Situacion de *Xylella fastidiosa* en Francia: interceptiones, prospecciones, metodologia de analisis y diversidad de cepas

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Anses - Plant health laboratory – Angers – Francia

Symposio:''*Xylella fastidiosa*: una especie compleja causante de enfermedades emergentes en Europa''
Palencia , 20 de septiembre de 2016 (GEDDI-SEF)

French agency for food, environmental and occupational health safety

General Directorate

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G. Lasfargues, Deputy Director General for Scientific Affairs
M. Mettendorff, Deputy Director General for Resources
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- Financial Affairs Department
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- Legal Affairs Unit

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3 core activities

2 Cross-functional Activities

- Department of Information, Communication and Dialogue with Society
- European and International Affairs Department

Veterinary Medicinal Products

- French Agency for Veterinary Medicinal Products

Risk Assessment

- Risk Assessment Department
- Regulated Products Department
- Research and Science Watch Department

Reference and Research Laboratories

- Department for Laboratory Affairs
- Dozulé Laboratory for Equine Diseases
- Fougères Laboratory
- Lyon Laboratory
- Maisons-Alfort Laboratory for Animal Health
- Food Safety Laboratory – Maisons-Alfort and Boulogne-sur-Mer sites
- Nancy Laboratory for Rabies and Wildlife
- Nancy Laboratory for Hydrology
- Niort Laboratory
- Ploufragan/ Plouzané Laboratory
- Sophia-Antipolis Laboratory
- Plant Health Laboratory

11 laboratories, conducting reference & research activities on food safety, animal health & plant health

NRL

Risk assessment through collective expertise involving external experts

Different kinds of groups of experts (includes 16 Expert committees)

Around 350 requests/year

Opinions published on the website

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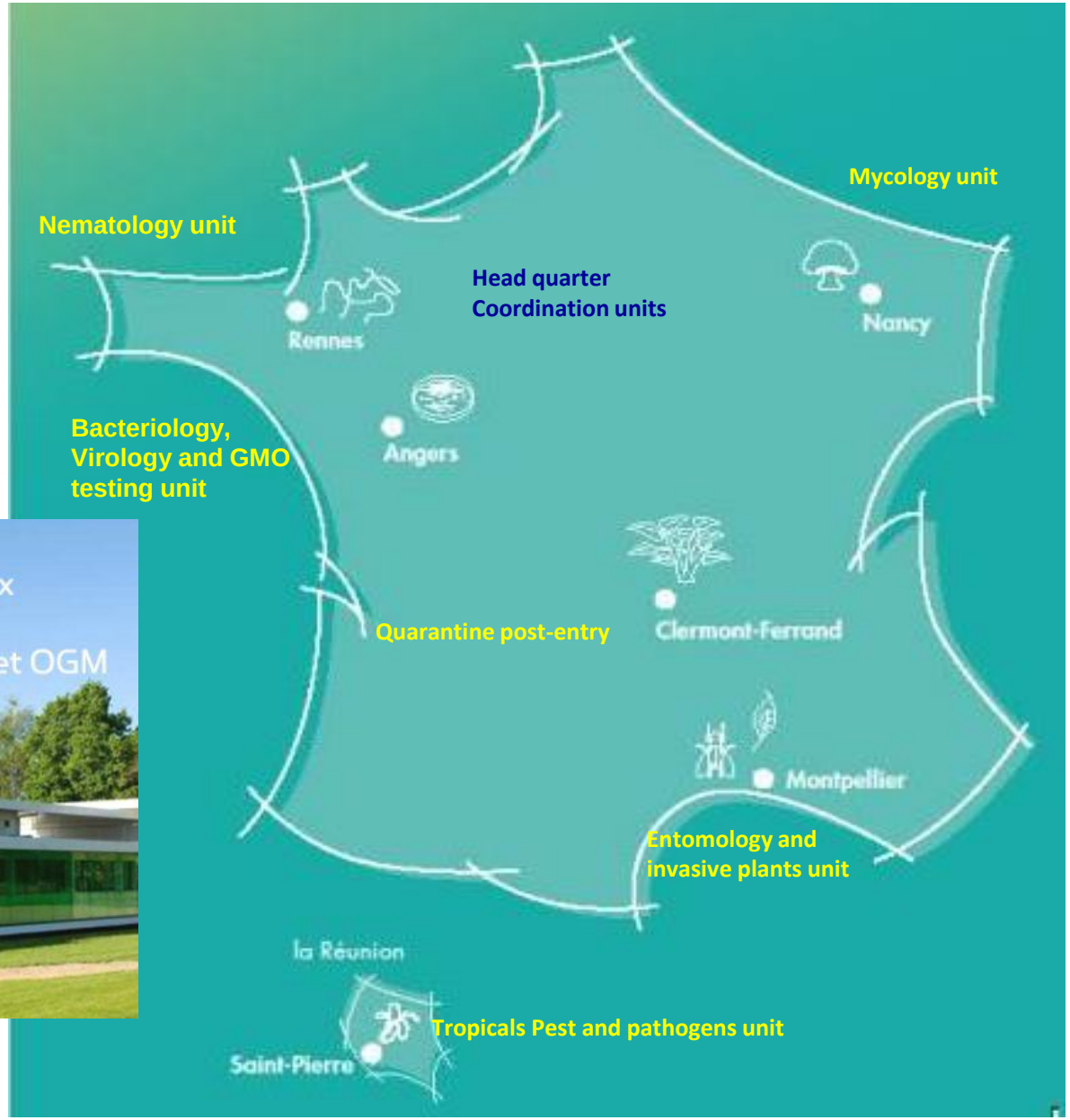
Plant health laboratory



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Bacteriology, virology, GMO unit
National reference laboratory - Angers

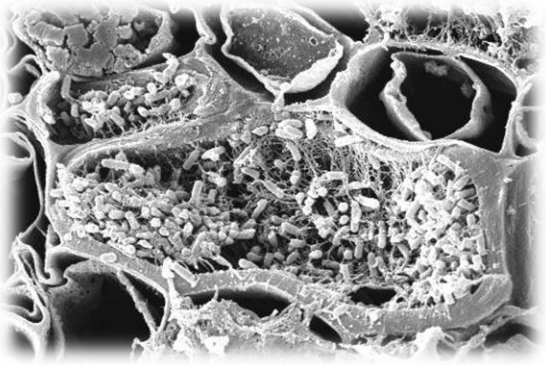
NRL- Bacteria

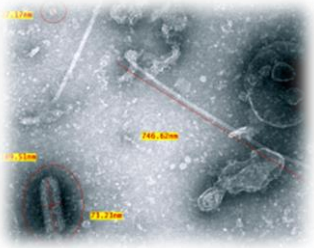
NRL- Phytoplasma

NRL- Virus

NRL- Viroïdes

NRL- GMO





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Key issues in the framework of reference missions

The intensification and the globalization of the international trade contribute to increasing risks for distribution of harmful organisms to plants from one region of the world to another.

The NRL has to:

- develop reliable methods of analysis to improve the monitoring and control of the sanitary state of the territory,
- develop the best tools for early detection of new harmful organisms and to be able to detect any emergence of harmful species,
- coordinate the network of laboratories in charge of official analysis by training organizing proficiency testing.

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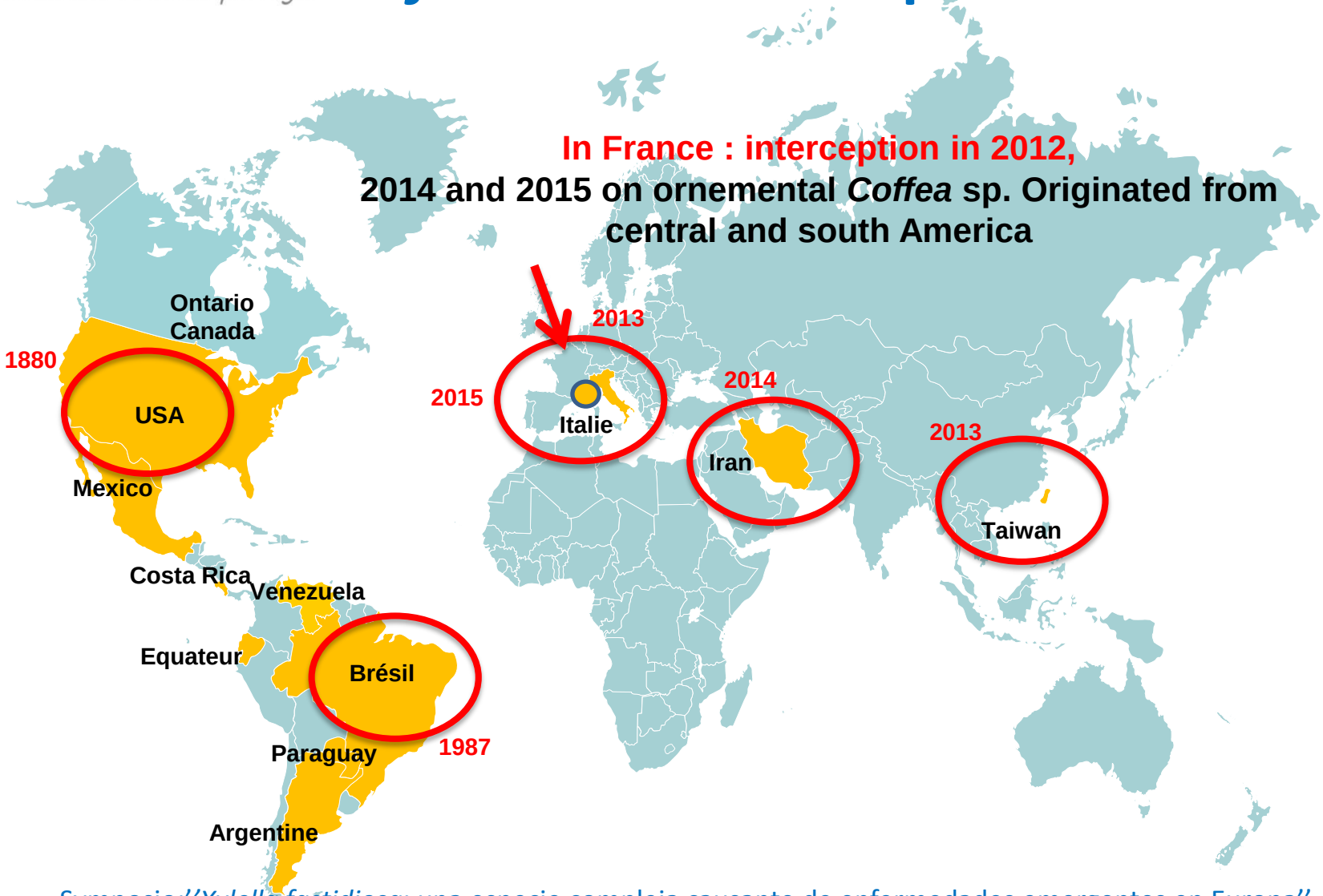
Facing an emergence of pest like *X. fastidiosa* in Europe

In France : interception in 2012,
2014 and 2015 on ornemental *Coffea* sp. Originated from central and south America



First detection on coffee trees imported

2012: France

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

In April 2012, *Xylella fastidiosa* (EPPO A1 List) was identified on *Coffea* spp. plants kept under confinement by a breeding company which regularly imports plant cuttings, in particular from South America where the bacterium is known to occur. The bacterium was detected during tests carried out at the initiative of the breeding company itself. 84 samples collected from 84 plants had been sent to a private laboratory. Out of the 84 samples, 5 tested positive by ELISA. These positive samples were tested by PCR for confirmation by the French official reference laboratory. The presence of *X. fastidiosa* was confirmed in 1 sample. The French NPPO then collected additional samples from 20 plants and the presence of the bacterium was finally confirmed in 3 plants. Strict eradication measures were taken: all 84 plants kept in the containment facility were destroyed, the

2014: The Netherlands, Italy, Germany

DISEASE NOTE

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

M. Bergsma-Vlami, J.L.J. van de Bilt, N.N.A. Tjou-Tam-Sin, B.T.L.H. van de Vossenberg and M. Westenberg

2015: Switzerland

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

Following the detection of *Xylella fastidiosa* (EPPO A1 List) by the Dutch NPPO in imported *Coffea* plants, tracing-forward studies were conducted in Switzerland on re-exported lots. In September 2015, the presence of *X. fastidiosa* was confirmed in 4 *Coffea* plants

Test methods and method validation

"**Validation of methods (5.4.5):**
Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled.

Definition of intended use is needed (which purpose), and proofs should be given.

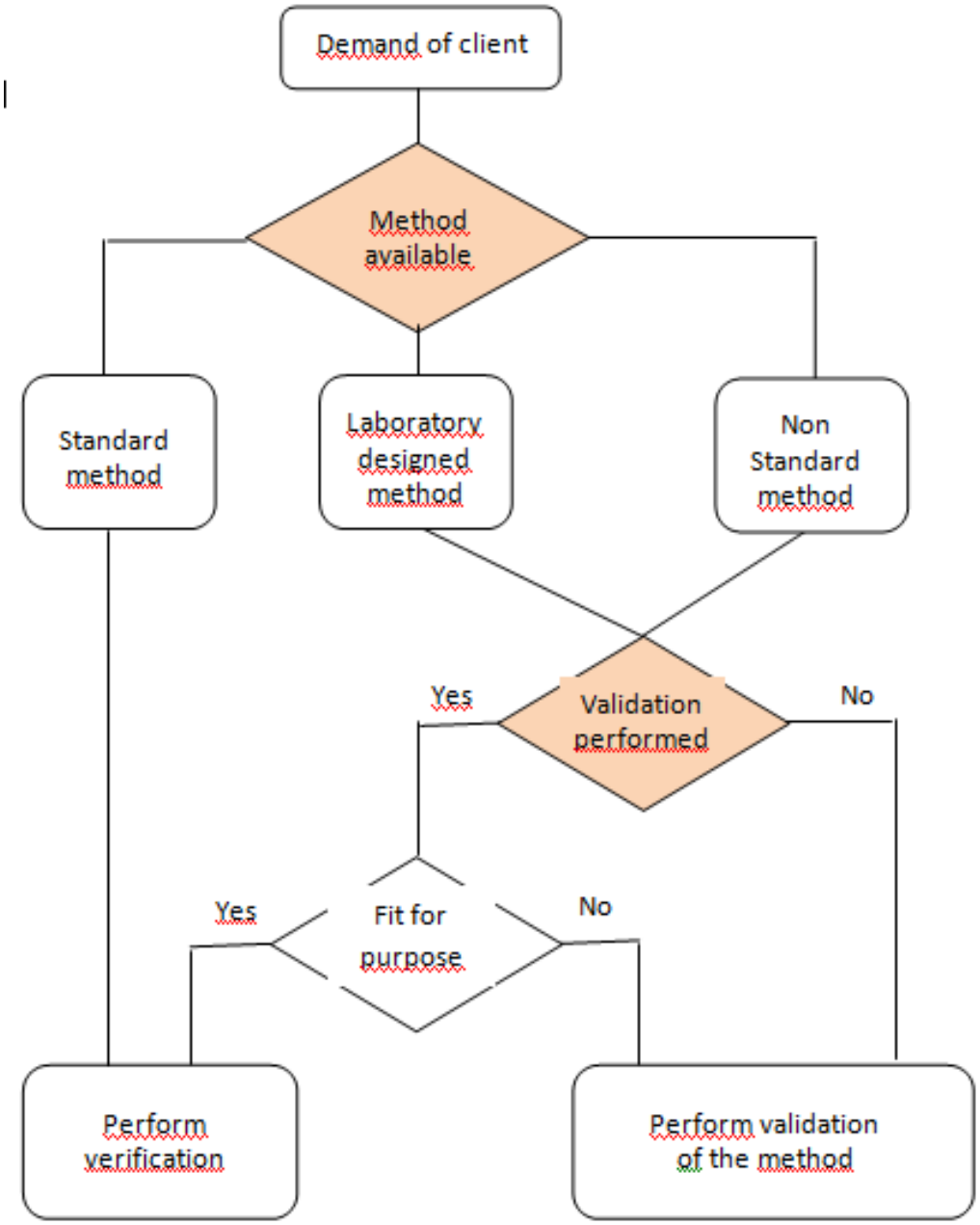
How to perform validation of methods:
Validation must be performed in the case of use of :
-Non standard methods,
-Home designed methods
-Standard methods uses outside their normal use

Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity EPPO PM 7/98

How to perform validation:
-using reference standards or reference materials;
-by comparison of results achieved with other methods;
-by interlaboratory comparisons;
-by systematic assessment of the factors influencing the result;

Test methods and method validation

Method selection process



Test methods and method validation

Evaluation of the performance criteria

Diagnostic sensitivity: Proportion of infected/infested samples testing positive compared to results from an alternative test (or combination of tests).

Sensitivity = true positives/(true positives + false negatives);

Specificity: Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from an alternative test (or combination of tests).
Specificity = true negative results/(true negatives + false positives);

Accuracy: closeness of agreement between a test result and the accepted reference value;

Repeatability: Level of agreement between replicates of a sample tested under the same conditions;

Reproducibility: Ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (time, persons, equipment, location, etc);

Limit of detection (analytical sensitivity): Smallest amount of target that can be detected reliably.

Test methods and method validation

Evaluation of the performance criteria by internal validation

		Reference results	
		Positive	**Negative**
Test result	**Positive**	PA (Positive agreement)	PD (Positive deviation)
	Negative	ND (Negative deviation)	NA (Negative agreement)

Test methods and method validation

Performance statistics

Performance criteria	Calculation
Accuracy (AC)	$AC = \frac{\sum PA + \sum NA}{N}$
Sensitivity (SE)	$SE = \frac{\sum PA}{N^+}$ *Comments: the result of the calculation (1-SE) gives the rate of false negatives obtained by the laboratory.*
Specificity (SP)	$SP = \frac{\sum NA}{N^-}$ *Comments: the result of the calculation (1-SP) gives the rate of false positives obtained by the laboratory.*
Repeatability (DA) (accordance)	DA = Percentage chance of obtaining the same result (positive, negative or indeterminate) from two identical samples analyzed in the same laboratory. *Calculation adapted from **ISO 16140.***

Test methods and method validation

Performance statistics by external validation: ring test

Example : For each laboratory, calculation of sensitivity, specificity and accuracy

Sample	Assigned value	Labortory result	Interpretation of laboratory result	Calculation of the performance statistics of the laboratory
A	+	+	PA	
B	+	+	PA	
C1	+	-	ND	
C2	+	-	ND	
C3	+	+	PA	
D	+	+	PA	
E	+	+	PA	
F1	+	+	PA	
F2	+	-	ND	
F3	+	-	ND	
G	-	-	NA	
H	-	-	NA	
I	-	-	NA	
J	-	-	NA	
K	-	-	NA	
L	-	-	NA	
M	-	ind	PD	
N	-	-	NA	
O	-	-	NA	
P	-	-	NA	

$$SE = \frac{\sum PA}{N^+}$$

= 6/10 x 100%

= 60%

$$SP = \frac{\sum NA}{N^-}$$

= 9/10 x 100%

= 90%

$$AC = \frac{\sum PA + \sum NA}{N}$$

= 15/20 x 100%

= 75%

Facing an emergence of pest like *X. fastidiosa*: validation of method

➢ **Need to rely on diagnosis methods with performant detection threshold to detect latent infections**

Role of the national reference laboratory : to select, evaluate and validate methods (molecular tools)

➢ First step : selection based on scientific publications

(methods able to detect all *X.f.* subsp.)

- PCR (Firrao & Bazzi, 1994)
- PCR (Pooler & Hartung, 1995)
- PCR (Minsavage *et al.*,1994)

- Real–Time PCR (Harper *et al.*, 2010, erratum 2013)

- LAMP (Harper *et al.*, 2010)

Note: ELISA not selected / not adapted to early detection (low sensitivity)

Facing an emergence of pest like *X. fastidiosa*: validation of method

➢ Second step: evaluation of methods on pure cultured strains

Inclusivity

Capacity of a method to detect all the target strains

• 15 strains of *X. fastidiosa* – 4 subspecies

Results :
100% for all the methods

Exclusivity

Capacity of the method to not give false positive results with non-target strains

• 29 non-target strains :

Genetical proximity 16 *Xanthomonas* spp.

Same host plants 1 *Xylophilus ampelinus*
1 *Ca. Liberibacter asiaticus*
1 *Ca. L. africanus*
6 *Coffea* spp. saprophytes and
4 *Citrus sinensis* saprophytes

Results :
100% for all the methods

Detection threshold

Enumeration by microscopy IF
DNA extraction by thermal lysis

Best results : **Real-Time PCR Harper (2010) > PCR Minsavage (1994)**

Facing an emergence of pest like *X. fastidiosa*: validation of method

➢ Third step: evaluation on spiked plant samples

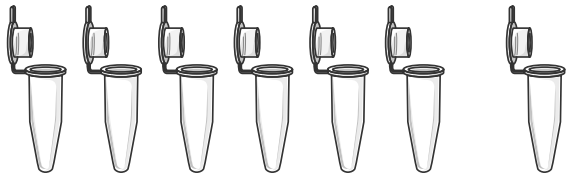
•5 plant species: 2 *Coffea* sp., grapevine, sweet orange and peach tree

Petioles and midribs → Crushing with Qiagen lysis buffer 1g/5mL

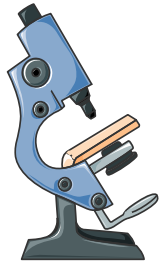
•4 *X.fastidiosa* strains

Tenfold dilution of bacterial suspension

Spiking with 5µL / tube + 395 µL /tube



(bact/mL)	1,25 .10^5	1,25 .10^4	1,25 .10^3	1,25 .10^2	1,25 .10^1	1,25 .10^0	NEG



Enumeration by microspcopy IF

X 3 assays / pair plant / strain
X 3 dilution series / assay

= 63 samples per plant species

DNA extraction with DNeasy Plant Mini Kit® (Qiagen)

↓

Amplification

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Facing an emergence of pest like *X. fastidiosa*: validation of method

➢ Third step: evaluation on spiked plant samples

Definitions:

•Diagnosis sensitivity: proportion of infected sample giving a positive result

•Repeatability: level of agreement between replicates of a sample tested under the same conditions

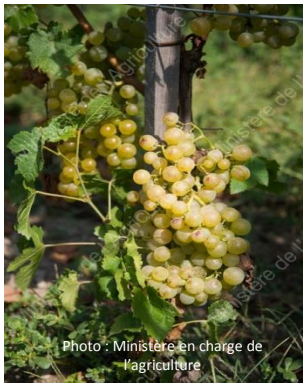
→ Best results with the Real-Time PCR Harper *et al.*, 2010

Criteria	Real-Time PCR Harper *et al.*, 2010	End point PCR Minsavage *et al.*, 1994
Diagnosis sensitivity (%)	77 to 97	65 to 82
Repeatability (%)	93 to 98	80 to 98
Detection threshold* (bact./g plant tissues)	5.10^2 to 5.10^3	5.10^2 to 5.10^4

→ But variability in the results according to plant matrices

➢ Fourth step: inter-laboratories evaluation

- **7 participating laboratories** (France, Italy, UK, New-Zealand, Netherland)
- **5 spiked matrices**



Samples	Plant and strain	Concentration (bact./mL)	Expected result
1	Coffee (Coffea arabica)	3,4E+04	Positif
2	X. f. subsp. pauca* CFBP8072	3,4E+03	Positif
3		3,4E+02	Positif
4		0 (matrice saine)	Négatif
5	Olive tree (Olea europaea)	2,8E+06	Positif
6		2,8E+05	Positif
7		2,8E+04	Positif
8	X. f. subsp. multiplex ATCC35871 (CFBP8173)	0 (matrice saine)	Négatif
9	Grapevine (Vitis vinifera)	1,1E+06	Positif
10		1,1E+05	Positif
11		1,1E+04	Positif
12	X. f. subsp. fastidiosa ATCC35879 (CFBP7970)	0 (matrice saine)	Négatif
13	Orange tree (Citrus sinensis)	3,1E+03	Positif
14		3,1E+02	Positif
15		3,1E+01	Positif
16	X. f. subsp. pauca* CFBP8072	0 (matrice saine)	Négatif
17	Peach tree (Prunus persica)	2,8E+04	Positif
18		2,8E+03	Positif
19		2,8E+02	Positif
20	X. f. subsp. multiplex ATCC35871 (CFBP8173)	0 (matrice saine)	Négatif

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Facing an emergence of pest like *X. fastidiosa*: validation of method

➢ Fourth step: inter-laboratories evaluation

➡ Confirmation of the performances of the method

Performance criteria	DNeasy® extraction + Real-time PCR Harper *et al.*, 2010
Diagnostic sensitivity	97%
Specificity	100%
Repeatibility	91%
Reproducibility	84%
Limit of detection (with detection probability of 100%)	Depending of matrices: ➢ **orange tree: 3.10^2 bact/mL** ➢ coffee tree: 3.10^4 bact/mL ➢ peach tree: 3.10^4 bact/mL ➢ **olive tree: 3.10^5 bact/mL** ➢ **grapevine: 3.10^6 bact/mL**

➡ Necessity to improve the limit of detection on complex matrices

Facing an emergence of pest like *X. fastidiosa*: validation of method

- 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)
Sensitivity	52,8	80,6	79,2
Specificity	100	100	100
Repeatability	97,8	97,8	96,6
Limit of detection	Orange≈ 10^2 bact./mL Grapevine≈10^6bact./mL Olive≈10^5 bact./mL	Orange≈ 10^2 bact./mL Grapevine≈10^3bact./mL Olive≈10^4 bact./mL	

Improvement of the limit of detection

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Facing an emergence of pest like *X. fastidiosa*: validation of method

Performance criteria of the method	DNA extraction: QuickPick™ + KingFisher™ mL Amplification Harper *et al.*, 2010 (Erratum 2013)		
Inclusivity	100% (23 targeted strains representing the 4 major subsp.)		
Exclusivity	100% (29 non-targeted strains: *Xylophilus, Xanthomonas, Liberibacter,* sap saprophytes)		
	Plant matrix (10^2 - 10^5 bact./mL, 15 samples)		
Matrix	**Orange tree** *+ X. f. pauca* CFBP 8072	**Grappe** *+ X. f. fastidiosa* CFBP 7970	**Olive tree** *+ X. f. multiplex* CFBP 8173
Sensitivity	100%	94%	67%
Specificity	100%	100%	100%
Repeatability	100%	96%	100%
Reproductibility	98%		
Detection threshold (with detection probability of 100%)	≈ 10^2 bact./mL	≈ 10^3 bact./mL	≈ 10^4 bact./mL

The presence of inhibitors (polyphenol, secondary metabolites,…) in some plant matrix (olive tree, oak) limits its sensitivity. → **To be aware of false negative in Olive tree**

Facing an emergence of pest like *X. fastidiosa*: detection

- The most performant method for early detection of *Xylella fastidiosa* in various matrices: DNA extraction with QuickPick™ (Bio-Nobile)+ Real-Time PCR (Harper *et al.*, 2010) → **French reference and official method**: MA039 .

https://www.anses.fr/fr/system/files/ANSES_MA039_Xylella fastidiosa_final.pdf

1 sample = 5 to 100 petioles = 0.5 to 1 g
grounding in sterile water (5 mL/g)

250 µL of macerate per replicate

2 DNA extractions / sample

- Automated DNA extraction
QuickPick™ Plant DNA kit (Bio-Nobile)
and KingFisher™ mL

4 amplifications

- Real-time PCR Harper *et al.*, 2010

Facing an emergence of pest like *X.fastidiosa* - First detection in Corsica – July 2015

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

The NPPO of France recently informed the EPPO Secretariat of the first record of *Xylella fastidiosa* (EPPO 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 ANSESreference 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

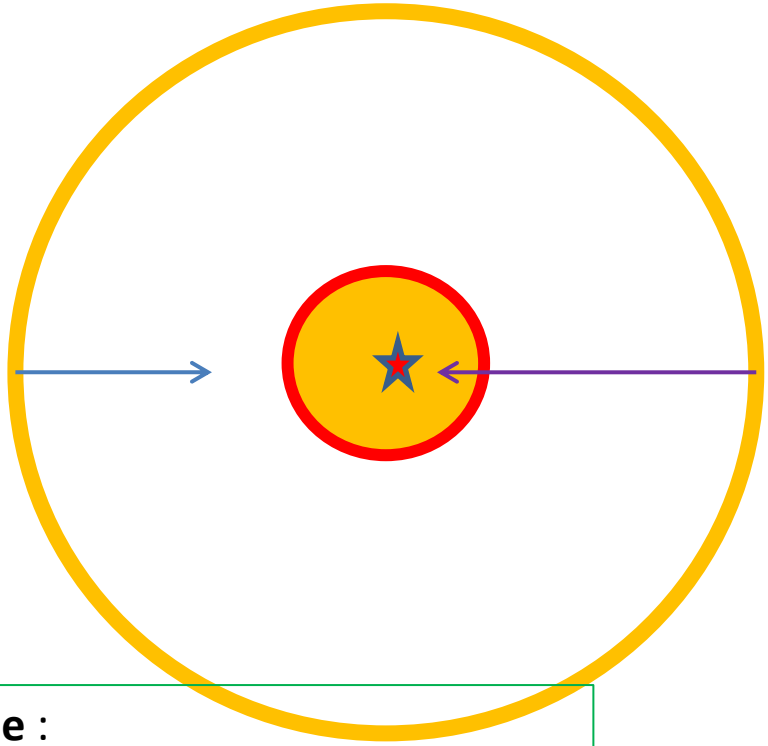
Additional key words: new record **Computer codes:** XYLEFA, FR

Polygala myrtifolia

Emergency plan and survey in infected zone

Buffer zone (Diameter 10 km):

- Visual observation of symptoms on specified plants
- Transfer of plants within this zone submitted to rules



In the infected zone (Diameter 200 m):

- vector treatment
- sampling of specified plants
- destruction (priority to Polygala and any plant with symptom)
- no plantation of host plants

In all delimitated zone :

- no circulation of specified plants : in particular, no specified plant for planting goes out the delimitated zone (including from nurseries)
- Surveillance and monitoring

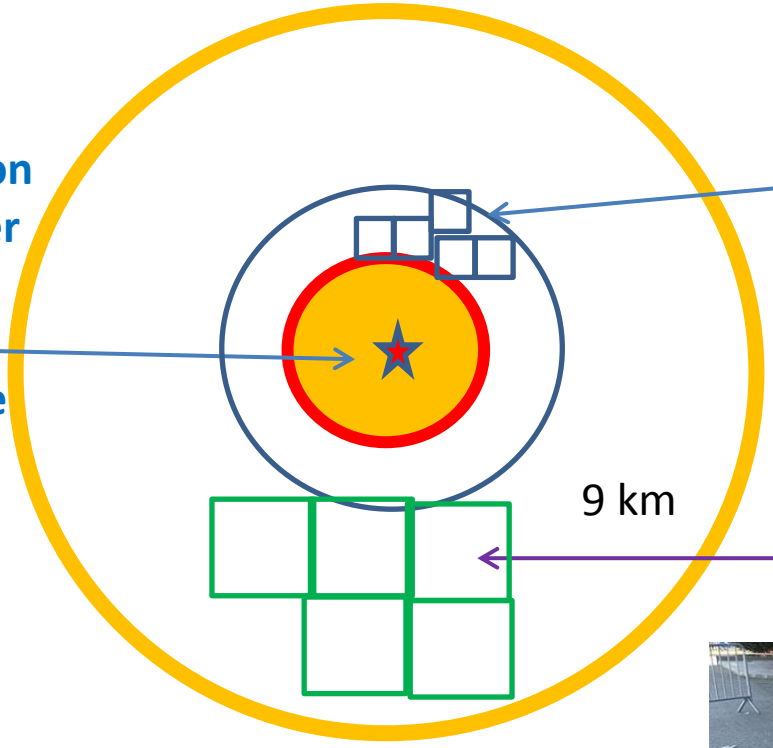
Source INRA

Emergency plan and survey in infected zone

New requirements

Objective: Identification of infected plants other than plants index

Representative sample of 100 plants of the specified plants (non-host) in the infected area of 100 m radius (~ 3ha)



2 visual observation/year of squares of 100mX100m

2 visual observation/year of squares 1kmX1km

Source FREDON/SRAL Corsica

Official and non official survey out of infected zone

Phytosanitary inspections within Officiel survey of regulated pest and specific to *Xylella fastidiosa* :

- assess the phytosanitary status and detect infections in susceptible plants, including those of economic importance or located in region with risk (south of France).

- Inspections non specific to *Xylella fastidiosa* .

- Inspection within framework of **(EPP)**:

- Control of retailers (garden centers, nurseries, etc.)

Visual inspections + systematic sampling on symptomatic plants

Special case: sampling on asymptomatic plants

- mother plants of host plants (5 samples / host specie)
- specified plants originating from a third country contaminated (5 samples / specie specified)

45 plants host of subsp multiplex, *pauca et fastidiosa* of *Xylella fastidiosa to survey*

Productions at risk

- **Fruit trees**
- **Grape Vines**
- **Ornementals**
- **Aromatic, medicinal and condiment plants**

Official and non official survey out of infected zone

- **Non official scheduled survey**

EU Decision 2015/789

Network of biological survey of territory : Grape Vine, *Citrus, Prunus,* Oaks, Platanus, Olive Tree

Forest Services : Different oaks (*Quercus suber, Quercus ilex*)

- **Phytosanitary controls on imports and exports**

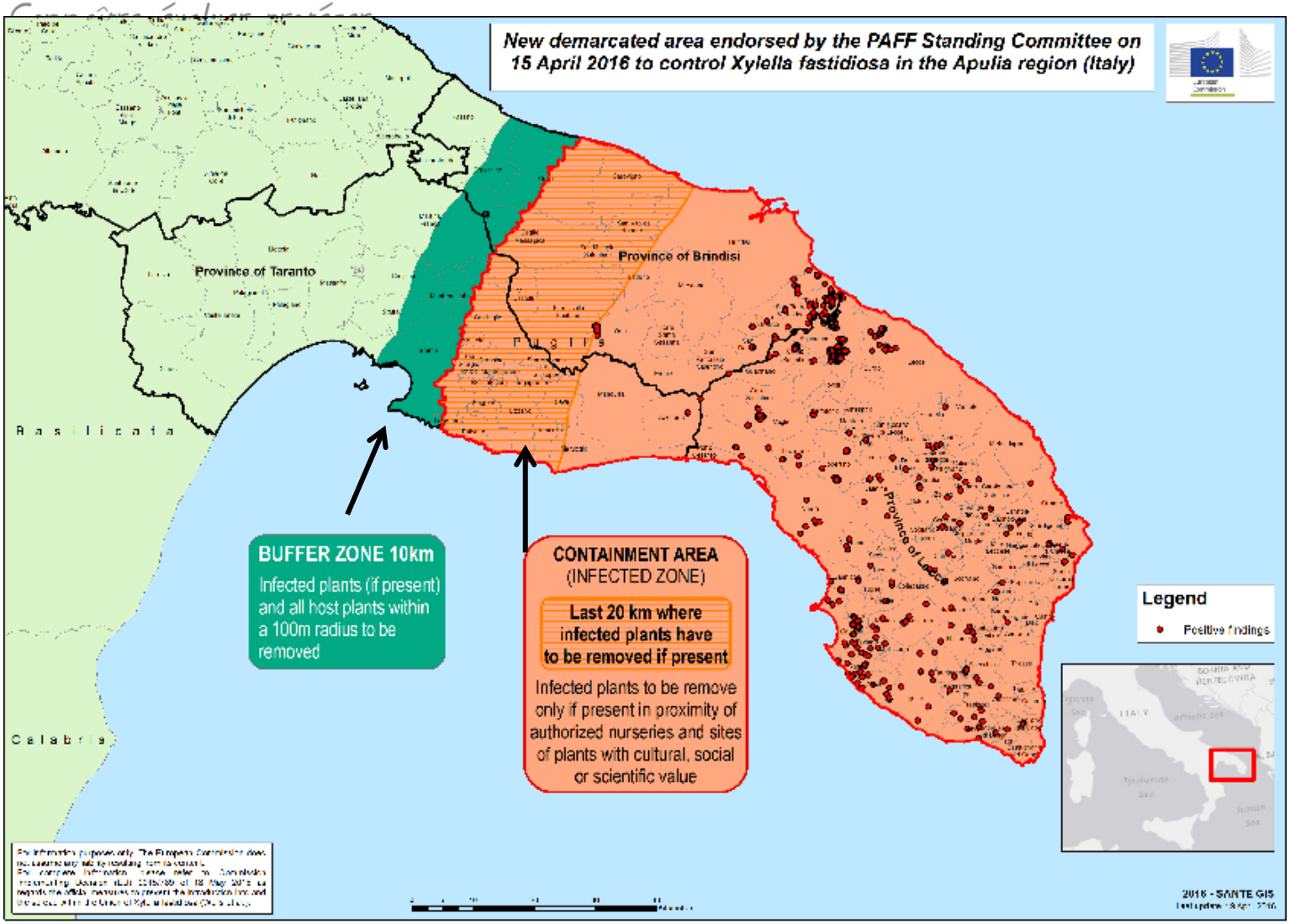
Coffea prohibited from Costa Rica/Honduras

- **The event-monitoring :** aims at **early detection** of infection in the country.

It is based on existing monitoring devices (regular monitoring within the epidemiological surveillance network), including other regulated pests
(Phytoplasma of the grapevine Flavescence dorée or PPV).
Observers of this network trained to recognize the symptoms, are encouraged to strengthen their vigilance during diagnostic susceptible plants at X. fastidiosa.

- **Reporting by individuals to official services**

Emergency plan and survey (Italy)



Results of survey in France September 2016

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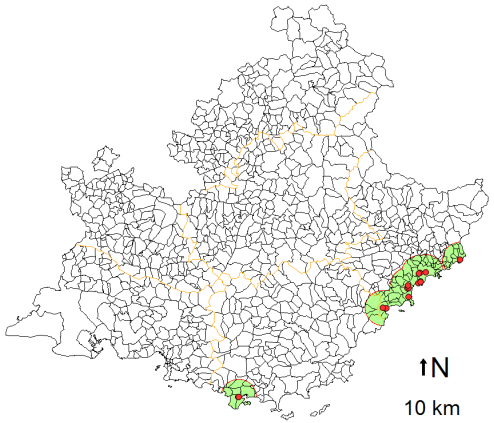
Provence, Alpes, Côte d'azur

14 foci

Zones tampons de 10 km autour des zones infectées par Xylella fastidiosa

Données entre le 21/07/2015 et le 06/09/2016



Zones délimitées
positif. n=45

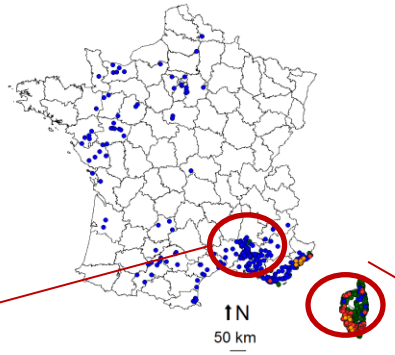
Detected from

- *Polygala myrtifolia and sp*
- *Spartium junceum*
- *Lavandula angustifolia*

Localisation de tous les végétaux prélevés

Données entre le 21/07/2015 et le 06/09/2016

INRA SCIENCE & IMPACT



Coffea sp. intercepted: 21/147

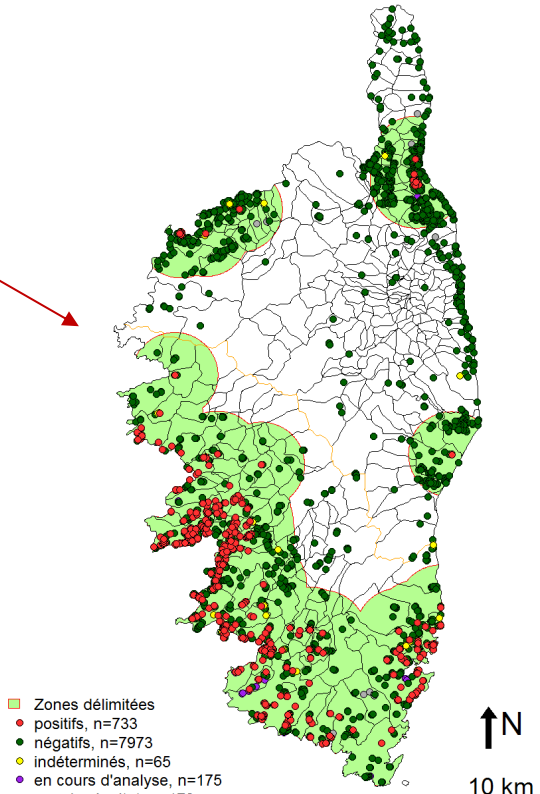
Rate of contamination: 14,3%

Corsica

282 foci

Zones délimitées et localisation de tous les végétaux prélevés

Données entre le 21/07/2015 et le 06/09/2016



Detected from 28 plant species

Symposio:''*Xylella fastidiosa*: una especie compleja causante de enfermedades emergentes en Europa''
Palencia , 20 de septiembre de 2016 (GEDDI-SEF)

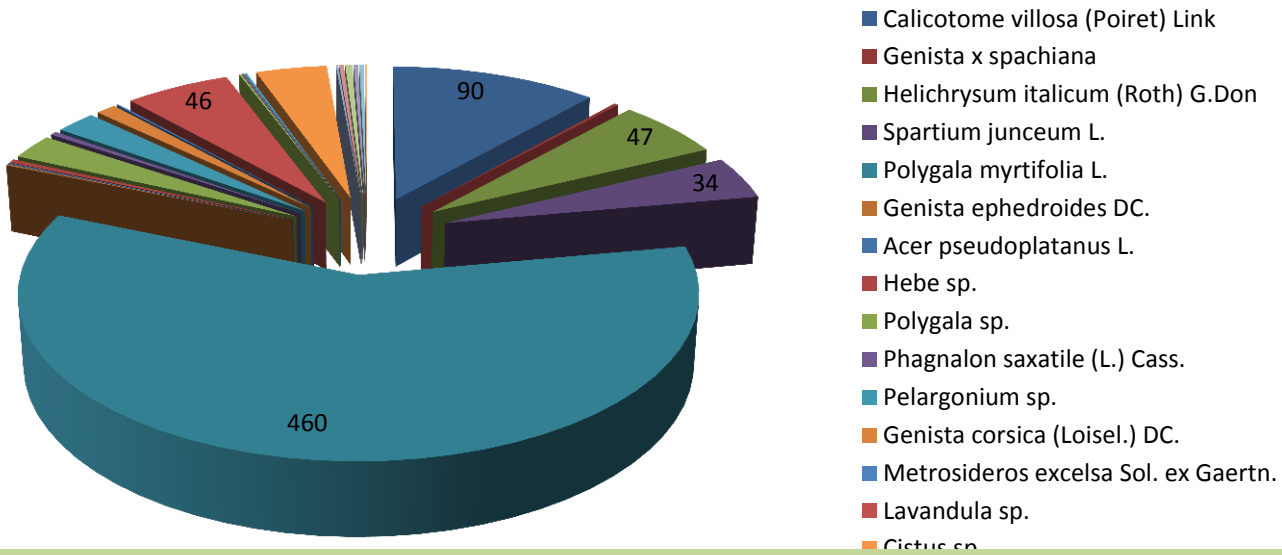
Results of survey in France

Host species sampled	Total samples	Positive samples	Negative samples	Undeterm samples	% positive samples
Calicotome villosa (Poiret) Link	273	90	164	8	33,0
Genista x spachiana	8	2	6	0	25,0
Helichrysum italicum (Roth)	206	47	146	2	22,8
Spartium junceum L.	158	34	115	0	21,5
Polygala myrtifolia L.	2202	460	1674	13	20,9
Genista ephedroides DC.	5	1	4	0	20,0
Acer pseudoplatanus L.	6	1	4	0	16,7
Hebe sp.	21	3	18	0	14,3
Polygala sp.	155	20	124	9	12,9
Phagnalon saxatile (L.) Cass.	24	3	21	0	12,5
Pelargonium sp.	158	18	133	4	11,4
Genista corsica (Loisel.) DC.	80	9	70	1	11,3
Metrosideros excelsa.	11	1	8	0	9,1
Lavandula sp.	607	46	537	3	7,6
Artemisia arborescens (Vaill.) L.	14	1	12	0	7,1
Rosa x floribunda	19	1	17	0	5,3
Prunus cerasifera Ehrh.	20	1	19	0	5,0
Cistus sp.	721	31	647	6	4,3
Coronilla valentina L.	25	1	24	0	4,0
Asparagus acutifolius L.	125	2	119	1	1,6
Myrtus communis L.	301	3	287	2	1,0
Quercus suber L.	270	2	262	3	0,7
Rosmarinus officinalis L.	663	2	645	0	0,3
Quercus ilex L.	449	1	428	4	0,2
TOTAL	**6521**	**780**	**5484**	**56**	**12,0**

Results of survey in France

Part of host plants contaminated by *X.f*

Number of positive samples in plant host



- On **12076 samples analysed**, 1/3 symptomatic, 1/3 asymptomatic, 1/3 no information
- **12% of plant host positive** at monitoring test (except coffea) (60% *Polygala myrtifolia*)
- **6,5% positive results on total plant sampled** (3% on symptomatic, 3% on asymptomatic samples)
- **More than 50% of samples identified with susbsp multiplex** (other part indertermined or under investigation)

anses
agence nationale de sécurité sanitaire
alimentation, environnement, travail
Connaître, évaluer, protéger

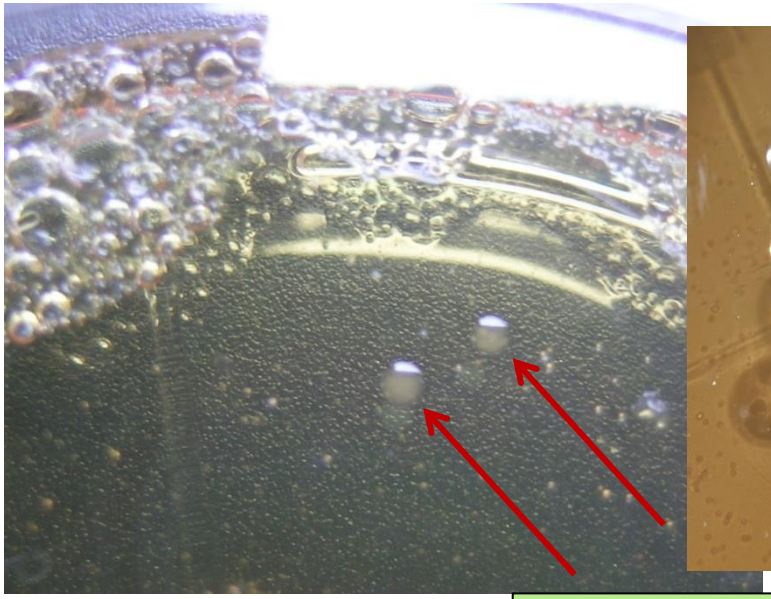
Results of survey in France

Plants never found infected in France

	# samples
Olea europaea	1359
Oleander spp.	636
Citrus sp.	417
Vitis sp.	155

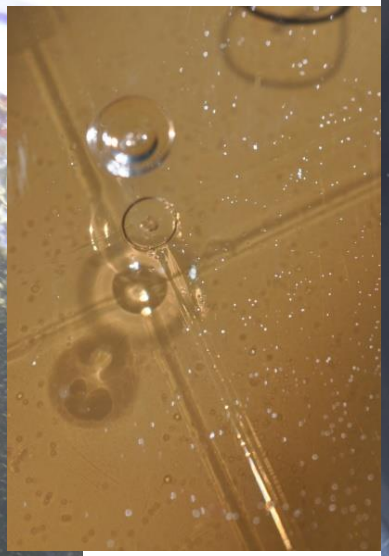
Identification of isolates of *Xylella fastidiosa*

On PWG modified after 20 days (*Coffea arabica)*



Source : Anses LSV

Isolation



Diameter of colonies around 2 mm

On BCYE medium After 10 days (*Coffea arabica)*

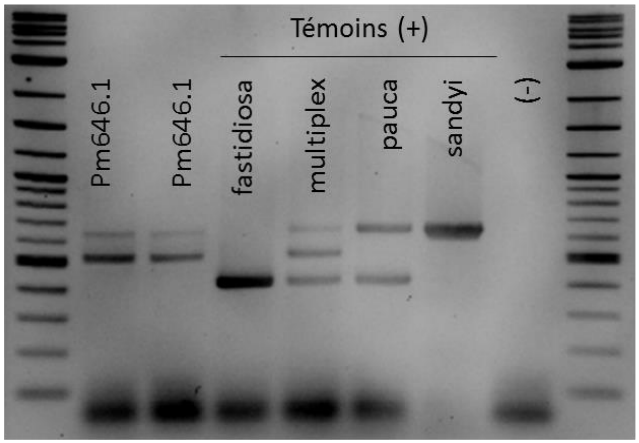
Source : Anses LSV

Identification of isolates of *Xylella fastidiosa*

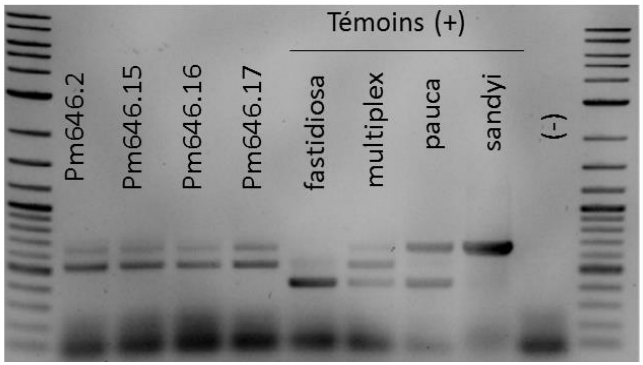
PCR multiplex

(Hernandez-Martinez *et al*, 2010)

a.



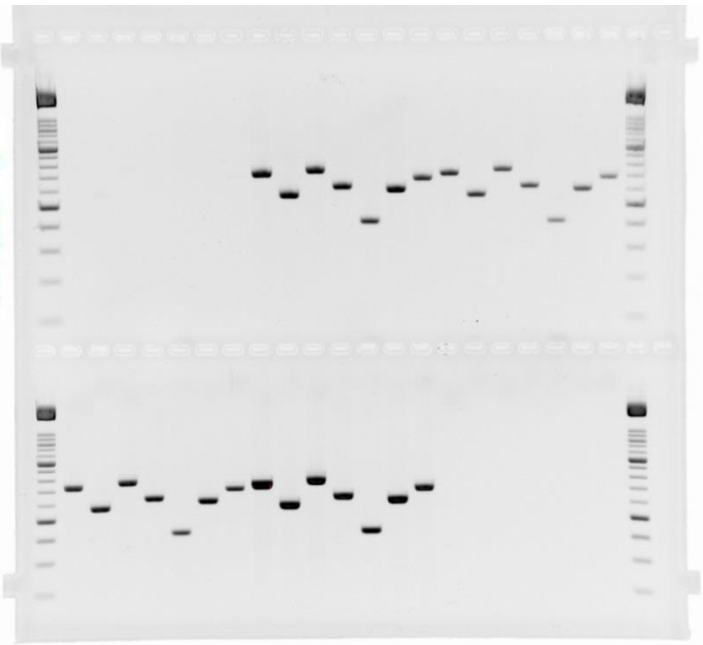
b.



MLSA/MLST

Partial sequences of 7 house keeping genes:
http://pubmlst.org/xfastidiosa/

cysG, gltT, holC, leuA, malF, nuoL, petC

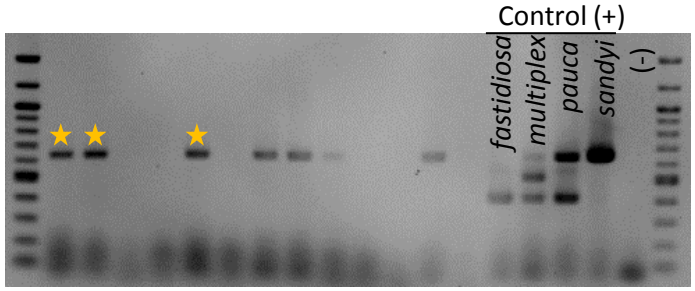


Analysis for MLSA/MLST done at INRA-Emersys

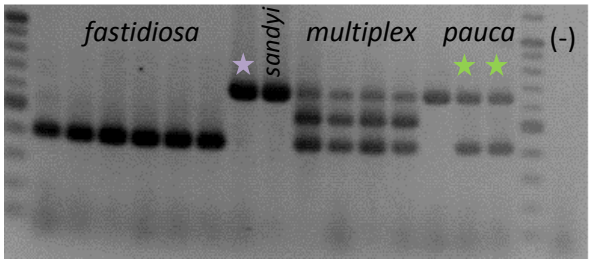
Identification of isolates of *Xylella fastidiosa (on Coffee)*

Multiprimer PCR test (3 genes)

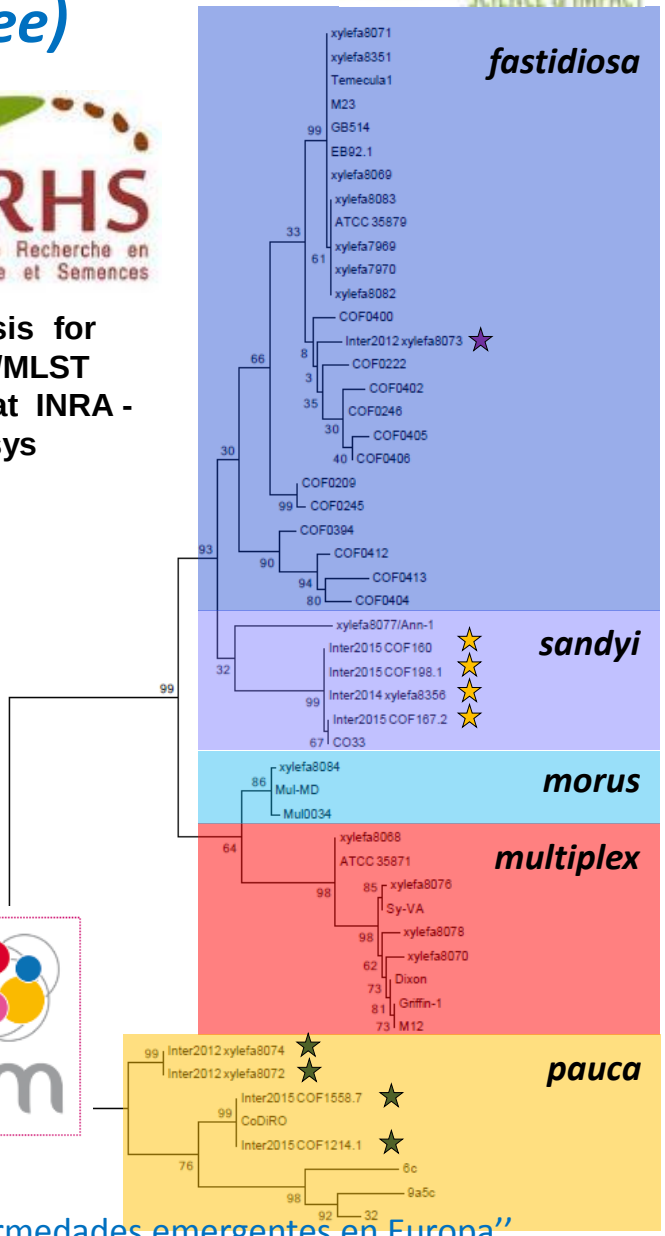
Hernandez-Martinez et al., 2006



Source INRA



Analysis for MLSA/MLST done at INRA - Emersys



MLSA: Partial sequences of **7 housekeeping genes:**

cysG, gltT, holC, leuA, malF, nuoL, petC as described in http://pubmlst.org/xfastidiosa/

Concatenated dataset; ML, 1000 bootstraps

★ = isolates from intercepted infected coffee plants

(Jacques *et al.* , 2016)

Identification of isolates of *Xylella fastidiosa (on Coffee)*

MLST scheme on strains (7 genes) (done at INRA-Emersys) http://pubmlst.org/xfastidiosa/

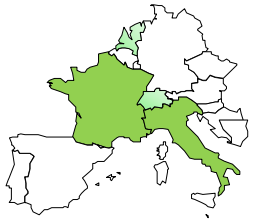
Date	Country	Strain	*cysG*	*gltT*	*holC*	*leuA*	*malF*	*nuoL*	*petC*	ST	subsp
2012	Mexico	CFBP 8073	29	1	1	9	10	19	1	75	*fastidiosa (sandyi)*
2014 2015 2015	Costa Rica ? ?	CFBP 8356 COF160 COF198.1	26	1	24	12	15	18	13	76*	*sandyi*
2015	Honduras	COF167.2	26	1	24	12	15	18	12	72*	*sandyi*
2012 2012	Ecuador Ecuador	CFBP 8072 CFBP 8074	28	8	25	7	8	16	6	74	*pauca*
2015	?	COF1214.1	24	14	10	7	16	16	6	53*	*pauca*

Novel alleles and STs

* Identical STs detected in France and Italy

Coffee plants

trade



Source INRA

Giampettruzzi et al., 2015; Jacques et al., 2016; Loconsole et al., 2016; Unpublished data

Identification of isolates of *Xylella fastidiosa (in Corsica and south)*

MLST (7 genes): (Analysis done at INRA-Emersys)

2 STs identical to subsp. ***multiplex*** US strains

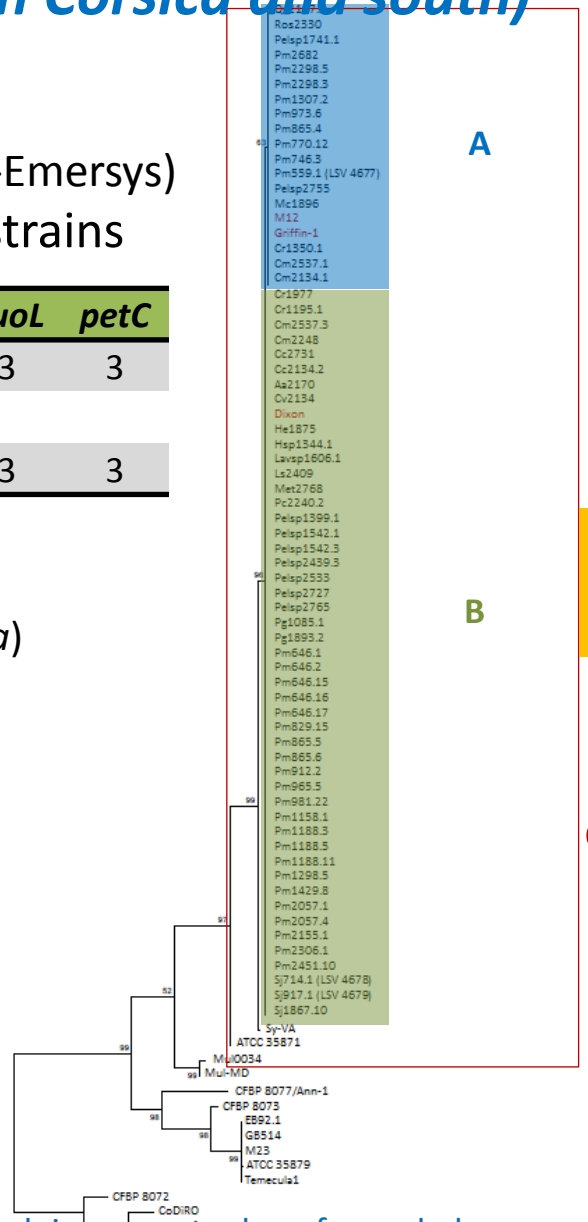
	ST	*cysG*	*gltT*	*holC*	*leuA*	*malF*	*nuoL*	*petC*
A	7	7	3	3	3	3	3	3
B	6	3	3	3	3	3	3	3

	ST
A	7
B	6

M12
(*Prunus dulcis*)

Dixon

Griffin-1
(*Quercus rubra*)



MLSA
(done at INRA-Emersys)

subsp. *multiplex*

No host specificity of the lineages

>400 samples

MLSA: Partial sequences of **7 housekeeping genes:**

cysG, gltT, holC, leuA, malF, nuoL, petC as described in http://pubmlst.org/xfastidiosa/

Concatenated dataset; ML, 1000 bootstraps

Identification of isolates of *Xylella fastidiosa (in Corsica and south)*

19 isolats from Corsica (2 ST *multiplex*)

Ref.	Subsp.	Plante hôte	Année
LSV4677	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV4678	*multiplex* ST6	*Spartium junceum*	2015
LSV4679	*multiplex* ST6	*Spartium junceum*	2015
LSV4706	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV4707	*multiplex* ST6	*Polygala myrtifolia*	2015
LSV4708	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV4710	*multiplex* ST6	*Lavandula sp.*	2015
LSV4713	*multiplex* ST6	*Prunus cerasifera*	2015
LSV4714	*multiplex* ST6	*Lavandula angustifolia*	2015
LSV4716	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV4717	*multiplex* ST6	*Polygala myrtifolia*	2015
LSV4718	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV4719	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV4720	*multiplex* ST7	*Cistus monspeliensis*	2015
LSV4721	*multiplex* ST7	*Pelargonium sp.*	2015
LSV4722	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV4723	*multiplex* ST6	*Coronilla valentina*	2015
LSV 47.60	*multiplex* ST6	*Helichrysum italicum*	2016
LSV 47.61	*multiplex* ST6	*Calicotome villosa*	2016

(MLSA/MLST done at INRA Angers - Emersys)

Identification of isolates of *Xylella fastidiosa*

4 isolats from south of France

Ref.	Subsp.	Host plant	Year
LSV4711	*multiplex* ST6	*Polygala myrtifolia*	2015
LSV4712	*multiplex ST6*	*Polygala myrtifolia*	2015
LSV4715	*multiplex* ST7	*Polygala myrtifolia*	2015
LSV 47.32	*multiplex* ST7	*Spartium junceum*	2016

8 isolats on coffee imported and intercepted

Ref.	Subsp.	Host plant	Year
LSV4103	***pauca ST74***	***Coffea arabica***	**2012**
LSV4209	***fastidiosa /sandyi ST75***	***Coffea canephora***	**2012**
LSV4627	*sandyi* (atypical) ST72	*Coffea arabica*	2015
LSV4628	*sandyi*	*Coffea arabica*	2014
LSV4639	*sandyi*	*Coffea arabica*	2014
LSV4659	*sandyi*	*Coffea sp.*	2014
LSV4709	*pauca* ST53	*Coffea arabica*	2015
LSV 47.33	*pauca* ST53	*Coffea arabica*	2016

(MLSA/MLST done at INRA Angers - Emersys)

Medium PWGm

Isolation of strain of Xylella is difficult

Caracterisation and identification by MLSA/MLST **directly on plant extract**

- Jacques *et al.* , New variants of coffee-infecting *Xylella fastidiosa* issued from homologous recombination. Appl. Environ. Microbiol., 2016.

Symposio:''*Xylella fastidiosa*: una especie compleja causante de enfermedades emergentes en Europa''
Palencia , 20 de septiembre de 2016 (GEDDI-SEF)

Identification of isolates of *Xylella fastidiosa* on plants

MLST scheme on strains (7 genes) (Work done at INRA)-Emersys

	ST	*cysG*	*gltT*	*holC*	*leuA*	*malF*	*nuoL*	*petC*
A	**7**	7	3	3	3	3	3	3
B	**6**	3	3	3	3	3	3	3

MLST direct on plant extract
50% of positive samples were assigned to Xylella fastidiosa subsp multiplex

Echantillon	*cysG*	*gltT*	*holC*	*leuA*	*malF*	*nuoL*	*petC*	ST	subsp
N°	7	3	3	3	3	3	3	**7**	*multiplex*
N°	ind	3	3	ind	3	3	Ind	**ind**	**Indetermined**

Adaptation of method MA039 to insects (*Philaneus spumarius*)

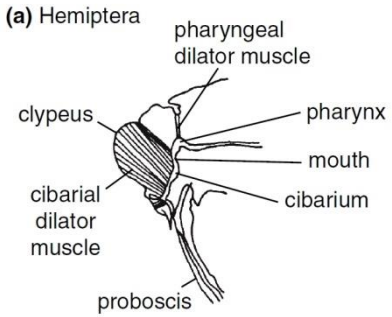
Xylella fastidiosa: bacteria in cibarium / pré-cibarium

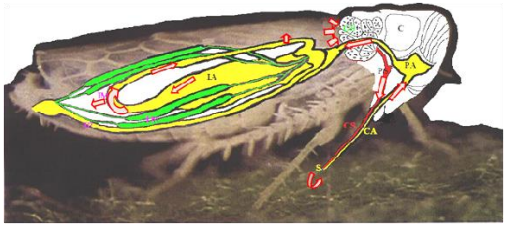
Testing of head after removing eyes

1 head

↓

Grinding with beads



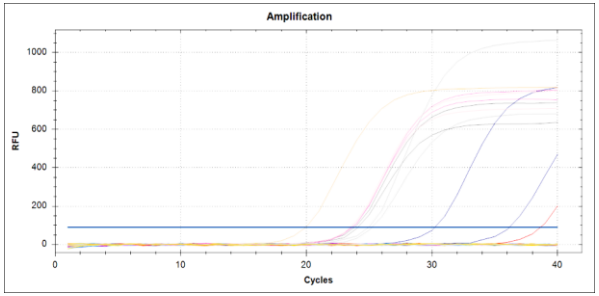


50 000 bacteria max (*Homalodisca vitripennis*) (Almeida, com. pers.)

↓

Kit QuickPick™ Plant DNA (Bio-Nobile)
Automate KingFisher™ mL

↓



Real Time PCR (Harper *et al.*, 2010, Erratum 2013=

Adaptation of method MA039 to insects (*Philaneus spumarius*)

Performance criteria	**Amplification Harper *et al.*, 2010 (Erratum 2013)**	
6 concentrations X 3 extractions X 2 PCR X 3 days, 108 PCR per method	***On Philaenus spumarius*** **(conc. bact. 0 à 10^5 bact./head)**	
DNA Extraction ***(by spiking X. f. subsp. fastidiosa)***	**QuickPick™ + KingFisher™ mL**	**Blood and Tissue KIT (Qiagen)**
Sensitivity	**60% (indetermined no included)**	**40% (indetermined no included)**
Detection limit **(with 100% of detection)**	**≈ 10^3 bact./head**	**≈ 10^4 bact./head**
Repeatability	**91%**	**87%**

Others parameters tested but no better effect: dilutions, other kits

Testing insects collected in Corsica in 2015

Site	*Philaenus spumarius* détected			
	tested	positive	indetermined	negative
Maquis 1	31	**2**	2	27
Maquis 2	40	**6**	1	33
Maquis 3	28	**2**	0	26
Pépinière	14	0	1	13
Jardin 1	18	**1**	2	15
Jardin 2	4	**1**	0	3
Espaces verts 1	21	**3**	1	17
Espaces verts 2	40	**2**	3	35
Total	196	17 (8,7%)	10 (5,1%)	169 (86,2%)

Others insects negative: 6 *Lepyronia coleoptrata*, 31 *Cicadellidae viridis*

Testing Philaenus spumarius (origine : Pouilles – Italy 2016)

- *X. fastidiosa* detected with method MA039 (Harper *et al*., 2010)
- Rate of contamination≈ 10% upon Maria Saponari (CNR)

P. spumarius R16942	Ct 1	Ct 2	Resultats
1	Ø	37,87	Ind.
2	Ø	38,69	Ind
3	38,38	38,72	Ind
4	Ø	Ø	Neg.
5	39,7	Ø	≈ Neg
6	38,86	Ø	Ind
7	38,7	38,83	Ind
8	Ø	Ø	Neg.
9	Ø	38,51	Ind
10	Ø	Ø	Neg.
11	Ø	38,59	Ind
12	39,22	38,78	Ind
13	Ø	Ø	Neg.
14	31,59	31,51	Pos.
15	Ø	Ø	Neg.
16	Ø	39,69	≈ Neg
17	Ø	Ø	Neg.
18	38,13	Ø	Ind
19	Ø	Ø	Neg.
20	38,5	Ø	Ind
21	38,45	Ø	Ind
22	Ø	38,86	Ind
23	Ø	Ø	Neg.
24	Ø	Ø	Neg.
25	Ø	Ø	Neg.

P. spumarius R16942	Ct 1	Ct 2	Resultats
26	38,6	Ø	Ind
27	38,8	Ø	Ind
28	39,56	Ø	≈ Neg
29	38,72	Ø	Ind
30	38,08	Ø	Ind
31	38,01	37,44	Ind
32	Ø	37,79	Ind
33	37,25	38,71	Ind
34	Ø	39,63	U≈ Neg
35	38,88	37,14	Ind
36	37,27	39,54	Ind
37	38,63	38,66	Ind
38	29,14	29,22	Pos.
39	37,19	38,7	Ind
40	38,74	Ø	Ind
41	32,74	32,64	Pos.
42	38,64	Ø	Ind
43	35,89	35,19	Ind
44	38,15	38,31	Ind
45	37,83	Ø	Ind
46	Ø	Ø	Neg.
47	36,89	Ø	Ind
48	Ø	38,17	Ind
49	Ø	Ø	Neg.
50	38,16	38,19	Ind

Documents and guides

List of plant hosts of Xylella fastidiosa subsp …

http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm

Guide for recognition of plant host of X.f subsp multiplex

http://agriculture.gouv.fr/le-point-sur-les-foyers-de-xylella-fastidiosa-en-france

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Fabrice Touzaint

Pauline de Jerphanion

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Maria Milagro Lopez Gonzalez
José Luis Palomo Gomez

Marie-Agnès Jacques
Nicolas Denancé
Martial Briand
Sophie Cesbron
Perrine Portier

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Thank you for your attention