Xylella fastidiosa in France: current situation in the region of Corsica and Provence-Alpes-Côte d'Azur.

Françoise Poliakoff
Anses – Plant health laboratory

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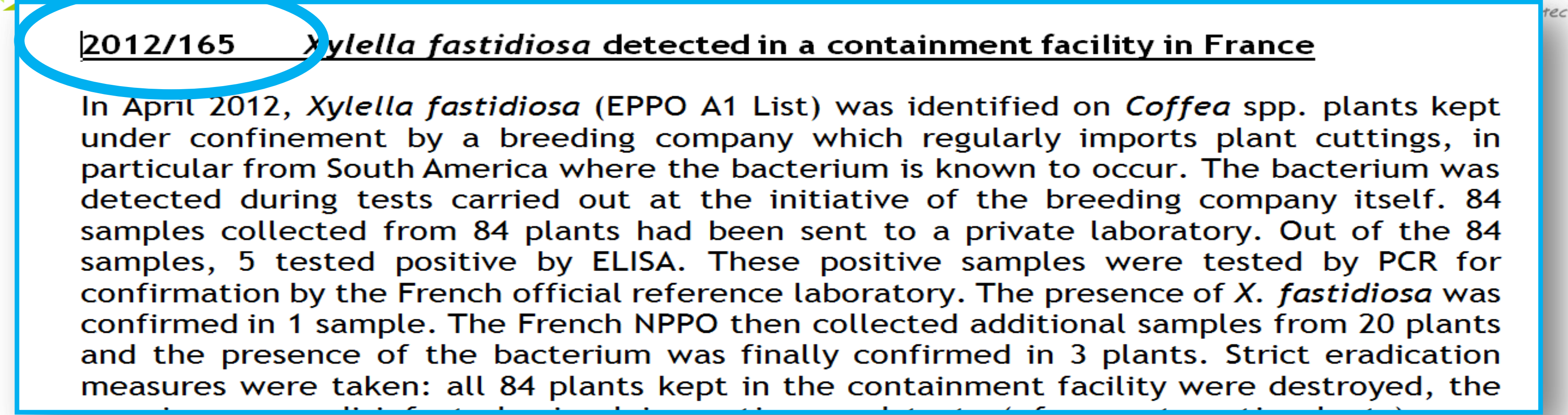
efsa European Food Safety Authority
UIB Universitat de les Illes Balears
European Commission
POnTE Pest Organisms Threatening Europe
XF ACTORS
Euphresco

Facing the emergence of *Xylella fastidiosa (Xf)*

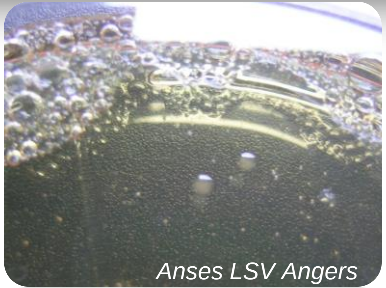


2012/165 *Xylella fastidiosa* detected in a containment facility in France

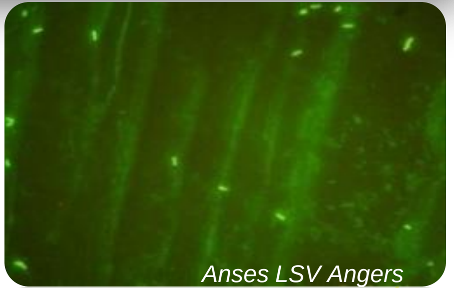
In April 2012, *Xylella fastidiosa* (EPPO A1 List) was identified on *Coffea* spp. plants kept under confinement by a breeding company which regularly imports plant cuttings, in particular from South America where the bacterium is known to occur. The bacterium was detected during tests carried out at 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

Coffee leaf scorch on *Coffea canephora*

Colonies of Xf subsp *pauca* isolated from *Coffea arabica* on BCYE



Colonies of Xf subsp *pauca* isolated from *Coffea arabica* on mPWG



Xf shapes by immunofluorescence

Validation method process for *Xf* detection

➢ Protocol of method validation

Intra-laboratory evaluation (NRL)

- Analytical specificity
- Analytical sensitivity (limit of detection)
- Diagnostic specificity (true negatives)
- Diagnostic sensitivity (true positives)
- Repeatability

Inter-laboratories evaluation (NRL)

- Analytical sensitivity (limit of detection)
- Diagnostic specificity (true negatives)
- Diagnostic sensitivity (true positives)
- Reproducibility

Transfert to laboratories
(Training – Proficiency test)

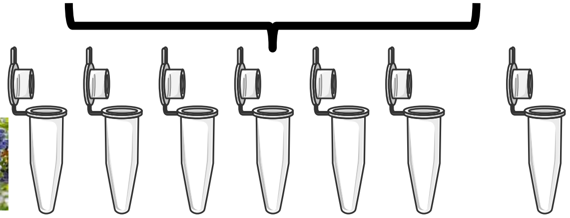
➢ Samples for evaluation

Standard for validation EPPO PM 7/98 – Guide ANSES/PR3/7/1

Spiking with tenfold dilutions of Xf suspension 5µL / tube

\+

Plant extract 395 µL /tube

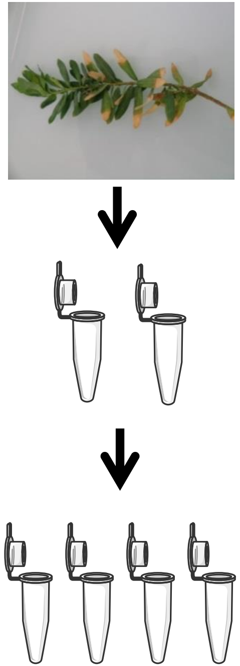


(bact/mL) 1,25 .10^5 1,25 .10^4 1,25 .10^3 1,25 .10^2 1,25 .10^1 1,25 .10^0 NEG

X 3 assays / plant / strain
X 3 dilution series / assay
= 63 samples per plant species

Validation method process for *Xf* detection

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



1 sample = 5 to 100 petioles = 0.5 to 1 g

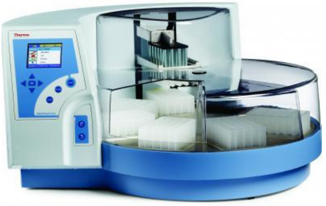
grounding in sterile water (5 mL/g)

2 DNA extractions / sample

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

4 amplifications

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

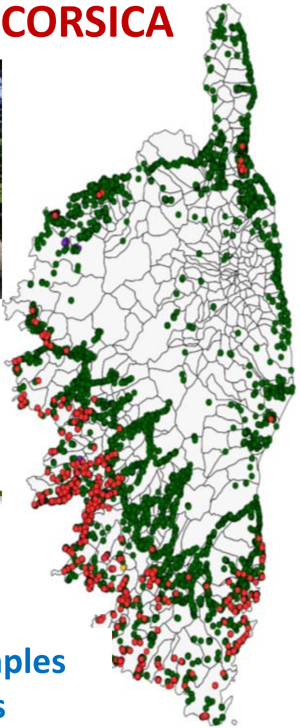


Validation method process for *Xf* detection

Performance criteria of the method	**DNA extraction: QuickPick™ + KingFisher™ mL Amplification Harper *et al.*, 2010 (Erratum 2013)**		
Inclusivity	**100% (45 targeted strains representing the 4 major subsp.)**		
Exclusivity	**100% (29 non-targeted strains: Xylophilus, Xanthomonas, Liberibacter, sap saprophytes)**		
	Spiked plant host (10^2 - 10^5 bact./mL, 15 samples)		
Host Plant	**Orange tree** **+ *X. f. pauca* CFBP 8072**	**Grappe** **+ *X. f. fastidiosa* CFBP 7970**	**Olive tree** **+ *X. f. multiplex* CFBP 8173**
Diagnostic sensitivity	**100%**	**94%**	**67%**
Diagnostic specificity	**100%**	**100%**	**100%**
Repeatability	**100%**	**96%**	**100%**
Reproducibility	**98%**		
Analytical sensitivity (with probability detection of 100%)	**≈ 10^2 bact./mL**	**≈ 10^3 bact./mL**	**≈ 10^5 bact./mL**

- The presence of inhibitors (polyphenol, secondary metabolites,…) in some plant host (olive tree, oak) limits its sensitivity.

French situation: survey results 2015- 2017



More than 50% of the area included in a Demarcated Area (DA)

Outbreaks located in urban (private and public gardens) and natural areas (maquis, forest) difficult to access.

Strategy of eradication not adapte

Containment strategy

will be adopted (end of 2017)

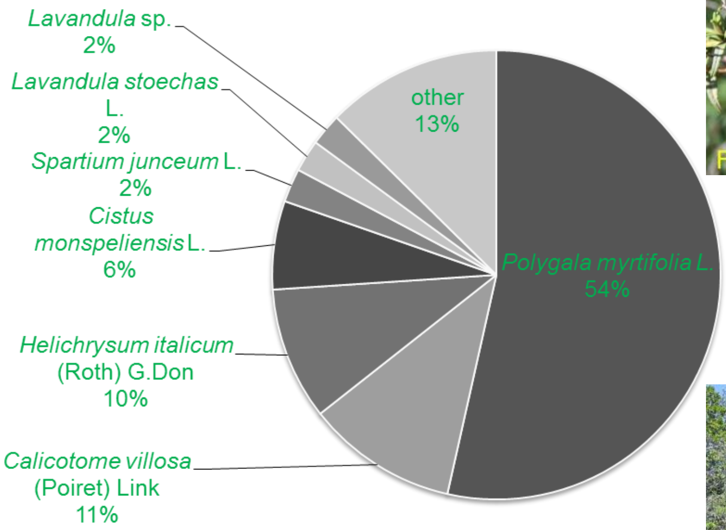
About 15000 samples

And 900 positives

Rate of positive samples ~ 6%

(Harper et al, 2010)

French situation: survey results 2015- 2017



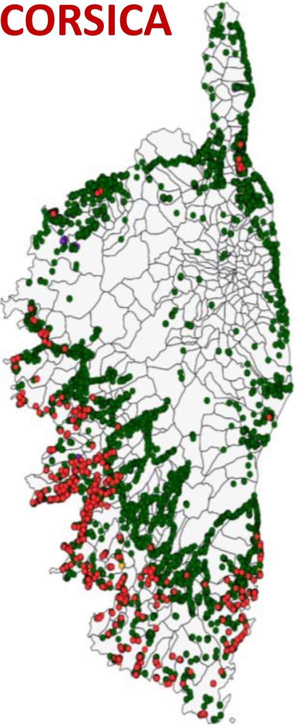
Repartition of positive host on 327 species sampled

Cistus monspeliensis

Helichrysum italicum

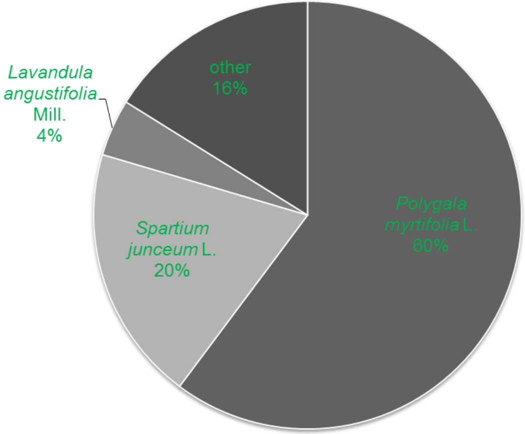
Calicotome villosa

CORSICA



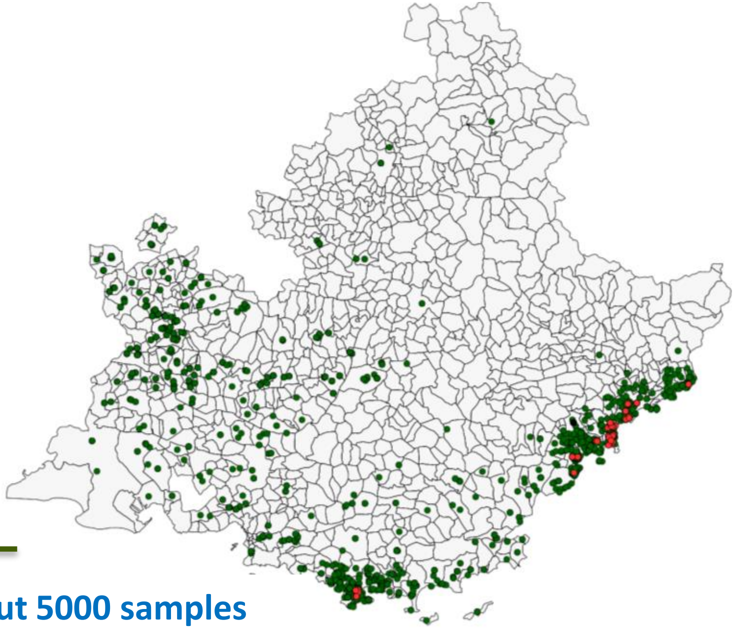
Rate of positive samples ~ 6%
(Harper et al, 2010)

French situation: survey results 2015- 2017



Repartition of positive host on 164 species sampled

PROVENCE ALPES COTE D'AZUR



About 5000 samples and 100 positives

**Rate of positive samples 2%
(Harper et al, 2010)**

Outbreaks located in urban and peri-urban areas (private and public gardens)

Stragegy of eradication

French situation: survey results 2015- 2017

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Host plant	Total samples	% positive	Number of positive
Calicotome villosa (Poiret) L.	299	33,1	99
Polygala myrtifolia L.	2761	19,0	522
Spartium junceum L.	236	16,9	40
Helichrysum italicum (Roth)	694	12,5	87
Cistus monspeliensis L.	565	10,2	58
Genista corsica (Loisel.) DC.	209	7,7	16
Lavandula stoechas L. sp	815	5,4	44
Cistus creticus L.	298	4,4	13
Cistus salviifolius L.	303	3,6	11
Lavandula angustifolia Mill.	749	1,7	13
Asparagus acutifolius L.	227	1,3	3
Cytisus scoparius (L.) Link	137	1,5	2
Quercus suber L.	419	0,9	4
Lavandula dentata L.	130	0,8	1
Myrtus communis L.	504	0,6	3
Cytisus villosus Pourr.	406	0,5	2
Prunus dulcis (Mill.) D.A W	299	0,3	1
Rosmarinus officinalis L.	1101	0,2	2


Polygala myrtifolia


Calicotome villosa


Spartium junceum


Helichrysum italicum

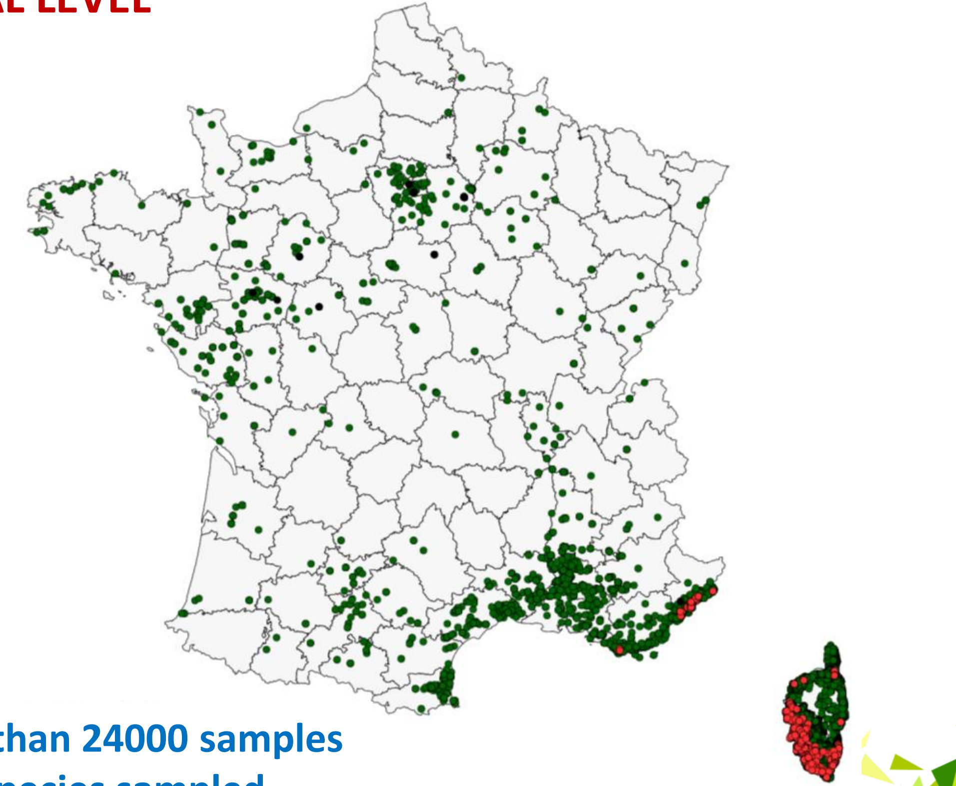

Cistus sp.

37 host plant for *Xylella fastidiosa*

French situation: survey results 2015- 2017

NATIONAL LEVEL

- ☐ No detection of Xf in cultivated zones (Nurseries, Orchards)
- ☐ More than 80% of contaminated samples belong to endemic plant species from Mediterranen flora present in maquis Corsica
- ☐ Since 2 years, boundaries of demarcated aeras do not evolve



More than 24000 samples
414 species sampled

Identification of strains of Xf present in France: survey results 2015- 2017

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In collaboration with INRA Emersys team, identification scheme based on MLSA/MLST has been developed directly *in plantae* for official testing:

INRA
SCIENCE & IMPACT

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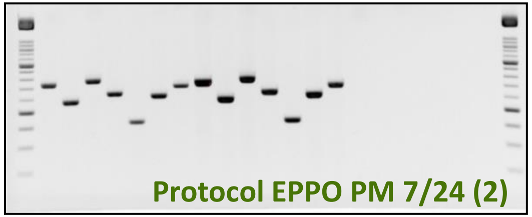
PCR (Yuan *et al.*, 2010) to amplify partial sequences of housekeeping genes: *cysG*, *gltT*, *holC*, *leuA*, *malF*, *nuoL*, *petC*


Protocol EPPO PM 7/24 (2)

Multi Locus Sequence Analysis (MLSA): the concatenated partial sequences of the 7 housekeeping genes: http://pubmlst.org/xfastidiosa/

Samples are infected with subsp. *multiplex* strains

Multi Locus Sequence Typing (MLST):

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

2 ST (ST6-ST7) identical to subsp. *multiplex* strains

Identification of strains of Xf present in France: survey results 2015 - 2017

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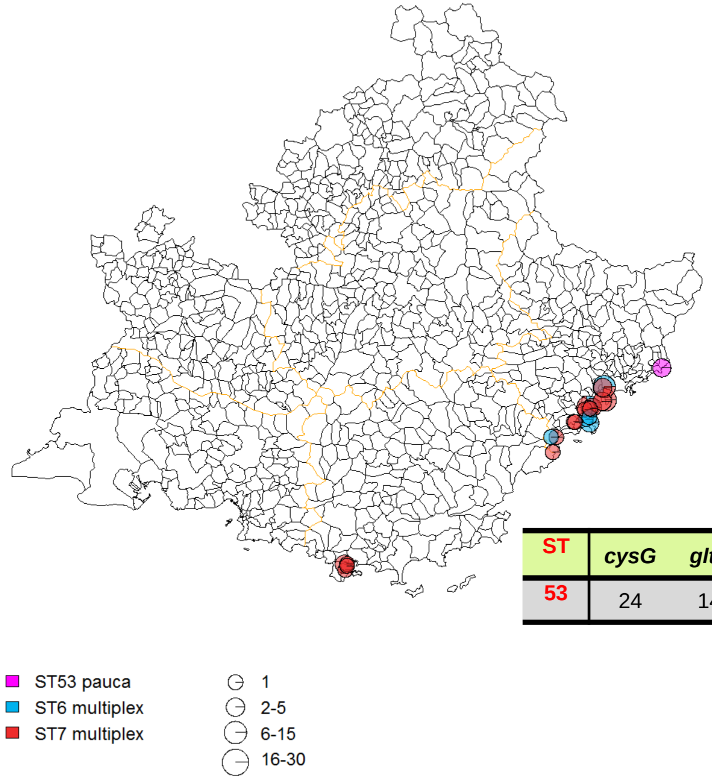
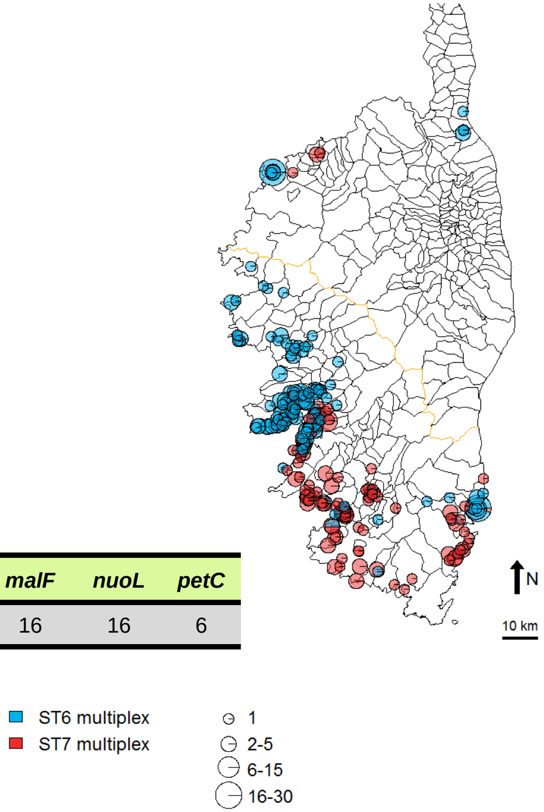


ST	*cysG*	*gltT*	*holC*	*leuA*	*malF*	*nuoL*	*petC*
53	24	14	10	7	16	16	6

CORSICA



Co-infection

Identification by MLST of multiplex strains of Xf France: survey results 2016- 2017

Plant species	Positive samples (Harper *et al.*, 2010)	Number/% Success in MLST sequencing 7 genes	Number/% Success in MLST sequencing 1 to 6 genes	Number/% Total failure in sequencing genes
***Calicotome* sp.**	88	29 / **33%**	14 / 16%	45 / **51%**
		Cq: 23,1 – 31,4	Cq: 24,3– 31,5	Cq: 23,1 – 35,1
***Polygala* sp.**	79	67 / **85%**	10 / 13%	2 / **2,5%**
		Cq: 19,2 – 33,5	Cq: 21,8 – 30,2	Cq: 34,5 – 34,8
***Helichrysum* sp.**	72	53 / **74%**	8/ 11%	11 / **15%**
		Cq: 18,6 – 29,0	Cq: 17,5 – 29,2	Cq: 20,4 – 31,7
***Spartium* sp.; *Genista* sp.**	42	33 / **76%**	5 / 12%	4 / **10%**
		Cq: 21,5 – 34,9	Cq:22,6– 34,3	Cq: 26,0 – 34,9
***Lavandula* sp.**	35	6 / **17%**	4 / 11%	25 / **71%**
		Cq: 24,3 – 30,8	Cq: 24,6 – 27,3	Cq: 20,5 – 35,0
***Cistus* sp.**	30	11 / **39%**	5 / 17%	14 / **47%**
		Cq: 19,8 – 30,1	Cq: 27,8 – 36,1	Cq: 24,3 – 36,0
Others species (15 different species)	25	15 / **62,5%**	6 / 24%	4/ **16%**
		Cq: 18,5 (*Phagnalon*) – 33,3 (*Cercis*)	Cq: 23,7 – 31,3	Cq: 25,2 (*Phagnalon*) – 33,2 (*Quercus* s.)
Total	371	214 / **58%**	52 / **14%**	105 / 28%
		Cq: 18,5 – 34,9	Cq: 17,5 – 36,1	Cq: 20,4 – 36,0

See Poster on limits and Improvement

PERSPECTIVES

- Evaluation and validation of other methods to improve:
- - *Xf* detection on complex matrices
- - identification of *Xf* subspecies by MLSA/MLST, genes sequençing
- - *Xf* detection in vectors (TPS on going)
- - *Xf* detection kits to use in fields

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