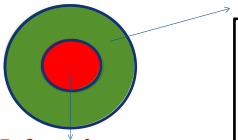


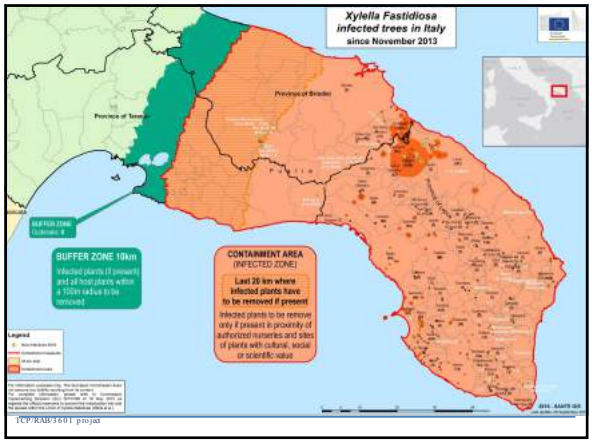
Buffer zone (10 km)

- Intensified monitoring of specified plants
- Vector control (e.g. agricultural practices)
- Movement restrictions out of the BZ

Infected zone

- Removal of plants (eradication/containment)
- Free movement of specified plants
- Planting prohibition of host plants (except for scientific purposes – Apulia)
- Monitoring

TCP/RAB/3601 projet



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Eradication measures (Art. 6)	Containment measures - Lecce, Brindisi, Taranto - (Art. 7)
In a radius of 100 m around infected plants, **clear-cut** of: • Infected and symptomatic plants • Host plants, regardless of their health status	**Removal of at least infected plants** located in : • Proximity of nurseries and growing sites; • Proximity of plants with cultural, social or scientific value • Within the upper 20 km adjacent to the buffer zone;
Sampling and testing specified plants (100 m around infected plants)	**Sampling and testing host plants** (100 m around infected plants)
Appropriate phytosanitary treatments	
Agricultural practices	
Planting prohibition of host plants (except for scientific purposes in South of Apulia)	

TCP/RAB/3601 projet

Import + EU movements of specified plants

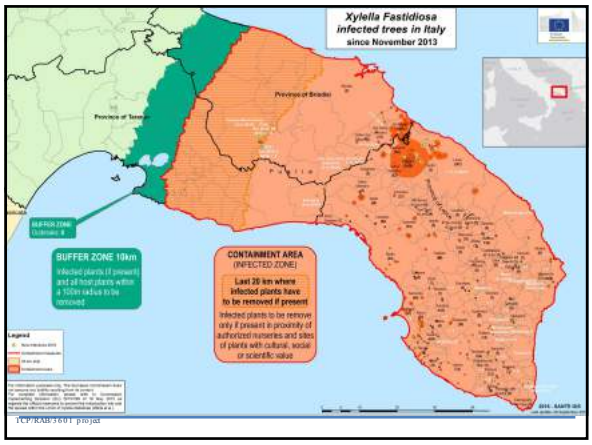
- Strict conditions for movement of specified plants out of the demarcated area:
  - e.g. insect proof conditions,
  - 200 m buffer zone around the nursery free from Xf,
  - Sampling, testing and phytosanitary treatment prior to movement
  - Traceability (for internal movement);
- Hot water treatment for movement of *Vitis* plants for planting;
- Derogations for ***in vitro*** planting material;

Import + EU movements of specified plants (II)

- **Plant passport** for all host plants grown outside the demarcated areas (e.g. in other parts of the EU) as well.
- **Declaration** of the Xf status to be sent by non-EU Countries prior to export!
  - Pest Free Country
  - Pest Free Area
  - Pest Free Production Site
- **Enhanced controls** on import from infected non-EU Countries
- Import ban of *Coffea* planting material from Costa Rica and Honduras

Xylella fastidiosa in Italy (I)

- Subspecies ***pauca*** – first outbreak notified on 21 October 2013, in the province of Lecce (Apulia);
- Extensive resources made available so far by Italy;
- Continuous spread of ***Xf*** out of the original infected area;
- **Movement of specified plants** out of the demarcated area is not authoritised (except *Vitis* under thermotherapy);



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Xylella fastidiosa in Italy (II)

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- 4 COM audits carried out so far, with one more planned in the week of 14 October;
- Limited implementation of EU measures since the first outbreak (e.g. removal of infected plants, survey activities);
  - Appeals to the Regional Administrative Court + Suspension Order by the Prosecutor of Lecce (removed in August 2016);
  - EU Court of Justice's ruling in favour of the proportionality and scientific basis of the EU measures (June 2016);
  - Ongoing infringement procedure against Italy;
- Infected plants subject to felling provisions are now very limited (currently being removed) and survey activities expected to be finalised by end of the year.

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Xylella fastidiosa in France (I)

CIHEAM

- Subspecies ***multiplex***, first notification in Corsica on 27 July 2015;
- 289 outbreaks (Corsica) – 15 outbreaks (PACA, France mainland);
- Plant species affected are increasing over time (30 so far) (e.g. *Polygala myrtifolia; prunus cerasifera; quercus suber, rosmary*);
- Analysis of the insect vectors and trace-back activities ongoing;
- Subspecies ***pauca*** recently reported in the outbreak of Menton (PACA) on *Polygala myrtifolia*

TCP/RAB/3601 projet

Xylella fastidiosa in France (II)

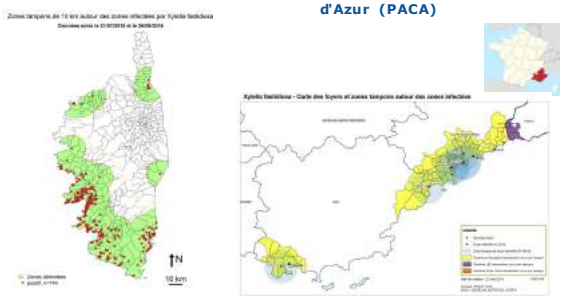
- EU emergency measures currently being implemented;
- COM audit carried out in February 2016:

  Shortcomings currently being addressed by FR

  - Survey activities in the buffer zones to be further stepped-up;
  - Movement restrictions out of the demarcated areas;
  - Newly identified host plants to be removed in all identified outbreaks;

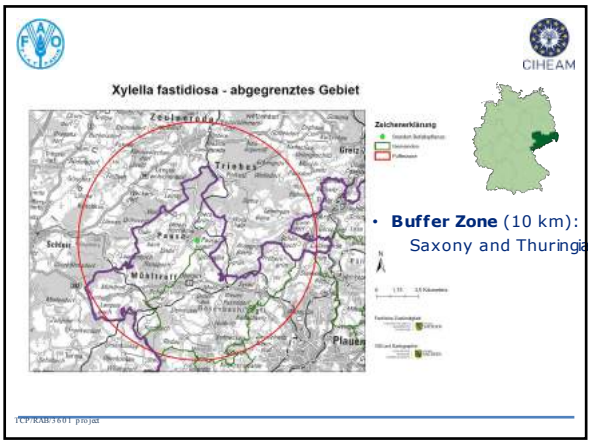
Corsica

Région Provence-Alpes Côte d'Azur (PACA)



Xylella fastidiosa in Germany

- Subspecies ***fastidiosa***, reported on 11 July 2016 in Saxony Region;
- One demarcated area: 1 potted oleander plant + 1 potted rosemary plant located in a small nursery
- Eradication measures are being taken;
- Survey activities currently being carried out, including analysis of the insect vectors;
- Trade restrictions in place;
- COM audit carried out in Oct 2016 – report published soon.



- **Buffer Zone** (10 km): Saxony and Thuringia

Survey of Xf in the Union territory

- No additional findings reported so far by other Member States for 2015 and 2016 (so far);
- Just the last case in Spain;
- EU Guidelines available to harmonise survey activities;

https://ec.europa.eu/food/sites/food/files/plant/docs/ph_biosec_legis_guidelines_xylella-survey.pdf

EU co-financing

- Co-financing possibilities based on certain conditions for:
  - General survey activities;
  - Implementation of EU Measures (e.g. removal of plants, monitoring, phytosanitary treatments, etc.);
  - Compensation to operators for the value of the destroyed material (from January 2017);
- DG AGRI funding (Rural Development Programmes) – possible support

EU funded research

1. **Pest Organisms Threatening Europe (POnTE)** – (Nov. 2015 – Nov. 2019)

   40% of the project on *Xylella fastidiosa* EU Contribution: 6,85 mln euro

   http://www.ponteproject.eu/

2. ***Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy (XF-ACTORS)"** (Nov 2016 – Oct 2020)

   EU contribution: 7 mln euro

Thank you

CIHEAM

"Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries"

TCP/RAB/3601 project

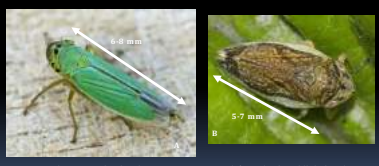
Main insect vectors of *X. fastidiosa* worldwide & in Italy

Khaled Djelouah, Franco Valentini, Thaer Yaseen

CIHEAM-Mediterranean Agronomic Institute of Bari, Italy

Transmission methods and diffusion

- ***X. fastidiosa is transmitted naturally from plant to plant with the help of vectors, mainly leafhoppers and cercopidae (feed on xylem).***



Potential vectors of X. fastidiosa in Europe A: Cicadella viridis (Cicadellidae) and B: Philaenus spumarius (Cercopidae). Russell F. Mizell, Peter C. Andersen, Christopher Tipping, Brent Brodbeck (University of Florida)

Xylem-sap feeders

3

Cicadellini

Cicadella viridis (L.)

Graphocephala fennahi Young

Anoterostemmatini

Anoterostemma ivanoffi (Lethierry)

Evacanthini

Evacanthus acuminatus (Fabricius)

Evacanthus rostagni (Picco)

Evacanthus interruptus (Linnaeus)

Aphrophora salicina (Goeze)

Aphrophora conicae (Germar)

Lepyronia coleoptrata (Linnaeus)

Aphrophora alni (Fallen)

Neophilaenus lineatus (Linnaeus)

Neophilaenus limpidus (Wagner)

Neophilaenus italosignus Drosopoulos & Remane

Neophilaenus infumatus (Haupt)

Neophilaenus exclamationis (Thunberg)

Aphrophora major Uller

Aphrophoridae

Philaenus spumarius (L.)

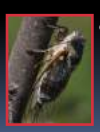
Neophilaenus campestris (Fallen)

Neophilaenus minor (Kirschbaum)

Aphrophora pectoralis Matsumura

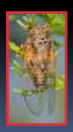
Peuceptyelus coriaceus (Fallen)

Neophilaenus albipennis (Fabricius)



Cicadidae

Tibicinidae



Cercopidae

Cercopis sanguinolenta (Scopoli)

Cercopis intermedia Kirschbaum

Cercopis sabaudiana Lallemand

Cercopis arcuata Fieber

Cercopis vulnerata Rossi

Haematoloma dorsata (Ahrens)

Transmission methods and diffusion

More than 20 species in Brazilian citus cited as vectors

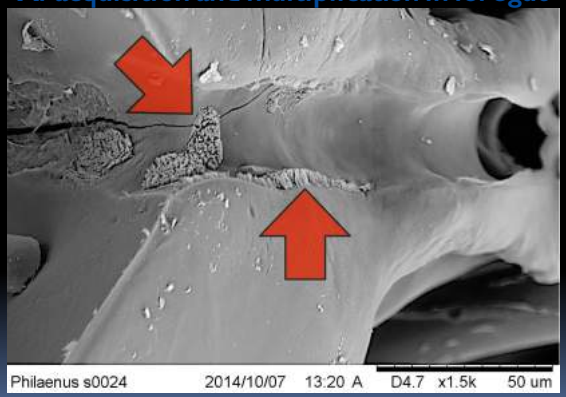
- The specificity between the bacterium and the vector is usually very low, so virtually any kind of insect that feeds on the xylem, could be a potential vector of the bacteria.

Transmission methods and diffusion

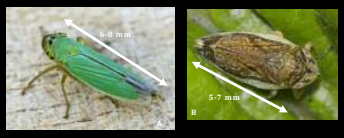
- This insects, however, only act as vectors of the disease at close range (their flight is around 100 m, although they can move longer distances aided by the wind).
- The main way of spreading the bacteria is contaminated plants trade.

Xf acquisition and multiplication in foregut

6



Transmission methods and dispersion



Potential vectors of X. fastidiosa in Europe A: Cicadella viridis (Cicadellidae) and B Philaenus spumarius (Cercopidae). Russell E Mizell, Peter C. Andersen, Christopher Tipping, Brent Brodbeck (University of florida)

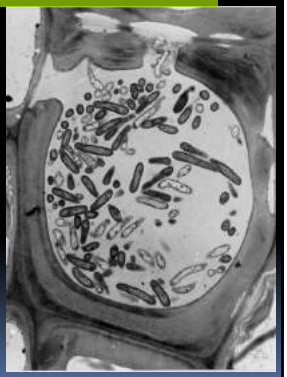
- The transmission of the bacterium is persistent and does not require a period of dormancy.
- Transmission is done by feeding of nymphs and adults on infested plants, and then on healthy ones.
- The bacterium is not transmitted to eggs and neither persists in the nymphal stages after molting.

Transmission methods and dispersion

- Potential vectors in the Mediterranean Basin
- Cicadella viridis (Cicadellidae: Cicadellinae) (Redak et al 2004)
- Philaenus spumarius (Cercopidea: Aphrophoridae) (Saponari et al, 2014.
- Neophilaenus campestris (Froghopper: Aphrophoridae) (Elbeaino et al, 2014.)
- (Euscelis lineolatus Cicadellidae: deltocephalinae) (Elbeaino et al, 2014.)

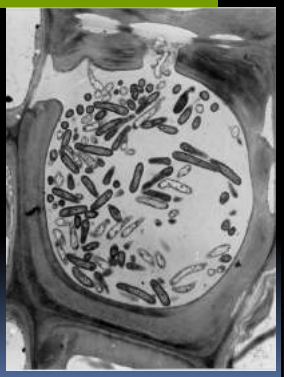
Biology

- The bacteria overwinter in weeds, grasses or wood of trees near the crops, or in host crops.
- These places also provide shelter for vectors during the winter season.
- In general transmission occurs between cultivated and wild hosts, but also between wild hosts can be given.

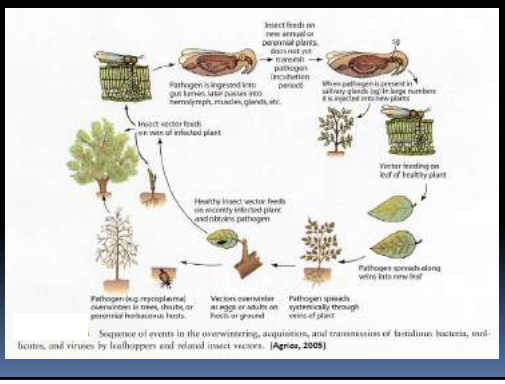


Biology

- In America, some species of insect vectors of *X. fastidiosa*, overwinter as adults, and thus maintain their infectivity during this season.
- However, these insects in Europe hibernate in the egg, so they lose their ability to transmit the bacteria.



Biology



Sequence of events in the overwintering, acquisition, and transmission of fastidious bacteria, mollicutes, and viruses by leafhoppers and related insect vectors. (Agrios, 2005)

IN ITALY

Philaenus spumarius (Linné, 1758)

juvenile and adult, the vector of *Xylella fastidiosa pauca* OQDS

Xylem-sap feeder

Juveniles live in spittle

Palaearctic, introduced in North America

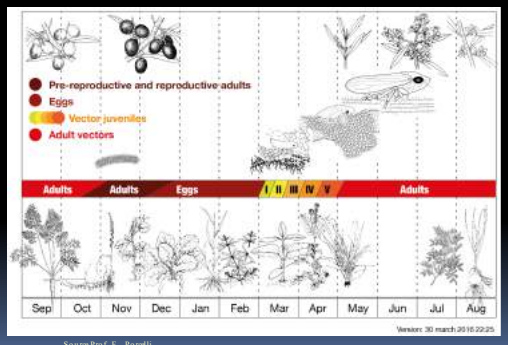
Philaenus spumarius L. (Hemiptera Aphrophoridae) hemimetabolous: eggs, juveniles (naiads & nymphs), adult

Philaenus spumarius

Adult colour forms

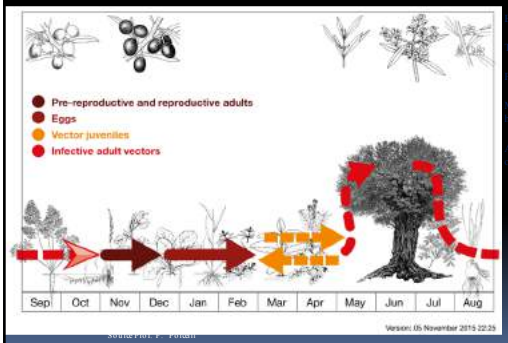
Source: Prof. F. Porcelli

Philaenus spumarius



Source: Prof. F. Porcelli

Philaenus spumarius



Ethology

-topics-

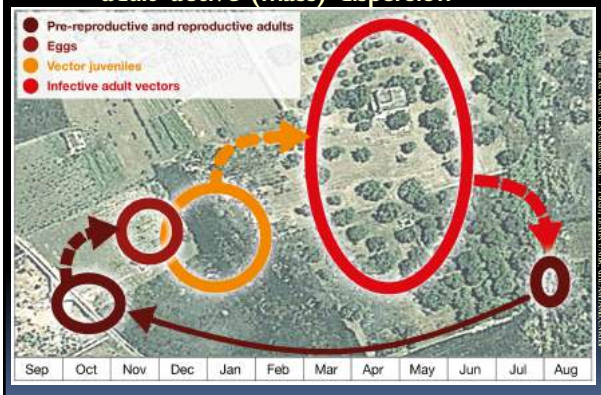
Polyphagous

Move to sprouting host plant

Adults prefer to feed on tips and twigs

16

adult active (mass) dispersion



17

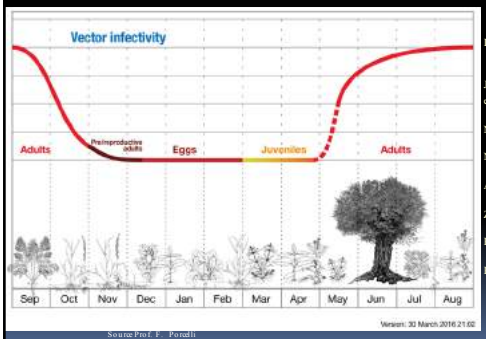
Philaenus spumarius

Hitchhiking

Passive adult dispersion

Source Prof. F. Porcelli

Philaenus spumarius



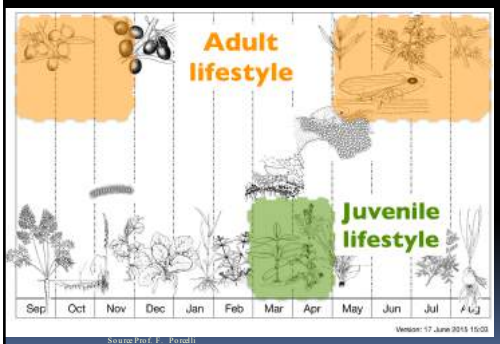
Source:Prof. F. Porcelli

- Infectivity
- Juveniles irrelevant for Xf epidemiology
- Not transtadial
- Not transovarial
- Adults are vectors
- Zero latency
- Persistent transmission
- High adult % is infective

20

Sampling

LIFE CYCLE



Sampling for *Philaenus spumarius*

Different sampling approaches for juveniles and adults

Juveniles host-plant preference

Source: Prof. F. Porazli

SAMPLING

Sampling for *Philaenus spumarius* juveniles

One square metre transect

Young individuals are gregarious

Nymphs are solitary

Juveniles host-plant preference

Collect spittle with host plant part in vials with data

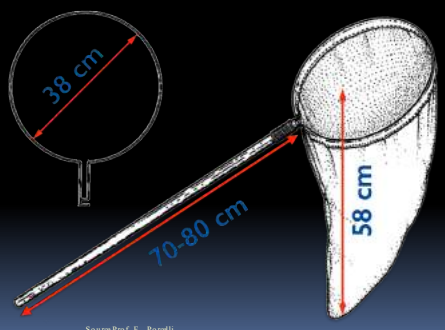
Preserve in 75% EtOH

Do not move living individuals from site to site

Courtesy: dr. Vincenzo Cavalieri

23

Sweeping NET



Sampling for *Philaenus spumarius* adults

Normalized sweeping net

Use on dry plants during warmer daytime

Collect adults into the net by vials

Promptly transfer in 75% EtOH with data

Do not move living adults from site to site

Source: Prof. F. Porazli

Sweeping NET

Sampling for *Philaenus spumarius* adults

Sweeping on ground

Sweeping on tree fronds

Sample size and number of sweeps

Seeking adults in not homogenous fields/environments

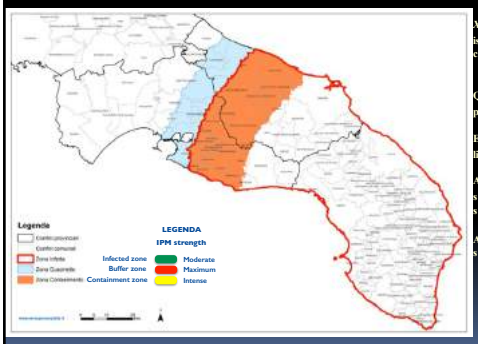
Source Prof. F. Porcelli

Aspirators

Economic and action threshold, control means and strategies

27

MANAGMENT STRATEGY



Xylella fastidiosa invasion is due to missing vector control

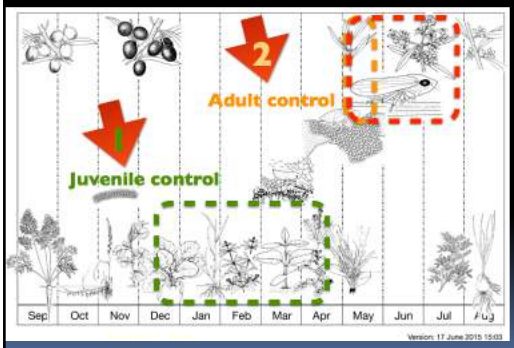
Quantitative functional pest knowledge

EFSA candidate Xf vector list

Appropriate key and secondary pest control strategy

Active survey by citizen scientists

CONTROL



Philaenus spumarius control

Key point(s)

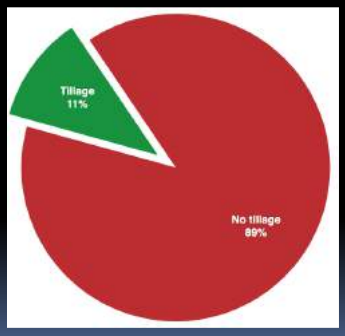
Life table

Antagonist knowledge

Active survey by citizen scientists

29

CONTROL



Source Prof. F. Porrelli

Philaenus spumarius juveniles control

Mechanical control on spontaneous herbs

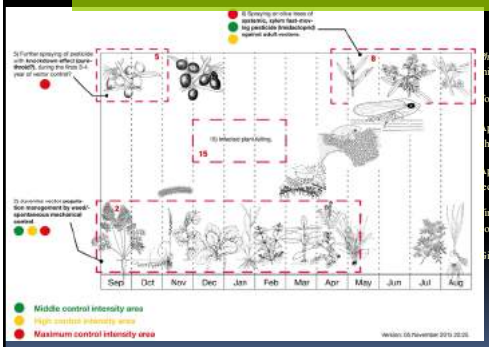
Cheap

Zero impact

Easily assessed

Given by citizen scientists

Philaenus spumarius



Philaenus spumarius minimum IPM

Topics:

Appropriate formulate chose

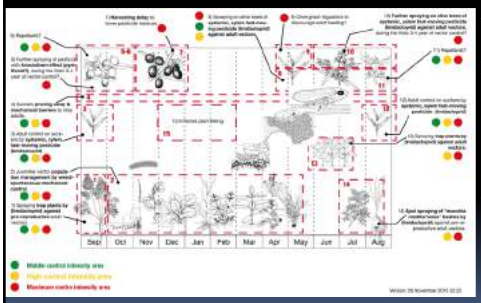
Appropriate distribution technique

Timing in execution of control component

Given by citizen scientists

Source Prof. F. Porrelli

Philaenus spumarius



Philaenus spumarius intense IPM

Additive topics to minimum option:

Useful as first transient control approach

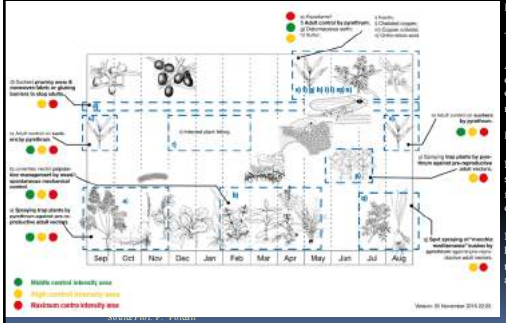
More complex

More effective

Need intense knowledge transfer to citizen scientists operators

Source Prof. F. Porrelli

Philaenus spumarius



Philaenus spumarius organic IPM

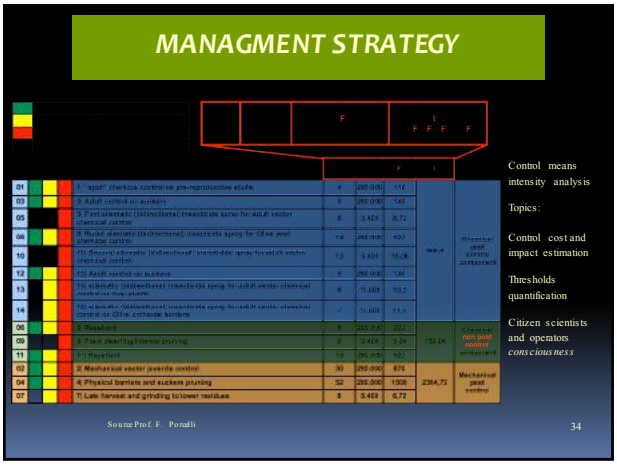
Topics:

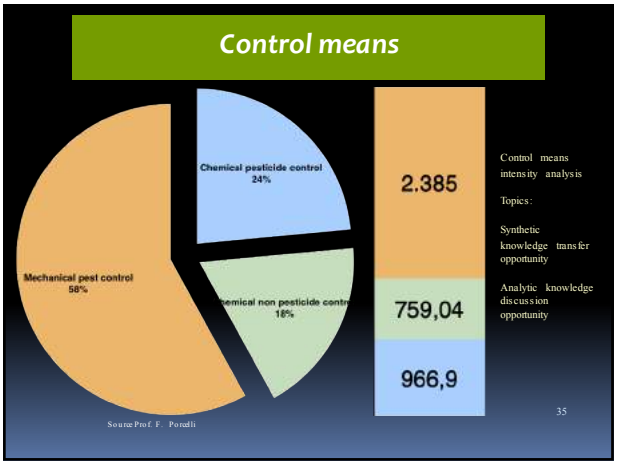
Alien invasive organism disrupt organic management

Effective?

Needs intense studies on formulates and control means

Needs intense knowledge transfer to citizen scientists and operators


MANAGMENT STRATEGY
Control means intensity analysis
Topics:
Control cost and impact estimation
Thresholds quantification
Citizen scientists and operators consciousness
Source Prof. F. Porcelli
34


Control means
Chemical pesticide control 24%
Mechanical pest control 58%
Chemical non pesticide contr 18%
2.385
759,04
966,9
Control means intensity analysis
Topics:
Synthetic knowledge transfer opportunity
Analytic knowledge discussion opportunity
Source Prof. F. Porcelli
35


Thank you for your attention

History and current situation of Xf in the EU-Mediterranean area

Thaer YASEEN, Franco Valentini & Khaled Djelouah

CIHEAM
Mediterranean Agronomic Institute of Bari (MAIB)
Division of Integrated Pest Management

The Olive Quick Decline Syndrome (OQDS)

is a syndrome of olive which appeared for the first time in the South of Apulia (Gallipoli district)

The disease, denoted *"Complesso del disseccamento rapido dell'olivo (CoDiRO)"*, may be caused by the concomitant action of different factors

WHEN?

Probably between 2008 and 2010

WHY?

SOME OF THE EARLY HYPOTHESES

(iii) Pollution of the ground water

(iv) Abandonment of the "good agricultural practices"

(v) Effects of the massive use of glyphosate

(vi) Root rot

ALL UNSUBSTANTIATED

INITIAL , SOME OF THE EARLY HYPOTHESES

OQDS was associated to:

- ***Zeuzera pyrina*** ***(the leopard moth)***

- ***Severe attacks of olive antrachnose (Colletotrichum* spp.)**
- **Tracheomycothic fungi**

Verticillium, Phaeoacremonium, Phaeomoniella spp.

TCP/RAB/3601 projet

Journal of Plant Pathology (2013), 95 (3), 659-668
Disease Note

IDENTIFICATION OF DNA SEQUENCES RELATED TO *XYLELLA FASTIDIOSA* IN OLEANDER, ALMOND AND OLIVE TREES EXHIBITING LEAF SCORCH SYMPTOMS IN APULIA (SOUTHERN ITALY)

M. Saponari, D. Boscia, F. Nigro and G.P. Martelli

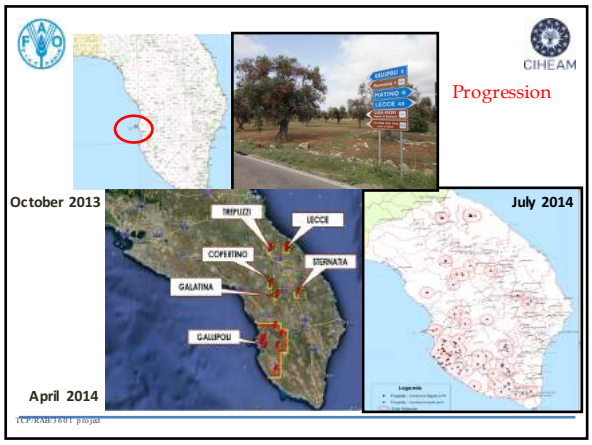
1st report on *X. fastidiosa* in Europe & Mediterranean region

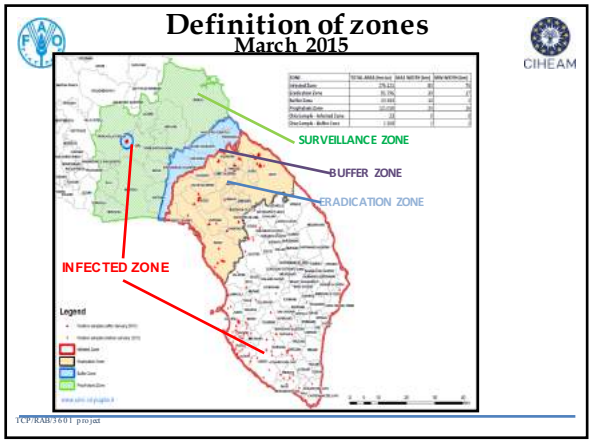
X. fastidiosa was the main cause associated to OQDS trees

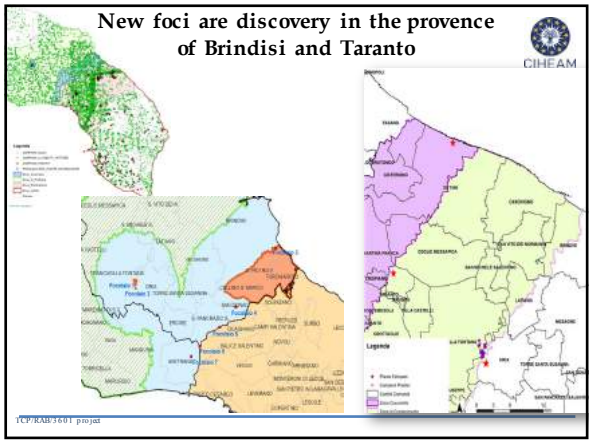
X. fastidiosa has been identified in coffee tree plants without symptoms for ornamental or industri use, imported by Germany, Slovakia, France, Holland and Italy.

TCP/RAB/3601 projet

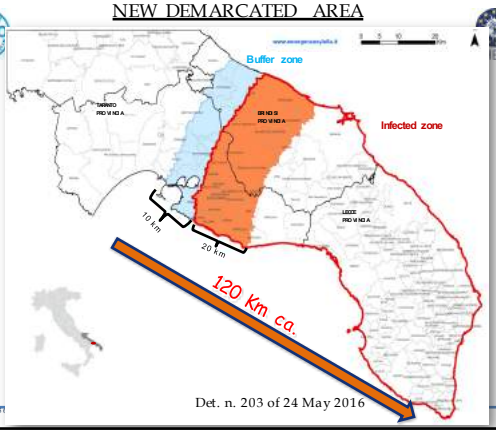
Apulia is the first olive producer region in Italy: 41% of the total olive production (ISTAT 2013)


CIHEAM
Progression
October 2013
July 2014
April 2014
TRIPUZZI
LECCE
COPERTINO
SERNATIA
GALATINA
GALLIPOLI
TCP/RAB/3601 projet


Definition of zones
March 2015
CIHEAM
SURVEILLANCE ZONE
BUFFER ZONE
ERADICATION ZONE
INFECTED ZONE
Legend
TCP/RAB/3601 projet


New foci are discovery in the provence of Brindisi and Taranto
CIHEAM
TCP/RAB/3601 projet

NEW DEMARCATED AREA



Det. n. 203 of 24 May 2016

Ministerial Decree 18 February 2016 on PFA of *Xylella fastidiosa*

- The results of the monitoring plan *has allowed the Italian Authorities to officially recognize as* ***pest-free areas*** *all the rest of the Italian Regions.*

- **Plants and plant products from the green areas guarantee maximum security in terms of health**.

Ministero delle politiche agricole, alimentari e forestali

X. fastidiosa: interceptions of infected plants in EU

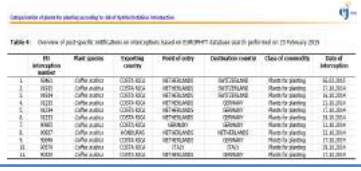
BEFORE 2013

X. fastidiosa has been identified in coffee tree plants without symptoms for ornamental use in France 2011 (EPPO)

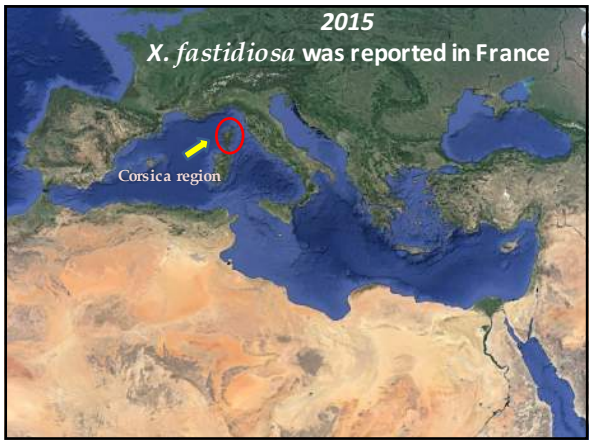
X. fastidiosa has identified in grape plants in Kosovo 1997 this data was publish but not confirmed

AFTER 2013

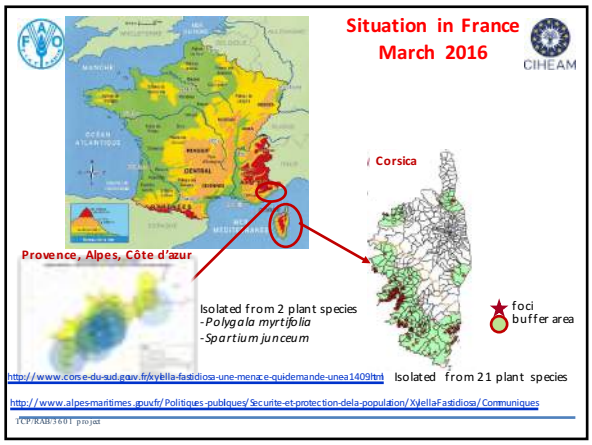
X. fastidiosa has been identified in coffee tree plants without symptoms for ornamental or industrial use, imported by Germany, Slovakia, France, Holland and Italy



and.....


2015
X. fastidiosa was reported in France
Corsica region


X. fastidiosa outbreak in Corsica
X. fastidiosa were detected in Propriano
CIHEAM
Ajaccio
Propriano
Sartene
Porto-Vecchio
July 22nd 2015
Phytosanitary measures were applied
CONTROLE PHYTOSANITAIRE

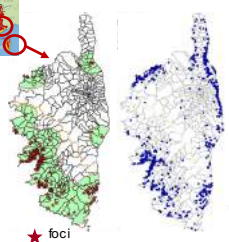

Situation in France
March 2016
CIHEAM
Corsica
Provence, Alpes, Côte d'azur
Isolated from 2 plant species
-Polygala myrtifolia
-Spartium junceum
foci
buffer area
Isolated from 21 plant species
http://www.corse-du-sud.gouv.fr/xylella-fastidiosa-une-menace-quidemande-unea1409.html
http://www.alpes-maritimes.gouv.fr/Politiques-publiques/Securite-et-protection-de-la-population/XylellaFastidiosa/Communiques
TCP/RAB/3601 projet

French situation

October 3rd, 2016

PACA:
Provence, Alpes, Côte d'azur
15 foci

Corsica
289 foci
highest focus: 530m



foci
buffer area
sampled

Isolated from 3 plant species
-*Polygala myrtifolia*
-*Spartium junceum*
-*Lavandula angustifolia*

Isolated from 27 plant species

http://www.corse-du-sud.gouv.fr/xylella-fastidiosa-une-menace-qui-demande-une-a1409.html

TCP/RAB/3601 projet
http://www.alpes-maritimes.gouv.fr/Politiques-publiques/Securite-et-protection-de-la-population/Xylella-Fastidiosa/Communiques

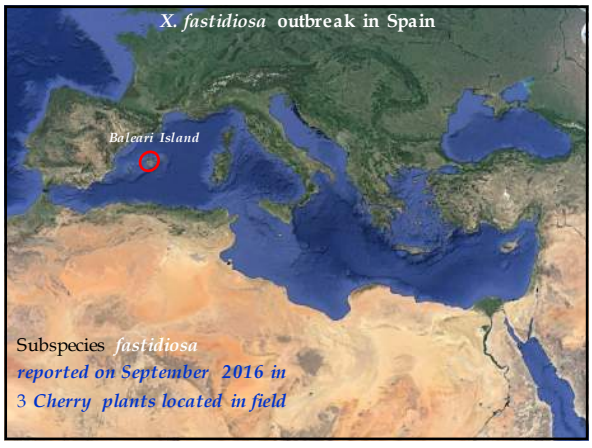
X. fastidiosa outbreak in German

- Subspecies ***fastidiosa, reported on 11 July 2016 in Saxony Region;***
  1 potted oleander plant + 1 potted rosemary plant located in a small nursery

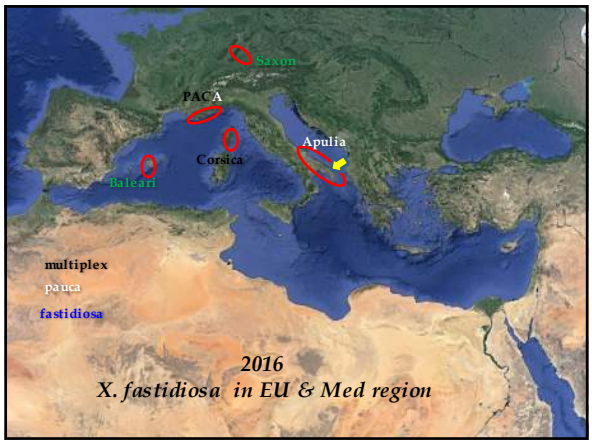
Buffer Zone (10 km): Saxony and Thuringia

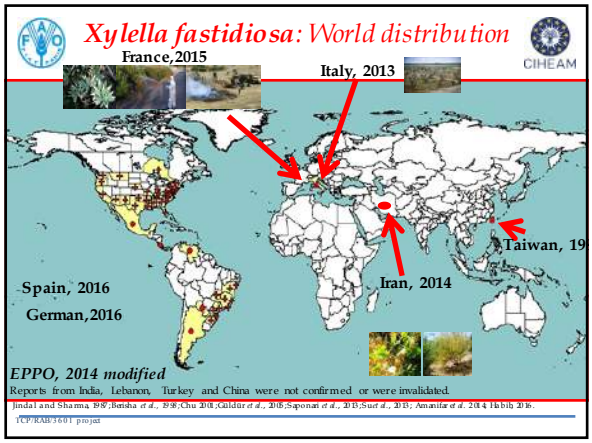
TCP/RAB/3601 projet

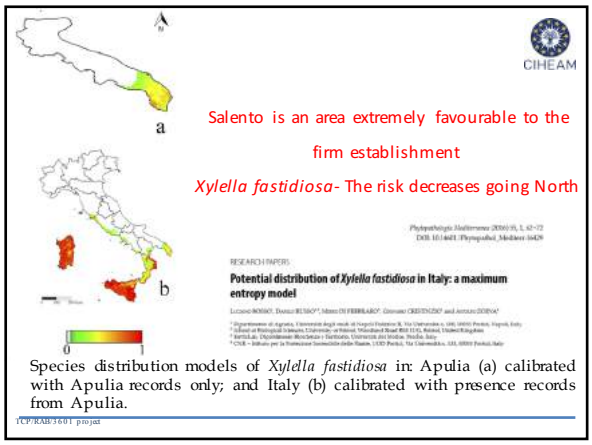
X. fastidiosa outbreak in Spain

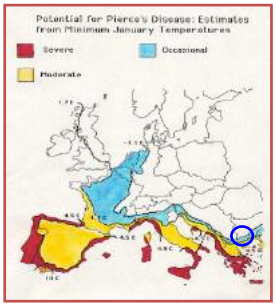


Subspecies *fastidiosa*
reported on September 2016 in
3 Cherry plants located in field


Saxon
PACA
Apulia
Corsica
Baleari
multiplex
paca
fastidiosa
2016
X. fastidiosa in EU & Med region

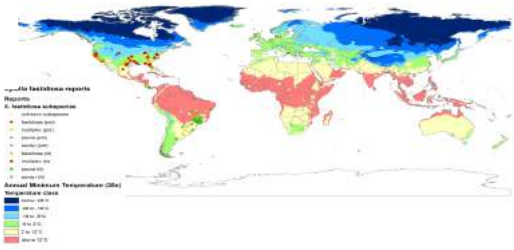

Xylella fastidiosa: World distribution
CIHEAM
France,2015
Italy, 2013
Taiwan, 19
Iran, 2014
Spain, 2016
German,2016
EPPO, 2014 modified
Reports from India, Lebanon, Turkey and China were not confirmed or were invalidated.
Jindal and Sharma, 1987; Barisha et al., 1998; Chu 2001; Güldür et al., 2005; Saponari et al., 2013; Suri et al., 2013; Amanifar et al. 2014; Habib 2016.
FCP/RAB/3601 projet


a
b
CIHEAM
Salento is an area extremely favourable to the firm establishment
Xylella fastidiosa- The risk decreases going North
RESEARCH PAPERS
Potential distribution of Xylella fastidiosa in Italy: a maximum entropy model
Species distribution models of Xylella fastidiosa in: Apulia (a) calibrated with Apulia records only; and Italy (b) calibrated with presence records from Apulia.
FCP/RAB/3601 projet



This prediction by A. Purcell, and the realization that OQDS was spreading faster to the South than the North of the Province of Lecce has suggested to resort to interventions for containing the infection within the boundaries of the affected area

World Annual minimum temperatures



(EFSA PLH Panel, 2015 WorldClim database http://www.worldclim.org)

Climatic conditions for establishment of *X. fastidiosa* are suitable in many places

Hypothesis: Possible pathogen introduction in Italy through infected material

The globalisation of plants trade has facilitated the import from exotic countries without evaluation of phytosanitary risks

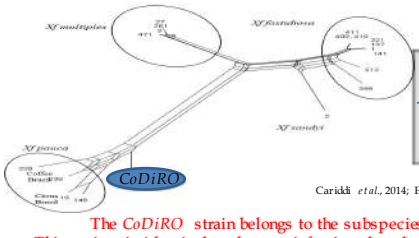
The European Union (and other countries) have imported in the last ten years a large number of plants of *X. fastidiosa* hosts, from areas where the bacterium was present and **that did not analyse the imported plants**
In the majority of the countries, the phytosanitary certificates for exports are provided without any analysis, just after visual inspection of the plants

Xylella fastidiosa in Apulia

ISOLATION

Bacterial colonies of *Xylella fastidiosa* isolated on BYCE/PWG medium, ca 20 days after plating from infected olive tissue and other.

Taxonomic allocation of the Salentinian strain of *X. fastidiosa*



MST; the Salentinian strain of *X. fastidiosa*
- ✓ genetically homogeneous
- ✓ highly identical

Cariddi *et al.*, 2014; Elbeaino *et al.*, 2014

The *CoDiRO* strain belongs to the subspecies *pauca*
This variant is identical to that one infecting oleander in CostaRica

ICP/RAB/3601 projet

Xylella fastidiosa: origin of the *CoDiRO* strain

CIHEAM

Costarica

How many *Xylella fastidiosa* introductions in France ?

CIHEAM

Interceptions :
Coffee plants : sandy/fastidiosa ST72, ST75, ST76;
pauca ST53, ST74

in field : multiplex ST6, ST7,
recombinant multiplex x sandy,
pauca ST53

Hypotheses:
Several introductions
Diverse plant material among which **coffee plants**
Recombinants : imported ou locally generated?

ICP/RAB/3601 projet

Host plant found to be susceptible to *Xylella fastidiosa* subsp. *pauca* in the Union territory

Acacia saligna(Labill.) Wendl.
Asparagus acutifolius L.
Catharanthus
Cistus creticus L.
Dodonaea viscosa Jacq.
Eremophila maculata F. Muell.
Euphorbia terracina L.
Grevillea juniperina L.
Laurus nobilis L.
Lavandula angustifolia Mill.
Myoporum insulare R.Br.
Myrtus communis L.
Nerium oleander L.
Olea europea L.
Phillyrea latifolia L.
Polygala myrtifolia L.
Prunus avium (L.) L.
Prunus dulcis (Mill) D.A. Webb
Rhamnus alaternus L.
Rosmarinus officinalis L.
Spartium junceum L.
Vinca
Westringia fruticosa(Willd.) Druce
Westringia glabra L.


Olea europea


Polygala myrtifolia


Nerium oleander


Prunus avium

.....(26)

TCP/RAB/3601 projet

Host plants found to be susceptible to *Xylella fastidiosa* subsp. *multiplex* in the Union terrirory

Acer pseudoplatanus L.
Artemisia arborescens L.
Asparagus acutifolius L.
Calicotome villosa (Poiret) Link
Cistus monspeliensis L.
Cistus salvifolius L.
Coronilla valentina L.
Cytisus scoparius (L.) Link
Genista x spachiana (syn. *Cytisus racemosus* Broom)
Genista corsica (Loisel.) DC.
Genista ephedroides DC.
Hebe
Helichrysum italicum (Roth) G. Don
Lavandula angustifolia Mill.
Lavandula dentata L.
Lavandula stoechas L.
Lavandula x allardii (syn. *Lavandula x heterophylla*)
Metrosideros excelsa Sol. ex Gaertn.
Myrtus communis L.
Pelargonium graveolens L'Hér
Phagnalon saxatile (L.) Cass.
Polygala myrtifolia L.
Prunus cerasifera Ehrh.
Quercus suber L.
Rosa x floribunda
Rosmarinus officinalis L.
Spartium junceum L.


Polygala myrtifolia

TCP/RAB/3601 projet

Host plant found to be susceptible to *Xylella fastidiosa* subsp. *fastidiosa* in the Union territory

Nerium oleander L.
Prunus

Host plants found to be susceptible to several subspecies of *Xylella fastidiosa* in the Union territory

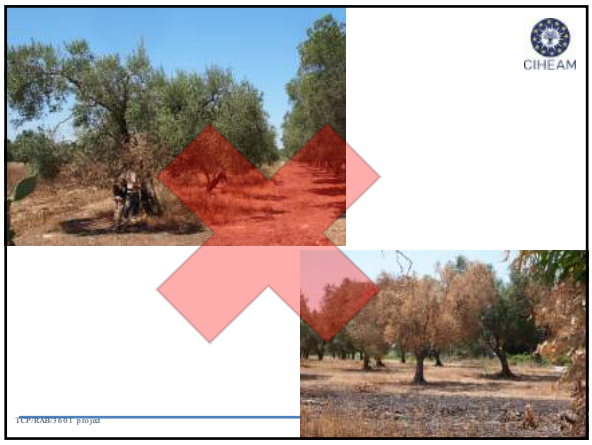
Coffea

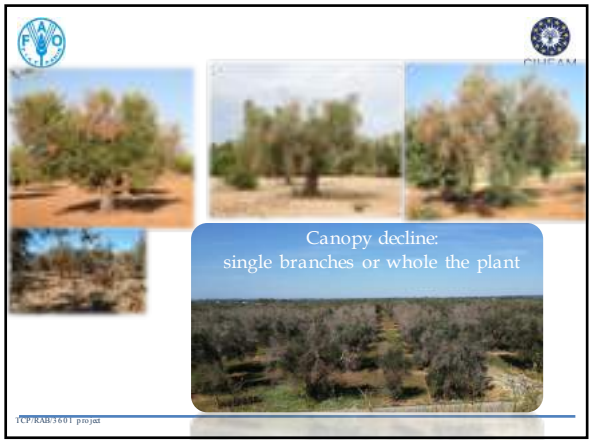
TCP/RAB/3601 projet

Symptomes

Xylella fastidiosa associated to the Olive Quick Decline Syndrome

Sectorial infection


CIHEAM
TCP/RAB/3601 projet


Canopy decline:
single branches or whole the plant
TCP/RAB/3601 projet


All the trees could be symptomatic
CIHEAM
TCP/RAB/3601 projet

The final end of ancient olive trees showing severe OQDS

Trees were heavily pruned for the elimination of dead branches: suckers show again leaf scorch symptoms

TCP/RAB/3601 projet

Millenarian trees (estimated age: 1500 years)

(Gigante di Alliste, March 2015)

TCP/RAB/3601 projet

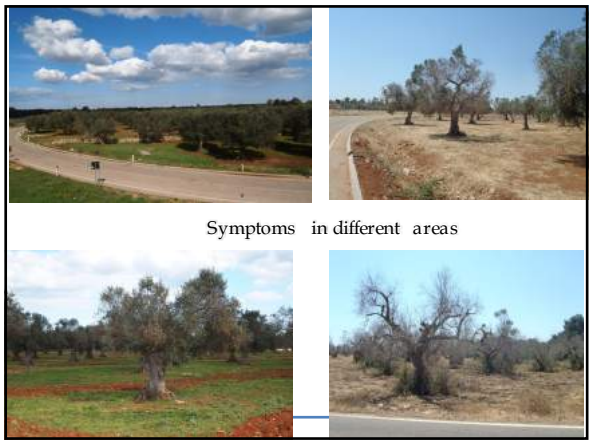
Same tree:

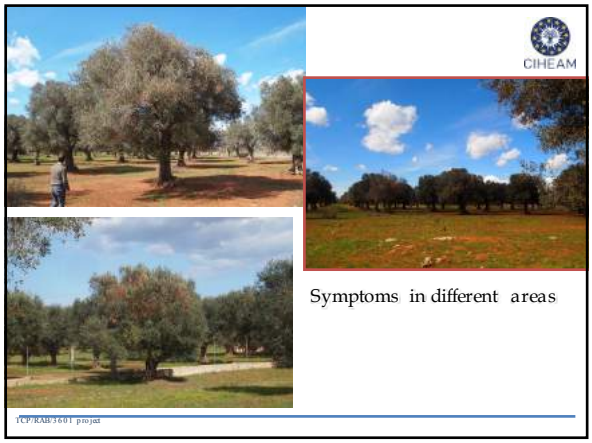
in September 2015

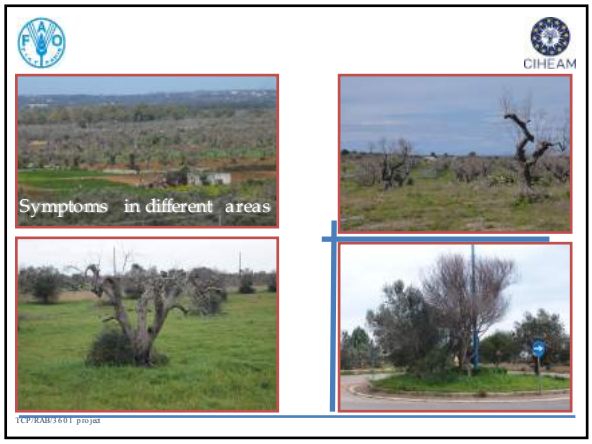
in September 2016

The disease affects equally well (and eventually kills) young trees (15-year-old orso)

TCP/RAB/3601 projet


Symptoms in different areas


CIHEAM
Symptoms in different areas
TCP/RAB/3601 projet


CIHEAM
Symptoms in different areas
TCP/RAB/3601 projet

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Symptoms in different areas

November 2016

CIHEAM

Italy

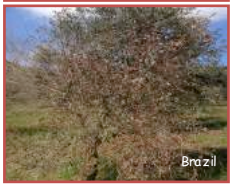
pauca

Argentina

Same symptom are present in

California (USA)

multiplex



Brazil

Symptomatic hosts of *X. fastidiosa* subsp. *pauca*, CoDiRO in Salento

Oleander
Nerium oleander

Symptomatic hosts of *X. fastidiosa* subsp. *pauca*, CoDiRO in Salento

Cherry – *Prunus avium*

Westringia fruticosa

Polygala myrtifolia

Symptomatic hosts of *X. fastidiosa* subsp. *pauca*, CoDiRO in Salento

Acacia saligna

Broom- *Cytisus scoparius*

Rosemary
Rosmarinus officinalis

Myrtus communis

Grevillea juniperina

Laurus nobilis

Cystus creticus

Lavandula angustifolia

Dodonaea viscosa purpurea

Pelargonium

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Acacia saligna

TCP/RAB/3601 projet

Grapevine and Citrus were never found infected in Apulia

To non hosts, 100 herbaceous species of 40 mono- dicotyledonous families (more than 1000 samples), conifers, palms, Cicadaceae e Musaceae and succulent plants added

TCP/RAB/3601 projet

All these hosts and a few more were infected by the same bacterial strain (ST53) found on olive in APULIA

Sectorial infection

Leaf scorch

In affected groves most of the trees can be symptomatic

In affected groves the totality of the trees can be symptomatic

A devastating sight

The disease affects also young trees

Centenarian trees

Final stage of the disease. Trees have been severely pruned to force them to re-vegetate

New shoots are few and desiccating.
Suckers (arrows) will live until the roots are alive

2010

TCP/RAB/3601 projet

2013

TCP/RAB/3601 projet

October 2013

TCP/RAB/3601 projet

Gallipoli, 9 July 2015

TCP/RAB/3601 projet

Gallipoli, October 2013

TCP/RAB/3601 projet

Gallipoli, March 2016

TCP/RAB/3601 projet

April 2014 June 2015 November 2016



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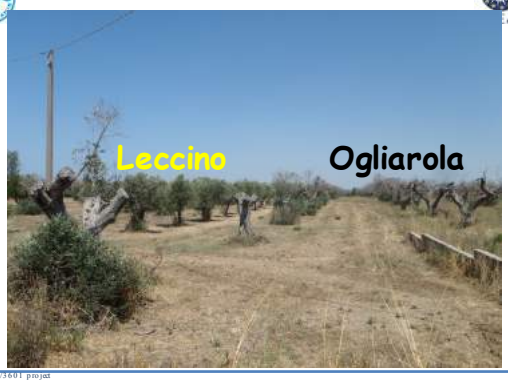
Evidences of genetic tolerance

TCP/RAB/3601 projet



TCP/RAB/3601 projet


CIHEAM
Thank you
CIHEAM
TCP/RAB/3601 projet

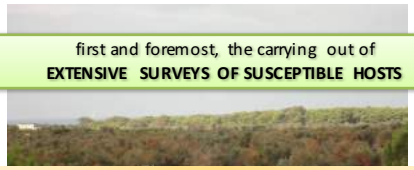
Eradication methods as foreseen in the EU Decision 2015/789 & discussion

Thaer YASEEN, Franco Valentini & Khaled Djelouah

CIHEAM
Mediterranean Agronomic Institute of Bari (MAIB)
Division of Integrated Pest Management

- Establishment of demarcated areas
- Eradication measures
- Containment measures
- Contingency plans

first and foremost, the carrying out of
EXTENSIVE SURVEYS OF SUSCEPTIBLE HOSTS



To identify the contaminated area and determine the most appropriate management strategies

Establishment of demarcated areas
(article 4)

- When the presence of *Xylella fastidiosa* is confirmed:
  - infected zone
  - buffer zone
  - notification to EC => list of demarcated areas
- Derogation: "interception"
  - *Xf* recently introduced
  - plants infected before introduction
  - absence of vectors
  - annual survey for 2 years

4

Infected zone

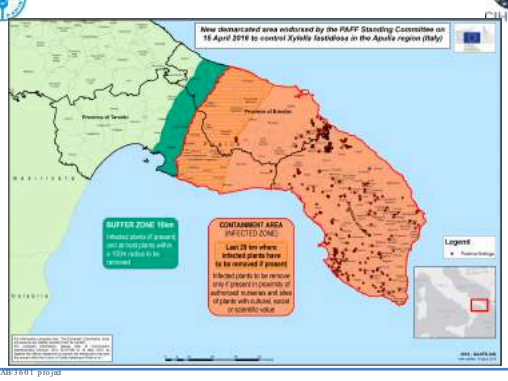
- All plants known to be infected by *Xylella fastidiosa*
- All plants showing symptoms indicating possible infection
- All other plants liable to be infected:
  - close proximity
  - common source of production

5

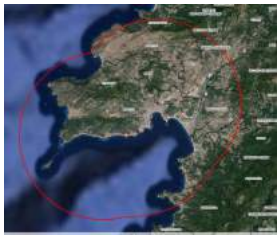
Buffer zone

- Surrounding the infected zone: width of 10 km (at least)
- Taking into account biology of the specified organism and its vectors, the level of infection, the presence of the vectors, and the distribution of specified plants in the area
- Delimitation to be reviewed if the presence of *Xf* is confirmed in the buffer zone

6



Demarcated areas in Ajaccio (Corsica)



Eradication measures

(article 6)

- Within a radius of 100 m around the infected plants:
  - remove host plants, regardless of their health status
  - remove plants known to be infected by
  - remove plants showing doubtful symptoms
  - apply appropriate phytosanitary treatments against the vectors prior to the removal of plants and on plants that may host those vectors
  - sample and test the specified plants in accordance with ISPM n°31
  - destroy the plants and parts of plants (*in situ* or nearby)
  - epidemiological investigations (trace back / forward)
  - appropriate agricultural practices

First outbreak in Corsica




10

First outbreak in Corsica (ctd)




11

First outbreak in Corsica (ctd)




12

Containment measures
(article 7)

- In the province of Lecce and listed municipalities
- Removal measures limited to infected plants:
  - in the proximity of sites approved for moving plants
  - in the proximity of plants with particular cultural, social or scientific value
  - in the "containment area" = situated within a distance of 20 km from the border of that infected zone with the rest of the Union territory.
- Sampling around infected plants, phytosanitary treatments, appropriate agricultural practices
- Monitoring of the presence of *Xf* in the containment area and the buffer zone (100mx100m squares)

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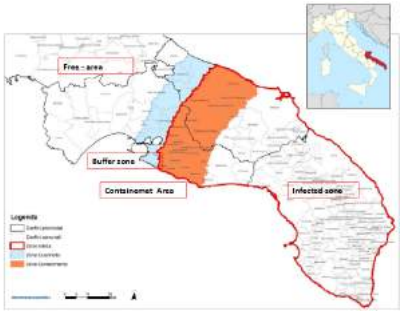
Containment measures in Apulia (Italy)



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Removal of infected plants

TCP/RAB/3601 projet

Contingency plans

(article 3a)

- Each Member State shall establish a contingency plan in case of a confirmed or suspected presence of *Xf*:
  - roles and responsibilities
  - approved laboratories
  - communication
  - protocols
  - training
  - Resources
- Set up by 31 December 2016 and communicated to the European Commission at its request

16

Thank you

CIHEAM

EU Surveillance methods (guidelines, survey plan and diagnostic)

Thaer YASEEN, Franco Valentini & Khaled Djelouah

CIHEAM
Mediterranean Agronomic Institute of Bari (MAIB)
Division of Integrated Pest Management

DECISION (EU) 2015/789

&
Guidelines for the survey of *X. fastidiosa* (Wells et al.) in the Union territory

Xylella fastidiosa, one of the most harmful plant pathogenic bacteria worldwide, is subject to a regular survey activity within the Union territory.
In this respect (in accordance with Decision EU 2015/789 Art. 3), Member States conduct annual surveys on a list of regulated plants (also known as 'specified plants') for the presence of *X. fastidiosa*.

DECISION (EU) 2015/789

- Definitions
- Article 3 Surveys of the specified organism in the territories of the Member States
- Article 3a Contingency plan
- Article 4 Establishment of the demarcated area
- Article 7 Containment measures
- Annex I List of specified plants

Guidelines for the survey of *X. fastidiosa*
(Wells et al.) in the Union territory

Definitions

a Specified organism means any subspecies of *Xylella fastidiosa (Xf)*

b host plants means plants for planting, other than seeds, belonging to the genera or species listed in the Commission database of host plants susceptible to *Xf* in the Union territory, as having been found to be susceptible in the Union territory to the specified organism or, where a Member State has demarcated an area with regard to only one or more subspecies of the specified organism pursuant to the second subparagraph of Art. 4 (1), as having been found to be susceptible to that or those subspecies; to the specified organism, or, where a Member State has demarcated an area with regard Annex I;

c specified plants means host plants and all plants for planting, other than seeds, belonging to the genera or species listed in Annex I;

d professional operator means any person involved professionally in one or more of the following activities concerning plants:
planting; breeding;
production (*including growing, multiplying, maintaining*);
introduction into, and movement within, and out of the Union territory;
making available on the market

4

Article 3
Surveys of the specified organism in the territories of the Member States

Member States shall conduct annual surveys for the presence of the specified organism in their territory on the specified plants.

Those surveys shall be carried out by the responsible official body, or under official supervision of the responsible official body.

They shall consist of visual examinations and, in the case of any suspicion of infection by the specified organism, collection of samples and testing.

Those surveys shall be based on sound scientific and technical principles, and shall be carried out at appropriate times of the year with regard to the possibility to detect the specified organism.

Those surveys shall take account of the **available scientific and technical evidence**, the biology of the **specified organism and its vectors**, the presence and **biology of specified plants**, and any other appropriate information, concerning the presence of the specified organism.

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Article 3a
Contingency plans

1. By 31 December 2016, each Member State shall establish a plan setting out the actions to be taken in its territory in accordance with Articles 4 to 6a, and Articles 9 to 13a, in case of a confirmed or suspected presence of the specified organism (hereinafter "the contingency plan"). L 333/144 EN Official Journal of the European Union 19.12.2015 (1) EFSA PLH Panel (EFSA Panel on Plant Health), 2015.

6

Article 3a
Contingency plans

2. The contingency plan shall also set out the following:
(a) the roles and responsibilities of the bodies involved in those actions and the single authority;
(b) one or more laboratories specifically approved for the testing of the specified organism;
(c) rules on the communication of those actions between the bodies involved, the single authority, the professional operators concerned and the public;
(d) protocols describing the methods of visual examinations, sampling and laboratory testing
(e) rules on training of personnel of the bodies involved in those actions;
(f) minimum resources to be made available and proceedings to make available additional resources in case of a confirmed or suspected presence of the specified organism.

3. Member States shall evaluate and review their contingency plans as necessary.
4. Member States shall communicate their contingency plans to the Commission at its request.'

7

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Article 4
Establishment of demarcated areas

1. Where the presence of the specified organism is confirmed, the Member State concerned shall without delay demarcate an area in accordance with paragraph 2, hereinafter "demarcated area".

By way of derogation from the first subparagraph, where the presence of one or more particular subspecies of the specified organism is confirmed, the Member State concerned may demarcate an area with regard to that or those subspecies only.'

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Article 4
Establishment of demarcated areas

2. The demarcated area shall consist of an infected zone and a buffer zone.
The infected zone shall include all plants known to be infected by the specified organism, all plants showing symptoms indicating possible infection by that organism, and all other plants liable to be infected by that organism due to their close proximity to infected plants, or common source of production, if known, with infected plants, or plants grown from them.

As regards the presence of the specified organism in the province of Lecce, the infected zone shall at least include that entire province.
The buffer zone shall be of a width of at least 10 km, surrounding the infected zone.

The exact delimitation of the zones shall be based on sound scientific principles, the biology of the specified organism and its vectors, the level of infection, the presence of the vectors, and the distribution of specified plants in the area concerned.

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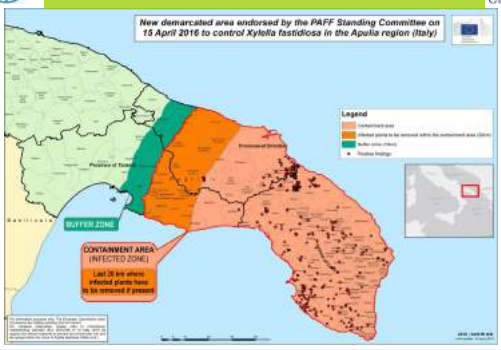
X. fastidiosa: the demarcated area in Apulia region in 2016



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Article 4
Establishment of demarcated areas

3. If the presence of the specified organism is confirmed in the buffer zone, the delimitation of the infected zone and buffer zone shall immediately be reviewed and changed accordingly.

4. On the basis of the notifications by Member States in accordance with Commission Implementing Decision 2014/917/EU(1), the Commission shall establish and update a list of the demarcated areas and communicate that list to the Member States.

5. Where based on the surveys referred to in Article 3 and on the monitoring referred to in paragraph 7 of Article 6 the specified organism is not detected in a demarcated area for a period of 5 years, this demarcation may be lifted. In such cases, the Member State concerned shall notify the Commission and other Member States.

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Article 4
Establishment of demarcated areas

6. By way of derogation from paragraph 1, the Member State may decide not to establish a demarcated area immediately where all of the following conditions are fulfilled:

(a) there is evidence that the specified organism was recently introduced into the area with the plants on which it was found;

(b) there is an indication that those plants were infected before their introduction into the area concerned;

(c) no vectors carrying the specified organism have been detected, on the basis of tests carried out in accordance with internationally validated testing methods, in the vicinity of those plants.

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Article 4
Establishment of demarcated areas

7. In the case referred to in paragraph 6, the Member State shall:
(a) carry out an annual survey for at least 2 years to determine whether any plants have been infected other than those on which the specified organism was first found to be present;

(b) on the basis of that survey, determine whether there is a need to establish a demarcated area;

(c) notify to the Commission and the other Member States the justification for not establishing a demarcated area, and the outcome of the survey referred to in point (a) as soon as they become available

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Article 7
Containment measures

3. The Member State concerned shall, within a radius of 100 m around the plants referred to in paragraph 2 and which have been found to be infected by the specified organism, sample and test the host plants, in accordance with the International Standard for Phytosanitary Measures ISPM No 31.
That testing shall be carried out at regular intervals and, at least, twice a year.

4. The Member State concerned shall apply appropriate phytosanitary treatments prior to the removal of plants referred to in paragraph 2 against the vectors of the specified organism and plants that may host those vectors. Those treatments may include, as appropriate, removal of plants.

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Article 7
Containment measures

5. The Member State concerned shall, *in situ* or in a nearby location designated for this purpose within the containment area, destroy the plants and parts of plants referred to in paragraph 2, in a manner ensuring that the specified organism is not spread.

6. The Member State concerned shall apply appropriate agricultural practices for the management of the specified organism and its vectors.

7. The member State concerned shall monitor the presence of the specified organism by annual surveys at appropriate times during the year in the area situated within the distance of 20 km as referred to in point (c) of paragraph 2

Those surveys shall be carried out in accordance with the provision of Article 6(7)

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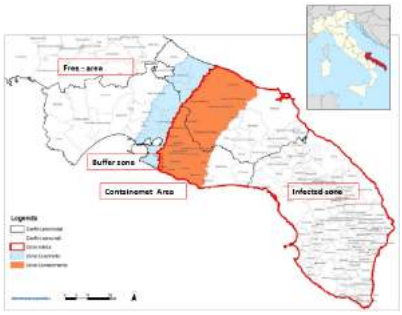
Containment measures in Apulia (Italy)



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Removal of infected plants

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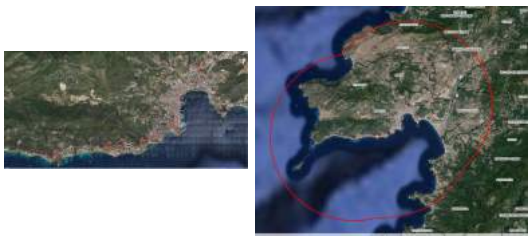
Demarcated areas in Ajaccio (Corsica)



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First outbreak in Corsica




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First outbreak in Corsica (ctd)




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First outbreak in Corsica (ctd)




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DECISION (EU) 2015/789

- Definitions
- Article 3 Surveys of the specified organism in the territories of the Member States
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Guidelines for the survey of *X. fastidiosa* (Wells et al.) in the Union territory

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Guidelines for the survey of X. fastidiosa in the Union territory

These general survey activities consist of visual examinations and, in the case of any suspicion of infection, collection of samples and testing.

They are carried out at appropriate times of the year, taking into account available scientific and technical evidence, the biology of the pest, its vectors, the presence and biology of the specified plants, as well as any appropriate information, concerning the presence of *X. fastidiosa*

For demarcated areas, where the pest is known to be present, different survey provisions apply.

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Guidelines for the survey of X. fastidiosa in the Union territory

REPORTING ON MEASURES

According to Article 14 of Decision (EU) 2015/789, Member States shall by 31 December of each year communicate to the Commission and to the other Member States a survey plan with the measures, including the scheduled time period of each measure, to be taken in the following year.

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Guidelines for the survey of X. fastidiosa in the Union territory

OBJECTIVES

- Ensure the highest possible level of early detection of outbreaks of *X. fastidiosa* in the Union territory.

- Harmonise survey activities across the Union, taking into consideration the level of Member States' specificities as regards risk factors

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Guidelines for the survey of X. fastidiosa in the Union territory

AIMS

- Identify key elements to be taken into consideration during the national assessment of the risk factors;

- Outline plant species of particular importance for the potential spread of *X. fastidiosa* in the EU;

- Achieve a common approach about the methodology used by Member States for visual surveys and laboratory detection of *X. fastidiosa*, including its subspecies and strains;

- Outline means for obtaining information about suspected cases of *X. fastidiosa* in addition to official surveys.

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IDENTIFICATION OF LOCATIONS AT MOST RISK FOR THE SURVEY

Traffic ways (in proximity of demarcated areas only)

Factors to be considered:

- Roads;
- Airports;
- Ferry Ports.

Nurseries

Factors to be considered:

- Imported plants;
- Origin of the stock materials;
- Sites where mother plants are grown;
- Ready for sale stocks;
- Open production fields;
- Protected environment production sites.

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IDENTIFICATION OF LOCATIONS AT MOST RISK FOR THE SURVEY

Human assisted spread pathways and risky points of introduction in an area

Chance of establishment due to climatic & environmental conditions (climate suitability, open air/greenhouse)

Member States shall identify the locations at most risk taking into consideration the climatic and environmental conditions, common production and growing practices in their territory, as well as the available information on the world-wide distribution of *X. fastidiosa*

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Potential Pathways

HLB control in California

Murraya and other Rutaceous plants used as greenfiller

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IDENTIFICATION OF LOCATIONS AT MOST RISK FOR THE SURVEY

HUMAN ASSISTED SPREAD PATHWAYS AND RISKY POINTS OF INTRODUCTION IN AN AREA

Existing trade patterns

Factors to be considered, in relation to the major entry pathways:

- Third Countries - the risk is different and related to the pest status of the country-widespread, pest free areas, reliability of Third Country declarations according to Article 16 of Decision 2015/789/EU4;
- EU internal trade in relation (directly or not) to demarcated areas;
- Volume of trade;
- Seasonality of trade;
- Plant species traded;
- Potential presence of vectors associated to trade.

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IDENTIFICATION OF LOCATIONS AT MOST RISK FOR THE SURVEY

HUMAN ASSISTED SPREAD PATHWAYS AND RISKY POINTS OF INTRODUCTION IN AN AREA

Sites where plants originating in risky areas are grown or kept

Factors to be considered:

- Recently established plantations, such as commercial orchards, parks and landscape developments;
- Retailers of plants for planting (nursery stock);
- Garden centres;
- Markets (based on the assessment of their trade practice);
- Plant collections (based on the assessment of the risk of plant import and plant exchange they are involved).

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IDENTIFICATION OF LOCATIONS AT MOST RISK FOR THE SURVEY

HUMAN ASSISTED SPREAD BY VECTORS

Hitch-hiking insects on different transport means (cars, lorries, ferries) may transfer the bacterium for long distances to new areas, out of the demarcated areas. Each Member State should identify the risk of this type of spread individually, particularly considering entry points with high traffic or trade.

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PRIORITISATION OF THE SPECIFIED PLANTS TO BE SURVEYED

Priority plants in relations to the different subspecies and strains of *X. fastidiosa* currently causing outbreaks and/or frequently intercepted in the EU:

In general trees, shrubs or perennial host plant species can be assumed having a higher risk for introduction and spread of *X. fastidiosa* because of:

- a higher chance of propagating vegetative asymptomatic infected plants;
- strict certification and testing systems are not applied to all species; and
- a longer life cycle increasing the chance of successful transfer by insect vectors, when plants are grown outdoors.

CoDiRO

Pierce's disease

CVC

TCP/RAB/3601 project

PRIORITISATION OF THE SPECIFIED PLANTS TO BE SURVEYED

Indicator plants:

The definition of indicator plants may be of help in maximizing the efficiency of detecting *X. fastidiosa* in the Union territory. Such plant species may be selected on the basis of the following criteria:

- Well expressed and clearly identifiable symptoms, at an early stage, under EU climatic conditions;
- Susceptible for a range of subspecies and strains.

SURVEY/SAMPLING IN A RISKY LOCATION

Plant species to be selected

Appropriate number of visual checks, samples to be taken

Appropriate period for the survey

Visual checks

a) Open fields – annual and perennial plant species should be **inspected during the vegetative season** (not during the dormancy stage), with the optimal period being from late spring to early autumn;
b) Protected environment sites – may be checked all year around,
c) Sites receiving multiple consignments may require more than one inspection annually, based on the assessment of risks

Sampling for laboratory testing

Sampling for laboratory testing should be made at the same time as visual checks.
The sampling period is in general advised to be in the vegetation period.
Samples

- **Symptomatic plants**

Asymptomatic plants

Sampling is theoretically possible during the entire vegetation period However, sampling after warm periods (e.g. late summer-early autumn) increases the probability for an accurate bacterial detection.

Grinded xylem tissue from dormant cuttings

Vectors

Member States may decide to sample and test insect vectors for the presence of *X. fastidiosa* outside of demarcated areas based on their risk assessment, and taking into account the occurrence of the potential insect vectors[14] in their territory.

They may share information about the used sampling technique with other Member States.

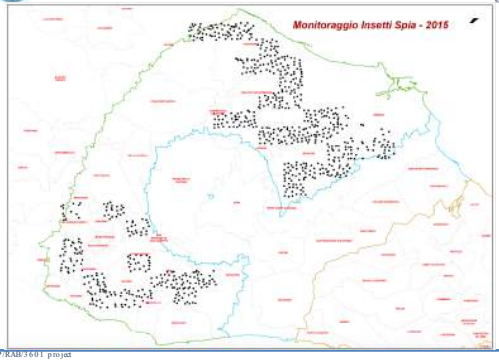
Net sweeping method is usually used for sampling adult specimen of *Philaenus spumarius*. Specimen may be collected directly from host plants or from the ground vegetation.


Neophilaenus campestris Fallen


Euscelis lineolatus Brullé

Example of vectors monitoring in Italy

More than 2000 individual insect (spy insects)



SURVEY/SAMPLING IN A RISKY LOCATION

APPROPRIATE NUMBER OF VISUAL CHECKS, SAMPLES TO BE TAKEN

It has to be noted that symptoms depend on specific host and *X. fastidiosa* strain combinations.

Generally, symptoms include **leaf scorch, defoliation, chlorosis, withering and desiccation of branches, and dwarfing.**

However, these can be confused with other biotic or abiotic causes (environmental stresses, water deficiencies, salt, air pollutants, nutritional problems, etc.).

The number of visual checks has to be defined in proportion to the existing risk identified by the Member State.

Details on the sampling strategy have to be provided on a preliminary basis in the survey plan and later confirmed in the final report.

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SURVEY/SAMPLING IN A RISKY LOCATION

APPROPRIATE PERIOD FOR THE SURVEY

CIHEAM

Repetition frequency

- The repetition frequency for risky locations should be defined based on the appropriate risk factors;
- For open fields, in pest free areas, where only asymptomatic plants have been detected during the visual check, sampling once per year in the optimal period (see chapter 4.3.2) is sufficient;
- For nurseries and sites with protected environment, the sampling frequency should relate to the number of production cycles;

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TCP/RAB/3601 projet

How to take the sample

CIHEAM

The samples should consist of:

- mature tissues (branches and leaves), collection of shoot portions in active growth should be avoided;
- mature leaves with petioles and woody twigs for perennial plant species;
- stem and the mature leaves in the case of herbaceous species, where possible.

These types of tissues could ensure the highest possible level of detection of the target pathogen. In general, it is recommended to follow the general principles of the good practice for sampling of plants and plant parts for laboratory analysis (e.g. registration of the spot and the sample, disinfection of the tools). Appropriate tools have to be provided for the sampling, e.g. in the case of large trees telescopic pruners are necessary.

TCP/RAB/3601 projet

How to preserve, transport samples

CIHEAM

Samples should be:

- shaken to ensure that no vectors are moved with the plant materials (e.g. adults vector will fly away, when the leaves, twigs are shaken). It is important to check that the sample does not contain any adult or juveniles of the vector species;
- put in closed container (e.g. plastic bag, etc.);
- kept at cool temperatures avoiding to expose samples for prolonged periods to sun or hot temperatures;
- transferred to the diagnostic laboratory as soon as possible, before the plant tissues deteriorate.

In the laboratory, samples should be stored at 4 – 10 °C, and general principles of the good laboratory practices for analysis plant samples for bacterial infection should be followed.

TCP/RAB/3601 projet

SAMPLING OF A PLANT

HOW TO TAKE THE SAMPLE

In general, it is recommended to follow the general principles of the good practice for sampling of plants and plant parts for laboratory analysis (e.g. registration of the spot and the sample, disinfection of the tools).
Appropriate tools have to be provided for the sampling

Plastic bags
Pruning shears
Telescoping tree pruner
Grafting knives
Sodium hypochlorite, ethanol 70%, etc.
Backpacks and fanny packs
Fridge and ICE bags
Markers
Labels for coding plants and samples
Sweeping net, aspirator
Containers for insects
GPS or Tablet (XylApp)

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IDENTIFICATION OF LOCATIONS AT MOST RISK FOR THE SURVEY
5. SAMPLING OF A PLANT

How to take the sample

Monitoring team

1. – Visual inspection
2. – Host plant identification
3. – Samples collection
4. – Coding and GPS data registration
5. – Sample delivery to the laboratory

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SAMPLING OF A PLANT

HOW TO TAKE THE SAMPLE

From a symptomatic plant

Take the twigs, branches or the stems with symptomatic leaves (at least 10 to 25 leaves depending on leaf size), or close to the symptoms, avoiding dead tissues or sections of the plants at an advanced stage of the symptom expression.

Guidelines related to appropriate samples from symptomatic plants for the diagnosis of *X. fastidiosa* are provided in the EPPO diagnostic protocol PM 7/24(2) *X fastidiosa*

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DIAGNOSTICS

Diagnostics should be executed in accordance with the new EPPO diagnostic protocol for *X. fastidiosa*

Thank you

CIHEAM

"Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries"

TCP/RAB/3601 project

Surveillance methodology and sampling procedures adopted in Apulia

Thaer YASEEN, Khaled Djelouah & Franco Valentini

CIHEAM- Mediterranean Agronomic Institute of Bari, Italy

CIHEAM IAM BARI

1

REGIONE PUGLIA
AREA POLITICHE PER LO SVILUPPO RURALE
SERVIZIO AGRICOLTURA
OSSERVATORIO FITOSANITARIO REGIONALE

The Apulian Phytosanitary Service (APS) has organized a strategic plan for monitoring the whole territory in order to know the distribution and incidence of the quarantine pest *Xylella fastidiosa* associated to the "Complex of Quick Decline of Olive " (CoDiRO) by coordinating the different "actors" involved in monitoring actions.

ARIF Agenzia Regionale attività Irrigue e Forestali

ASSOCODIPUGLIA Associazione Regionale Consorzi Difesa Puglia

CIHEAM IAM BARI

CRSFA

Consiglio Nazionale delle Ricerche Istituto per la Protezione Sostenibile delle Piante

CoDiRO

The monitoring of *X. fastidiosa* engages a large territory that includes not only the infected olive growing area of Salento and the surrounded area, but also all the free areas of the region with expenditure of economical and human resources

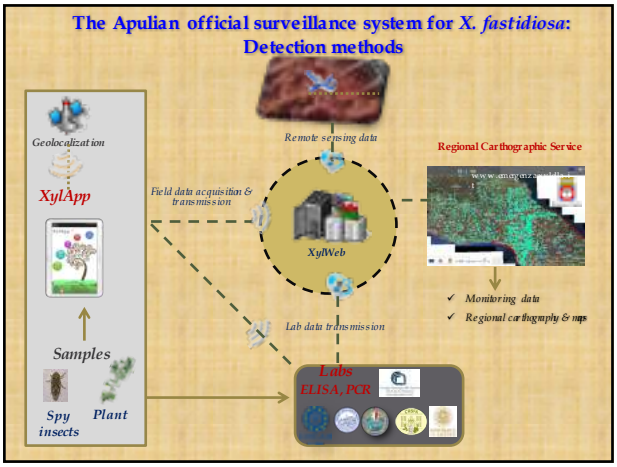
Furthermore, it is very complex because of the characteristics of the pathogen that infect many plant species and can be transmitted by polyphagous insect vectors

The RPS, in collaboration with other scientific institutions, has defined and established:

1. The sampling methods to adopt in "the infected zones", "buffer zones", "free zones" and "nurseries";
2. The list of "susceptible" plant species;
3. The sample material for testing;
4. Measures to be taken for the movement of the plant material to the laboratories;
5. The list of accredited laboratories for the analysis;
6. The rules for mapping and geo-localization of the infected foci;
7. The training courses to be organized for the personnel involved in the different phases of the monitoring activity.

Training courses organized for the personnel involved in the different levels of the monitoring activity

The Apulian official surveillance system for *X. fastidiosa*: Detection methods



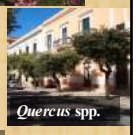
In the preliminary step, the monitoring activity has also involved the list of "susceptible" plant species

- *Palms*
- *Conifers*
- *Citrus spp.*
- *Grapevine*
- *Cactaceae spp.*
- *Ornamentals plant*
- *Other.....*

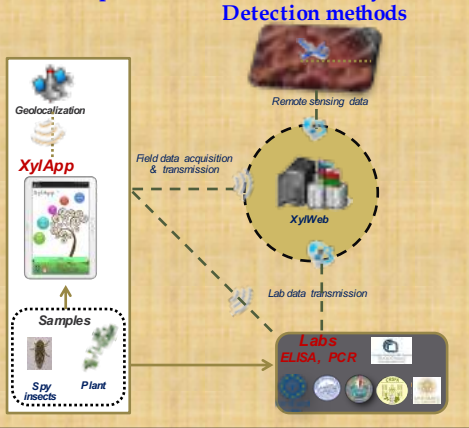

Olea europea


Nerium oleander


Amygdalus communis


Quercus spp.

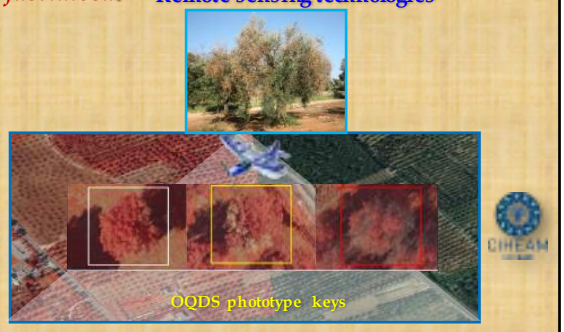
The Apulian official surveillance system for *X. fastidiosa*: Detection methods



Steps of the current monitoring program

- Photo-interpretation of satellite images of the territory
- Identification of areas to investigate
- Sampling of plants and insects
- Collection and transmission of field data using an Applet
- Laboratory testing
- Elaboration and archiving of information in a data base
- Drawing up infection maps in GIS environment
- Application of control measures

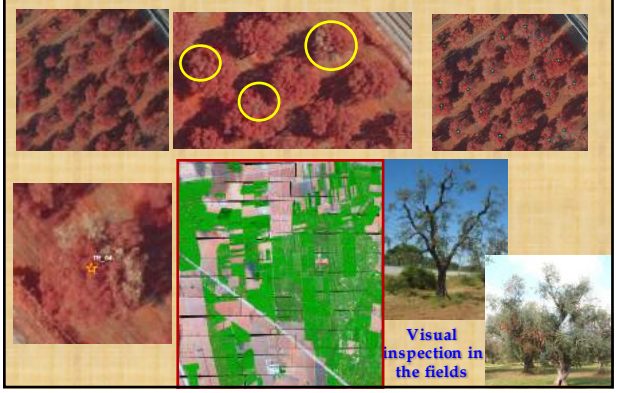
X. fastidiosa: Remote sensing technologies



Photointerpretation of very high resolution (10 cm) aerial images (visible and near-infrared region)

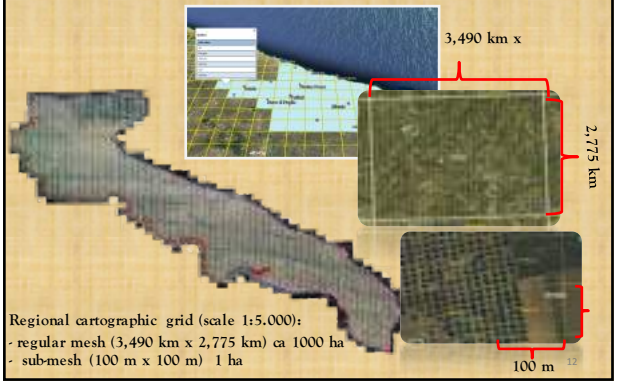
Gualano *et al.*, 2014

X. fastidiosa: The photointerpretation process of high resolution aerial images



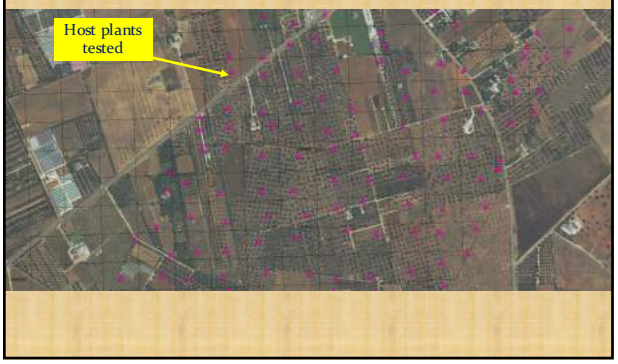
Monitoring organization in Apulia region

The APULIA regional territory has been subdivided into a grid



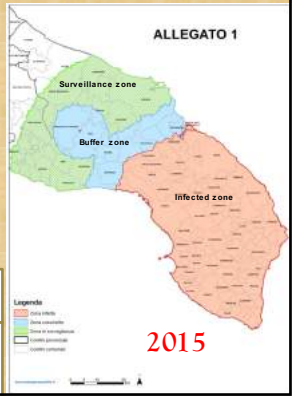
Regional cartographic grid (scale 1:5.000):
- regular mesh (3,490 km x 2,775 km) ca 1000 ha
- sub-mesh (100 m x 100 m) 1 ha

100 x 100 m in buffer zone



Identification of the sites of monitoring

- Pathogen-free area
- Surveillance zone
- Buffer zone
- Foci of Oria & Torchiarolo
- Containment zone
- Infected zone
- Containment zones around nurseries
- Undefined sites of monitoring (sensitive zones, *etc*)



NEW DEMARCATED AREA

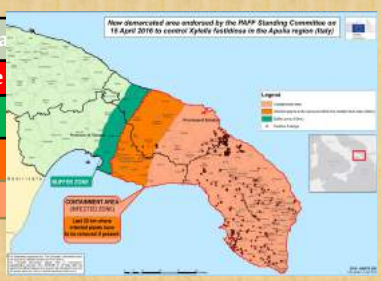
Pathogen-free area

Surveillance zone

Buffer zone

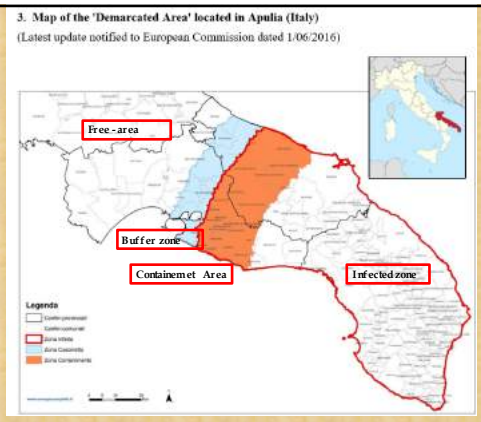
Containment Area

Infected zone



3. Map of the 'Demarcated Area' located in Apulia (Italy)

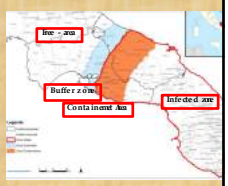
(Latest update notified to European Commission dated 1/06/2016)



List of demarcated areas established in the Union territory for the presence of *Xylella fastidiosa* as referred to in Article 4(1) of Decision (EU) 2015/789- Brussels, 23 June 2016

Free area

Sampling is conducted in a homogenous manner inside the "regular mesh", 5 sub-meshs to be inspected every 1000 ha selected on a risk basis



•host plants should be observed visually

• Many attention on olives, fruit trees and other host plants of *X. fastidiosa*

• Symptomless plants are taken randomly

•Suspected plants are sampled and geo-referenced

Samples were collected mainly from plants with "suspicious" symptoms focusing on those located in the vicinity of roads, Nursery and channels.

Containement area (20 km infected zone) and buffer zone

Sampling is conducted in a capillary manner in the all sub-*mesh (1Ha)*

All specified plants (potential hosts of *Xf* - Annex I ND) must be observed; particular attention must be paid on susceptible host plants of *CoDiRO*

During sampling host plants showing suspected symptoms must by collected for testing

In absence of symptomatic plants, sampling is made randomly

CONTAINMENT ZONE OF 20 KM

CRITICAL SITES LOCATED IN THE INFECTED AREA

In the containment zone or in the area of 200 m around the nurseries, sampling of host plants should be capillary

Presence of infected plants
all host plants present in a radius of 100 m around the infected plant should be observed visually and vegetative material from symptomatic and asymptomatic plants around those with symptoms are **collected**.

Foci zone

In foci site out of Lecce province, after the eradication of the infected plants, all host plants present in a radius of 100 m should be sampled.

Preparative activities

Equipments

- Plastic bags of different size
- Pruning shears
- Telescoping tree pruner
- Grafting knives
- Sodium hypochlorite and ethanol 70%, *etc.*
- Backpacks and fanny packs
- Fridge and siberine bags
- Office materials
- labels to codify the plants and the samples
- Net, aspirator and container for insects
- GPS or Tablet

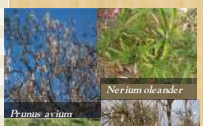
The team in the field

Monitoring teams are composed of 2 or 3 units:

1. – Visual inspection
2. – Choise of plants
3. – Collection of samples
4. – Coding and georeferencing of samples
5. – Sample delivery to the laboratory

Host species of X*f*CoDiRO object of monitoring

N	Scientific name	Family
1	*Acacia saligna* (Labill.) Wend.Sin. *Acacia cyanophylla* Lindl.	*Fabaceae*
2	*Asparagus acutifolius* L.	*Asparagaceae*
3	*Catharanthus roseus* (L.) G. Don.	*Apocynaceae*
4	*Cirsus creticus* subsp. *Eriacphalus* (Vis.)Greuter & Burdet sin. *Cirsus incanus*	*Cistaceae*
5	*Dalechampia roseae* Jacq	*Sapindaceae*
6	*Euphorbia terracina* L. (asintomatica)	*Euphorbiaceae*
7	*Grevillea Juniperina* R.Br.	*Proteaceae*
8	*Laurus nobili* L.	*Lauraceae*
9	*Lavandula angustifolia* Mill.	*Lamiaceae*
10	*Myoporum insulare* R.Br.	*Scrophulariaceae*
11	*Myrtus communis* L.	*Myrtaceae*
12	*Nerium oleander* L.	*Apocynaceae*
13	**Olea europea L.**	*Oleaceae*
14	*Polygala myrtifolia* L.	*Polygalaceae*
15	*Prunus avium* (L.) L.	*Rosaceae*
16	*Prunus dulcis* (Mill) D.A.Webb	*Rosaceae*
17	*Rhamnus alaternus* L.	*Rhamnaceae*
18	*Rosmarinus officinalis* L.	*Lamiaceae*
19	*Spartium junceum* L.	*Fabaceae*
20	*Vinca minor*	*Apocynaceae*
21	*Westringia fruticosa* (willd.) Druce	*Lamiaceae*
22	*Westringia glabra* R. BR.	*Lamiaceae*



http://ec.europa.eu/food/plant/docs/ph_biosec_legis_emergency_db-host- plants_update 05.pdf

..... and

N.	Scientific name
1	*Phillyrea latifolia* L.
2	*Lavandula stoechas* L.
3	*Eremophila maculata* F. Muell.
4	*Pelargonium spp.*

Three new plant species found infected by Xylella fastidiosa in Apulia 20.06.2016

July 2016

http://www.ponteproject.eu

Host plants found to be susceptible to *Xylella fastidiosa* subsp. *Pauca* n. 26

COMMISSION DATABASE OF HOST PLANTS FOUND TO BE SUSCEPTIBLE TO *XYLELLA FASTIDIOSA* IN THE UNION TERRITORY – UPDATE 6 – 15/07/2016

Host plant found to be susceptible to *Xylella fastidiosa* subsp. *fastidiosa*
Nerium oleander L.

Host plants found to be susceptible to *Xylella fastidiosa* subsp. *multiplex* -

Acer pseudoplatanus L., *Artemisia arborescens* L., *Asparagus acutifolius* L., *Calicotome villosa* (Poiret), Link , *Cistus monspeliensis* L., *Cistus salvifolius* L., *Coronilla valentina* L., *Cytisus scoparius* (L.) Link, *Genista x spachiana* (syn. *Cytisus racemosus* Broom), *Genista corsica* (Loisel.) DC., *Genista ephedroides* DC., *Hebe*, *Helichrysum italicum* (Roth) G. Don, *Lavandula angustifolia* Mill., *Lavandula dentata* L. , *Lavandula stoechas* L., *Lavandula x allardii* (syn. *Lavandula x heterophylla*), *Metrosideros excelsa* Sol. ex Gaertn., *Myrtus communis* L., *Pelargonium graveolens* L'Hér, *Phagnalon saxatile* (L.) Cass., *Polygala myrtifolia* L., *Prunus cerasifera* Ehrh., *Quercus suber* L., *Rosa x floribunda*, *Rosmarinus officinalis* L., *Spartium junceum* L.

Host plants found to be susceptible to several subspecies of *Xylella fastidiosa*
Coffea

Visual inspection

Symptoms associated to CoDiRO (quick decline and leaf scorch of olive)

ALS - Almond Leaf Scorch

OLS – Oleander Leaf Scorch and scald

Visual inspection

Prunus avium

Spartium junceum

Westringia fruticosa

Polygala myrtifolia

Acacia saligna

XylApp

Compiling of a description sheet for each sample using a field applet named XylApp

Santoro *et al.*, 2014

What is XylApp

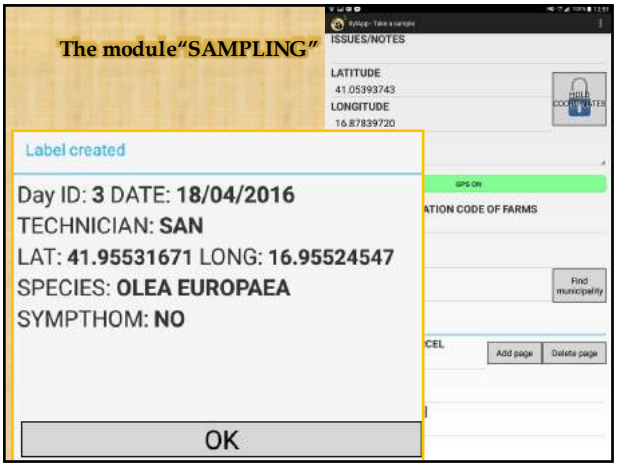
It is an application (App) for "***android systems***", designed and developed by the Mediterranean Agronomic Institute of Bari, with the aim to *facilitate*, *optimize* and *rationalise* the *collection*, the *geolocalisation* and the *storage* of the data (population, agronomic, territorial, photography, etc.) related to plant tissue material and/or insect samples (vectors or spy insects) collected in the field during the monitoring of *Xylella fastidiosa*.

The daily data of the collected samples acquired by ***XylApp*** are *transmitted* in real time to the central Database (***XylWeb***).

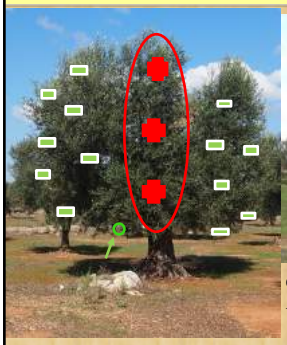
XylApp modules

The application consists of five independent modules:

1) Sampling
2) Explore & Sampling
3) Find
4) Archive
5) Vademecum



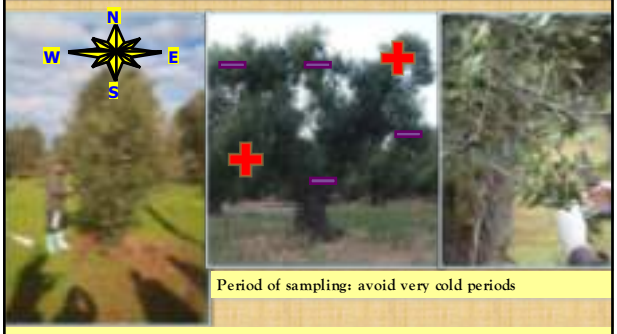
Samples collection :
collecting samples near symptomatic parts



Olive sampling:
A) Plants with symptoms clearly established: Sampling from parts of the canopy showing symptoms
B) Plants with slight symptoms or asymptomatic: sampling from mid to high parts of the canopy, taking at least 8 twigs along all the parts of the plant

Symptomatic trees:
collecting samples near symptomatic parts excluding necrotic tissues

Samples collection: Symptomless trees



Period of sampling: avoid very cold periods

Plants with slight symptoms or asymptomatic : collecting samples from the four cardinal points in particular from mid to high parts of the canopy

Regardless of the sampling season, avoid new growth and suckers

Sampling from deciduous trees, shrubs or herbaceous species

Trees: twig samples from 4 cardinal points or as alternative 10-12 mature leaves with small parts of lignified twigs during summer / autumn.

Shrubs: mature leaves with small lignified twigs.

Herbaceous and annual plant species: portion of the stem with basal leaves; when it is possible all the plant is taken with eventually main root/s

Plant samples collection

Each single sample is composed by at least 8 twigs per tree of about 15-20 cm or alternatively 10-12 mature leaves with small lignified twigs

No collected young sucker, flesh or leaves

Each sample is "enveloped and closed" in a plastic bag paying attention that insects are not present

SAN 04122013 01 N40°12'34" E18°12'34" A01

Samples are coded and geo- referenced

Type of sample

The cutting should be beaten in order to eliminate insects and sealed in well closed plastic bags

Official monitoring of *Xf* using the 'Spy insects' approach

The monitoring of *Xf* is going to be carried out in the pathogen-free areas, in the surveillance zone and in the buffer zone (Annex I, Atto Dirig. Regione Puglia, 195/2015)

In 2015 the SFR has indicated the **CIHEAM-MAIB** as laboratory for sampling about 2000 samples of 'spy insects' in the surveillance zone and for detecting the pathogen using the real time LAMP.

- This activity is going to be carried out from June to January (depending on the area)
- Sampling will be preferably carried out along the borders of the groves of host plant species (primarily olive groves)
- Sampling will be conducted during the day hours when insects are active

"Insect spy" in *Xf*-free area could be very effective for the early *Xf*-detection and the prevention of its spread

Monitoring phases

Insects captures & storage

- ✓Insects captures using the sweeping net
- ✓Captures the insects in a radius of 10/20 m from the georeferenced point
- ✓Aspiration of about 2-3-4(..) selected spy insects/site in glass tubes
- ✓Identification of the spy insects based on general features (*size, shape, color, movement mode*)
- ✓Addition of ethanol
- ✓At the end of the day all glass tubes, containing the dead insects, are stored in a container (coded with the date and sampling team)
- ✓Each glass tube is coded/1 capturing site (data generated by *XylApp*).

Insects captures & storage

Sampling team : 2 persons

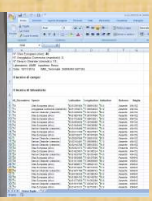
Safe movement of samples

Transportation must be carried out in closed containers or cooled bags

Measures to be taken to prevent pathogen spread

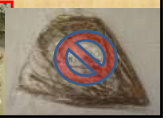
- cleaning of vehicles, tools and clothing used for sampling
- closing windows of the vehicle in order to limit the risk of vectors spread

Laboratory



The sample (plant materials or insects) delivered to the accredited laboratory should be intact, not dry, accompanied by the **DELIVERY REPORT** and file generated by *XylApp*

Non suitable samples are refused



Procedures of phytosanitary analyses for Xf in plant material

Sample arival and storage

- Sample general **controls** (i.e. number, label, bag status, status of plant material)
- **Anomalies** are described in the acceptance report
- Sample **storage** in Xyl-chamber at 4 ±2 C° for at least 12h

Sample coding and storage for analyses

- **Data control** of labels, list of samples generated by XylApp and/or enclosed with the delivery report; mistakes/anomalies are reported to the SFR
- **Opening** of the sample bags to exclude the presence of insect
- Sample **storage** in Xyl-chamber

43

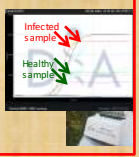
Immediate delivery to CNR of SUSPECTED & POSITIVE samples (and relative documentation) for confirmation tests

Delivery report which is signed by IAMB and CNR

PCR or RT-PCR

At the end of the analyses all material (tubes, bags etc.) are sterilized in autoclave at 121°C, 1 atm, per 15 min.

RT- LAMP



Identification of the 'spy insects' and analyses

Delivery of all results to SFR

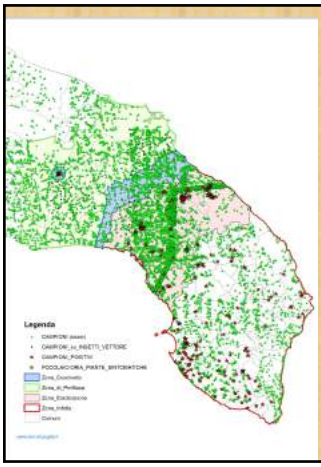
The Apulian official surveillance system for *X. fastidiosa*

XylWeb is a webserver designed and developed by CIHEAM of Bari for the collection, storage and management of the monitoring data flow for Xf.

- Filtered
- Analyzed
- Processed
- Exported

This software represents the core of this innovative surveillance system for *Xf* in which all data converge.

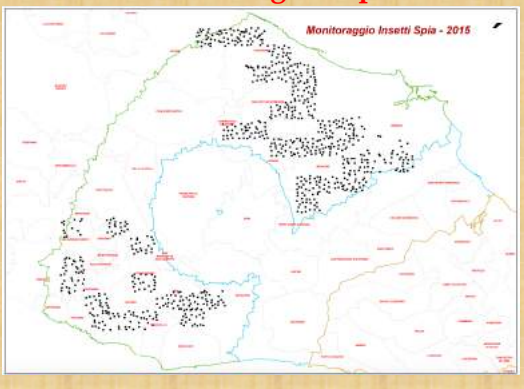
XylWeb allows data traceability and real time analyses for producing reports and other elaborates. Its implementation with the regional cartography provides a clear map on the distribution of the samples, infected plants etc..



Monitoring 2013-2015

www.emergenzaxylella.it

vectors monitoring in Apulia



More than 2000 individual insect (spy insects)

Survey activities 2016

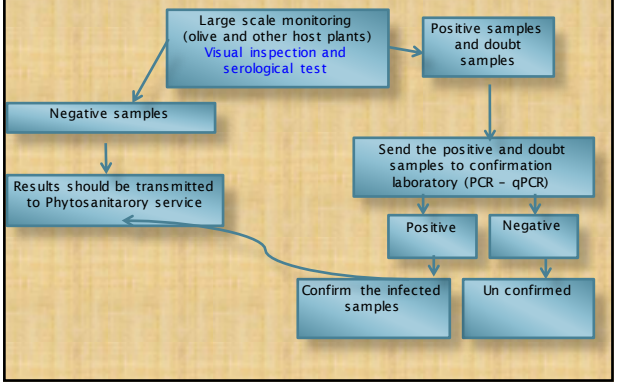
Staff resources

Monitoring activities initially carried out by regional inspectors and Consortium technicians

New resources: selection for about 200 regional technicians (ARIF)

Training course (symptom recognition, sampling, geo-referencing of plants, labeling and storage of samples)

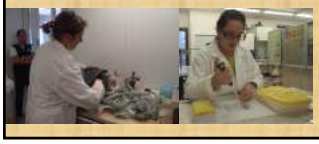
General schema for survey activity in Puglia



Survey activities 2016

Laboratories

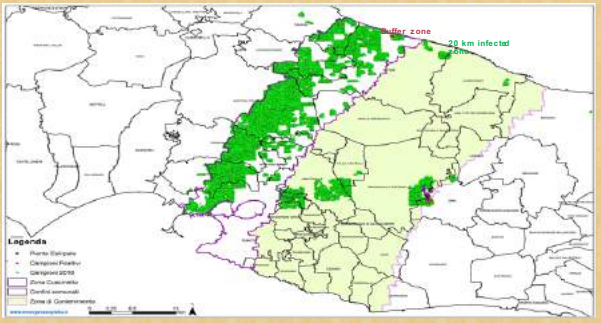
1. CNR - Institute for Sustainable Plant Protection, Bari
2. CRSFA 'Basile Caramia' - Center for Research, Experimentation and Training in Agriculture, Locorotondo
3. IAMB Mediterranean Agronomic Institute, Bari
4. University of Foggia (DiSAAA), Foggia
5. University of Salento (DiSTeBA), Lecce



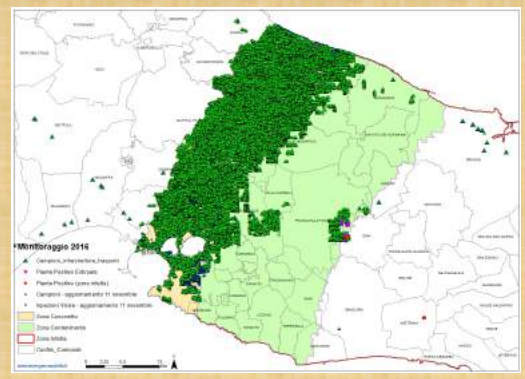
JULY – DEC 2016

	sub mesh to be inspected	Minimum number of samples
Buffer zone (10 km)	20.786	20.786
Infected area (20 km)	58.566	58.566
Free area	6.878	6.878
TOTAL	**86.230**	**86230**

Survey results updated to 14 October 2016



Survey results updated to 11 November 2016



11 nov2016

Monitoring 2016 – updated to 11 November	Total number 100x100 mesh inspected	Number of samples	Positive samples	Eradicated plants	Eradicated plants in100 m
Buffer zone	45.706	41.520	1	1	15
Containment zone	12.333	12.816	18	18	
Free zone (1000 h mesh)	32	77	0		
Infected zone (excluding the containment area)		138	70		
TOTAL	**58.071 (*)**	**54.551**	**89**	**19**	**15**

(**) di cui 6.209 maglie ispezionate visivamente senza prelievo di campione

Buffer zone

SPECIE	N° Campioni
Acacia Saligna	105
Amygdalus Communis (mandorlo)	482
Arbutus Unedo L. (corbezzolo)	2
asparagus	1.352
Catharanthus Roseus (vinca rosea)	1
Cistus	320
Cynodon Dactylon (gramigna)	103
Dodonaea Viscosa Purpurea	2
eucalipto	1
Laurus Nobilis (alloro)	207
Lavanda	6
macchia mediterranea	1
Malva sylvestris L. (malva)	81
Myoporum Insulare	1
Myrtus Communis (mirto)	608
Nerium Oleander (oleandro)	975
Olea Europaea (olivo)	35.477
Pelargonium Fragrans (geranio)	25
Phillyrea (fillirea)	746
Pistacia Lentiscus (lentisco)	10
Polygala Myrtifolia	40
Prunus Armeniaca (albicocco)	2
Prunus Avium	231
Prunus Persica (pesco)	1
Pyrus Pyraster L. (perastro)	3
Quercus spp	18
Rhamnus Alaternus (alaterno)	178
Rosmarinus Officinalis (rosmarino)	458
Spartium Junceum (ginestra)	76
tamerici	3
Vinca Minor (pervinca minore)	3
Westringia Fruticosa	2
TOTALE	41.520

Containment Area

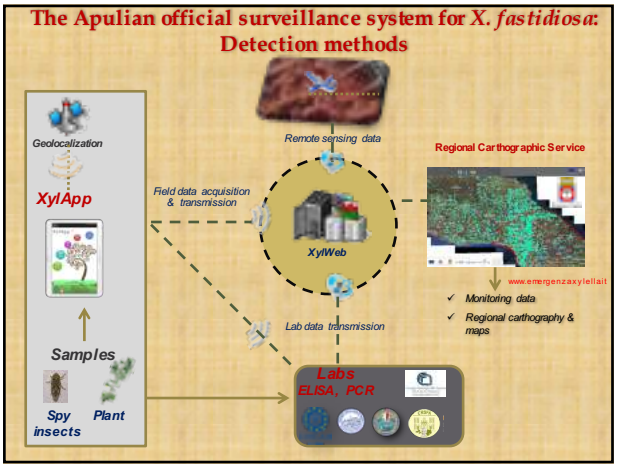
SPECIE	N° Campioni
Acacia Saligna	8
Amygdalus Communis (mandorlo)	148
asparagus	272
Catharanthus Roseus (vinca rosea)	12
Cistus (Cisto)	13
Cynodon Dactylon (gramigna)	40
Grevillea Juniperina	1
Laurus Nobilis (alloro)	30
Lavandula Stoechas L.	4
Malva sylvestris L. (malva)	7
Myrtus Communis (mirto)	58
Nerium Oleander (oleandro)	206
Olea Europaea (olivo)	11.853
Pelargonium Fragrans (geranio)	11
Phillyrea (fillirea)	8
Polygala Myrtifolia	10
Prunus Avium	86
Prunus Persica (pesco)	1
Pyrus Pyraster L. (perastro)	1
Quercus spp (querce di ogni specie)	9
Rhamnus Alaternus (alaterno)	10
Rosmarinus Officinalis (rosmarino)	55
Spartium Junceum (ginestra)	14
TOTALE	12.816

Infected zone

SPECIE	N° Campioni
Eremophila Maculata F. Muell.	1
Laurus Nobilis (alloro)	1
Lavandula Stoechas L.	1
Myrtus Communis (mirto)	1
Nerium Oleander (oleandro)	18
Olea Europaea (olivo)	116
Phillyrea Latifolia L.	1
Polygala Myrtifolia	1
TOTALE	138

Free zone

SPECIE	N° Campioni
Cynodon Dactylon (gramigna)	4
Malva sylvestris L. (malva)	1
Nerium Oleander (oleandro)	8
Olea Europaea (olivo)	62
Quercus Ilex (leccio)	2
TOTALE	77



Thank you for your attention

"Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries"

TCP/RAB/3601 project

Innovative surveillance system for Xylella fastidiosa in Apulia

PhD Franco Valentini, Thaer Yaseen, Khaled Djelouah

CIHEAM- Mediterranean Agronomic Institute of Bari, Italy

CIHEAM IAM BARI

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

Remote sensing:
Photointerpretation of aerial images

The Spy insects approach

On site early detection:
real time LAMP & DTBIA

Softwares:
XylApp & XylWeb

2

Xylella fastidiosa & the Olive Quick Decline Syndrome (OQDS) in the Southern part of Apulia region, Italy

Apulia region

the largest olive producer region

Acacia saligna (Labill.) Wendl.
Asparagus acutifolius L.
Calluranthus
Cistus creticus L.
Dodonaea viscosa Jacq.
Eremophila maculata F. Muell.
Euphorbia terracina L.
Grevillea juniperina L.
Laurus nobilis L.
Lavandula angustifolia Mill.
Myoporum insulare R.Br.
Myrtus communis L.
Nerium oleander L.
***Olea europea* L.**
Phillyrea latifolia L.
Polygala myrtifolia L.
Prunus avium (L.) L.
Prunus dulcis (Mill) D.A. Webb
Rhamnus alaternus L.
Rosmarinus officinalis L.
Spartium junceum L.
Vinca
Westringia fruticosa (Willd.) Druce
Westringia glabra L.
.....26

The list of host plants of *X. fastidiosa* subspecies *pauca* strain CoDiRO in Apulia region

X. fastidiosa: vector & potential vectors

Philaenus spumarius is the only assessed vector of *Xf* in Apulia. It is a polyphagous insect, showing an high density population in the *Xf*-infected area of Apulia (Saponari *et al.*, 2014)

Potential vectors of *X. fastidiosa* in Apulia (Elbeaino et al., 2014)

Neophilaenus campestris Fallen

Euscelis lineolatus Brullé

X. fastidiosa the surveillance programme in Apulia

A difficult challenge

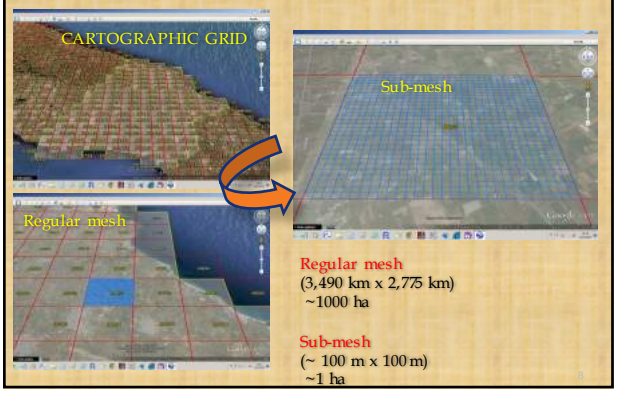
- Numerous host species worldwide (about 359 spp)
- More than 22 species as hosts of the *Xf pauca* strain CoDiRO (ST53)
- Many cultivated and wild species
- *P. spumarius* a poliphagous vector

6

The surveillance programme of *Xf* in Apulia

The regional cartographic grid a tool for planning the surveillance of *X. fastidiosa* in the Apulian territory

A tool for distributing the samples in a rational and representative way

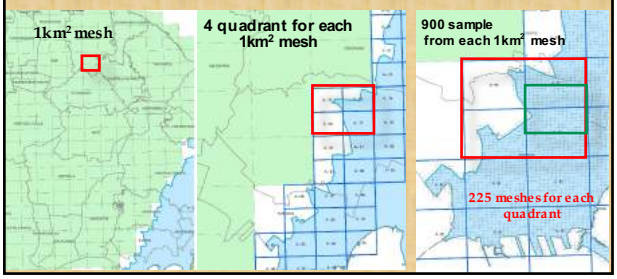


Regular mesh
(3,490 km x 2,775 km)
~1000 ha

Sub-mesh
(~ 100 m x 100 m)
~1 ha

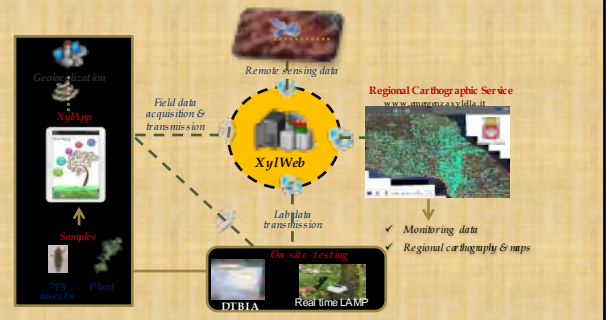
Sample collection in Apulia region

- Buffer zone and contaminated area were divided to 1km² mesh
- Each mesh was divided into 4 quadrant
- Each quadrant was further divided to small meshes 1 h (100mX100m) area (a total of 225 meshes for each quadrant) 225 X 4quadrant=900 small mesh for each 1km² .
- Each small mesh will be monitored visually and 1 sample will be collected.
- A total of **900 sample of each 1km²** mesh



Each small mesh will be monitored visually and 1 sample will be collected
A total of 225 sample for each

The innovative system for early surveillance of Xf designed & developed by CIHEAM-IAMB D'Onghia et al, 2015

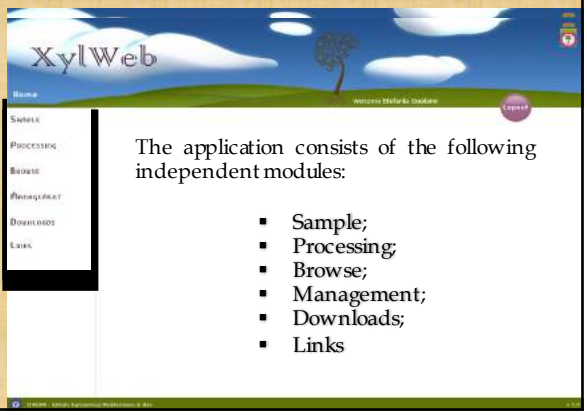


It's a software web-based for the collection, storage and management of surveillance data for *X. fastidiosa*.

This software represents the core of the surveillance system in which all data converge.

XylWeb allows data traceability and real time analyses for producing reports and other elaborates. Its implementation with the regional cartography provides a clear map on the distribution of the samples, infected plants etc..

What is XylWeb

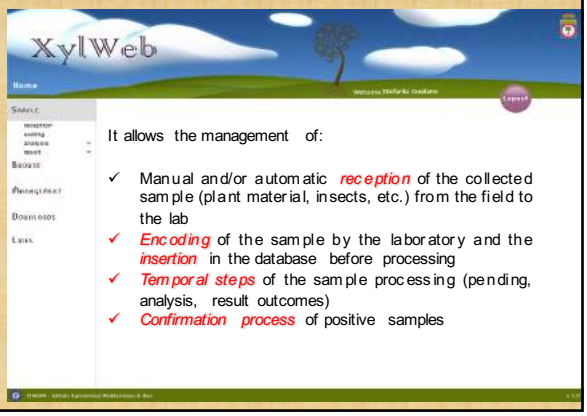


The application consists of the following independent modules:

- Sample;
- Processing;
- Browse;
- Management;
- Downloads;
- Links

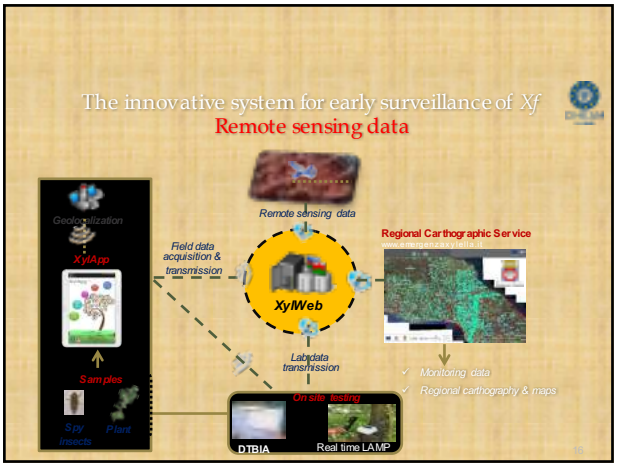
The module "Sampling"

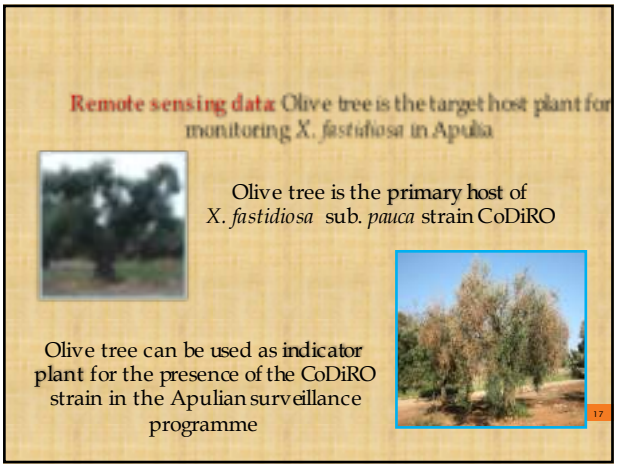
The module "Sampling"

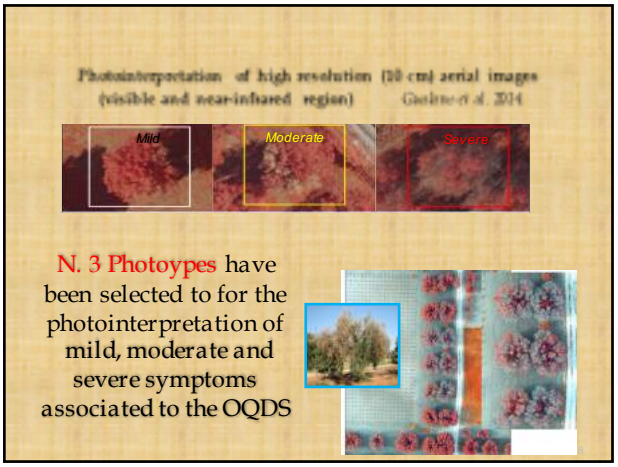


It allows the management of:

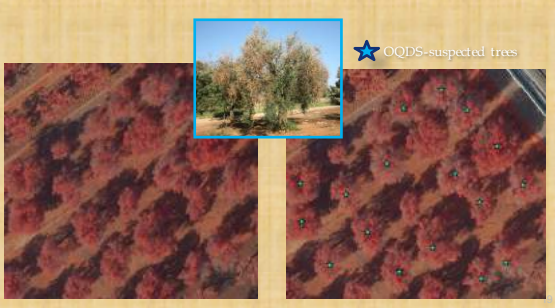
- ✓ Manual and/or automatic *reception* of the collected sample (plant material, insects, etc.) from the field to the lab
- ✓ *Encoding* of the sample by the laboratory and the *insertion* in the database before processing
- ✓ *Temporal steps* of the sample processing (pending, analysis, result outcomes)
- ✓ *Confirmation process* of positive samples


The innovative system for early surveillance of Xf
Remote sensing data
Remote sensing data
Geolocalization
XylApp
Samples
Spy insects
Plant
Field data acquisition & transmission
XylWeb
Regional Carthographic Service
Labdata transmission
On site testing
DTBIA
Real time LAMP
Monitoring data
Regional carthography & maps


Remote sensing data: Olive tree is the target host plant for monitoring X. fastidiosa in Apulia
Olive tree is the primary host of X. fastidiosa sub. pauca strain CoDiRO
Olive tree can be used as indicator plant for the presence of the CoDiRO strain in the Apulian surveillance programme
17


Photointerpretation of high resolution (10 cm) aerial images (visible and near-infrared region)
Mild
Moderate
Severe
N. 3 Photoypes have been selected to for the photointerpretation of mild, moderate and severe symptoms associated to the OQDS

Manual photointerpretation of the OQDS-suspected trees on high resolution aerial images



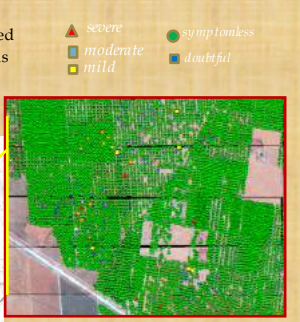
Analysis of a large area requires the creation of an image mosaic, which is composed of several aerial photographs

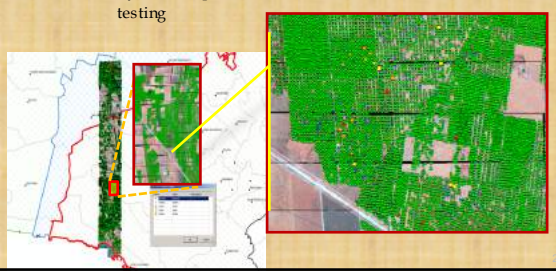
Mosaicking of swath images sequences acquired from aircraft is a critical task.

Geometric distortions and radiometric differences, which interfere with the mosaicking process, are corrected and the position of each image in the mosaic improved by the aircraft company

Results of photointerpretation in GIS environment

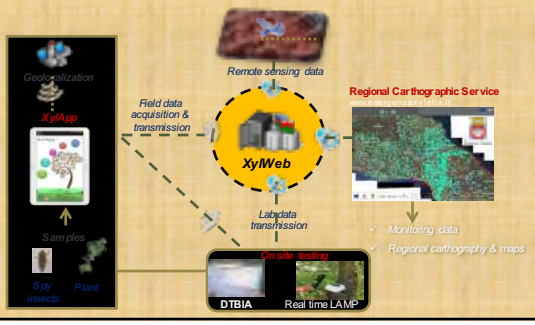
Photointerpreted trees are inspected for visual observations. If OQDS is confirmed, they are sampled for testing





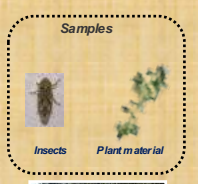
Visualization in XylApp of the suspected OQDS-trees by photointerpretation which will be inspected

The innovative system for early surveillance of Xf
Field data acquisition : the spy insects

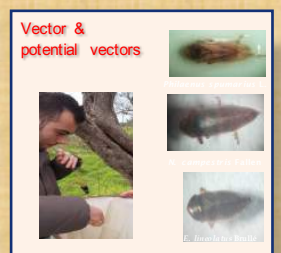


FIELD SAMPLING FOR ASSESSING THE PRESENCE OF X. FASTIDIOSA

Plant material and insects (vector or potential vectors)



Vector & potential vectors



The "Spy insect" approach for monitoring *X. fastidiosa* in symptomless olive orchards in Southern Italy

Spy insects harbour *X. fastidiosa* and may indicate its presence before symptoms development in host plants.

The monitoring of the 'Spy insects' can increase the probability to early detect the presence of *Xylella* in areas which are apparently pathogen-free because of the absence of symptoms

The assessed Spy insects in Southern Apulia

Philaenus spumarius L.

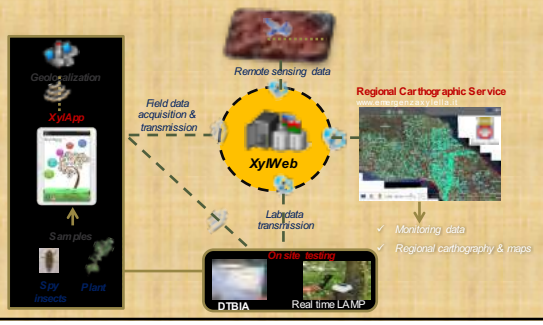
N. campestris Fallen

E. limbatus Stalls

25

The innovative system for early surveillance of Xf

Field data acquisition: XylApp



What is XylApp?

It is an application (App) for "*android systems*", designed and developed by CIHEAM-IAMB, with the aim to *facilitate, optimize and rationalise* data *collection*, the *geoloadisation* and the *storage* related to plant tissue material and/or insect samples (vectors or spy insects) collected in the field during the monitoring of *Xylella fastidiosa*.

The daily data of the collected samples acquired by *XylApp* are *transmitted* in real time to the central Database (*XylWeb*).

XylApp modules

The application consists of five independent modules for data acquisition by inspectors

1) Sampling

2) Explore & Sampling

3) Find

4) Archive

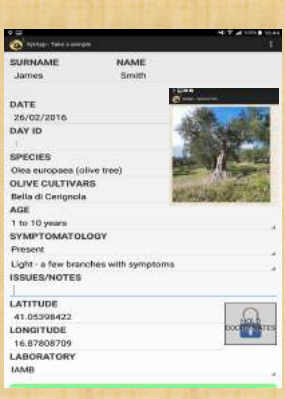
5) Vademecum

XylApp
The **module"SAMPLING"**

Sampling

It allows to acquire data and geo-locate the samples collected in the field, *WITHOUT* the need to "look for the sample in the territory" (eg. It is known the location of the field in which the olives trees are sampled).

Finally, click "Save" to confirm the insertion of the individual sample data



XylApp
The module "ARCHIVE"

Archive

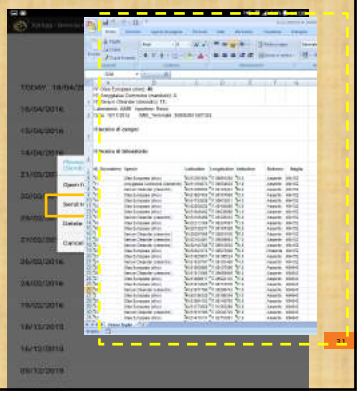
It allows you to *store* (in a renamed folder with monitoring data) and *send* to the central *DataBase* (XylWeb).
It can *generate* a report to excel, *edit* and/or *delete* individual samples, etc.

XylApp
The module "ARCHIVE"

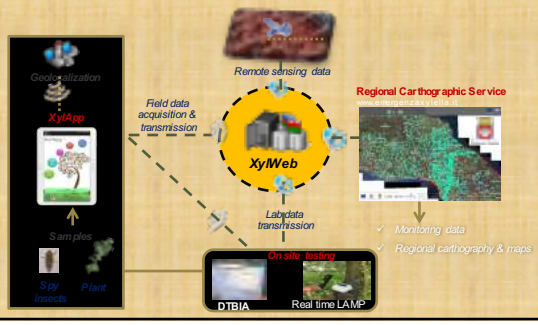
Archive

Delivery to laboratories of **the list of the** collected samples as excel file

Laboratories can plan the analyses in advance based on this list



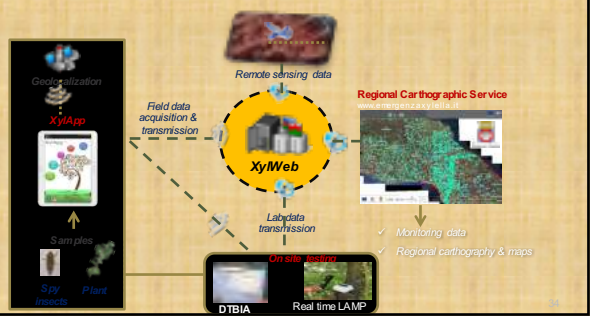
The innovative system for early surveillance of Xf
Laboratory data: DTBIA & real time LAMP

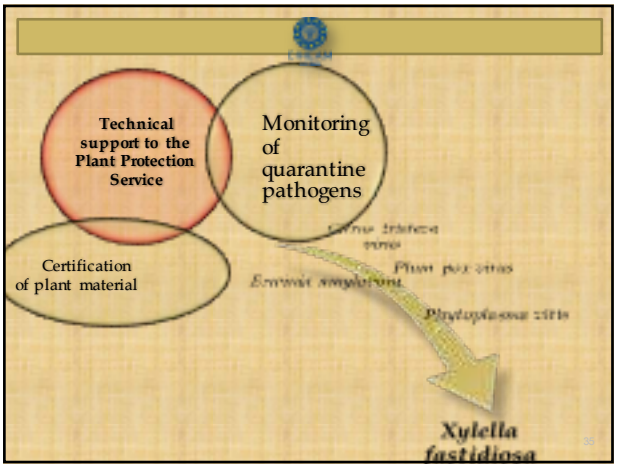


On site detection methods for *X. fastidiosa* to avoid the movement of infected plant material and insect vectors

The innovative system for early surveillance of Xf

From XylWeb data are mirrored for visualization to the Regional Carthographic website





X. fastidiosa: Legislation

COMMISSION IMPLEMENTING DECISION (EU) 2015/2417 amending the

COMMISSION IMPLEMENTING DECISION (EU) 2015/789

as regards measures to prevent the introduction into and the spread within the Union of *X. fastidiosa*

COUNCIL DIRECTIVE 2000/29/EC and implementations as regards on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community.

National and Regional (Apulia)

Ministerial Decree – MD 26/02/2016. Definition of the areas free from the harmful organism *X. fastidiosa* (Wells et al.) within the teritory of the Italian Republic.

MD 19/06/2015. Emergency measures to prevent, control and eradicate *X. fastidiosa* within the territory of the Italian Republic

Ministerial Council 10/02/2015. Declaration of the Emergency status for the spread of the bacterium *X. fastidiosa* in Apulia

DAG 289 12/08/2016

IPPC/FAO	EPPO PM7/24
International Standards for Phytosanitary Measures	Diagnostic Protocol for *X. fastidiosa*

Thank you for your attention

Laboratory diagnostic methods for *Xylella fastidiosa* (EPPO standards)

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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/24 (2)

Diagnostics
Diagnostic

PM 7/24 (2) *Xylella fastidiosa*

Specific scope

This Standard describes a diagnostic protocol for *Xylella fastidiosa*.[1]

It should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols*.

Specific approval and amendment

First approved in 2004-09.
Revised in 2016-09.[2]

Introduction

EPPO code: XYLEFA

Phytosanitary categorization:
EPPO A1 list no. 166

EU Annex designation:
II/A1 as *Xylella fastidiosa*
II/A1 as citrus variegated chlorosis
IV/A1 as peach phony rickettsia

Introduction

TCP/RAB/3601 project

Symptoms

Source: Agdes, APS, Hopkins, Purcell, Boscia, Landa, Navas-Cortés, de la Fuente, etc.

TCP/RAB/3601 project

Symptoms

- Symptoms depend on the particular combination of host and *Xylella fastidiosa* strain.
- Symptoms include: leaf scorching, wilting of the foliage, defoliation, chlorosis or bronzing along the leaf margin and dwarfing, yellow spots on leaves, chlorotic foliage (pronounced yellow discoloration between healthy and necrotic tissues), irregular lignification of bark, stunting, premature leaf drop, reduction of production and dimension of fruits, fruit distortion, crown dieback .
- Symptoms usually appear on just a few branches but later spread to cover the entire plant.
- Bacterial infections can be so severe as to lead to the death of the infected plant.
- Infections are frequently asymptomatic in several hosts. Plants can remain asymptomatic for long periods.

TCP/RAB/3601 project

Symptoms

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- Symptoms can be confused with those caused by other biotic or abiotic factors (other pathogens, environmental stresses, water deficiencies, salt, air pollutants, nutritional problems, sunburn, etc.)
- Illustrations of possible confusions can be seen at: http://agriculture.gouv.fr/sites/minagri/files/xylella_fastidiosa_symptomes_et_risques_de_confusions_biotiques_et_abiotiques_dgal-1.pdf

Olmus

TCP/RAB/3601 projet

Source: https://gd.eppo.int/taxon/XYLEFA/photos

Symptoms

Olive Quick Decline Symdrome

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TCP/RAB/3601 projet

Symptoms

Olive Quick Decline Symdrome

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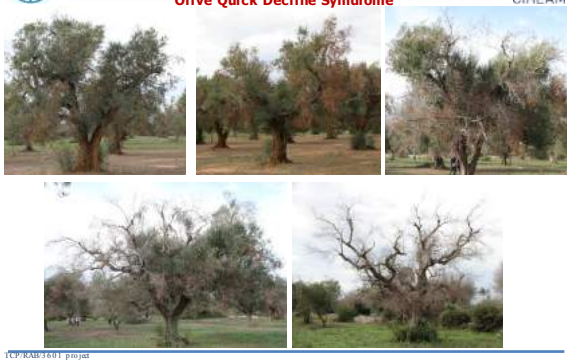

TCP/RAB/3601 projet

Symptoms

Citrus Variegated Chlorosis

TCP/RAB/3601 projet

Symptoms

Pierce Disease

Fotos cortesía de A.H. Purcell and C. Clark

TCP/RAB/3601 projet

Inspection of consignments

General elements:

- Useful information referring to phytosanitary inspections to be carried out for imported consignments
  - *EPPO Standard PM 3/72* (2) Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification (EPPO, 2009).
  - ISPM no. 23 *Guidelines for Inspection* (IPPC, 2005). http://www.acfs.go.th/sps/downloads/ISPM_31.pdf
- The most relevant **pathway for introduction** of *X. fastidiosa* is the importation of **plants for planting** and infectious insects (**vectors**) originating from areas where the pest is present.
- Plants for planting present a high risk of pest introduction as:
  - *Xf* can survive, and multiply, on the introduced plant
  - *Xf* might then transfer to a suitable host (outdoors plants)

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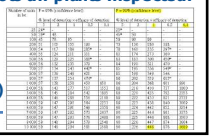
Inspection of consignments

- When plants are imported or moved in active growth, with leaves, an adequate proportion of plants of a consignment should be subjected to a systematic examination in order to detect the presence or signs of pests in a lot.
- The size of the unit of inspection in a specified lot size is indicated in Tables 1, 3 and 4 of ISPM no. 31 Methodologies for sampling of consignments (IPPC, 2009).
- For *X. fastidiosa*, the level of confidence should allow reliable detection of the lowest possible level of infestation.
- All lots which include symptomatic plants should be sampled for testing, with the sample including a representative range of symptoms.

Inspection of consignments

- For consignments originating from countries where *X. fastidiosa* is not known to occur:
  - 448 plants should be inspected from a lot of 10 000 which will provide 99% confidence of detecting evident symptoms present in 1% of the plants.
- For consignments originating from countries where *X. fastidiosa* is known to occur:
  - 3689 plants should be inspected from a lot of 10 000 which will provide 99% confidence of detecting evident symptoms present in 0.1% of the plants. Small lots all plants inspected.

- Similar values for sampling in place of production
  - National survey (1)
  - Phytosanitary certificate (2)



Inspection of consignments

Number of units in lot	P = 95% (confidence level)					P = 99% (confidence level)				
	% level of detection × efficacy of detection					% level of detection × efficacy of detection				
	5	2	1	0.5	0.1	5	2	1	0.5	0.1
25	24*	-	-	-	-	25*	-	-	-	-
50	39*	48	-	-	-	45*	50	-	-	-
100	45	78	95	-	-	59	90	99	-	-
200	51	105	155	190	-	73	136	180	198	-
300	54	117	188	285*	-	78	160	235	297*	-
400	55	124	211	311	-	81	174	273	369	-
500	56	128	225	380*	-	83	183	300	450*	-
600	56	132	235	379	-	84	190	321	470	-
700	57	134	241	445*	-	85	195	336	549*	-
800	57	136	248	421	-	85	199	349	546	-
900	57	137	254	470*	-	86	202	359	615*	-
1 000	57	138	258	450	950	86	204	368	601	990
2 000	58	143	277	517	1553	88	214	410	727	1800
3 000	58	145	284	542	1805	89	220	425	792	2263
4 000	58	146	288	556	2108	89	222	433	821	2735
5 000	59	147	290	564	2253	89	223	438	840	3009
6 000	59	147	291	569	2358	90	224	442	853	3214
7 000	59	147	292	573	2437	90	225	444	861	3373
8 000	59	147	293	576	2498	90	225	446	868	3500
9 000	59	148	294	579	2548	90	226	447	874	3604
10 000	59	148	294	581	2588	90	226	448	878	3689

Sampling

Plant material

- **Sampling period** for symptomatic or asymptomatic plants:
  - Tropical plants indoors (all year)
  - Outdoor plants (late spring-early autumility)
    - *Polygala*
    - *O. europaea* & *Nerium oleander*
    - Deciduous & dormant species (*Prunus* spp.)

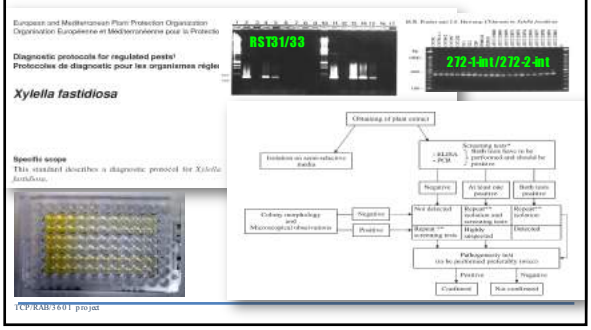
- **Sample collection**
  - Dormant Plants: tested for latent infections or held in contained conditions, wait to next growing season
  - Symptomatic plants: representative of symptoms (10-25 leaves)
  - Asymptomatic plants: random & representative of aerial part

Sampling of insects (Xylem-sucking hemiptera)

- Container surface (floor, walls)
- Plants, foliage, leaves
- Sweeping nets (adults) or aspirators better than sticky traps

Detection

Recommended screening tests (EPPO 2004)



Detection

Recommended screening tests on plant material

Symptomatic/asymptomatic plants

Screening tests - at least two tests (based on different biological principles or targeting different parts of the genome)
Serological tests
Conventional PCR/ real-time PCR test/ LAMP

- All tests negative → *X. fastidiosa* not detected
- Inconsistent tests results → Resampling recommended
- At least two tests positive → Sample with suspected presence of *X. fastidiosa*

For testing of (a)symptomatic plants in an outbreak area or in buffer zone a single test including serological tests may be performed

It is advised to include molecular tests for detection on asymptomatic plants material in a pest free area

Unlike other EPPO protocols for bacteria, isolation is not recommended as a screening test

Samples should be considered as '*samples with suspected presence of X. fastidiosa*' when at least two screening tests are positive

Detection

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Recommended screening tests on plant material

Vectors

Screening tests - at least two tests (targeting different parts of the genome)
Conventional PCR/ real-time PCR test/LAMP

- All tests negative → *X. fastidiosa* not detected
- Inconsistent tests results → Resampling recommended
- At least two tests positive → Sample with suspected presence of *X. fastidiosa*

Samples should be considered as '**samples with suspected presence of *X. fastidiosa***' when at least two screening tests are positive

Detection & Identification

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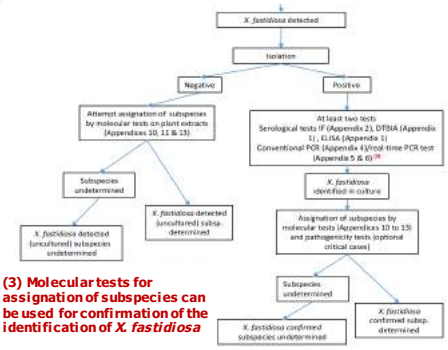

(3) Molecular tests for assignation of subspecies can be used for confirmation of the identification of *X. fastidiosa*

Detection & Sample preparation

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- Preservation and transport of samples should be done appropriately
- Samples should be processed as soon as possible after arrival
- Petioles and midribs are the best source for diagnosis (Xylem vessels)

Type of sample	Host plants/ Type of tissue	Minimum number of leaves per laboratory sample	Approximate weight of the laboratory sample
Samples from individual plants with leaves	Petioles and/or midribs of leaves of large size such as Coffea sp., Ficus sp., Vitis sp., Nerium oleander.	5	0.5-1g
	Petioles and/or midribs of leaves of small size such as Polygala myrtifolia and Olea sp.	25	0.5-1 g
	Plant species without petioles or with small petiole and midrib	25	0.5-1 g
Composite sample from several plants from a single lot with leaves	Samples of asymptomatic plants collected from e.g. imported consignments or nursery monitoring	100-200	10 to 50 g
Dormant plants or cuttings	Xylem tissue	Not applicable	0.5-1 g

Source:

Detection & Culture-Isolation

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Isolation and culturing on agarized media (EPPO, 2004)

- Isolation and cultivation of Xf strain CoDiRO from diseased plants (olive, oleander, etc.)

Detection & Culture-Isolation

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- *Xylella fastidiosa* is very difficult to isolate and grow in axenic culture, even from symptomatic plants.
- The bacterium does not grow on most common culture media, and requires specific media. PD2 (Davis et al. 1980), BCYE (Wells et al., 1981) or PWG (modified after Hill & Purcell, 1995) are widely used for the isolation from different host species. Media are described in Appendix 8.
- The use of at least two different media is recommended, in particular when isolation is attempted for new hosts or in the case of a first detection.
- It is very important to surface disinfect the sample to avoid growth of saprophytes because *X. fastidiosa* grows very slowly (the colonies can take up to 28 days to be visible) and can be readily overgrown by other microorganisms in the plates.
- Procedures for isolation from plant material are presented in Appendix 9.
- As a control, whenever possible a suspension of a *X. fastidiosa* strain at a concentration of about 10^6–10^7 cfu/mL should be plated onto the same medium.
- Plates should to be sealed or kept in plastic bags to prevent desiccation during incubation.

Detection & Culture-Isolation

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Difficult, time consuming and labor-intensive, when a large number of samples are processed

Detection & Critical issues

- Tissue preparation: disinfestation of tools and plant material
- ELISA and DNA extraction procedures for detection
- Evaluation of DNA extraction procedures and their effectiveness
- Evaluation of the presence of PCR inhibitors in tissue plants for each matrix
- Estimation of the detection limits

Detection & Sample preparation

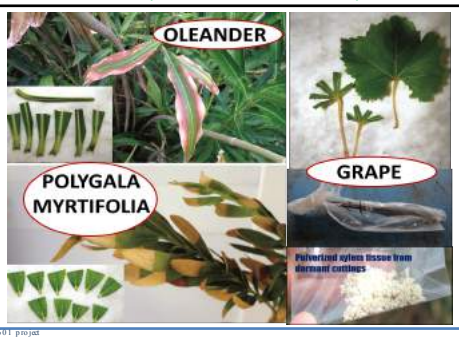
1. SAMPLE PREPARATION for DNA purification and ELISA-LAMP crudesap

OLIVE

Olive twigs (at least 10) collected from a sampled tree. Leaves showing leaf scorching and symptomless leaves (N. 8 -10 corrisponding to 0.5-1 gr) selected for the sample preparation, petioles and midveins excised for the extraction.

Detection & Sample preparation

1. SAMPLE PREPARATION for DNA purification and ELISA-LAMP crudesap



Detection & Sample preparation

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1. SAMPLE PREPARATION for DNA purification and ELISA-LAMP crude sap

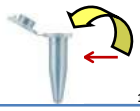
0.5-1 gr of midveins and petioles excised from 10 leaves (size/symptoms/no symptoms/ecc.)

+ 5/10 ml of buffer (1:10)

small pieces of midveins and petioles (0.5-1 g) homogenized using the semi-automated homogenizer Homex 6

Advantage: process 0.5-1 gr of tissue Representative of the sample

DNA extraction or ELISA/LAMP sap preparation



1 ml crude sap

ICP/RAB/3601 projet

Detection & DNA Extraction

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CTAB based extraction (for plants and insects)

1 ml of sap in CTAB Buffer

insect heads removed

homogenized in microcentrifuge tubes containing tungsten carbide beads or with pestle

+ 0.5 ml of CTAB buffer

•Up to 5 heads of insect can be pooled to perform one test

Chloroform treatment

cold 2-PROPANOL PRECIPITATION

PURIFIED DNA

Modified DNeasy® mericon™ Food Standard Protocol (Qiagen) (for plants)

1 ml of sap in Lysis buffer /1 spin column

designed for the extraction of total DNA from a large-scale sample of raw or processed food material

performed manually or automatized using a dedicated workstation

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Direct Tissue Blot Immunoassay (DTBIA) as an alternative to ELISA in large scale analysis directly in field

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DTBIA: protein antigens from a freshly cut plant tissue transferred to nitrocellulose membrane, incubated and detected with enzyme-labelled specific antibodies

Previous works on other pathogens

Direct Tissue Blot Immunoassay (DTBIA) for Detection of Citrus Tristeza Virus (CTV)*

S. M. Garnsey, T. A. Permar, M. Cambra, and C. T. Henderson

Experience of CIHEAM-IAMB

Journal of Plant Pathology (2001), 83 (2), 139-142 Edizioni ETS Pisa, 2001 139

SHORT COMMUNICATION

DETECTION OF CITRUS ISOROSIS VIRUS BY DIRECT TISSUE BLOT IMMUNOASSAY

A.M. D'Onghia[1], K. Djelouah[1], D. Frasheri[1] and O. Potere[2]

ICP/RAB/3601 projet

DTBIA recently validated for Xf detection as an alternative to ELISA in large scale analysis directly in field

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Direct tissue blot immunoassay for detection of *Xylella fastidiosa* in olive trees

Explants
twigs (positive and negative controls)

Nitrocellulose membranes
0.45 µm poresize

Blocking solutions 'Saturation of protein-binding sites' (Fat milk,)

Antisera (dilutions)

Incubation times

Processing times * within few hours ofsample collection/ after 1week'

PROTOCOL STEPS

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1. MEMBRANE PREPARATION


Premarked with a grid of suitable size so as to record the positions of individual sample


Selected 4 twigs

2.Print in the field/ laboratory


Smooth cut sections are gently pressed to the membrane, 2 prints for each twig (8 prints) (Menbrane stored in the dark)

3. MEMBRANE BLOCKING


Saturation of protein-binding sites by incubating the membrane in 1% of Fat Milk solution for 1h at RT on an orbital shaker

4. MEMBRANE WASHING


The membrane is washed with PBS (0.05% Tween 20) on a orbital shaker 3 times x 3min each

PROTOCOL STEPS

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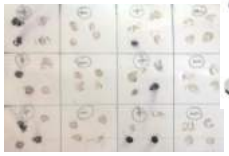
5. Addition of xf specific antibodies conjugated with alkaline phosphatase solution (Loewe, Agritest)

6. MEMBRANE DEVELOPMENT


The membrane is stained by immersion in developing solution "dissolving one tablet (BCIP - NBT.Sigma Fast) in 10ml distilled water, Membrane dried on filter paper RT

7. READING




The membrane is observed under a low power magnification lens (X10-X20)

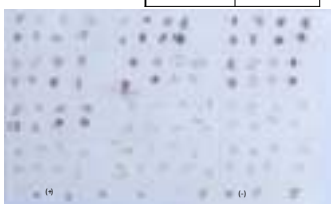
Purple coloration indicates positive reactions
The absence of purple coloration in the spot(s) indicates negative reactions

XYLELLA FASTIDIOSA INTERLABORATORY VALIDATION JULY 2015

With different kits

Test	OLIVE
DTBIA	PA=100% NA=100%

CIHEAM-IAM Bari
CNR Bari
University of Foggia
CRSFA Locorotondo



Following the developed procedure the olive samples were correctly categorized as positive and negative in four laboratories. Reactions recovered were consistently stronger and easy to assess and interpret, even without observation of the imprinted membrane under the microscope

TCP/RAB/3601 projet

DTBIA could represent an important contribution to further improvement of the serological detection at large scale of XF

ADVANTAGES

- Easy to handle, no sophisticated equipment is required
- Very short time of execution (less than 3 hours)
- Its cost is lower than ELISA
- Possibility to send printed membranes or store them at RT for several days before testing
- Printing of samples directly in the field, thus avoiding the movement of infected plant material thereby reducing the risk of spreading the bacterium in new areas

Interpretation of results could be a limiting factor

TCP/RAB/3601 projet

Detection & PCR

Generic detection (*X. fastidiosa sensu lato*)

Molecular protocols	Target gene
Conventional PCR	
PCR Minsavage *et al.*,1994	RNA polymerase sigma factor gene
Rodrigues et al., 2003	16S rDNA, DNA gyrB gene
LAMP Harper et al., 2010	16S rDNA
Real-Time PCR	
Francis et al., 2006	HL gene
Harper *et al.*, 2010	16S rDNA
LAMP Harper *et al.*, 2010 *	16S rDNA

Serological tests	ELISA (Sherald & Lei, 1991)
IFA	(Carbajal et al., 2004)
SEROLOGICAL kits	kit AGDIA – kit Loewe - Agritest

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Detection & Identification

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Generic detection
(*X. fastidiosa* specific subspecies)

Molecular protocols	Target subspecies
PCR amplification + sequencing	
Multi Locus Sequence Typing (MLST) (Yuan et al., 2010) Seven genes	all
PCR amplification	
Conventional PCR and Multiplex PCR (Hernandez-Martinez et al., 2006)	fastidiosa, multiplex, sandyi
Conventional PCR (Pooler & Hartung, 1995)	pauca

Detection & PCR

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Controls	Purpose
Negative isolation control (NIC)	to monitor contamination during nucleic acid extraction: nucleic acid extraction and subsequent amplification preferably of a sample of uninfected matrix or if not available clean extraction buffer
Positive isolation control (PIC)	to ensure that DNA of sufficient quantity and quality is isolated: nucleic acid extraction and subsequent amplification of the plant matrix sample that contains the target organism (e.g. naturally infected host tissue or host tissue spiked with the target organism).
Negative amplification control (NAC)	to rule out false positives due to contamination during the preparation of the reaction mix: amplification of molecular-grade H2O
Positive amplification control (PAC)	to monitor the efficiency of the amplification: Nucleic acid of XF (DNA extracted from XF, total DNA extracted from infected host tissue, whole-genome amplified DNA or a synthetic control cloned)

Detection & Conventional PCR

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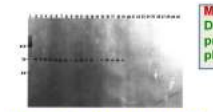
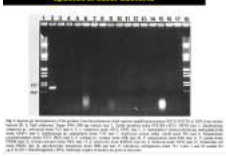
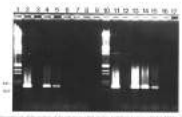


Southern hybridization assay of labeled DNA of plasmid pX10-2 as a probe (7.6 kb) against EcoRI-digested DNA of 19 *X. fastidiosa* strains from different origin and several species of other bacteria



Minsavage et al. 1997.
Development of polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84:456-461.

OH). Oligonucleotide primers used in PCR amplification tests were synthesized with a 394 DNA synthesizer (Applied Biosystems, Foster City, CA) at the University of Florida, ICBR Facility, Gainesville. Primers synthesized were RST29 (5'-GCAACAAACAACGAACAAAGGCAATGC-3'), RST30 (5'-GTGTAAGTTTGAACACCGTCGTGG-3'), RST31 (5'-GCGTTAATTTTCGAAGTGATTCGATTGC-3'), RST32 (5'-CGAACAAAGGCAATGCCGC-3'), and RST33 (5'-CACCATTCGTATCCCGGTG-3').



Detection & Conventional PCR

- About 80 manuscripts using them
- Different host plants
- Strains from different countries
- Insect samples

Minsavage et al. 1997.
Development of polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84:456-461.

Detection & qPCR

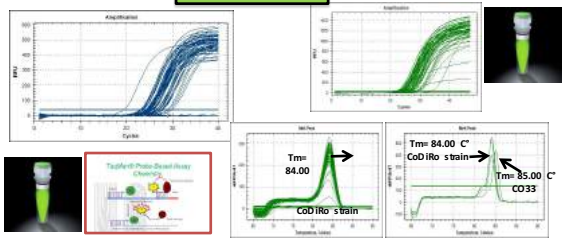
Harper et al., 2010 (Taqman probe)

Target sequence located at rimM open reading frame of *X. fastidiosa* 16S rRNA

ON FIELD SAMPLES

SYBR green version - Francis et al., 2006

Target sequence in internal transcribed spacer (ITS) regions between the genes coding for 16S-23S rRNA.



Detection & qPCR

qPCR: Interpretation of results

Harper et al., 2010 (Taqman probe)

- **Verification of the controls:**
  - The PAC amplification curves should be exponential;
  - The NAC should give no amplification
- **Negative samples:**
  If a sample does not produce an amplification curve or if it produces a curve which is not exponential
- **Positive samples:**
  If a sample produces a an exponential amplification curve

Tests should be repeated if any contradictory or unclear results are obtained

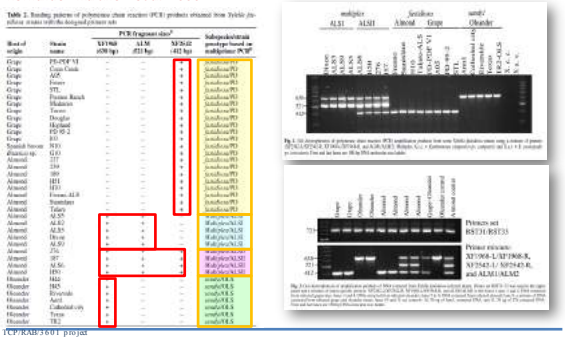
SYBR green version - Francis et al., 2006

- **Verification of the controls:**
  - The PAC amplification curves should be exponential;
  - The NAC should give no amplification
- **Negative samples:**
  If a sample does not produce an amplification curve or if it produces a curve which is not exponential
- **Positive samples:**
  If a sample produces a an exponential amplification curve and the specific melting peak should be in the range of 83-85 C°

Frequently curves with values of Ct 35 - 40 do not exhibit a characteristic curve. The result is interpreted as being UNDETERMINED. Tests should be repeated if any contradictory or unclear results are obtained.

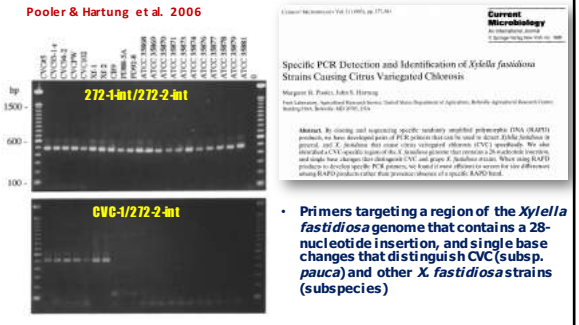
Identification & Conventional multiplex PCR

Hernandez-Martinez et al. 2006



Identification & Conventional PCR

Pooler & Hartung et al. 2006

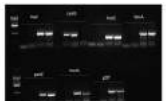


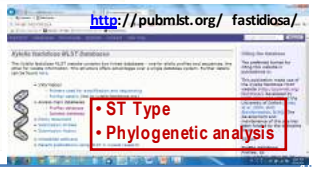
Specific PCR Detection and Identification of *Xylella fastidiosa* Strains Causing Citrus Variegated Chlorosis

- Primers targeting a region of the ***Xylella fastidiosa*** genome that contains a 28-nucleotide insertion, and single base changes that distinguish CVC (subsp. ***pauca***) and other ***X. fastidiosa*** strains (subspecies)

Identification & PCR + sequencing

MULTILOCUS SEQUENCE TYPING (MLST) *Xylella fastidiosa*





http://pubmlst.org/ fastidiosa/

- ST Type
- Phylogenetic analysis

Identification & PCR + sequencing

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MULTILOCUS SEQUENCE TYPING (MLST)
Xylella fastidiosa

Please note, a perfect match requires 100% identity, no mismatches, no gaps, and an alignment over the full length of the locus.

Locus	Allele	Identity	Mismatches	Gaps	Alignment
leuA	13	100.00%	0	0	11/708
petC	No matches	-	-	-	-
malF	16	100.00%	0	0	12/730
cysG	2	Exact match	-	-	-
holC	No matches	-	-	-	-
nuoL	17	100.00%	0	0	13/667
gltT	No matches	-	-	-	-

Identification & PCR + sequencing

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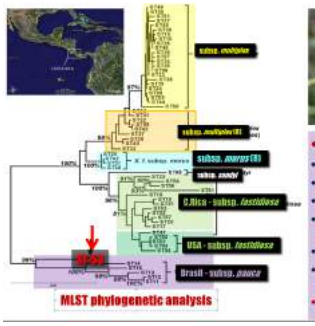

•ST=53 (7 genes):
- Origin Costa Rica
- Is *X. fastidiosa* f.sp. *pauca*
- Primera cita en el país
- Isolates 5 from oleander + 1 coffee
- 5/7 alleles = *X. f. pauca*
- 2/7 alleles = close to *X. f. pauca* (non described, 2014)
- Origin recombination? New taxa?

The Need

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Ideal diagnostic test suitable for developing, underdeveloped and undeveloped countries should be

"Reliable"

- Affordable
- Sensitive
- Specific
- User-friendly
- Robust and rapid
- Equipment free
- Deliverable to the end user

LAMP (Loop mediated isothermal amplification)

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© 2000 Oxford University Press Nucleic Acids Research, 2000, Vol. 28, No. 12 e63

Loop-mediated isothermal amplification of DNA

Tsugunori Notomi[1,2,*], Hiroto Okayama[2], Harumi Masubuchi[1], Toshihiro Yonekawa[1], Keiko Watanabe[1], Nobuyuki Amino[3] and Tetsu Hase[1]

[1]Eiken Chemical Co. Ltd, 1381-3 Shimoishigami, Ohtawara, Tochigi 324-0036, Japan, [2]Department of Biochemistry and Molecular Biology, The University of Tokyo, Graduate School of Medicine, Bunkyo-ku, Tokyo 113-0033, Japan and [3]Department of Laboratory Medicine, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

- Originally reported by **Notomi *et al*** in 2000 of EIKEN Chemical Co. Ltd., Japan Eiken Chemical Co., Ltd. Eiken GENOME SITE **(http://www.eiken.co.jp/en/)**
- As of 17th April 2016, PubMed database has listed more than 1536 articles on this topic

LAMP characteristics

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- ***Bst DNA polymerase*** with strand displacement activity at 65℃
- No need for a step to denature double stranded into a single stranded form
- The amplification efficiency is extremely high
- Reduced total cost not require special reagents or sophisticated equipment
- Amplification can be done with RNA templates following the same procedure as with DNA templates, simply through the addition of reverse transcriptase (RT-LAMP)

Design of primers

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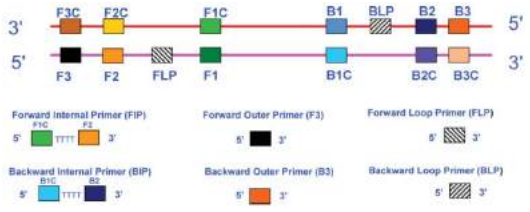
4 primers based on the 6 distinct regions of the target gene: the F3c, F2c and F1c regions at the 3' side and the B1, B2 and B3 regions at the 5' side

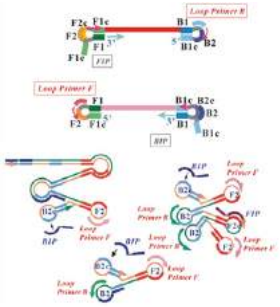


Softwares

(www.premierbiosoft.com/isothermal/lamp.html)

(http://loopamp.eiken.co.jp/e/lamp/primer.html)

Using Loop Primers



https://www.youtube.com/watch?v=L5zi2P4lggw

LAMP does not require thermal cycler

PCR	LAMP
• Cycle reaction	• Isothermal reaction
• Denature (95℃)	• 60- 65℃
• Annealing (50~60℃)	• Max 1 hour
• Polymerization (72℃)	
• ~ >1 hour	

Detection

1- Visual detection

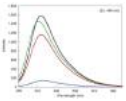
Turbidity

Positive reaction is turbid

Fluorescence

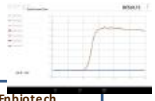
Positive reaction is green

2- Measurement of fluorescence/turbidity
Inoculum quantification



3-Gel electrophoresis of the amplification products

4- Real-time LAMP
Fluorescent detection in real-time



Icgene, Enbiotech
LAMP device
Real Time monitoring

Targets. . . any nucleic acid from target organism (DNA or RNA)

- Bacteria
- Viruses
- Fungi
- Protista
- Worms

Examples of LAMP application

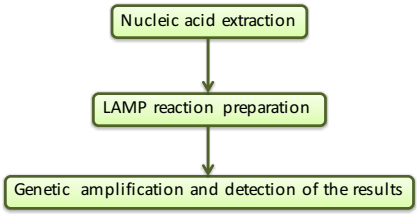
Disease	Pathogen	References
Tomato and potato late blight	*Phytophthora infestans*	Hansen et al., 2016
Fusarium wilt of chickpea	*Fusarium oxysporum* f.sp. *ciceris*	Ghosh et al., 2015
Grape powdery Mildew	*Erysiphe necator*	Thiessen *et al.*, 2013
Fire blight	*Erwinia amylovora*	Temple and Johnson, 2011; Bühlmann *et al.*, 2012; Moradi *et al.*, 2012
Citrus Bacterial Canker	*Xanthomonas spp.*	Rigano *et al.*, 2010
Grey Mould	*Botrytis cinerea*	Tomlinson *et al.*, 2010
Pierce's disease, citrus veinal chlorosis, almond leaf scorch, Olive Quick Decline	*Xylella fastidiosa*	Harper *et al.*, 2010 Yaseen *et al.*, 2015

LAMP vs. PCR

- LAMP does not require an expensive thermocycler
- Amplification specificity is extremely high as LAMP requires 4/6 oligonucleotide primers that recognize 6/8 distinct regions on the target DNA
- Detection limit : LAMP ≥ PCR and RT-PCR
- Detection time : LAMP < PCR and RT-PCR
- LAMP reaction: accelerated by two loop primers
- PCR reagent recommended storage temperature is -20°C, LAMP reagents can be stored at 4°C and can shipped at ambient temperature.
- Crude DNA preparation can be used as LAMP template DNA.

(Goto *et al.*, 2009)

Procedure



Simple and rapid DNA extraction from insect and plant material

Phytopathologia Mediterranea (2015) 54, 3, 488–496
DOI: 10.14601/Phytopathol_Mediterr-15250

RESEARCH PAPERS

On-site detection of *Xylella fastidiosa* in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method

THAER YASEEN[1], SANDRO DRAGO[2], FRANCO VALENTINI[1], TOUFIC ELBEAINO[1], GIUSEPPE STAMPONE[2], MICHELE DIGIARO[1] and ANNA MARIA D'ONGHIA[1]

[1] CIHEAM - Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy
[2] Bionat Italia s.r.l. via Aquileia, 34, 90144 Palermo, Italy

Summary. A recent severe outbreak of *Xylella fastidiosa* associated with 'olive quick decline syndrome' (OQDS) was reported in Apulia (Southern Italy). In this study an on-site real-time loop-mediated isothermal amplification (real-time LAMP) was developed for detecting *X. fastidiosa* in host plants and insects. A marked simplification of the DNA extraction procedure was obtained by heating the samples in a portable Smart-Dart device and using an optimized enhancer reaction buffer. The connection to a tablet or Smartphone allowed to visualize the results of the reaction in real time. Compared to PCR and ELISA, with which it showed comparable results in terms of sensitivity and reliability in the *X. fastidiosa* detection, this simplified real-time LAMP procedure proved to be "user friendly", displaying the advantages to be an on-site detection method of easy handling, rapid execution and low cost.

Key words: real-time LAMP, *Philaenus spumarius*, olive quick decline syndrome (OQDS).

1CP/RAB/3601 projet

T. Yaseen et al.

Figure 2. Overview of the on-site detection of *X. fastidiosa* in 'spy insects' using real-time LAMP in SMART DART device directly in the field: a) adult of *P. spumarius*; b) collected insects in 0.2 mL extraction buffer; c) DNA extraction; d) real-time LAMP results acquisition by the tablet.

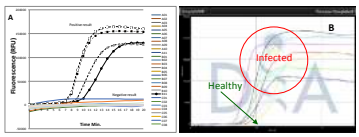


Figure 3. *Xf*-infected olive samples showing positive results in real-time LAMP with 20 min reaction time in both real-time IQ5 Bio-Rad device (A), and in Smart-Dart device equipped with D&A software (B). *Xf*-negative samples showed linear curves.

1CP/RAB/3601 projet

Xylella screen Glow

Extraction Strip

Primer MIX Strip

Budge Xylella screen glow

LAMP MIX

Mineral oil

Positive control

Negative control

1CP/RAB/3601 projet

Xylella screen Glow and icgene

TCP/RAB/3601 projet

Start the App

TCP/RAB/3601 projet

First screen

TCP/RAB/3601 projet

Run the App

Introduce user card

Activate the kit

Sample cutting 4-5 olive leaf peduncle
Or transfer the insect from 95% alcohol tube to filter paper, leave them to dry for 5 minutes.

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Insert peduncles or dried insects in extraction tube

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Insert the tube in icgene

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Run the extraction procedure

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Add 22,5ul of LAMP master mix to each primer mix tube

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Be sure that the primer dissolved in master mix and add 30ul of mineral oil

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Add 2,5ul of the extraction buffer for the relative sample

TCP/RAB/3601 projet

Nominate the experiment and each single sample

TCP/RAB/3601 projet

Run the amplification

TCP/RAB/3601 projet

Waite until “Insert samples” appears in the tablet

TCP/RAB/3601 projet

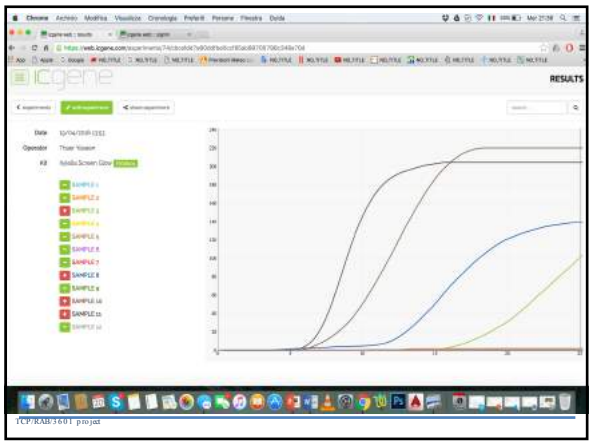
Insert samples

TCP/RAB/3601 projet

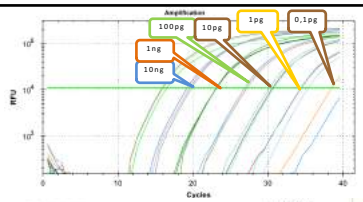
Amplification

TCP/RAB/3601 projet

Results

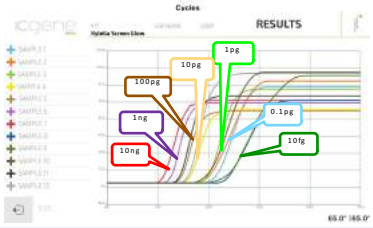


RT-PCR



X. fastidiosa
DNA diluted 1:10

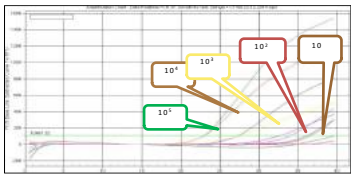
RT-LAMP



1CP/RAB/3601 projet

RT-LAMP

RT-PCR



Serial dilution of the pathogen from 10^5 up to 10 cells

1CP/RAB/3601 projet

Possibility to send the results a real time to a server to collect the data and the all related information

1CP/RAB/3601 projet

ICGEN gene with battery Autonomy of 9 courses

Real time LAMP represent the further of the serological molecular detection method of XF

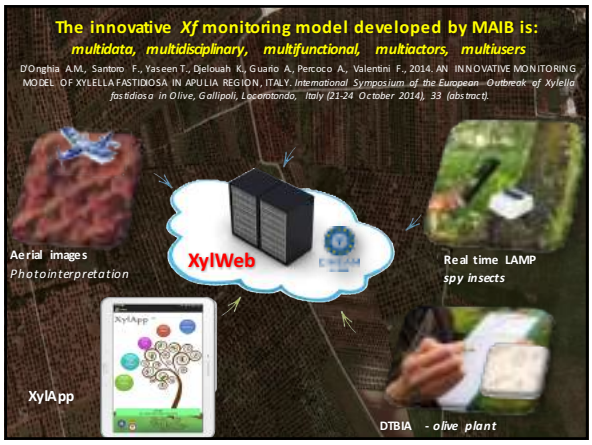
ADVANTAGES

- User friendly method, easy to handle, only a a simple portable equipment is required
- Ready-to-use extraction system that allows for total DNA extraction in only a few minutes and without the use of sophisticated laboratory instruments
- The only method molecular can work with crud extract
- Very short time of execution (less than 40 min) including extraction
- Its cost is lower than qPCR or the conventional PCR
- More sensitive than qPCR
- Possibility to send the results a real time to a server to collect the data and the all related information
- The only efficient method for the detection Xf in the possible vectors and other spy insects which can harbor the bacterium.

Real time LAMP represent the further of the serological molecular detection method of XF

ADVANTAGES

- Ready-to-use amplification and detection system composed of strips that contain freeze dried primers and a Master Mix with all the reagents necessary for the execution of the test
- Possibility of performing genetic tests directly on site
- Stable Kit at room temperature, transportation at room temperature and stored at +4°C
- Automatic interpretation of results
- Automatic detection of the kit through contactless badge


The innovative Xf monitoring model developed by MAIB is:
multidata, multidisciplinary, multifunctional, multiactors, multiusers
D'Onghia A.M., Santoro F., Yaseen T., Djelouah K., Guario A., Percoco A., Valentini F., 2014. AN INNOVATIVE MONITORING MODEL OF XYLELLA FASTIDIOSA IN APULIA REGION, ITALY. International Symposium of the European Outbreak of Xylella fastidiosa in Olive, Gallipoli, Locorotondo, Italy (21-24 October 2014), 33 (abstract).
Aerial images
Photointerpretation
XylWeb
Real time LAMP
spy insects
XylApp
DTBIA - olive plant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Thank you
TCP/RAB/3601 projet

Case and Management of the XF Outbreak in Apulia

Thaer YASEEN

CIHEAM
Mediterranean Agronomic Institute of Bari (MAIB)
Division of Integrated Pest Management

Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-Olive Quick Decline Syndrome in NENA Countries
TCP/RAB/3601 project

The first thing to do is

Trust the science

Practical aspect

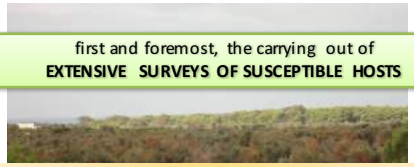
Case of outbreak of *Xylella fastidiosa*

- EXTENSIVE SURVEYS OF SUSCEPTIBLE HOSTS
- Identify the contaminated area and determine the most appropriate management strategies
- Identify *X. fastidiosa* diffusion factors
- Define human assisted spread and vectors
- Awareness raising campaigns
- The use of validation of diagnostic tools
- Eradication of the infected plants

Practical aspect

Case of outbreak of *Xylella fastidiosa*

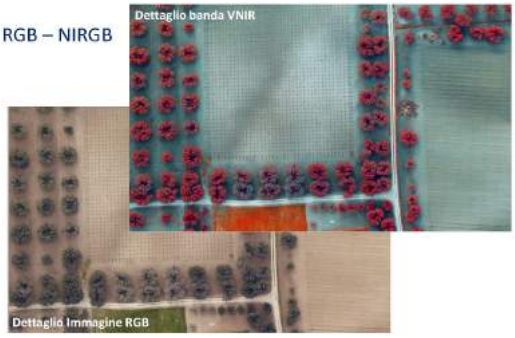
first and foremost, the carrying out of
EXTENSIVE SURVEYS OF SUSCEPTIBLE HOSTS



To identify the contaminated area and determine the most appropriate management strategies

TCP/RAB/3601 projet

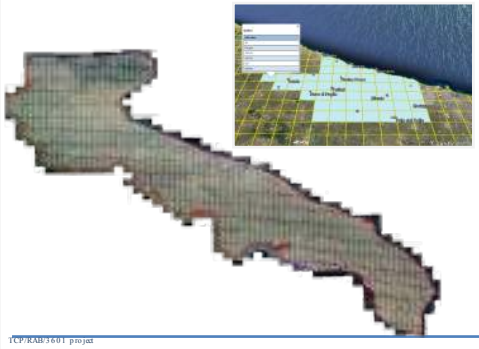
RGB – NIRGB



TCP/RAB/3601 projet

Monitoring organization in Apulia region

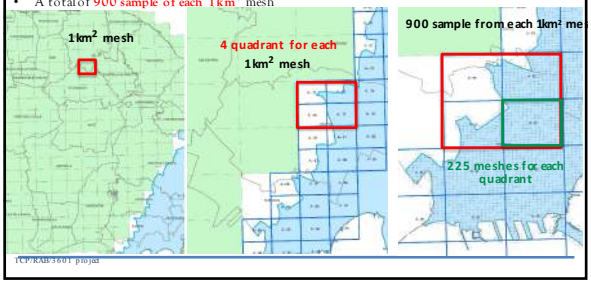
The APULIA regional territory has been subdivided into 1km^2 grid (mesh)



TCP/RAB/3601 projet

Sample collection in Apulia region

- Buffer zone and contaminated area were divided to 1km² mesh
- Each mesh was divided into 4 quadrant
- Each quadrant was further divided to small meshes 1 h (100mX100m) area (a total of 225 meshes for each quadrant) 225 X 4 quadrant=900 small mesh for each 1km² .
- Each small mesh will be monitored visually and 1 sample will be collected.
- A total of 900 sample of each 1km² mesh

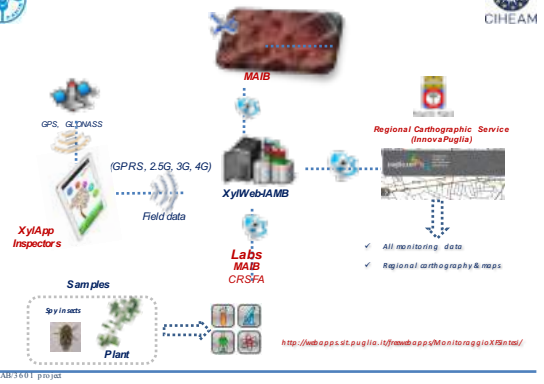


TCP/RAB/3601 project

Each small mesh will be monitored visually and 1 sample will be collected a total of 225 sample for each

TCP/RAB/3601 project

Under the coordination of the Regional Plant Protection Service and the Commissioner of the Forest Service

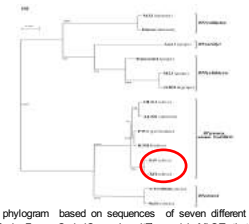


TCP/RAB/3601 project

Multilocus Sequence Typing of *Xylella fastidiosa* isolated from olive affected by "olive quick decline syndrome (OQDS)" in Italy

T. Elbeaino, F. Valentini, R. Abou Kubaa, P. Moubarak, T. Yaseen, M. Digiaro, 2014. *Phytopathologia Mediterranea*, 53 (3), 533-542

Bacterial colonies of *Xylella fastidiosa* isolated on BYCE/PWG medium, ca 20 days after plating from infected olive tissue.



Concatameric phylogram based on sequences of seven different loci (*leuA*, *petC*, *lacF*, *cysG*, *holC*, *nuoL*, *glfT*) used in MLST that were linked for each *Xylella fastidiosa* isolate.

Other research

MLST approach on Xf isolates from other host species

Plant metabolites associated to the infection

Monitoring Xf in the 'Spy insects' for assessing the infection before symptoms development

Ben Moussa I. E., Valentini F., Lonasso D., Mazzoni V. , Digiaro M., Varvaro L, D'Onghia A.M., 2015 . Evaluation of *Spy' Insect* approach for monitoring *Xylella fastidiosa* in symptomless olive orchards in the Salento peninsula (Southern Italy). 7th meeting of the IOBC/wprs WG "Integrated Protection of Olive Crops", *abstract*.

Philaenus spumarius L.

N. campestris Fallen

E. lineolatus Brullé

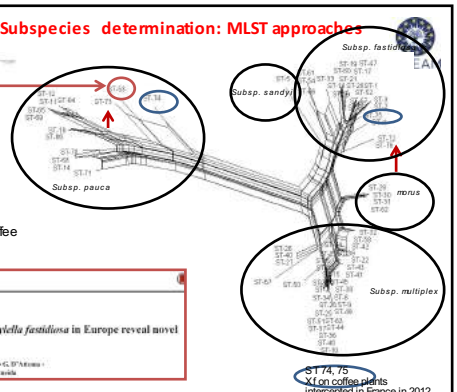
Very effective approach for assessing the pathogen free status areas

Other research

- Seasonal evaluation of *Xf* infection in the potential vectors/spy insects (paper submitted for publication)
- Efficient insect capture/control methods

Subspecies determination: MLST approaches

ST53 CoDIRO = ST53 from Costa Rica on coffee ad oleander



ST72,73,76 Xf Isolate intercepted in Europe on coffee plants in 2014

Intercepted isolates of *Xylella fastidiosa* in Europe reveal novel genetic diversity

ST 74, 75 Xf on coffee plants intercepted in France in 2012

TCP/RAB/3601 projet

The Olive Quick Decline Syndrome (OQDS) in Italy

Symptoms



Leaf scorching, foliar browning, and dieback of shoots, twigs and branches

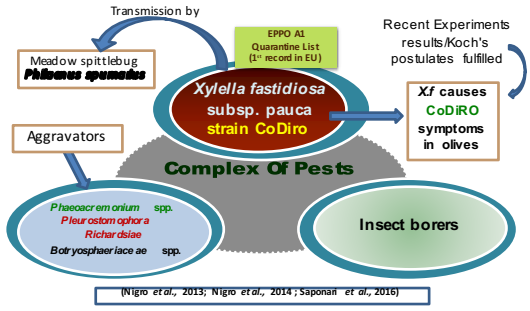

(Martelli *et al.*, 2016; Nigro *et al.*, 2014)

Death of the trees

Brown discolorations of the vascular system

ICP/RAB/3601 project

The Olive Quick Decline Syndrome (OQDS) in Italy



(Nigro *et al.*, 2013; Nigro *et al.*, 2014 ; Saponari *et al.*, 2016)

ICP/RAB/3601 project

Fungi associated to the OQDS

Kingdom	Fungi
Division	Ascomycota
Subdivision	Pezizomycotina
Class	Sordariomycetes
Subclass	Sordariomycetidae
Order	Togniniales
Family	Togniniaceae
Genus	*Phaeoacremonium*
Species	*Phaeoacremonium parasiticum* (Crous *et al.*, 1996)
	Phaeoacremonium scolyti (Mostert *et al.*, 2005)
	Phaeoacremonium sicilianum (Essakhi *et al.*, 2008)
	Phaeoacremonium italicum (Raimondo *et al.*, 2014)

(Mostert *et al.*, 2006a; Mostert *et al.*, 2006b; Damm *et al.*, 2008; Essakhi *et al.*, 2008; Sami *et al.*, 2014; Raimondo *et al.*, 2014)

- Well-known vascular pathogens causing wilt and dieback of various woody plants (Grape, plum, peach, nectarine, apricot, apple, pear, quince).
- Several species involved in Esca and Petri disease on grapevine, associated with other fungi.

(Carlucci *et al.*, 2015b; Nigro *et al.*, 2013)

In Italy, Seven *Phaeoacremonium* species (*Pm. parasiticum, Pm. minimum, Pm. alvesii, Pm. rubrigenum, Pm italicum, Pm sicilianum and Pm scolyti*) are known to affect olive trees showing decline in Apulia and Lecce.

ICP/RAB/3601 project

Fungi associated to the OQDS

Kingdom	Fungi
Division	Ascomycota
Subdivision	Pezizomycotina
Class	Sordariomycetes
Subclass	Sordariomycetidae
Order	Calosphaeriales
Family	Pleurostomataceae
Genus	*Pleurostomophora*
Species	*Pleurostomophora richardsiae* (Vijaykrishna *et al.*, 2004) Or *Pleurostoma richardsiae* (Réblová *et al.*, 2015)

(Vijaykrishna *et al.*, 2004;Levenstadt *et al.*, 2012; Eskalen *et al*, 2004 Halleen*et al.*, 2007; Rolshausen *et al.*, 2010)

- Previously known as *Phialophora richardsiae*.
- Can be found in soil, decaying wood and vegetation
- Reported as a pathogen of grapevines showing Petri and Esca disease symptoms in California, and South Africa.

(Carlucci *et al.*, 2013b; Carlucci *et al.*, 2015a)

In Italy, associated with wilt of apical foliage, brown streaking and cankers of branches in olive

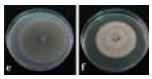
- Reported in 2015 on declined grapevines showing brown subcortical patches and streaking on the wood.

TCP/RAB/3601 project

Detection techniques of the fungi

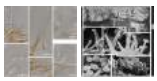
Isolation and Culturing

Field Symptoms



Pl. richardsiae (Vijaykrishna et al, 2004; Carlucci et al, 2013..)

Phaeoacremonium spp.
(Mostert *et al.* 2006; Gams *et al.* 2007; Essakhi *et al.* 2008)



Morphological features

Molecular Techniques (PCR based strategies)

LAMP –Loop Mediated Isothermal Amplification

- Sensitive, less time-consuming than conventional PCR, do not require skilled operators.
- Reaction occurs at isothermal conditions (65 °C), using one enzyme *Bst* DNA polymerase.
- Possible on-field application in a simple heat-block.

Examples of LAMP application in Plant pathology

Disease	Pathogen	References
Tomato and potato late blight	*Phytophthora infestans*	Hansen et al., 2016
Fusarium wilt of chickpea	*Fusarium oxysporum* f.sp. *ciceris*	Ghosh et al., 2015
Grape powdery Mildew	*Erysiphe necator*	Thiessen *et al.*, 2013
Fire blight	*Erwinia amylovora*	Temple and Johnson, 2011; Bühlmann *et al.*, 2012; Moradi *et al.*, 2012
Citrus Bacterial Canker	*Xanthomonas spp.*	Rigano *et al.*, 2010
Grey Mould	*Botrytis cinerea*	Tomlinson *et al.*, 2010
Pierce's disease, citrus veinal chlorosis, almond leaf scorch, Olive Quick Decline	*Xylella fastidiosa*	Harper *et al.*, 2010 Yaseen *et al.*, 2015

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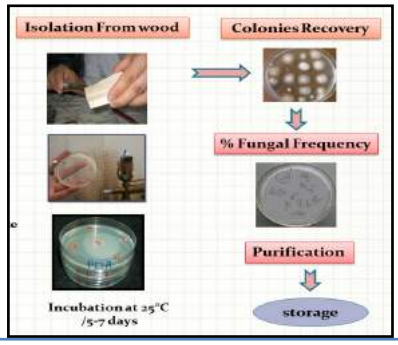
LABORATORY ANALYSIS- Classical methods

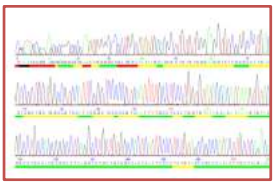


MOLECULAR CHARACTERIZATION

- **Molecular identification were performed at IAMBARI – Mycology Dept.**

DNA extraction → PCR amplification → Detection with gel electrophoresis.

DNA sequence-based identification methods



BLAST IN GENEBANK

Phaeoacremonium spp. associated with olive wilt and decline in southern Italy

(Carlucci *et al.*, 2015)


Wilt and browning


Discoloration of wood

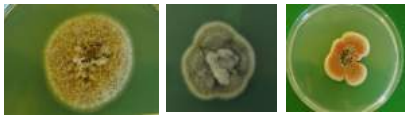

Wilting of shoots after artificial inoculation


Pm. *italicum*


Pm. *aleophilum*


Pm. *sicilianum*

Fungi isolates from OQDS olive trees - MAIB



Phaeoacremonium spp.

Phaeomoniella spp.

Other researches

- The role of fungi in the OQDS
- Development of primers for molecular detection of fungi species on large scale

Similar symptoms in other countries

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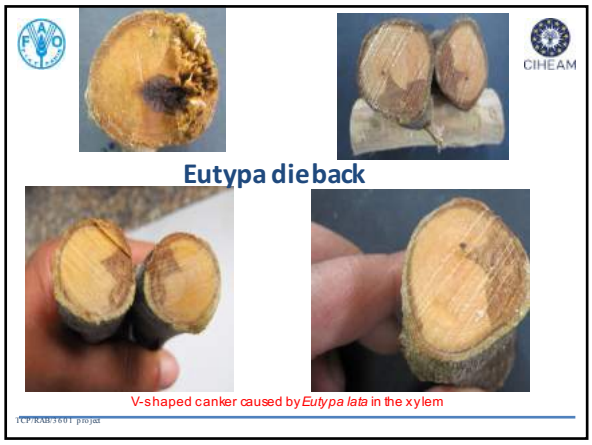

CIHEAM
Eutypa dieback
V-shaped canker caused by *Eutypa lata* in the xylem
TCP/RAB/3601 projet


CIHEAM
Thank you
CIHEAM
TCP/RAB/3601 projet

Capacity development and awareness raising on *X. fastidiosa* – stakeholder involvement and media control

Thaer YASEEN, Franco Valentini & Khaled Djelouah

CIHEAM
Mediterranean Agronomic Institute of Bari (MAIB)
Division of Integrated Pest Management

Preventive Measures for the Introduction and Spread of *Xylella fastidiosa*-CliveQuick Decline Syndrome in NENA Countries
TCP/RAB/3601 project

COMMISSION IMPLEMENTING DECISION (EU) 2015/789
of 18 May 2015
as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.)

COMMISSION IMPLEMENTING DECISION (EU) 2016/764
of 12 May 2016
amending Implementing Decision (EU) 2015/789 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.)
(notified under document C(2016) 2731)

Article 3a
Contingency plans

1. By 31 December 2016, each Member State shall establish a plan setting out the actions to be taken in its territory in accordance with Articles 4 to 6a, and Articles 9 to 13a, in case of a confirmed or suspected presence of the specified organism (hereinafter "**the contingency plan**"). L 333/144 EN Official Journal of the European Union 19.12.2015 (1) EFSA PLH Panel (EFSA Panel on Plant Health), 2015.

2

Article 3a
Contingency plans

2. The contingency plan shall also set out the following:
(a) the roles and responsibilities of the bodies involved in those actions and the single authority;
(b) one or more laboratories specifically approved for the testing of the specified organism;
(c) rules on the communication of those actions between the bodies involved, the single authority, the professional operators concerned and the public;
(d) protocols describing the methods of visual examinations, sampling and laboratory testing;
(e) rules on training of personnel of the bodies involved in those actions;
(f) minimum resources to be made available and proceedings to make available additional resources in case of a confirmed or suspected presence of the specified organism.
3. Member States shall evaluate and review their contingency plans as necessary.
4. Member States shall communicate their contingency plans to the Commission at its request.'

3

Article 6
Eradication measures

8. The Member State concerned shall raise public awareness concerning the threat of the specified organism and concerning the measures adopted to prevent its introduction into and spread within the Union.

It shall set up road signs indicating the delimitation of the respective demarcated area.

4

Awareness raising

Article 13a
Awareness campaigns

Awareness campaigns have been proved to be useful tools for detecting new outbreaks.

Member States shall make information available to **the general public, travelers, professional** and **international transport operators** concerning the threat of the specified organism for the Union territory.

They shall make that information **publicly available**, in the form of targeted **awareness campaigns on the respective websites** of the responsible official bodies or other websites designated by those bodies.

5

Awareness raising

Article 13a
Awareness campaigns

At the same time, **citizen initiatives may also generate additional suspect reports** for areas in the wider environment which may not be surveyed for plant health purposes (e.g. non-professionals have allowed detecting *X. fastidiosa* on plants of *Polygala myrtifolia* in the French territory).

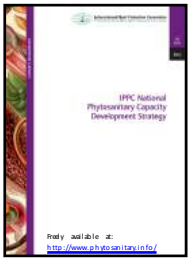
Early reporting of a suspected presence of *X. fastidiosa*, **both in open field** or **protected conditions**, increases the effectiveness of any control strategy and **prevents further spread** of the bacterium in the rest of the Union territory.

6

Capacity development

What is national phytosanitary capacity?

"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".



Freely available at: http://www.phytosanitary.info/

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Capacity development

The IPPC manual provides practical guidance on the establishment and on-going development of stakeholder relations, covering such topics as:

- institutional set-up
- the composition and tasks of policy advisory bodies and operational groups
- communication and involvement of stakeholders
- public awareness
- good reporting practices.

Stakeholders relations must be organized on a horizontal level throughout the government and public administration.

Freely available at: http://www.phytosanitary.info/

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Which are the Stakeholders?

http://www.clipartof.com/

"a person, group or organization that has interest in the phytosanitary activities of a National Plant Protection Organization (NPPO)"

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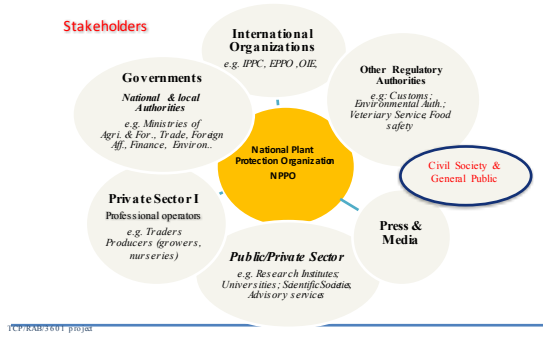
Capacity development



Capacity development

Ministry of Agriculture and Forestry could convene

- a National Scientific Committee with members drawn from Ministry experts, the NPPO, research institutes and other occasional expertise
- a Working Group with members drawn from the scientific Committee, professional operators (all stakeholders having a priority interest in the specified plants), civil society groups.

The high level and the multi-stakeholder component of these groups shows the importance placed by the government on the contingency plan and aims to minimize any opposition to a potentially expensive eradication plan.

The development process should be smooth because stakeholders, civil society included, should realize the dangers associated with *X. fastidiosa*, readily accepted the need to use severe eradication measures if the pest is found in a member country

Capacity development

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

Contingency plan

Management

Capacity development

Regulatory intervention points for which capacity development is needed

- ✓Pest Risk Assessment
- ✓Pest Risk Management
- ✓Pest Diagnostic
- ✓Import verification
- ✓Inspection
- ✓Surveillance & Monitoring
- ✓Eradication
- ✓Containment

Contingency plan

- Cost-benefit analysis of eradication/containment
- Stakeholders analysis
- Communication strategy

Capacity development

Capacity development *intervention points*	Stakeholders (non exhaustive list)
Pest Risk Assessment	Official body(NPPO), research Inst., experts and other professional resources
Pest Risk Management	Official body (NPPO), Authorities, legal experts, policy makers
Pest Diagnostic	Official body (NPPO), Diagnostic entity (reference laboratories)
Import verification	Official body (NPPO), Authorities (e.g. customs, immigration)
Inspection	Official body (NPPO), Diagnostic entity (reference labs)
Surveillance and monitoring	Official body (NPPO), extensions, researchers, professional operators, police, military, civil society, media
Eradication and containment	Official body (NPPO), Authorities (e.g. police, military), extensions, researchers, professional operators, civil society, media

Stakeholders involvement

e.g. **Implementation of the surveillance**

Pest surveillance lends itself to a range of possible partners and options, especially when the **Official Body (NPPO)** has a limited range of plant health experts or where there are specific benefits in having stakeholder participation.

Stakeholders can be involved in **many activities, for example**:

Producers and commodity associations may be engaged to:

Pest record: e.g. a document providing information concerning the presence or absence of symptomatic host plants at a particular location, at a certain time, within an area, monitor traps on their farms for vectors, collect information on pest occurrence etc.
They make their pest records available to the NPPO

Members of civil society and the general public may be involved in surveillance activities: they can keep insect traps in their own gardens, collect and send them to the NPPO.

However, when stakeholders are involved in surveillance activities, the NPPO should ensure they are trained and audited adequately in appropriate fields of plant protection and data management, including sampling methods, preservation and transportation of samples for identification and record-keeping associated with samples.

In all cases, data and records provided by stakeholders should be accurately verified by the NPPO

Capacity development

Capacity development at each intervention point through:

- Education & Training
- Legislation *(EU, National, Regional)*
- Technical protocols *(e.g. EPPO, ISPMs)*
- Communication strategy and advocacy
- Infrastructures & Equipments
- Human and other resources

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Capacity development

Training of inspectors on *X. fastidiosa* in Italy (2013-2016)

Training on pathogen monitoring: *symptomatology on different host species, sampling (plant material & insects), field data acquisition using XylApp, safe maintenance and transport of the samples*

>300 Inspectors from Plant Protection Services, Municipality and Forestry Police (Apulia region and other Italian regions)

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Training of EU & Foreign Plan Protection Officers/ Researchers in Italy (2013-2016)

Short-term training courses/workshops on *Xylella fastidiosa* (surveillance, detection & control) were also organized for plant protection officers/ researchers from EU & from Mediterranean countrie

BTSF Workshop by the EU Commission
~30 EU Plant Protection Officers

CIHEAM training courses
~30 Plant Protection Officers/ researchers *(Ministries of Agriculture of Croatia, Lebanon, Jordan)*
~40 Researchers *(France, Greece, Italy, Morocco, Spain, Tunisia, Turkey)*

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Capacity development

Information systems

are required to ensure effective management of the information as it moves from field to record keeping to reporting and mapping.

The Official Body should select **hardware** and **software** in terms of short- and long-term programmes goals

- **data flow structure** and **presentation**
- **work flow structure**
- **record keeping**
- **general guidelines** for information management

e.g. The Apulian information system developed for *Xylella fastidiosa*

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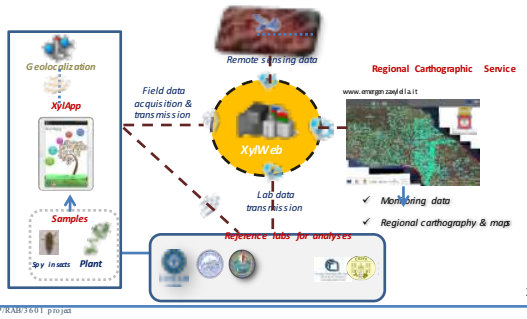
Capacity development

The Apulian information system for *X. fastidiosa*

Data acquistion, collection, storage, management, reporting and mapping



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Guidelines for the survey of *Xylella fastidiosa* (Wells et al.) in the Union territory, 2015

2.3 Awareness raising campaigns

Information sheets and pest alerts have proved to be useful in communicating with **professional operators** and the **wider public**, along with a **mechanism to report** and be able to follow up on suspected findings.

Training of professional operators in symptom recognition and those involved in conducting official surveys of land or crops for other purposes has also helped to identify additional outbreaks.

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Awareness raising

Awareness tools

Passive *(information)*

- Websites
- Newspaper articles
- Other news channels
- Posters
- Flyers
- Pamphlets
- List servers
- Draft policy documents

Active *(disussion)*

- Conference calls
- Video Conferencing
- Face-to-face meeting
- Questionnaires
- Social media (interactive)
- Advisory committees or councils
- Working groups
- Consultations

EPPO
Awareness campaign

EPPO
Awareness campaign

Awareness campaign

Xylella leaflet in Sicily

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Xylella
Awareness campaign

The website of the Apulian Phytosanitary Service on 'Xylella emergency'

www.emergenzaxylella.it

A passive and active tool for information and discussion
It could be used as an interactive platform

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The EC website - Plant Health & Biosecurity
Emergency measures on Xylella fastidiosa

http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa_en

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Awareness raising

Advisory Group with EU Stakeholders' representatives organised on a regular basis by the European Commission (DG SANTE/DG AGRI)

http://ec.europa.eu/dgs/health_food-safety/advisory_groups_action_platforms/advisory_group_en.htm

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Awareness raising

Several national & international initiatives

Seminars & Meetings

- International Symposium, Gallipoli – Apulia, Italy
- 14th Meeting of the Mediterranean Phytopathological Union – Istanbul, Turkey
- **TAIEX meeting** (Tirana, Albania; Ankara, Turkey; Podgorica, Montenegro)
- 7th IOBC/WPRS - Kalamata, Greece
- Seminars at different Italian Universities, Municipalities, Associations of Professional Operators etc.

- EFSA workshop, Brussel

X. fastidiosa knowledge gaps and research priorities for the EU

- 11th Commission of Phytosanitary Measures, FAO Rome

Side Session on *X. fastidiosa*

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Capacity development
Awareness raising

The FAO-IPPC-CIHEAM International Workshop on

***Xylella fastidiosa* & the Olive Quick Decline Syndrome (OQDS)**

Session 1 *CIHEAM/Istituto Agronomico Mediterraneo di Bari* 19-22 April, 2016

Xylella fastidiosa and its impact

Session 2

International collaboration and projects

Session 3

Legislative aspects

Session 4

Surveillance and diagnostic methods

Session 5

Surveillance and diagnostic methods (Demo in the FIELD)

Session 6

Surveillance and diagnostic methods III (Demo in the LAB)

Session 7

From knowledge to implementation, the need for capacity development

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TCP/RAB/3601 project International training workshop on Xylella www.iamb.it

Official Comunication
Press & Media

It is a very critical issue to provide media & press with a correct information.

Communication should be made only through the Official Body (*e.g. Communication office, the Website*)

Generally it is better to provide the information through clear & precise written texts

Information on advances of Research should be given by research institutions only after the communication to the Official Body

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CONCLUSION

Awareness of the phytosanitary threat posed by *X. fastidiosa*

- No treatment solutions are currently available to treat diseased plants in open field
- The death/elimination of a number of infected hosts species will affect severely the whole territory territory

Stakeholders and civil society from Member States should be aware of the need to protect their territories from the serious threat posed by *X. fastidiosa*.

To this aim they should become actors of the contingency programme, therefore actively involved in the **surveillance** for **early pathogen detection** and collaborative in the eradication and containment measures

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CONCLUSION

Awareness of the phytosanitary threat posed by *X. fastidiosa*

Once prepared in advance on the risk for the country posed by *X. fastidiosa*, when the introduction occurs they can contribute to the early detection and collaborate in the immediate eradication to avoid the establishment and spread because they will understand that is more convenient by the economical, environmental and social point of view to eliminate the organism than try to contain/or control.

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Thank you