

Xylella fastidiosa outbreaks in Europe: new genotypes in France

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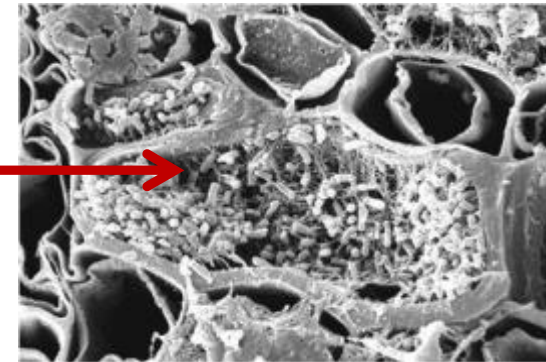
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Xylella fastidiosa

- Xylem-limited bacterium. Invades xylem vessels and produces biofilms
- First description of the disease in 1887 (USA)
- First isolation on media in 1978



X.f. within a xylem vessel in the leaf of affected Chardonnay grape. (ca. x 4,000). [E. W. Kitajima](#) (ESALQ/USP/Brazil).

• Transmitted by:

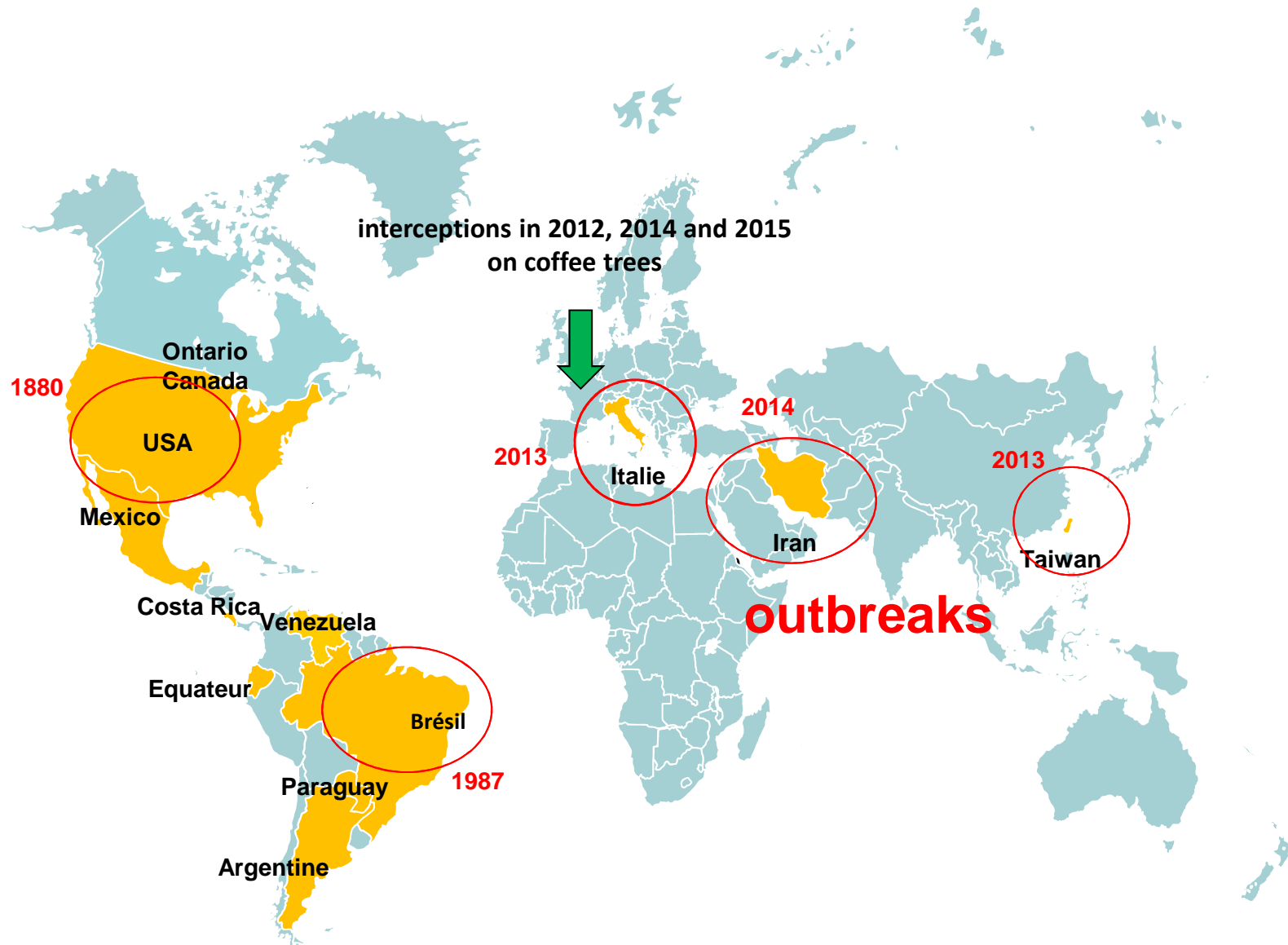
- Insect vectors (*Cicadellidae*, *Cercopidae*, *Cicadidae*)
- Infected plants for planting

• Quarantine disease (2000/29CE)



(Photo : Fred CHEVAILLOT – Source : INPN MNHN)

Xylella fastidiosa : distribution



A large range of hosts...6 subspecies

Pearce's disease
on grapevine
X. f. subsp. fastidiosa



Almond Leaf Scorch
X. f. subsp. multiplex



Oleander Leaf Schorch
X. f. subsp. sandyi



Citrus Variegated
Chlorosis
X. f. subsp. pauca

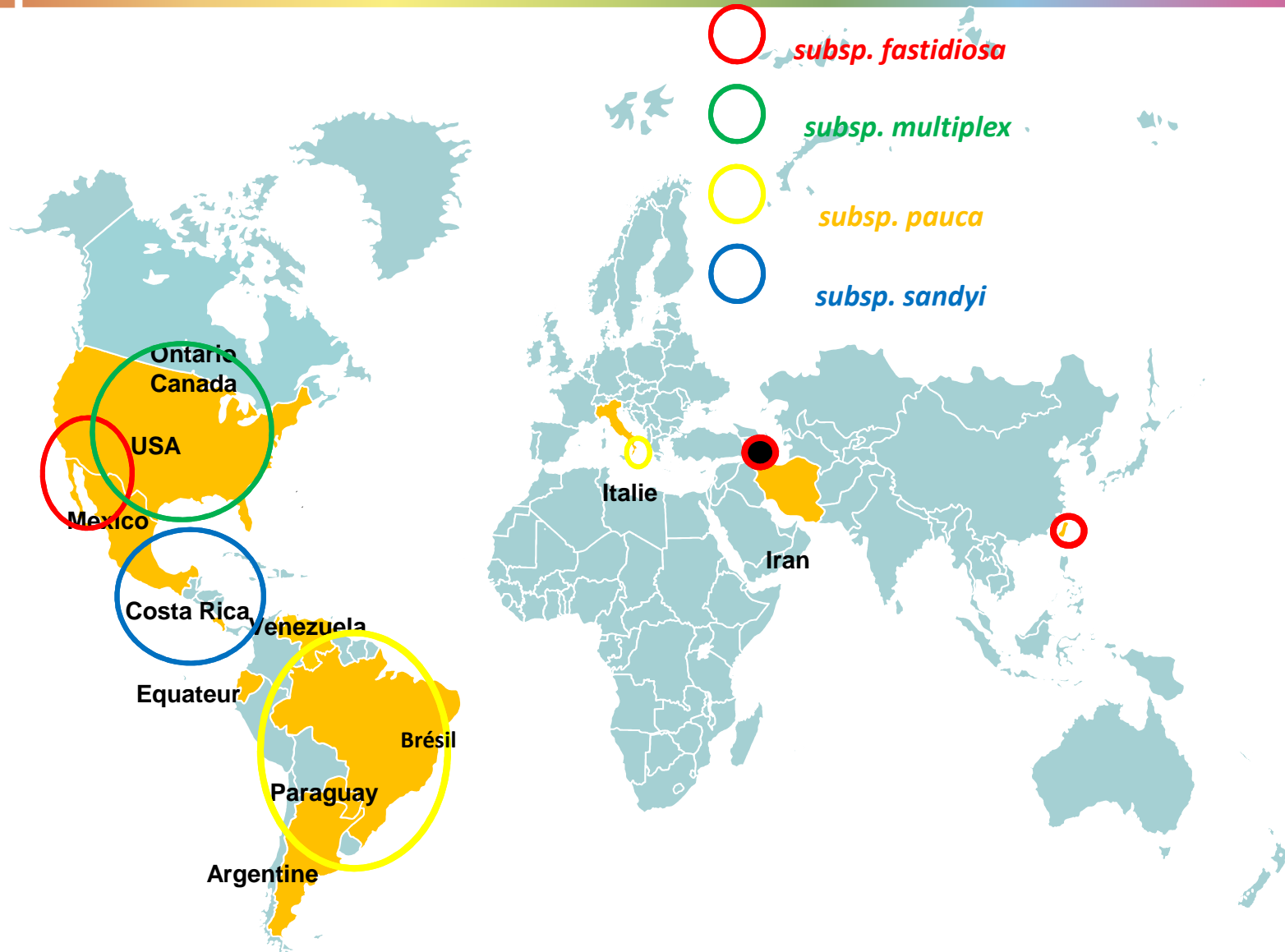


- subsp *morus*
- subsp *tashke*

CoDIRO
X. f. subsp. pauca



X. fastidiosa : distribution of subspecies



Very Serious Threat for agriculture in Europe!

Our experience on *Xylella* in France:

- (2000) IF tests for Quarantine on *Vitis*, *Citrus*, *Prunus*
- (2012; 2014; 2015) Import / survey of territory:
coffee plants intercepted

**Urgent need of sensitive tools
to prevent introduction**

Import / survey

Interception of infected coffee plants in France

2012/165 *Xylella fastidiosa* detected in a containment facility in France

2012:
France

Xylella fastidiosa (EPPO A1 List) was identified on *Coffea* spp. plants kept in a containment facility by a breeding company which regularly imports plant cuttings, in a region where the bacterium is known to occur. The bacterium was identified in a containment facility of the breeding company itself. 84 samples were sent to a private laboratory. Out of the 84 samples, 16 were confirmed and 16 were confirmed and the remaining 68 were not confirmed.

DISEASE NOTE

XYLELLA FASTIDIOSA IN COFFEA ARABICA ORNAMENTAL PLANTS IMPORTED FROM COSTA RICA AND HONDURAS IN THE NETHERLANDS

2015/181 *Xylella fastidiosa* detected in *Coffea* spp. plants in Switzerland

Following the detection of *Xylella fastidiosa* (EPPO A1 List) by the Swiss authorities in 2015, tracing-forward studies were conducted in Switzerland. In September 2015, the presence of *Xylella fastidiosa* was confirmed in a containment facility in Switzerland.

2014: The Netherlands, Italy, Germany

2015:
Switzerland

Isolation of strains from *Coffea*:

- 2012: subsp. *fastidiosa/sandyi* and subsp. *pauca*
(Jacques *et al.*, in revision)
- 2014 ; 2015: subsp. *sandyi*; subsp. *pauca*

Our experience on *Xylella* in France:

- (2000) IF tests for Quarantine on *Vitis*, *Citrus*, *Prunus*
- (2012; 2014; 2015) Import / survey of territory: coffee plants intercepted.
- Improvement of detecting scheme / test perf studies:
 - Intra laboratory study: diagnostic sensitivity, specificity, detection threshold..
 - 2014: Inter-laboratory comparison (6 labs; Italy, The Netherlands, New Zealand, France)
 - 2015: French reference method MA039

Comparison with an alternative extraction method: QuickPick™ SML Plant DNA (Bio-Nobile) vs DNeasy®

Performance criteria (%)	DNeasy® Plant mini kit Qiagen	QuickPick™ + Robot (BioSprint 15 = KingFisher™ mL)	QuickPick™ + magnets (manual protocol)
Diagnostic sensitivity	52,8	80,6	79,2
Diagnostic Specificity	100	100	100
Repeatability	97,8	97,8	96,6
Limit of detection	Orange ≈ 10 ² bact./mL Grapevine ≈ 10 ⁶ bact./mL Olive ≈ 10 ⁵ bact./mL	Orange ≈ 10 ² bact./mL Grapevine ≈ 10 ³ bact./mL Olive ≈ 10 ⁵ bact./mL	



Improvement of the limit of detection and sensitivity ;
Always some matrices with “inhibitors”

Official French protocol MA 039

1. Preparation of sample :

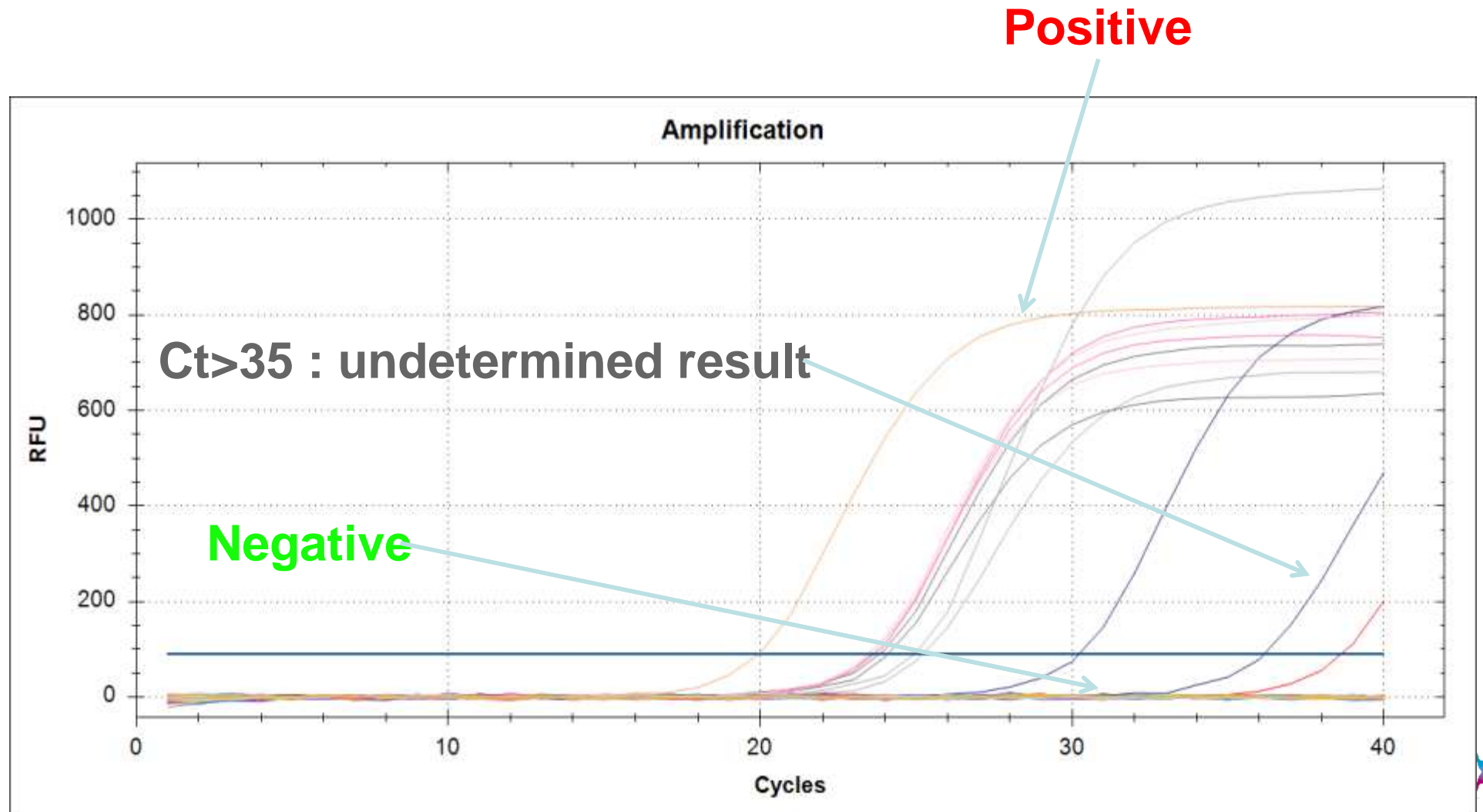
- Cutting and soaking of **0,5 to 1 g of petioles / midribs** in water; grinding (minimum 15 minutes under shaking)
 - Use of **250 µL** of macerate per replicate
 - **asymptomatic / symptomatic : min 2 replicates**
- Centrifugation 20 minutes / 20 000 g → pellet

2. DNA extraction with the QuickPick kit (Bio –Nobile)

3. Real-time PCR Harper *et al.* 2010, 2013 – 2 repeats per replicates

Official French protocol MA 039

Real-time PCR results



Our experience on *Xylella* in France:

- (2000) IF tests for Quarantine on *Vitis*, *Citrus*, *Prunus*
- (2012; 2014; 2015) Import / survey of territory: coffee plants intercepted
- Since 2012: Improvement of detecting scheme
- 2015: French reference method MA039
- **First detection in Corsica in July 2015**

2015/144 First report of *Xylella fastidiosa* in France

The NPPO of France recently informed the EPP0 Secretariat of the first record of *Xylella fastidiosa* (EPP0 A1 List) on its territory. During an official visual inspection, carried out in the framework of the surveillance programme against *X. fastidiosa*, a hedge of 31 desiccated plants of *Polygala myrtifolia* was observed on 2015-07-20, on the island of Corsica. This hedge was located along a wall, near a parking lot, in a commercial area of the municipality of Propriano (Corse du Sud department). Samples were collected and tested (real-time PCR, IF) by the ANSES reference laboratory. The identity of the bacterium was confirmed on 2015-07-22 and serological tests revealed a high concentration of the bacterium in tested plant tissues. The isolation of the bacterium on growing medium is under way and results will probably be obtained within 3 to 4 weeks. It is suspected that infected *P. myrtifolia* plants had been imported from another EU member state, but a study is being carried out to confirm this. All infected *P. myrtifolia* plants were destroyed by burning on 2015-07-23.

In accordance with a contingency plan, official phytosanitary measures were immediately taken to eradicate the disease (e.g. insecticide treatments, plant destruction). An infected area with a radius of 100 m around infected plants has been demarcated, as well as a buffer zone with a 10 km radius. Further studies are being made to determine the extent of the infected area. All known host plants of *X. fastidiosa* located in the infected area, as well as any plant showing suspicious symptoms, are being destroyed. In the infected area, the following host plants were present: *Rosmarinus officinalis*, *Westringia*, *Polygala myrtifolia* and *Olea europaea* (as explained above, only *P. myrtifolia* plants were found to be infected). On 2015-07-22, insects were collected by aspiration and specimens are being identified by the entomology laboratory of Anses. Finally, an information campaign has been launched, in particular to warn passengers that they should not bring plants into Corsica.

The pest status of *Xylella fastidiosa* in France is officially declared as:

Corsica: Transient, actionable, under eradication.

Mainland: Absent, intercepted only.

Source: NPPO of France (2015-07).

INTERNET

Avis aux voyageurs pour la Corse

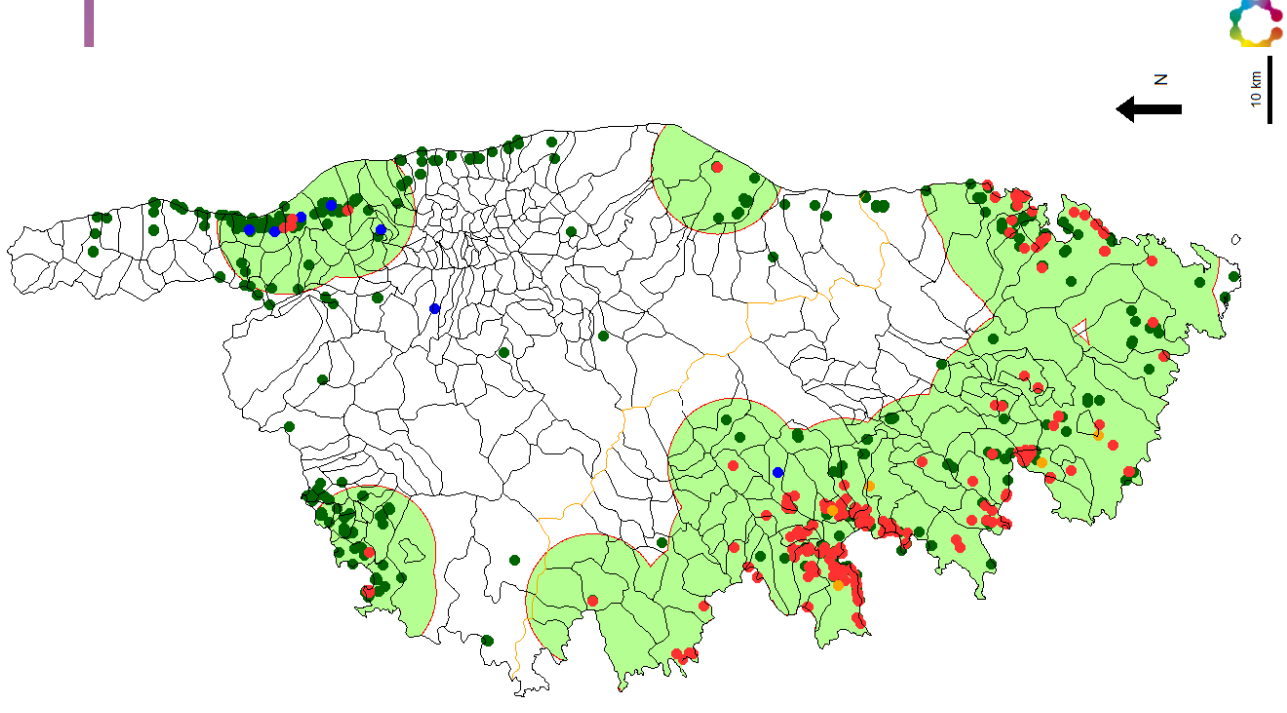
<http://www.gouvernement.fr/partage/4876-corse-avis-aux-voyageurs-transportant-des-vegetaux>

Arrêté du 24 juillet 2015 définissant une zone délimitée vis-à-vis de *Xylella fastidiosa* et les mesures de lutte applicables

http://www.corse-du-sud.gouv.fr/IMG/pdf/Arrete_24_juillet_XF.pdf

Additional key words: new record

Computer codes: XYLEFA, FR



- négatif (coord. GPS)
- négatif (coord. commune)
- positif (coord. GPS)
- positif (coord. commune)

Our experience on *Xylella* in France:

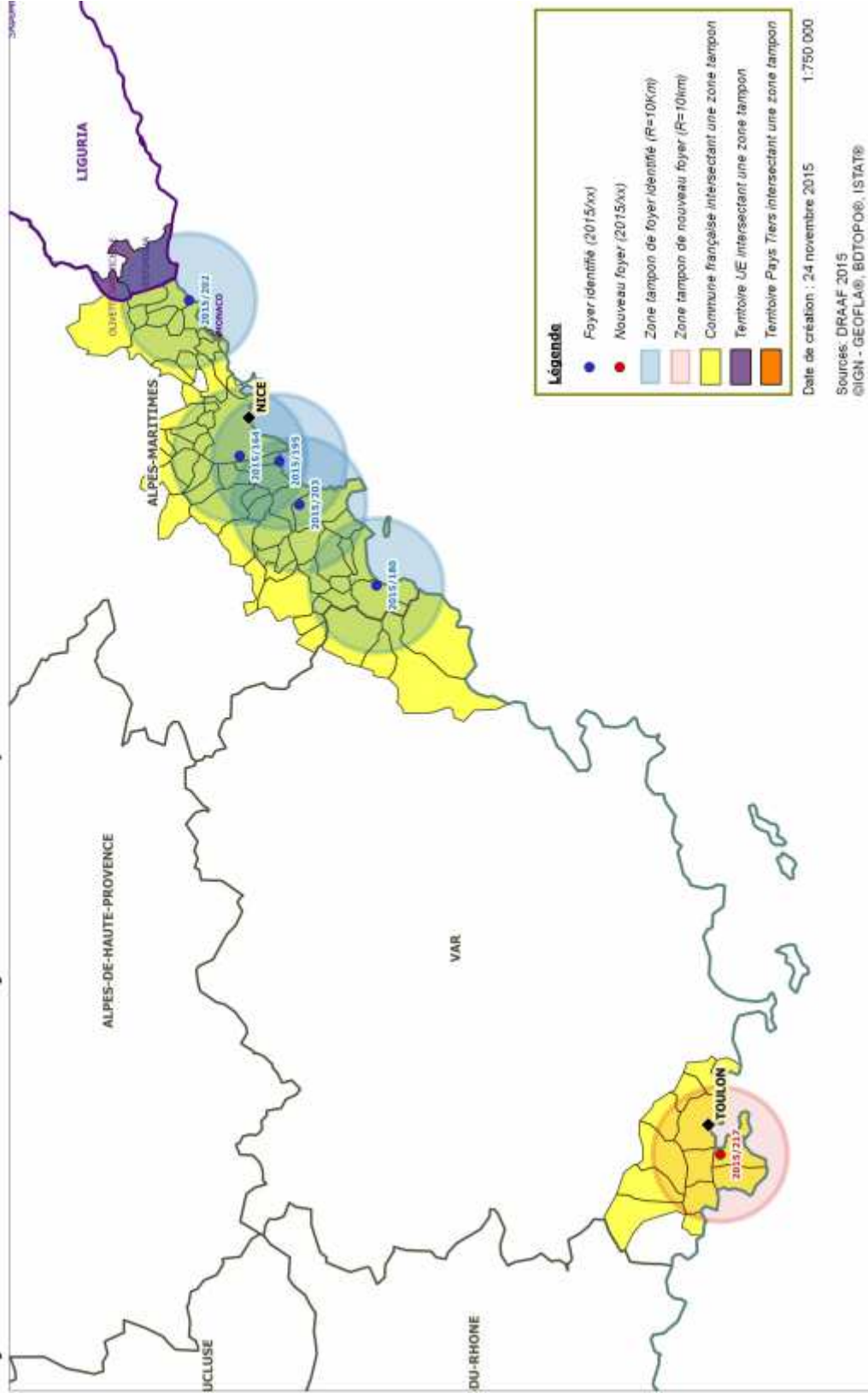
- (2000) IF tests for Quarantine on *Vitis*, *Citrus*, *Prunus*
- (2012; 2014; 2015) Import / survey of territory: coffee plants intercepted in
- Since 2012: Improvement of detecting scheme
- First detection in Corsica in July 2015
- **First detection in South – East of France in October**

2015/180 *Xylella fastidiosa* detected in Alpes-Maritimes, mainland France

In France, *Xylella fastidiosa* (EPPO A1 List) was first found in July 2015 on the island of Corsica, mainly on *Polygala myrtifolia* (EPPO RS 2015/144 and 2015/161). Since this initial record, new information has been published on the Internet by the official authorities and a map showing infected areas (174 foci as of 2015-10-30, and their buffer zones of 10 km radius) can be viewed on the Internet [\[Link\]](#).

In October 2015, *X. fastidiosa* was detected for the first time in Alpes-Maritimes department (Southern France). A first case (*X. fastidiosa* subsp. *multiplex*, same

Xylella fastidiosa - Carte des foyers et zones tampons autour des zones infectées



Host range in Corsica

First alert in Corsica:

Polygala myrtifolia



Host range in Corsica

First alert in Corsica: *Polygala myrtifolia* (sentinel plant)

Next to *Polygala*:

- *Spartium junceum*, *Cytisus racemosus*, *Genista ephedroides*
- *Myrtus communis*
- *Cistus creticus*, *C. monspeliensis*, *C. salviifolius*



Host range in Corsica

First alert in Corsica: *Polygala myrtifolia*

Next to *Polygala*

- *Spartium junceum*, *Cytisus racemosus*, *Genista ephedroides*
- *Myrtus communis*
- *Cistus creticus*, *C. monspeliensis*, *C. salviifolius*
- *Pelargonium* spp.



Host range in Corsica (November 2015)

First alert in Corsica: *Polygala myrtifolia*

Next to *Polygala*

- *Spartium junceum*, *Cytisus racemosus*,
Genista ephedroides
- *Myrtus communis*
- *Cistus creticus*, *C. monspeliensis*, *C. salviifolius*
- *Pelargonium* spp.
- *Hebe pinguifolia*
- *Lavandula dentata* hybr.
- *Rosa* sp.
- *Prunus cerasifera*
- *Acer pseudoplatanus*
- *Rosmarinus officinalis*
- *Quercus suber*



Host range in Corsica (November 2015)

First alert in Corsica: *Polygala myrtifolia*

- *Spartium junceum*, *Cytisus racemosus*,
Genista ephedroides
- *Myrtus communis*
- *Cistus creticus*, *C. monspeliensis*, *C. salviifolius*
- *Pelargonium* spp.
- *Hebe pinguifolia*
- *Lavandula dentata* hybr.; *L. angustifolia*
- *Rosa* sp.
- *Prunus cerasifera*
- *Acer pseudoplatanus*
- *Rosmarinus officinalis*
- *Quercus suber*



Samples tested in 2015 by Anses

2015	
N° of samples received	4648
Samples tested	4559
Samples refused	89
Positive samples	485 (10,4%)

<i>Polygala</i>	1234
<i>Olea spp</i>	589
<i>Citrus spp</i>	221
<i>Rosmarinus spp</i>	195
<i>Nerium oleander</i>	181
<i>Coffea spp</i>	127
<i>Quercus spp</i>	123
<i>Vigne</i>	109
<i>Myrtus</i>	91
<i>Ficus spp</i>	73
<i>Prunus spp</i>	68

Polygala sp.

Total of samples	1234
Positive	399 (32%)
Negative	811
Corsica	909
Out of Corsica	325

Coffea

Total of samples	127
Positive	15 (12%)
Negative	109

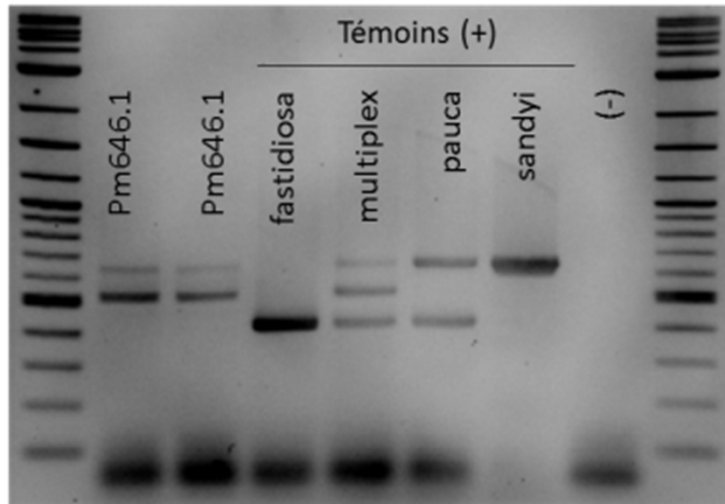
« *Genista* » family

Total of samples	42
Positifs	9 (21%)
Négatifs	33

Pelargonium spp.

Total of samples	70
Positive	16 (23%)
Negative	50

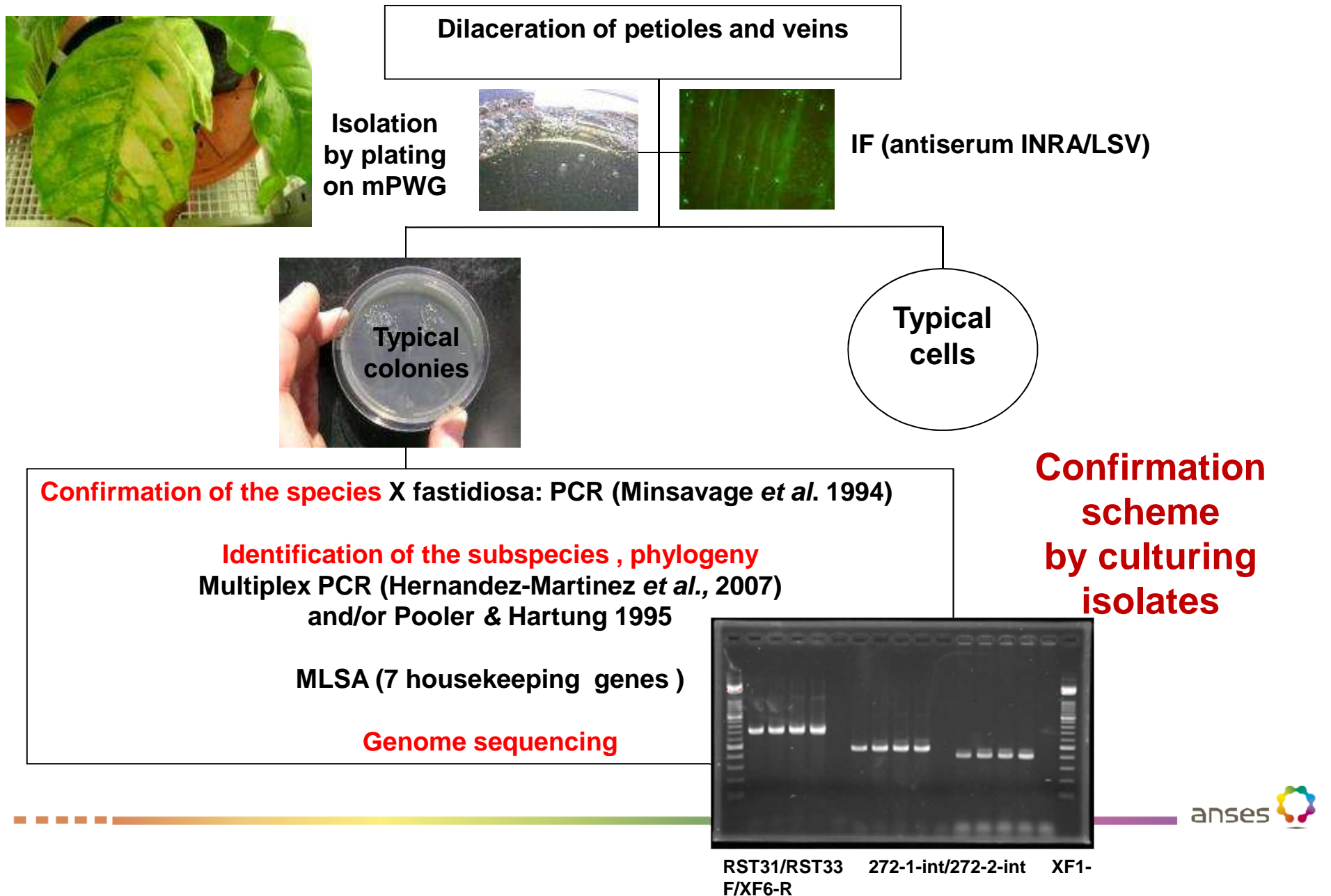
Identification of the X.f subspecies on plant samples



Multiplex PCR Hernandez-Martinez *et al.* (2006):

- subsp. *fastidiosa*
- subsp. *multiplex* with 2 profiles
 - ALSI :2 amplicons
 - ALSII: 3 amplicons
- subsp. *sandyi*
- subsp. *pauca*
(+ PCR Pooler and Hartung)

22 strains of *X fastidiosa* isolated from France



Conclusion



- **According to our experience,**
 - **Outbreaks in France are different of Italian**
 - **Introduction of plants from infected countries: very big threat for Europe**
 - **Survey of imported plants and of territory is necessary to prevent and protect agriculture**
- **Using of sensitive and reliable tools for early detecting : big challenge for EPPO members**
- **Next step: working on insect detection**



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Thank you for your attention

