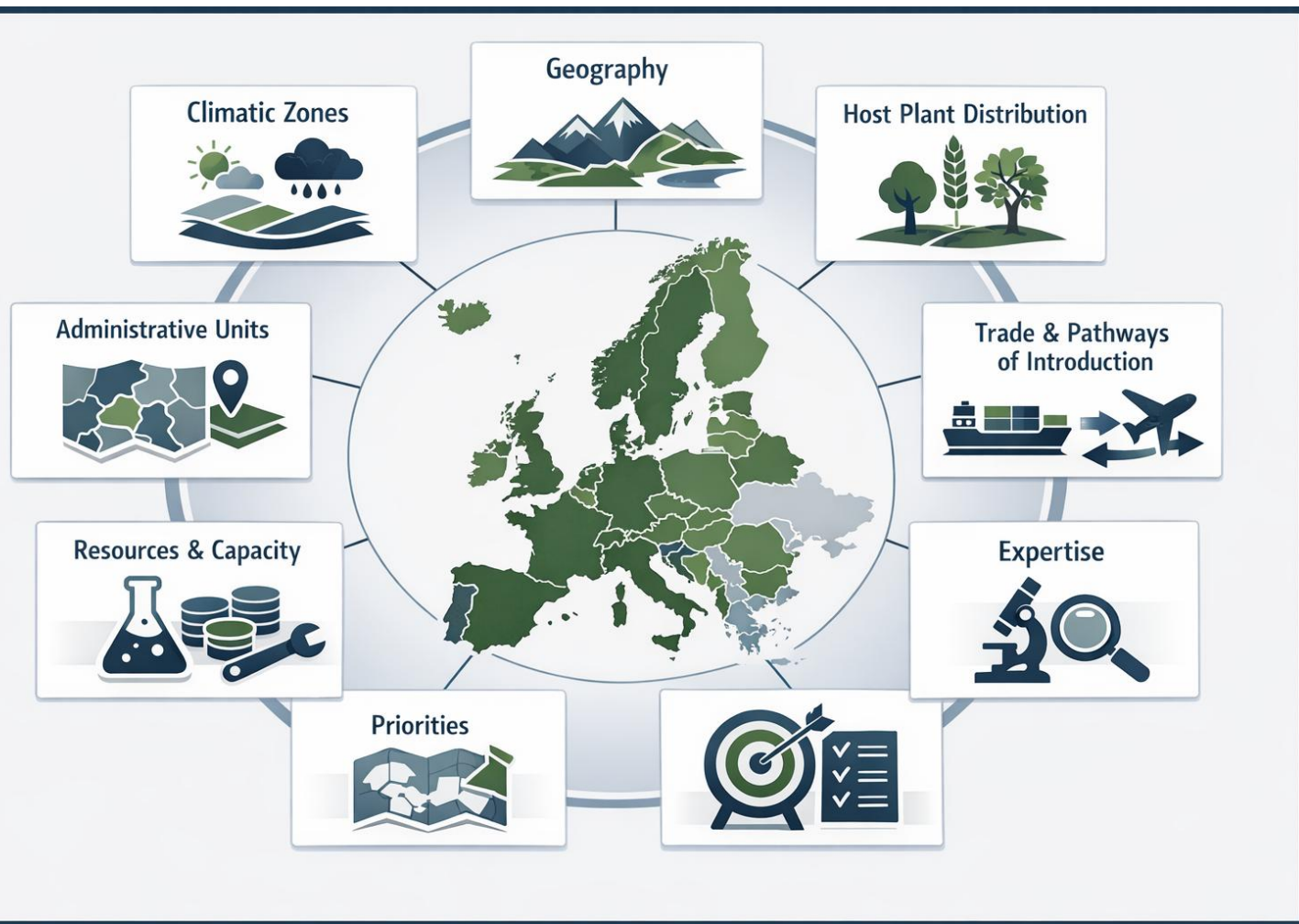


Xylella fastidiosa: EFSA toolkit for harmonisation of surveys in the European Union

Sybren Vos, Team leader EFSA Plant Health Monitoring

VARIABILITY IN THE EU MS THAT IMPACTS PEST SURVEILLANCE



Harmonisation of pest surveys
is a big challenge!

- Common rules
- Common framework
- Common tools

Training, training and
training!



COMMON RULES - LEGISLATION

Plant Health Law (EU) 2016/2031

Article 22 - UQP

- Risk based Scientific sound
- Reporting obligations

Article 24- **Priority pests**

- Annual survey & reporting
- high degree of confidence
- the timely detection of those pests

Commission Implementing regulation (EU) 2020/1201

Annual risk based surveys

Sampling and molecular testing plants and vectors

In line with EFSA guidelines and PSC

- Sufficient confidence a low prevalence
- Detection/Delimiting/Buffer zone

...



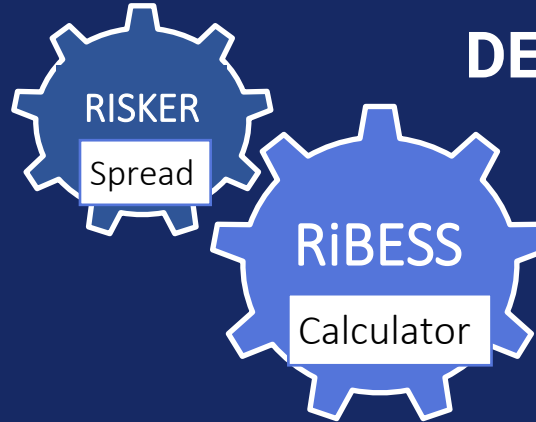
COMMON TOOLS FOR PEST SURVEYS

PREPARE



What? Where? How? When?

DESIGN



How much?



RiPEST

Expert system

OPTIMISE



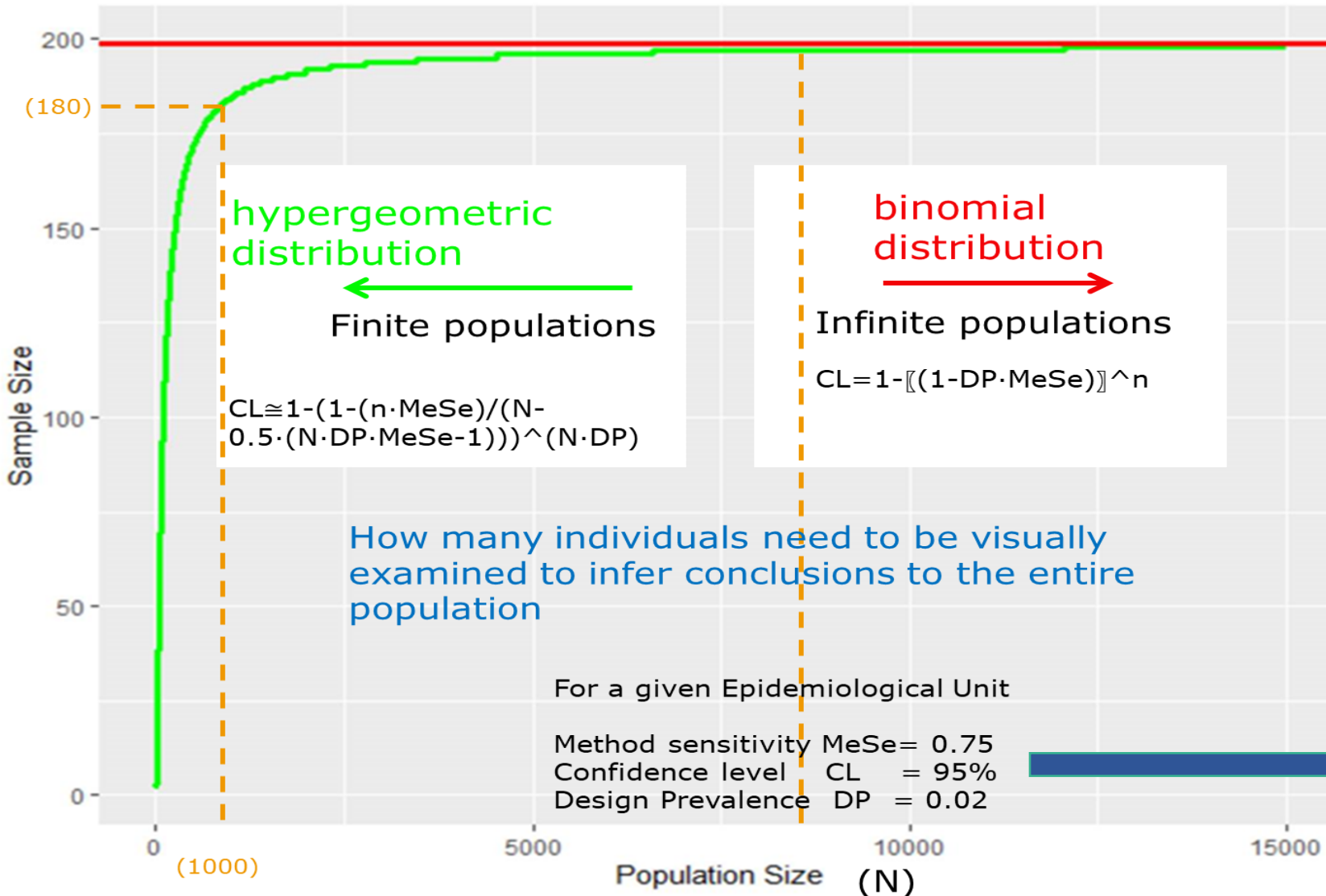
OptiPest

How much less?

- Adaptable and flexible toolkit valid for all pest
- Science based with full integration of statistics
- Better use of resources with
 - risk-based surveys and crop optimisation
- Comparable surveys in time and in space

- High confidence in conclusions
- Evidence based pest status

COMMON METHODOLOGY FOR PEST SURVEY DESIGN



efsa SUPPORTING PUBLICATIONS **OPEN ACCESS**

Technical report | [Open Access](#)

Update of general guidelines for statistically sound and risk-based surveys of plant pests

[European Food Safety Authority \(EFSA\)](#), [Elena Lázaro](#), [Stephen Parnell](#), [Antonio Vicent Civera](#), [Martijn Schenk](#), [Jose Cortiñas Abrahantes](#), [Juan Navas-Cortes](#), [Hans-Hermann Thulke](#), [Francesco Pecori](#), [Joshua Koh](#), [Jan Schans](#), [Marc Aerts](#), [Gabriele Zancanaro](#), [Sybren Vos](#), [Tomasz Kaluski](#)
... See fewer authors ^

First published: 03 December 2025 | <https://doi.org/10.2903/sp.efsa.2025.EN-9788>

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EFSA methodology for estimating pest survey parameters for detection surveys

[European Food Safety Authority \(EFSA\)](#), [Alexandre Nougadère](#), [Daria Rzepecka](#), [Eduardo de la Peña](#), [David Makowski](#), [Stephen Parnell](#), [Étienne Babin](#), [Jose Cortinas Abrahantes](#), [Tomasz Kaluski](#), [Sybren Vos](#)

First published: 13 April 2026 | <https://doi.org/10.2903/sp.efsa.2026.EN-10065> | [VIEW METRICS](#)

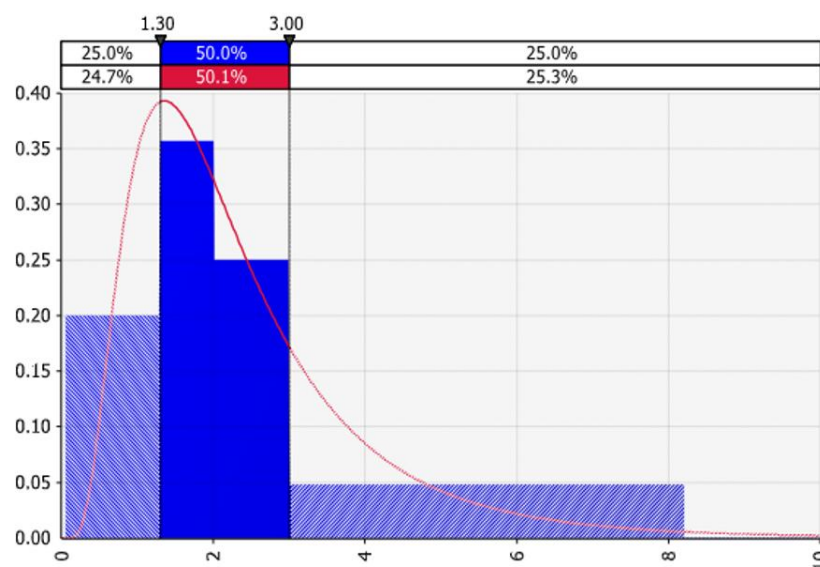
PEST SURVEY PARAMETERS

➤ RATE OF EXPANSION: EXPERT KNOWLEDGE ELICITATION for 47 UQP

Table 4: Fitted values of the probability distribution of the rate of expansion (km/year)

Percentile	1	2.5	5	10	17	25	33	50	67	75	83	90	95	97.5	99
Fitted distribution	0.5	0.6	0.7	0.9	1.1	1.3	1.5	2.0	2.6	3.0	3.6	4.4	5.5	6.7	8.4

Fitted distribution: Lognorm(2.4050,1.6435), @RISK7.6.



***Xylella fastidiosa* spread estimates to be revised in 2026/2027**

- new knowledge and
- ongoing research work in BEXYL and
- EFSA Risk Assessment for Xf



SUPPORTING PUBLICATIONS

OPEN ACCESS

Technical report | [Open Access](#)

EFSA methodology for assessing candidate priority pests under EU Regulation 2016/2031

European Food Safety Authority (EFSA) [✉](#) Eduardo de la Peña, Katharina Dehnen-Schmutz, Gianni Gilioli, Pablo González-Moreno, David Makowski, Alexander Mastin, Alexandre Nougadère, Maria Luisa Paracchini, Stephen Parnell, Alessandro Portaluri, Bethan Purse, Maria Ribaya, Berta Sánchez, Marica Scala, Sara Tramontini, Sybren Vos ... [See fewer authors](#) ^

First published: 22 January 2025 | <https://doi.org/10.2903/sp.efsa.2025.EN-9230> | [VIEW METRICS](#)

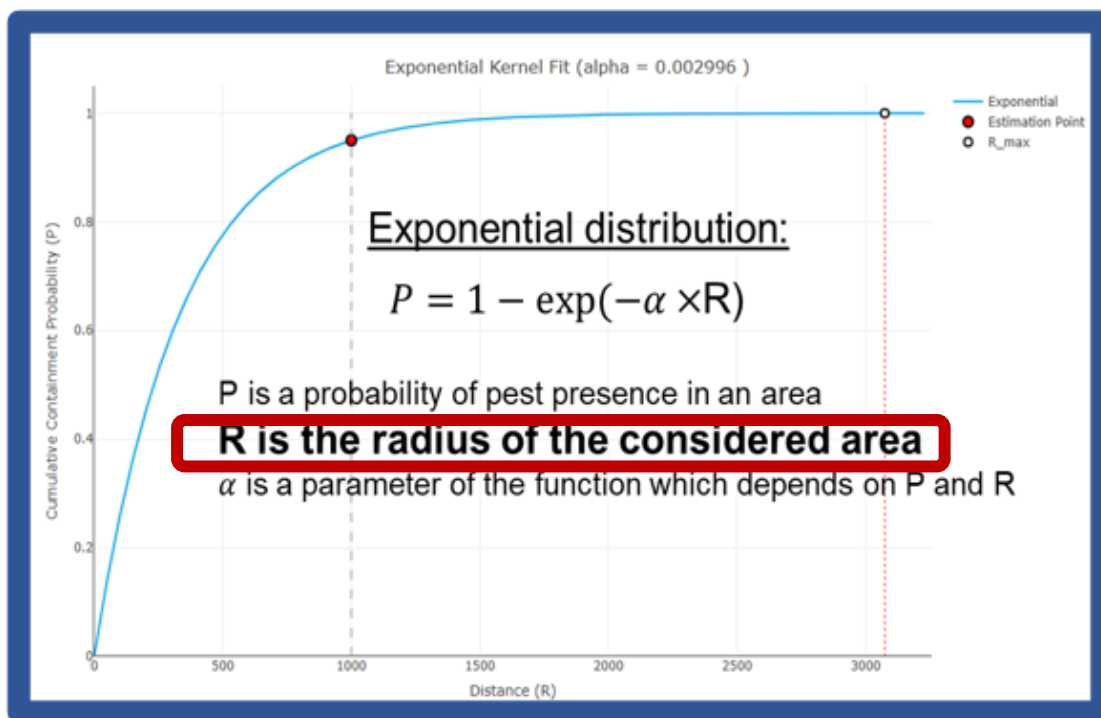
PEST SURVEY PARAMETERS

➤ RELATIVE RISK and RISK AREAS for 47 UQP:

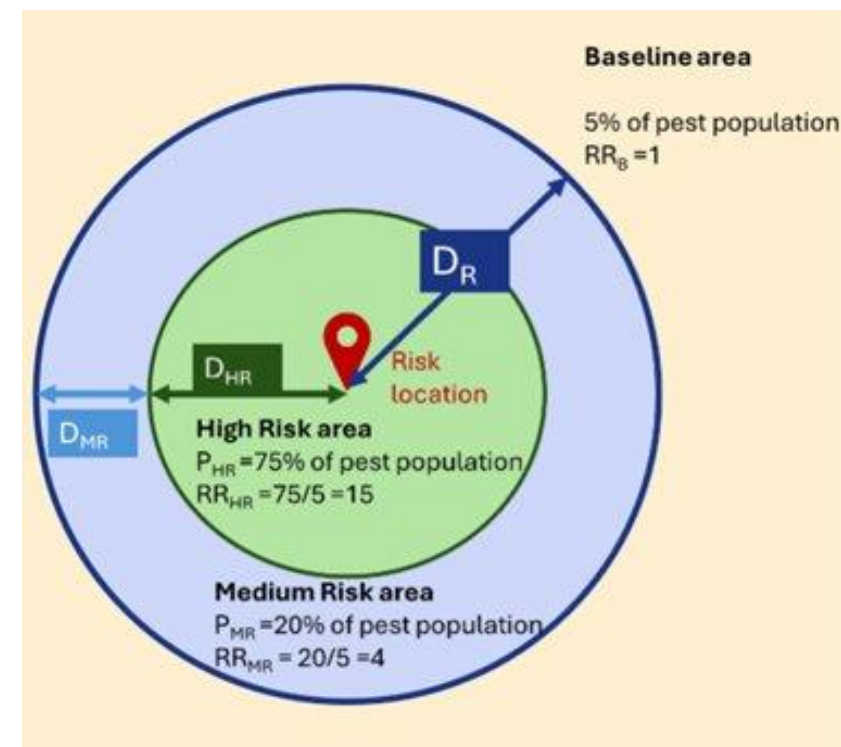
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Fitted distribution: Lognorm(2.4050,1.6435), @RISK7.6.



<https://r4eu.efsa.europa.eu/app/risker>



PEST SURVEY PARAMETERS

➤ RELATIVE RISK BASED ON EXPONENTIAL KERNEL DISTRIBUTION



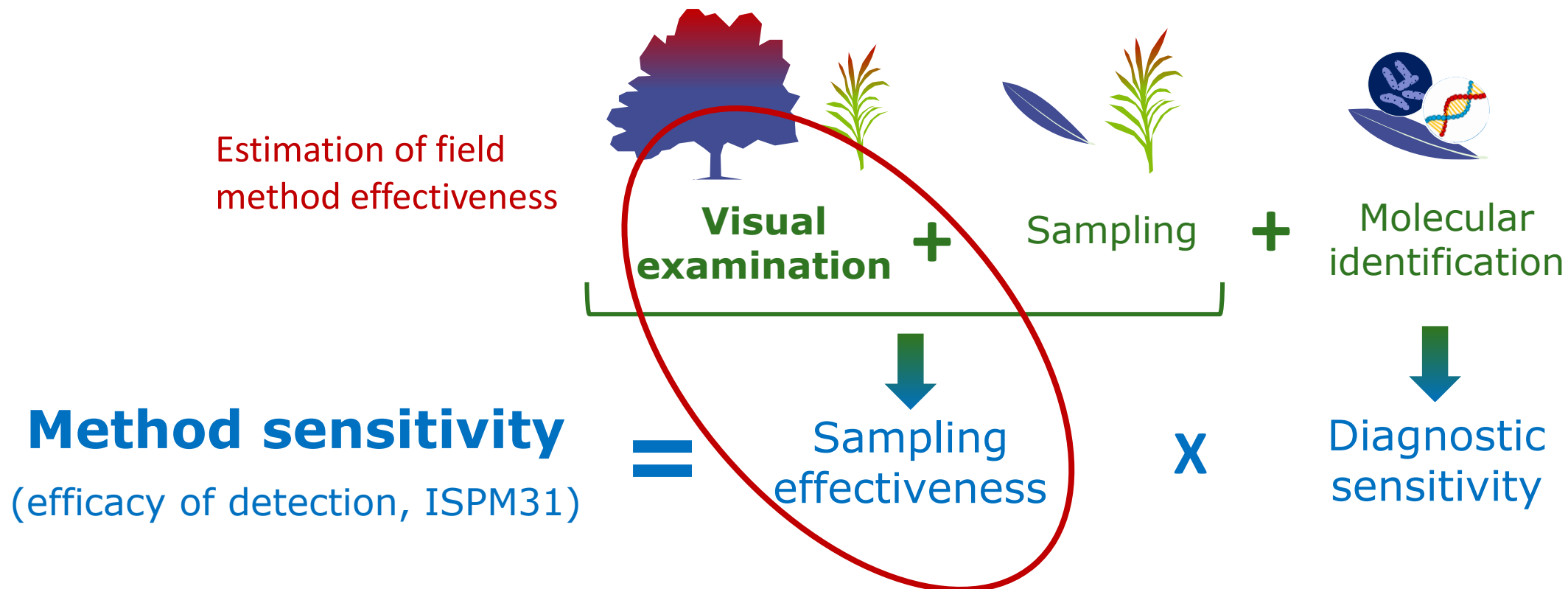
<https://r4eu.efsa.europa.eu/app/risker>

	Risk areas (distance in meters)	Proportion of pest population	Relative Risk compare to baseline (ratio)
High-Risk area	0 to 919	0.75	15 (= 0.75/0.05)
Medium-Risk area	919 to 1986	0.2	4 (= 0.2/0.05)
Baseline	1986 and further	0.05	1

PEST SURVEY PARAMETERS

➤ METHOD SENSITIVITY for 20 PRIORITY PESTS

Detection method = Sequence of operations for detecting and identifying the pest
from the field to the laboratory



PEST SURVEY PARAMETERS

➤ METHOD SENSITIVITY for 20 PRIORITY PESTS

Estimation of field method effectiveness for detection surveys

1. Visual examination

of symptoms and signs of pest,

Given that a plant is infested by the target pest, what is the probability that the visual examination will detect it?

2. Asymptomatic sampling

Given that a plant is infested by the pest, what is the probability that the asymptomatic sample will be infested?

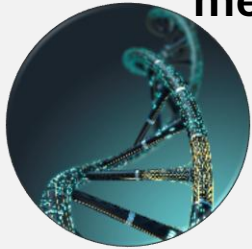
3. Trapping method

Given that the pest is present within the area covered by a single trap, what is the probability that the trap will capture at least one individual pest?

For *Xylella fastidiosa* EKE planned end 2026

CONCLUSIONS

**RESEARCH on
e.g. New detection
methods**



E-DNA



Remote Sensing



Trapping

**KNOWLEDGE
TRANSFER**

Pre-detection

to improve

**Early detection of
UQP**

EFSA PEST SURVEY TOOLKIT

Prepare



**EFSA Pest
Survey Cards**

**Database
for PP**

Design and Optimize



RiPEST

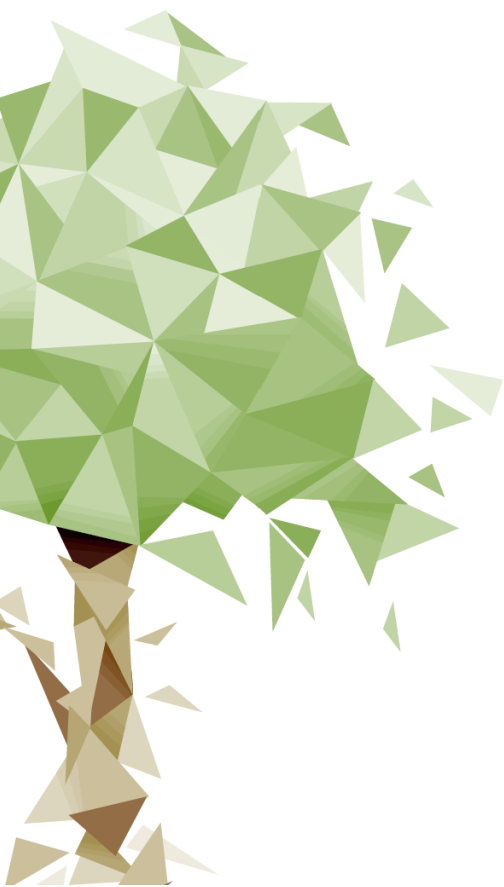


OptiPest



EFSA PLH MONITORING TEAM 2026





THE DATABASE OF 13 YEARS OF MONITORING IN APULIA – OVER 1,500,000 ANALYSIS – A POWERFUL TOOL FOR THE STUDY OF THE EPIDEMIC OF *XYLELLA FASTIDIOSA*

Salvatore Infantino, Anna Percoco, Michele Digiario, Donato Boscia





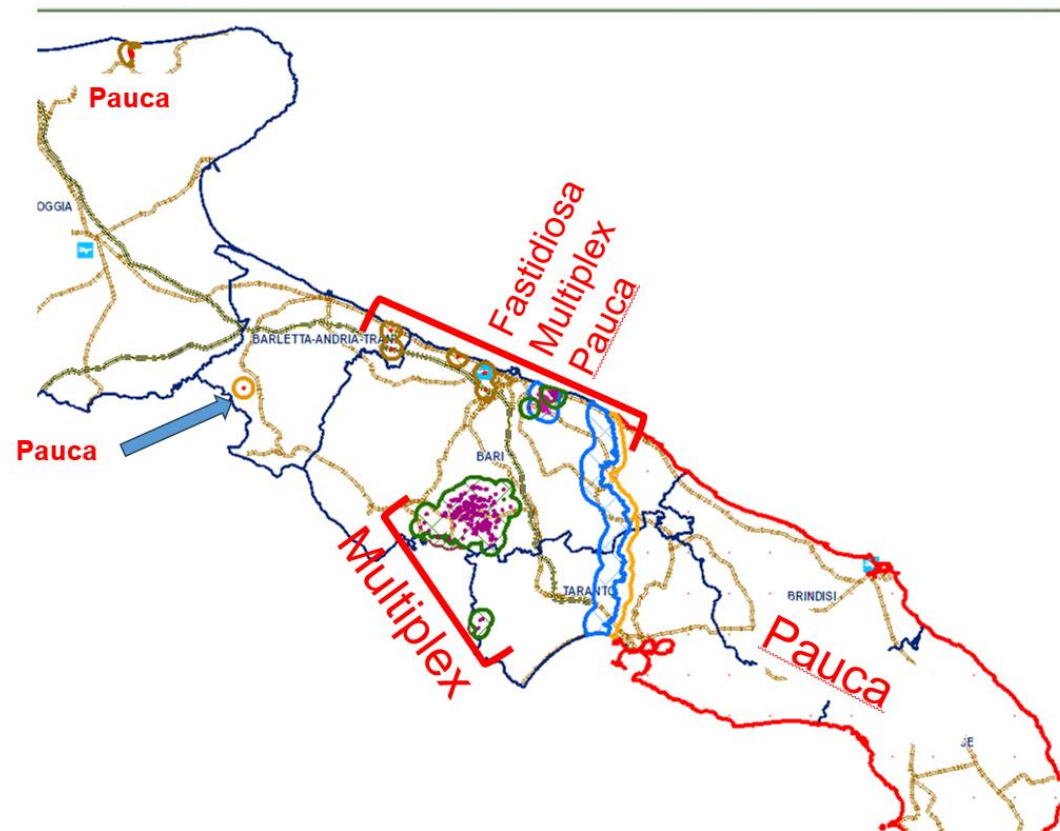
Soon afterward the discovery, in 2013, of the first European outbreak of *Xylella fastidiosa* in Apulia, the Regional phytosanitary authority has implemented a huge monitoring program, still ongoing, to support surveillance, and, where appropriate, to implement eradication or containment actions

From 2013 through the end of the 2025, **a total of 1,501,793 plants were sampled and analyzed!**

An effort unique internationally



Although the scale of the work makes it **difficult to replicate and sustain in the long term**, it has significantly advanced understanding of the spread and complexity of *Xylella fastidiosa*



The Regional Plant Protection Service of Apulia deemed it appropriate to make such a large **dataset accessible to both the scientific community and the general public through the institutional website www.emergenzaxylella.it of the Apulia Region**, which also provides maps and cartographic representations for each annual monitoring campaign

Emergenza
Xylella

[Home](#) [Registrati](#) [Contatti](#) [FAQ](#)

Connettiti

Osservatorio Fitosanitario

- Xylella fastidiosa subsp. PAUCA**
- Contenimento della diffusione - Misure Fitosanitarie
- Piante specificate sensibili alla Xylella fastidiosa sottospecie pauca riscontrate in Puglia
- Procedure Monitoraggio
- Materiale Didattico
- Questionari
- Normativa**
- Comunitaria
- Nazionale
- Regionale
- Estirpazione**
- Moduli
- Zone Interessate**
- Ricerca particelle catastali
- Ricerca particelle catastali Avviso Rigenerazione olivicola
- Monitoraggio**
- Download dati Monitoraggio

Download dati Monitoraggio Xylella f.

Download dati Monitoraggio Xylella f.

Di seguito è possibile accedere ai dati del monitoraggio "Xylella fastidiosa" messo in atto dalla Regione Puglia nell'ultimo decennio a seguito della scoperta del batterio agente causale del disseccamento rapido che ha investito la Puglia meridionale.

E' una mole di dati imponente, con i risultati di oltre un milione di analisi, che ha richiesto uno sforzo enorme da parte dei soggetti coinvolti nella gestione dell'emergenza.

Per una corretta interpretazione dei dati è necessario precisare gli scopi del monitoraggio ed i limiti del suo utilizzo:

- La normativa comunitaria relativa alle misure da attuare per prevenire l'introduzione e la diffusione nell'Unione della Xylella fastidiosa (Regolamento di esecuzione 2020/1201, preceduto da varie "decisioni") riporta tra le prescrizioni a cui ogni Stato membro deve attenersi l'esecuzione annuale di monitoraggi. Sin dalla Decisione 789 del maggio 2015 la Puglia meridionale interessata dall'epidemia non è soggetta a misure di "eradicazione" ma, preso atto dell'insediamento ormai endemico del batterio, a misure di "contenimento", ossia misure atte a contenere l'ulteriore espansione dell'area infetta.
- Conseguentemente l'obiettivo del monitoraggio, con cui si analizzano, in stragrande maggioranza, piante della zona "Indenne" e "cuscinetto" (anch'essa indenne per definizione) e dell'adiacente "zona di contenimento", ossia la parte terminale della zona infetta, dove in un'area ancora sostanzialmente libera dal batterio si ritrovano solo sporadici avamposti di piante infette, non è quello di inventariare le piante infette ma di precedere il batterio e identificare e contenere o eliminare sul nascere nuovi focolai per abbattere la pressione d'inoculo sulla zona indenne e rallentare il più possibile l'espansione della zona infetta. Pertanto i dati di monitoraggio non possono fornire indicazioni sulla frequenza del batterio nella zona infetta, in quanto la maggior parte della sua superficie non è più oggetto di sorveglianza da parte dell'Osservatorio Fitosanitario.
- Per ogni pianta, a partire dalla campagna 2014-2015, è indicata anche la presenza o assenza di sintomo di disseccamento, senza indicazione dell'eventuale grado di severità. Considerato che la presenza di porzioni, spesso piccole, di rami disseccati non è un sintomo specifico ed è frequente anche in piante sane, l'indicazione di "sintomo presente", pur includendoli, non è sinonimo di sintomi di OQDS (Olive Quick Decline Syndrome) notoriamente associati a piante infette da Xylella fastidiosa, a maggior ragione in un'area dove l'eventuale infezione è avvenuta in tempi recenti e non sufficientemente lunghi per lo sviluppo del "disseccamento rapido".

Download dati Monitoraggio Xylella f.	Dettaglio
Campagna monitoraggio 2013-2014: Xylella f. sub. Pauca	Vai al dettaglio
Campagna monitoraggio 2014-2015: Xylella f. sub. Pauca,	Vai al dettaglio
Campagna monitoraggio 2016-2017: Xylella f. sub. Pauca	Vai al dettaglio

European Food Safety Authority (EFSA)

- EFSA
- Commission database



The files of the database are preceded by an introductory note CONTAINING INSTRUCTIONS FOR THE CORRECT INTERPRETATION OF THE DATA, which are essential to avoid the risk of misinterpreting the database

Download dati Monitoraggio Xylella f.



Download dati Monitoraggio Xylella f.

Di seguito è possibile accedere ai dati del monitoraggio "Xylella fastidiosa" messo in atto dalla Regione Puglia nell'ultimo decennio a seguito della scoperta del batterio agente causale del disseccamento rapido che ha investito la Puglia meridionale.

E' una mole di dati imponente, con i risultati di oltre un milione di analisi, che ha richiesto uno sforzo enorme da parte dei soggetti coinvolti nella gestione dell'emergenza.

Per una corretta interpretazione dei dati è necessario precisare gli scopi del monitoraggio ed i limiti del suo utilizzo:

The purpose of monitoring is not to inventory infected plants, but to **anticipate the bacterium by identifying new outbreaks at an early stage**, thereby reducing inoculum pressure on uninfected areas and **slowing, as far as possible, the expansion of the infected zone.**

CONSEQUENTLY A VERY LOW PERCENTAGE OF POSITIVES IS THE EXPECTED RESULT





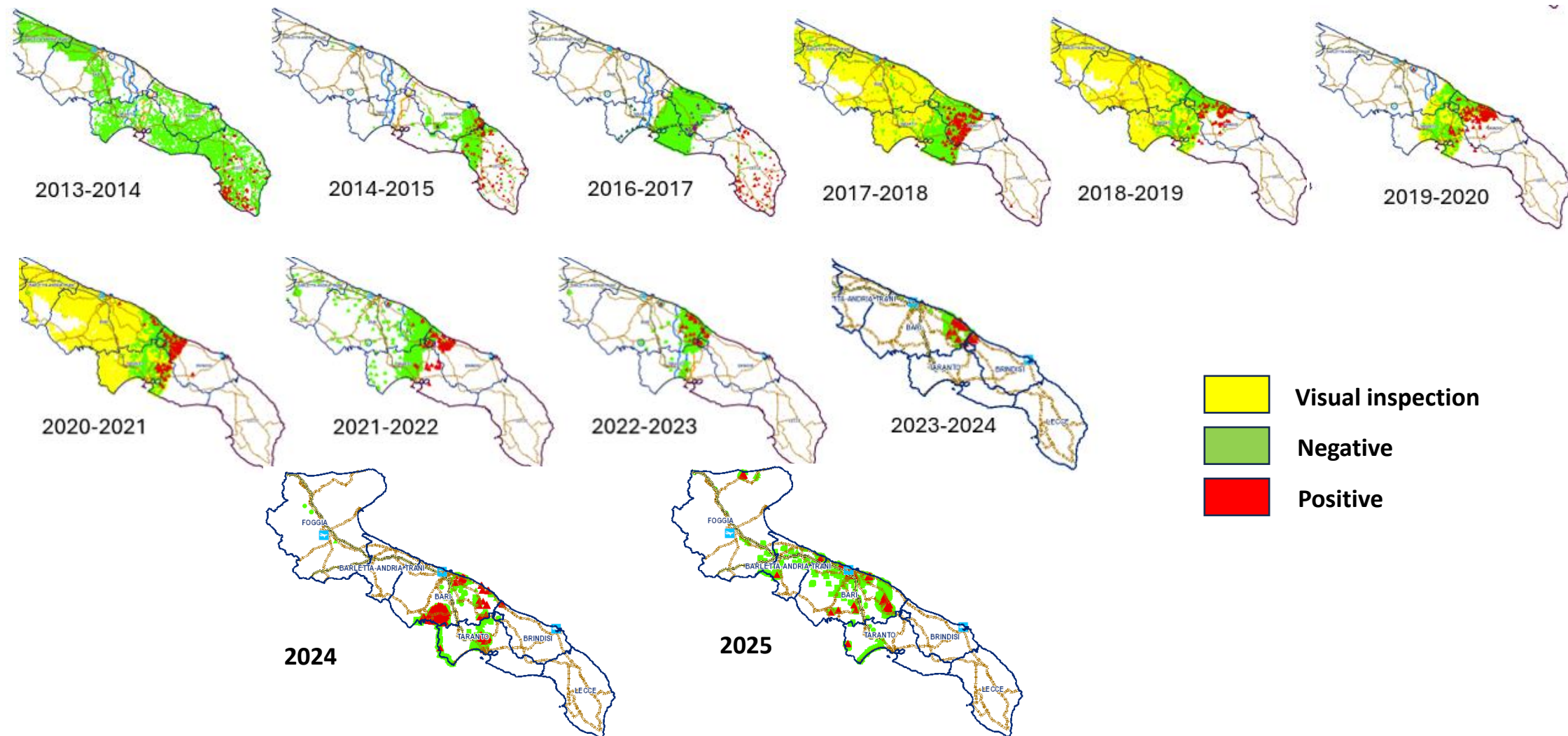
As expected, the percentage of the positive samples is relatively low

Monitoring campaigns	Nr. analyzed plants	Positives
2013/2014	11099	351 (3.16%)
2014/2015	51409	4191 (8.15%)
2016/2017	159109	1588 (1.00%)
2017/2018	198768	3835 (1.93%)
2018/2019	68500	1074 (1.57%)
2019/2020	50967	1396 (2.74%)
2020/2021	172005	1205 (0.7%)
2021/2022	219120	2800 (1.28%)
2022/2023	266352	366 (0.14%)
2023/2024	62876	189 (0.3%)
2024/2025	119723	1113 (0.93%)
2025	121865	401 (0.32%)
Total	1,501,793	18,510 (1.23%)

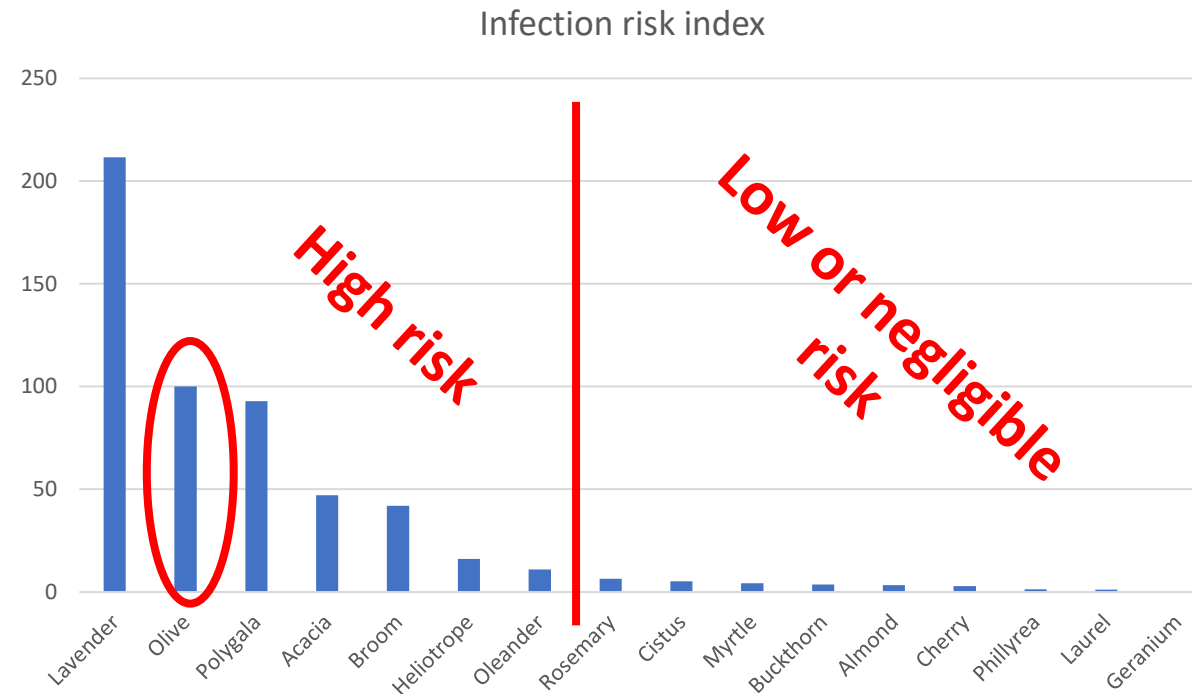
83.5% of the total (1,253,753) are olive trees, of which 17,313 (1.38%) were positives



The cartographic representation make it possible to visualize the pathogen's progression, from the initially rapid diffusion in southern Salento to a slower advance towards the north



The database also provides additional valuable insights as, i.e.
evidence of different degrees of susceptibility to Pauca ST53 among host species



"infection risk index" (IRI) given by the **ratio between the percentage of positives of each species and the percentage of positives in olive**, classified as "reference host species"

$$\text{IRI} = \% \text{ species pos.} / \% \text{ olive pos.}$$

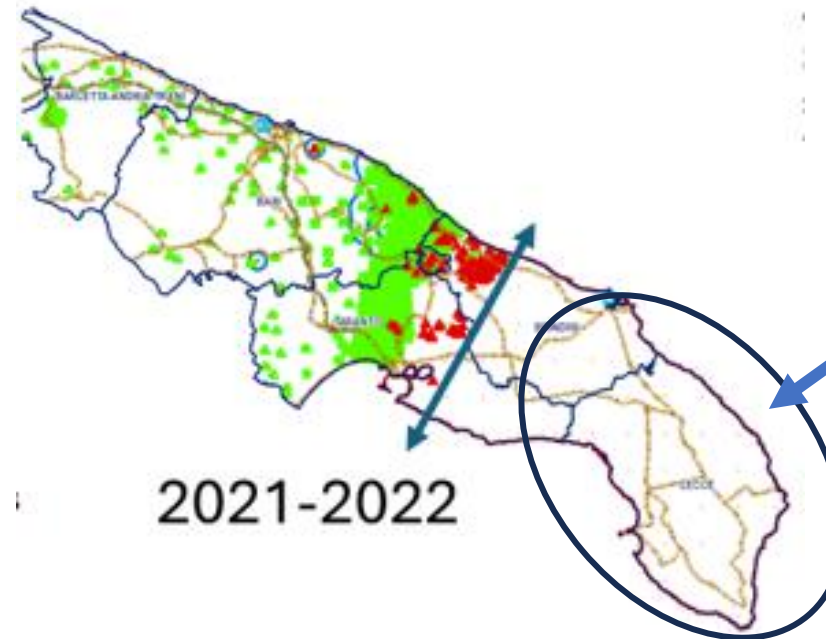
Other additional valuable insights:

further confirmation of the etiological role of subsp. *pauca* as the agent of Olive Quick Decline Syndrome (OQDS)

Example 1, northern area with partial impact

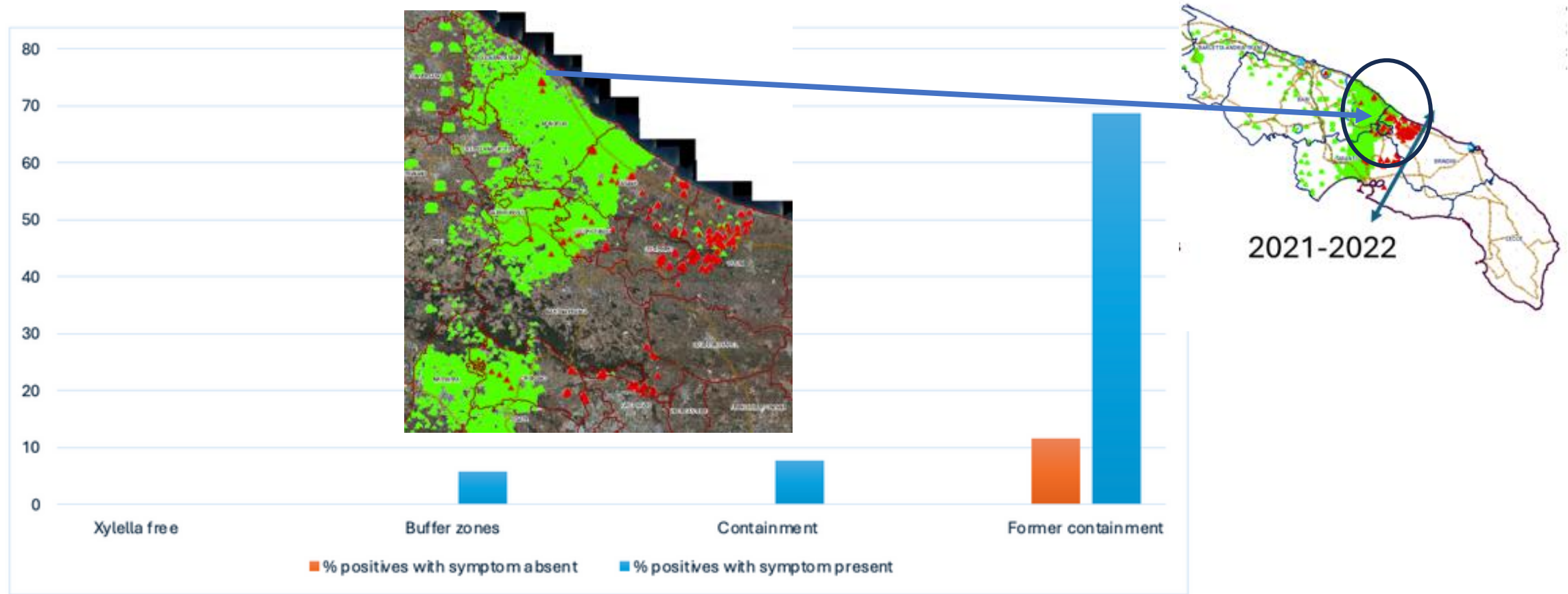
2021-2022:

***2791 Olives positives
out of 188,009 (1.48%)***



Area devastated by OQDS

further confirmation of the etiological role of subsp. *pauca* as the agent of OQDS



The percentage of infected olives is significantly higher in trees with "symptom present" and it increases almost exponentially moving from northwest to southeast

further confirmation of the etiological role of subsp. *pauca* as the agent of Olive Quick Decline Syndrome (OQDS)

Example 2, southern area with devastating impact

Although the monitoring was not intended to inventory infected trees, an exception was made in the 2017 campaign, when a **small monitoring** was also conducted in the devastated area, no longer subject to surveillance



In rosso l'indicazione degli impianti di Leccino, in giallo quella degli impianti di Cellina di Nardò e/o Ogliarola salentina.

500 severely affected olive trees sampled at 13 different sites and 100 asymptomatic olive trees of the resistant cv "Leccino" were analyzed

The map on the right, extracted from the article titled "Incidence of *Xylella* in olive groves with rapid desiccation" published in *L'Informatore Agrario* (https://cartografia.sit.puglia.it/doc/xylella/Informatore_Agrario_27_2017.pdf) showing the location of all 16 monitored sites.

further confirmation of the etiological role of subsp. *pauca* as the agent of Olive Quick Decline Syndrome (OQDS)

Example 2, southern area with devastating impact



The results, reported in the database, show that **all 500 severely compromised trees tested positive**, while only **15% of the 100 asymptomatic Leccino trees were positive**, as expected for resistant varieties



RIGENESI

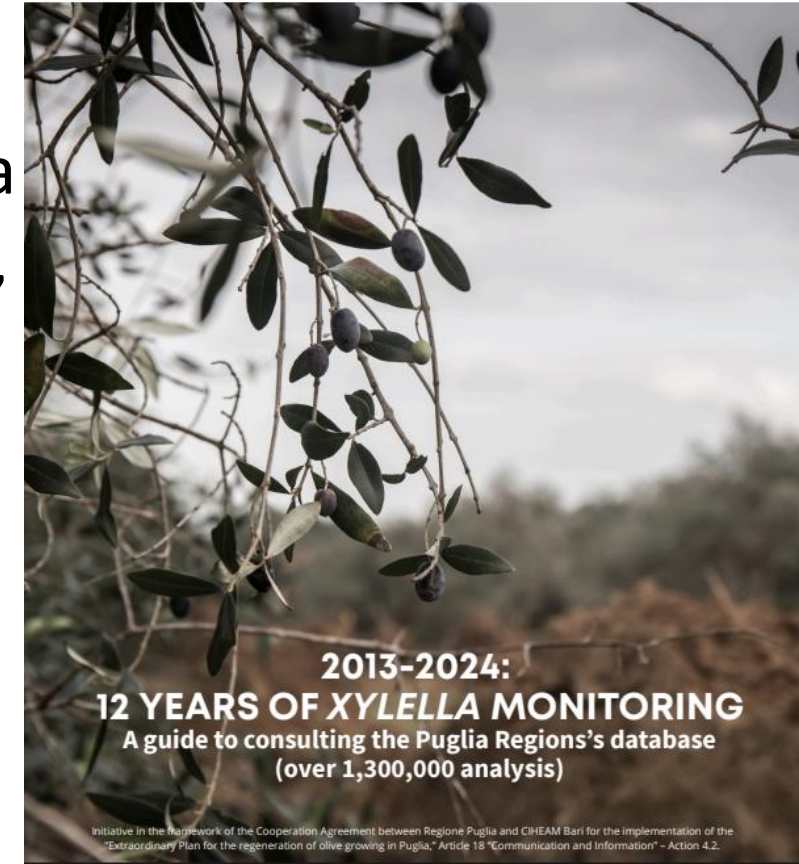
SCIENCE DIPLOMACY & COMMUNICATION FOR
OLIVE SECTOR REGENERATION

Briefing No. 1 – November 2025

Editors: Plant Protection Service of Regione Puglia and CIHEAM Bari

For those interested in exploring the entire database, a **detailed guide to its consultation has been published**, in the frame of the RIGENESI project (Briefing No. 1), by the Regional Phytosanitary Service and the CIHEAM and downloadable at the link

<https://rigenesi.iamb.it/en/reports/>





**THANK YOU FOR YOUR
ATTENTION**



Delimiting and managing the *Xylella fastidiosa* outbreak in Alicante (Spain): insights from surveillance and eradication data

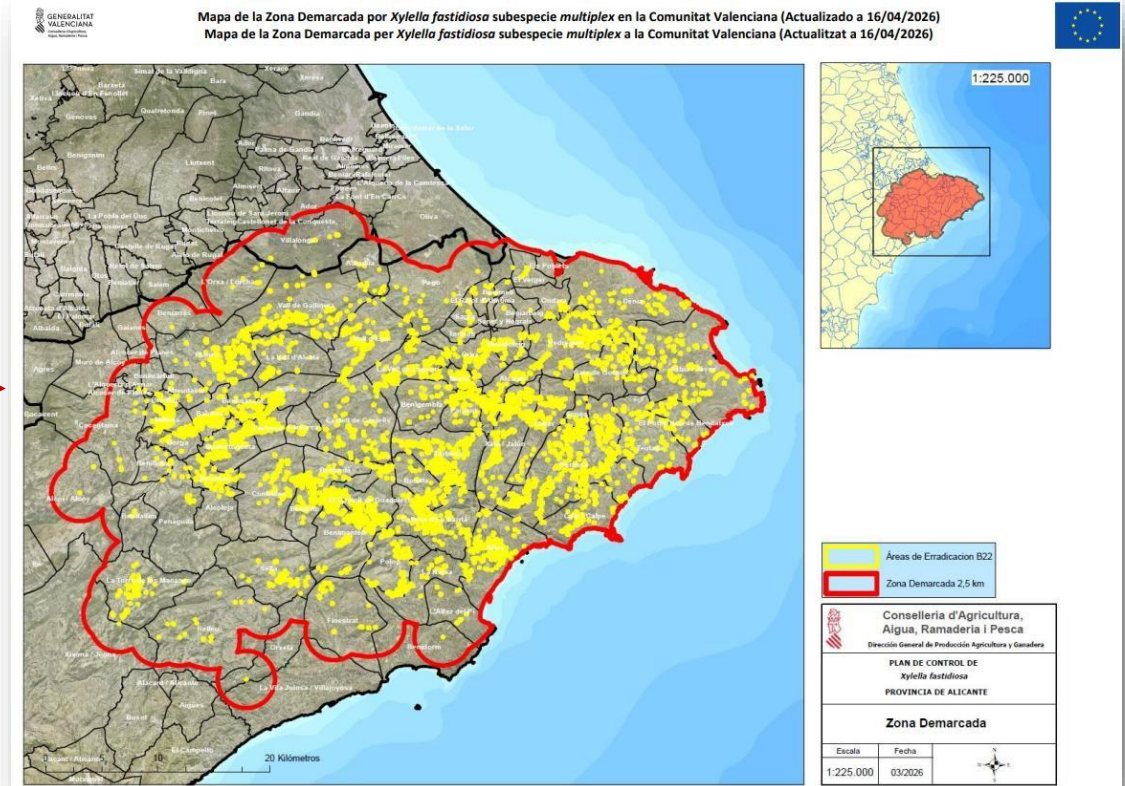
Martina Cendoya, David Cubillo, Vicente Dalmau, Antonio Vicent



Xylella fastidiosa outbreak in Alicante (Spain)

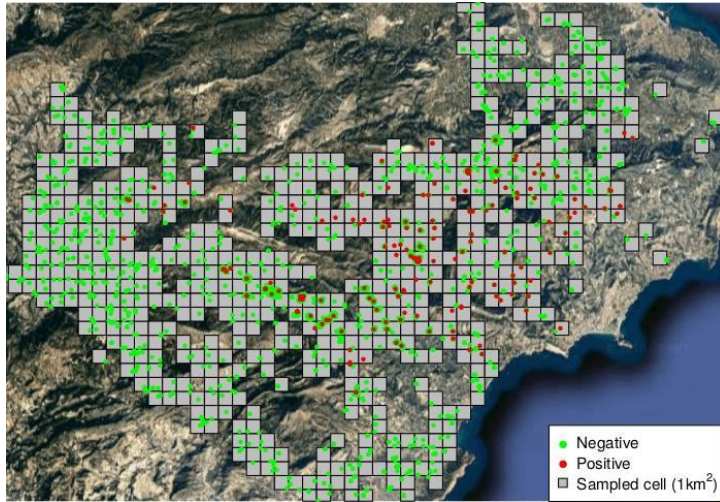


- First detection in 2017
- *Xf* subsp. *multiplex*
- Almond leaf scorch disease
- Situation in 2025:
 - Demarcated zone 170,721 ha
 - 4,260 ha have been eradicated with 315,166 almond trees removed

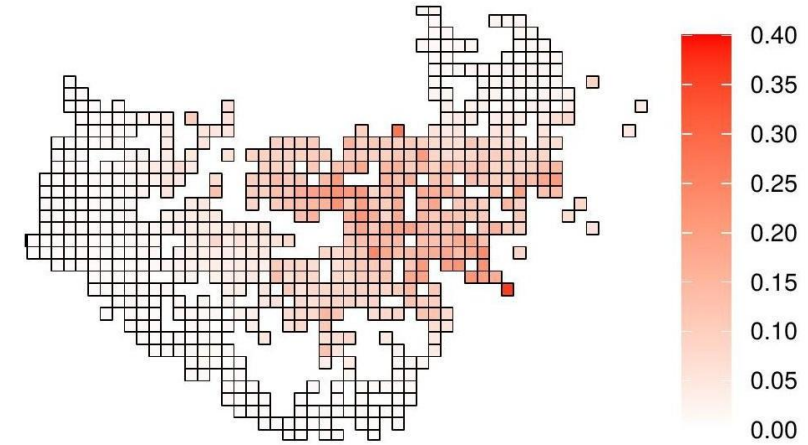


Effects of climatic and spatial factors

- Analysis of climatic and spatial effects → Bayesian spatial modelling

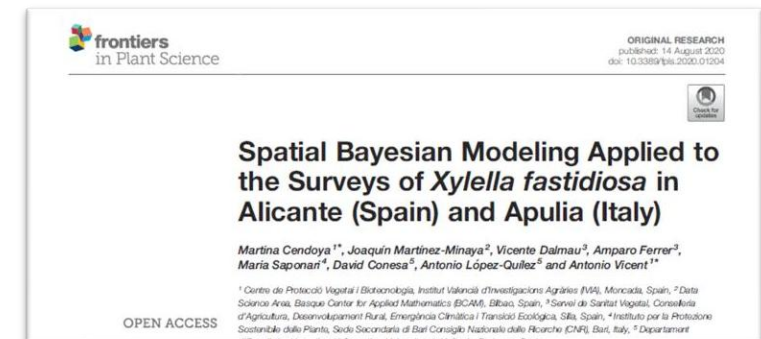


Official surveys



Mean of the predictive posterior distribution
of the **probability of presence**

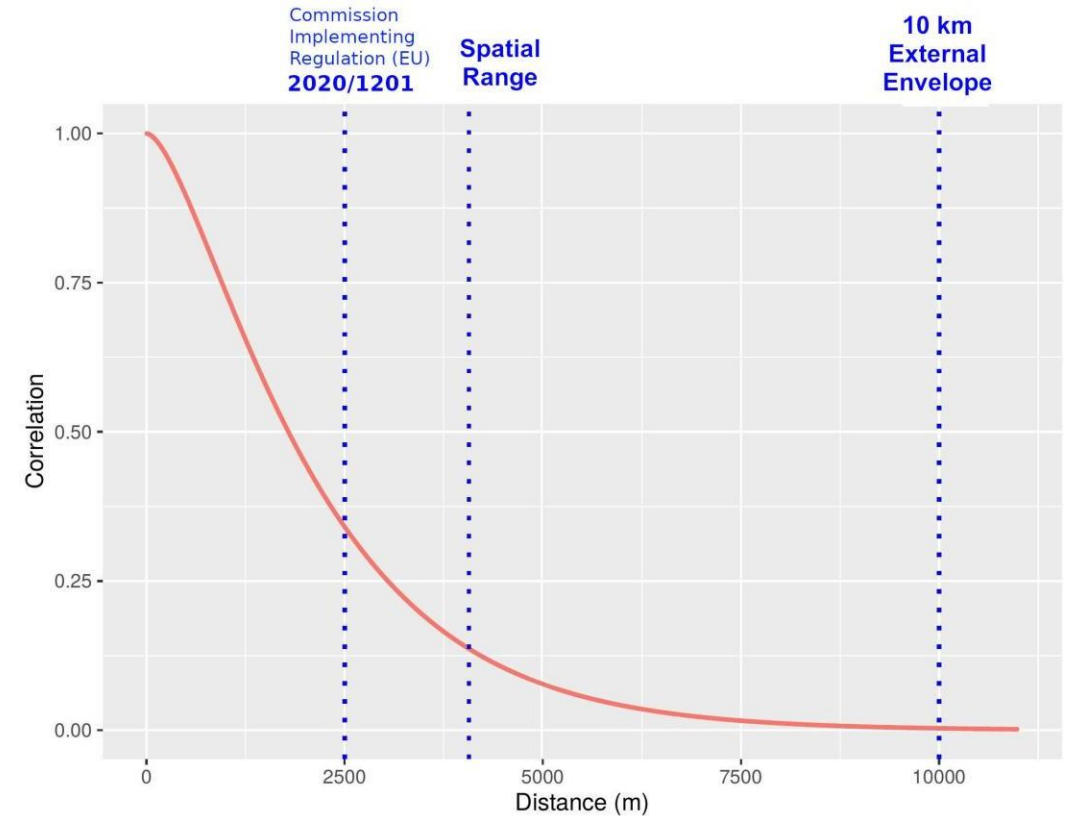
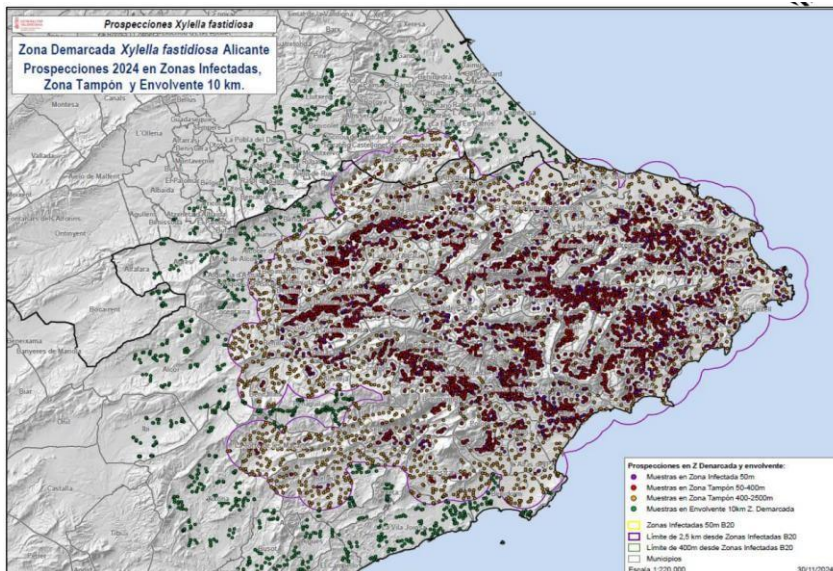
- No relevant effect of climatic variables on the probability of *Xf* presence
- Strong effect of the **spatial component**



Spatial distribution

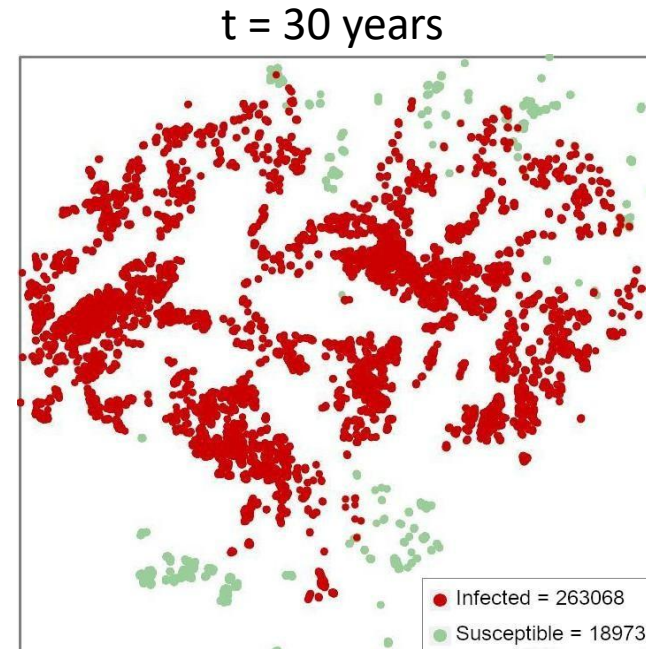
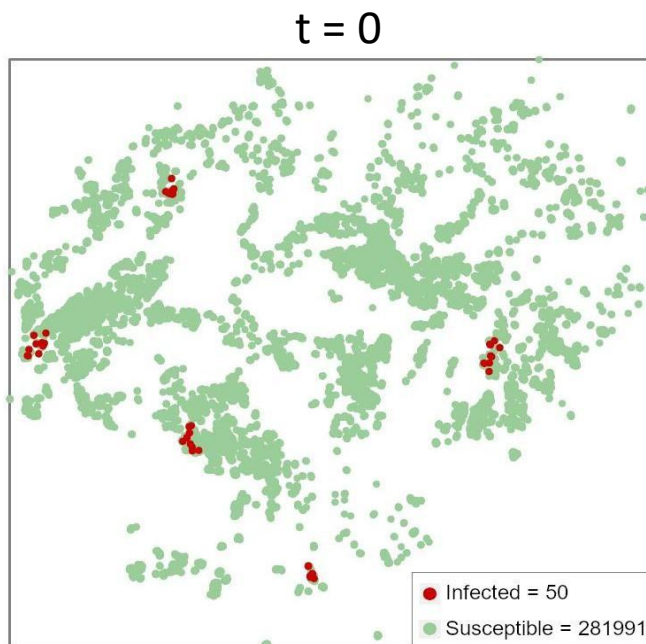
Spatial Range: maximum distance at which two observations are considered to be spatially correlated

	Mean	(95% CI)
Intercept	-1.68	(-2.21, -1.23)
σ_u	1.52	(1.28, 1.80)
Range (m)	4030.17	(2907.41, 5563.88)



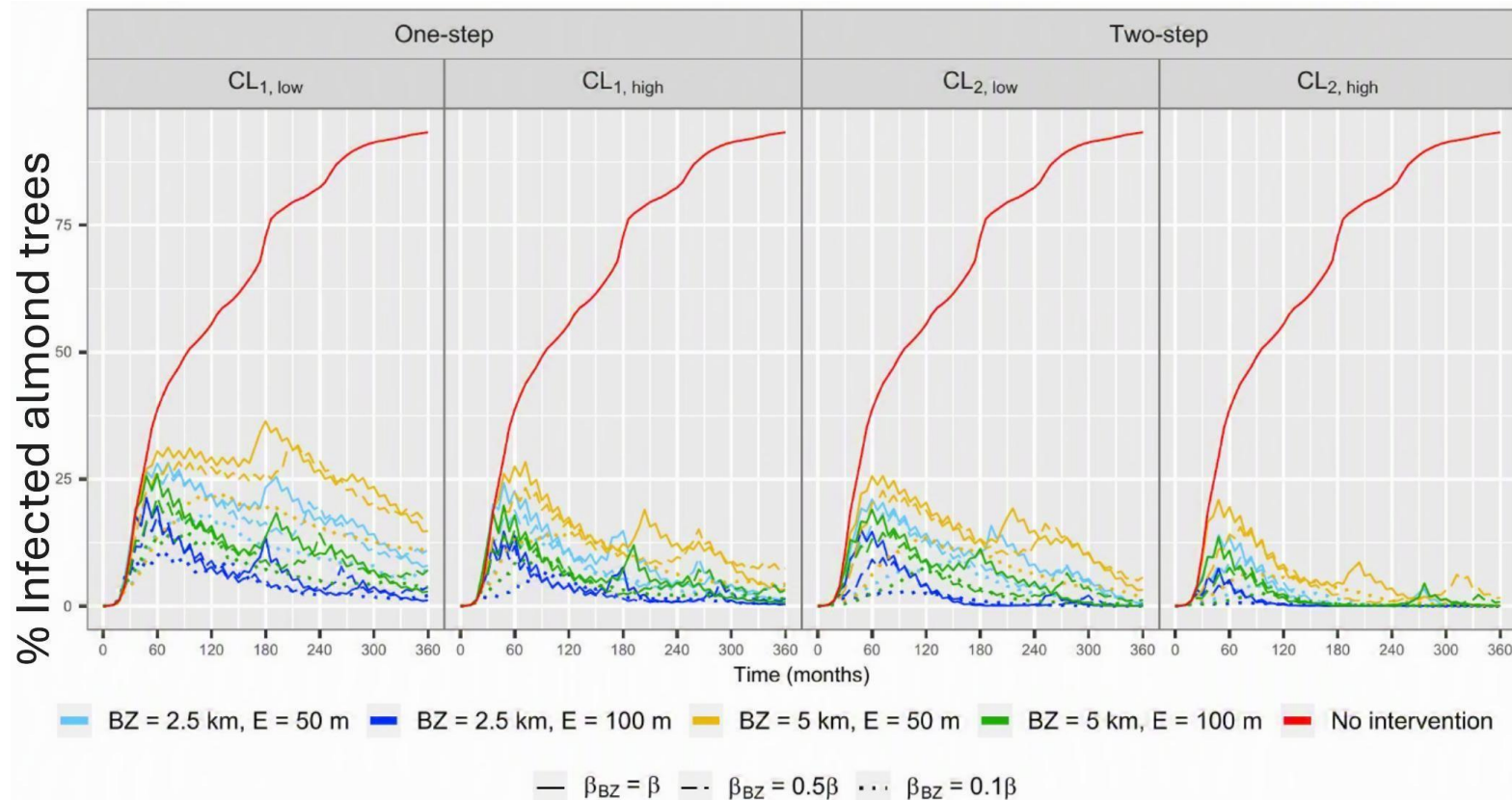
Outbreak management plans

- **Backtracking the outbreak to its most likely origin:**
 - Molecular epidemiological studies: Introduction mid-1990s (Landa et al. 2020; Moralejo et al. 2020)
 - Current extent of the infested area
 - Almond tree distribution
- **Baseline scenario:**
 - Individual-Based spatial spread model → Spread **without interventions** over 30 years



Outbreak management plans

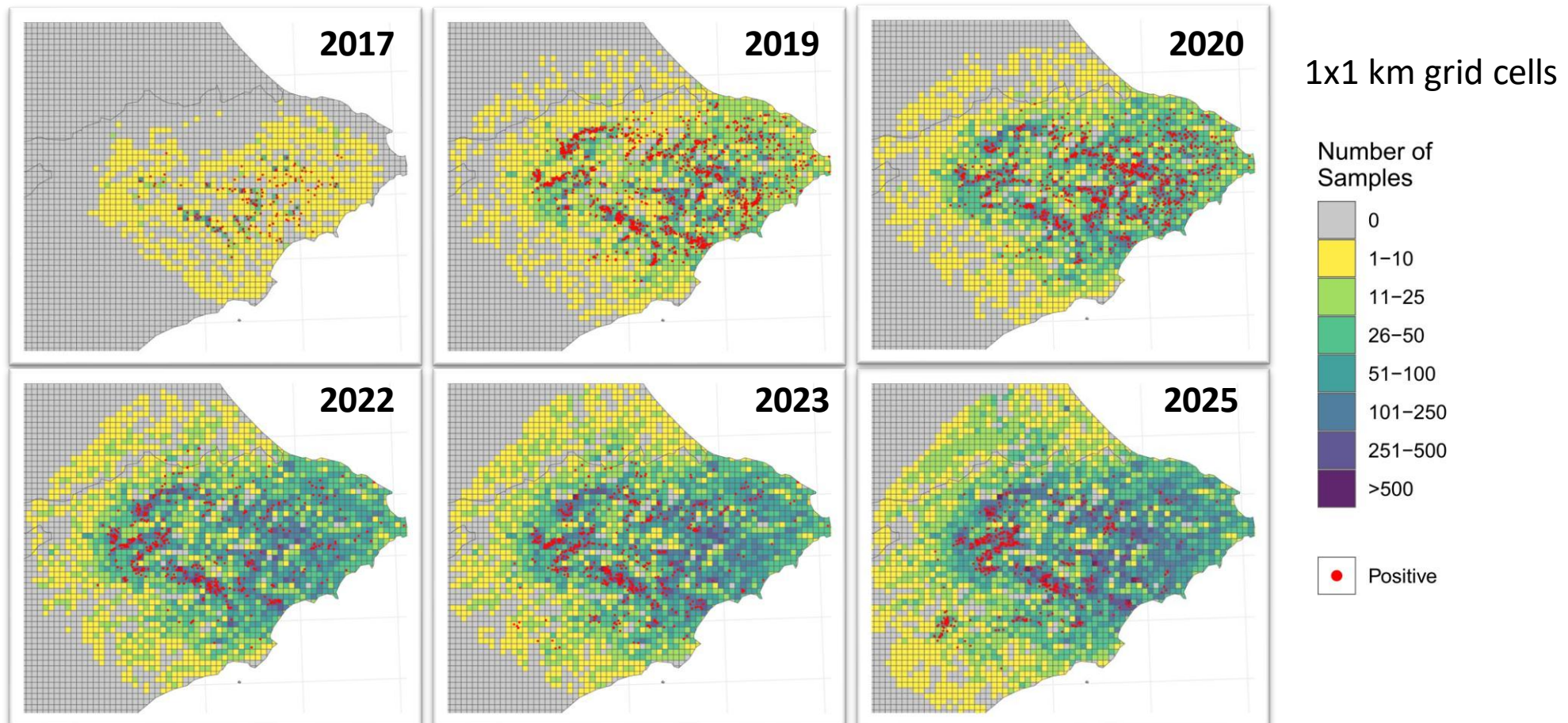
- Simulation of different combinations of management plans:
 - **Surveillance** (based on EFSA guidelines)
 - **Control**
 - Including those in Regulation (EU) 2020/1201



Progressive delimitation of the outbreak

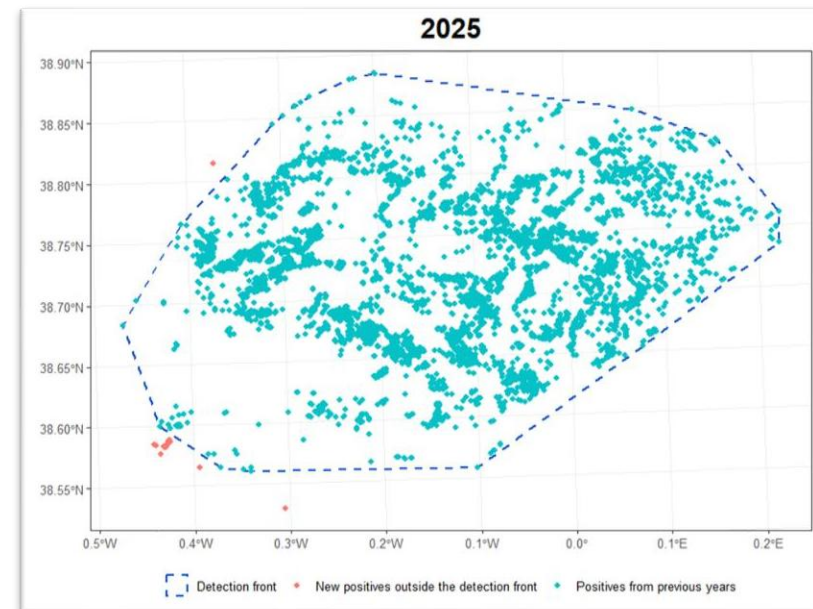
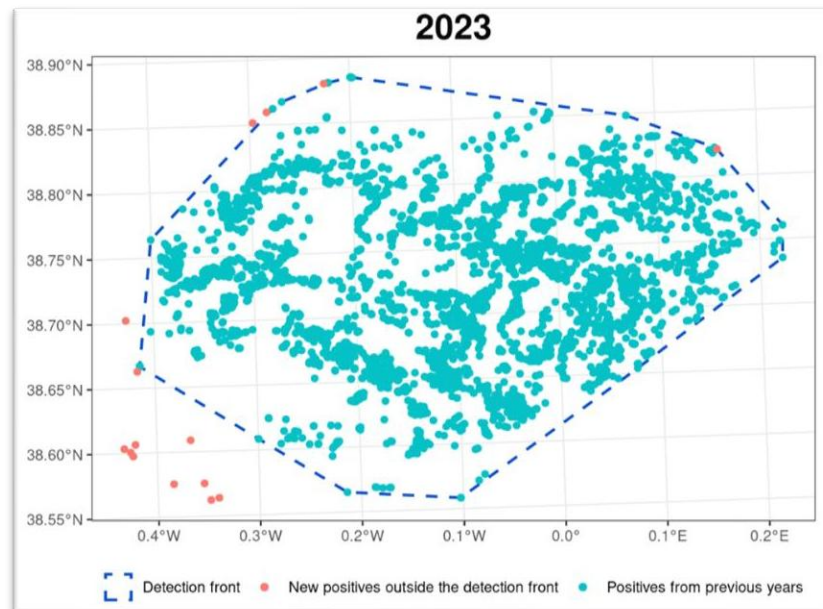
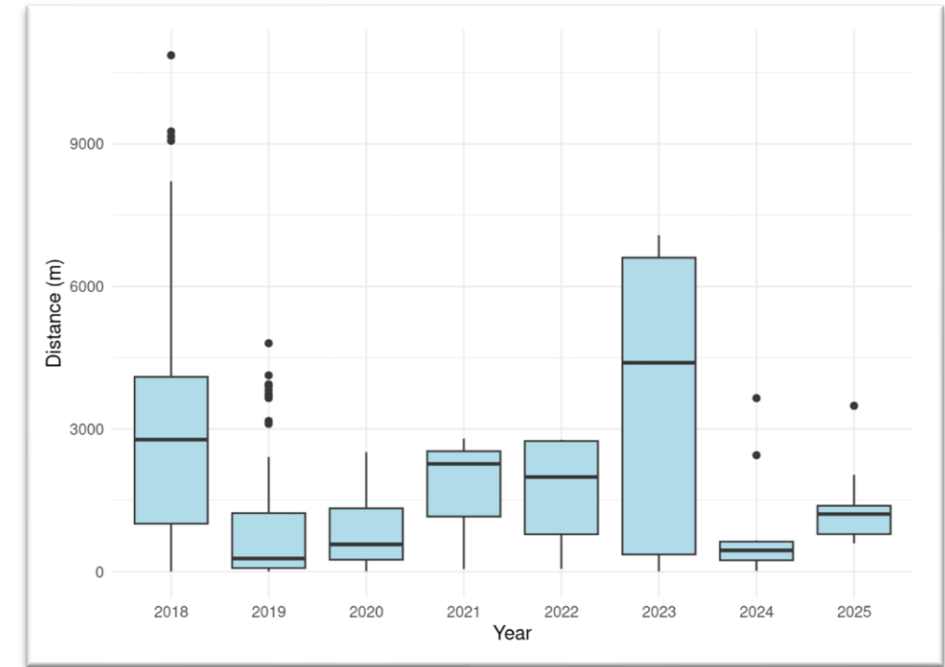
Cumulative sampling effort and new positives

- Progressive surveillance expansion led to the detection of new infected locations
- Heterogeneous surveillance (more intensive around positives in accordance with the regulation)

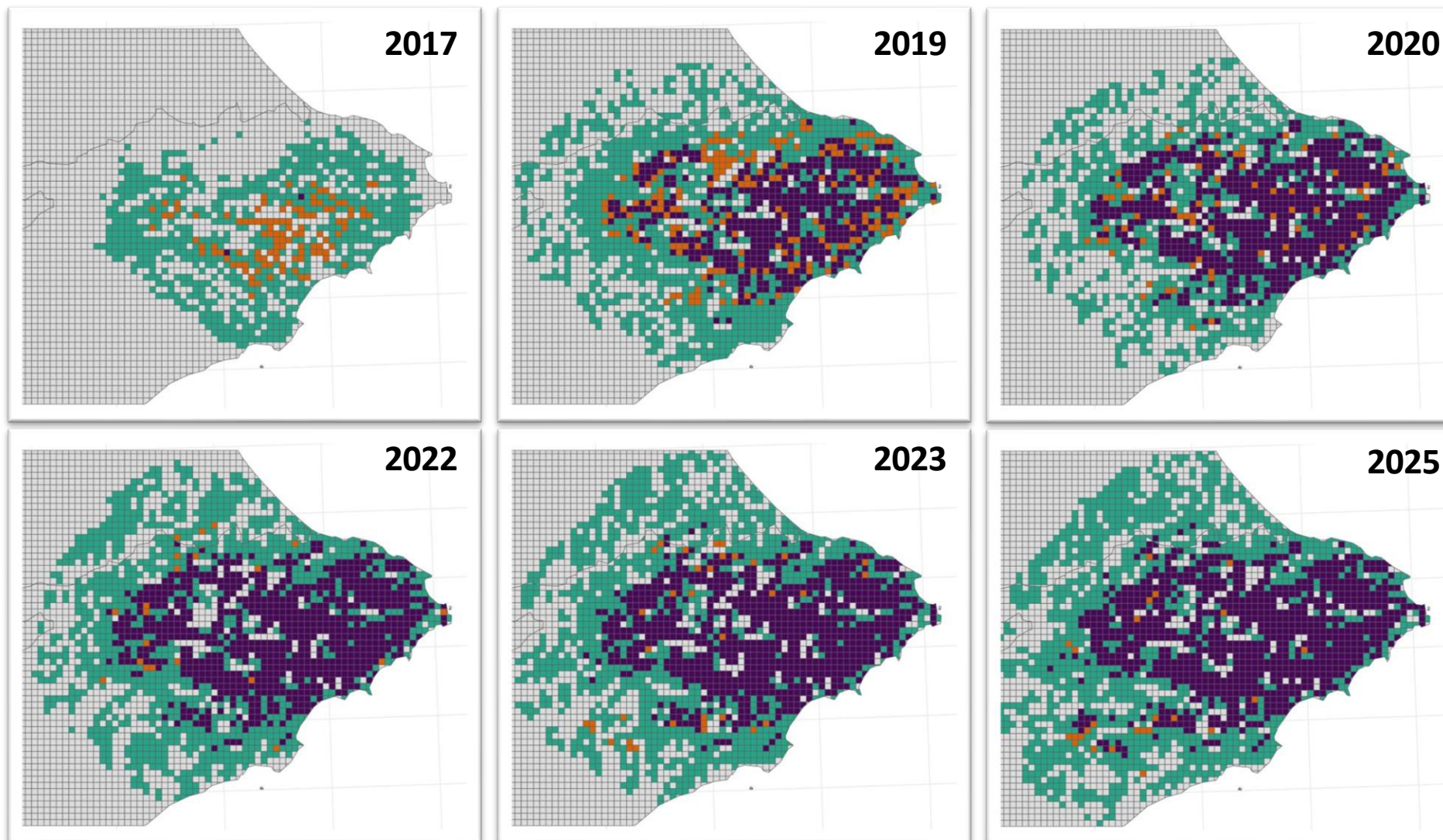


Detection front

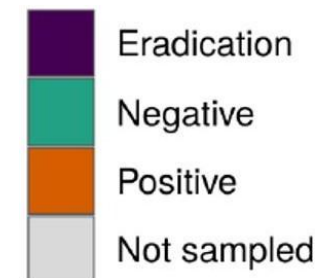
- Distance of a new positive from the **detection front** delimited by the cumulative positives from previous years
- Most of the new detections remained close to the previously delimited outbreak
- The observed variation is largely explained by the surveillance coverage rather than by further spread



Eradication progress

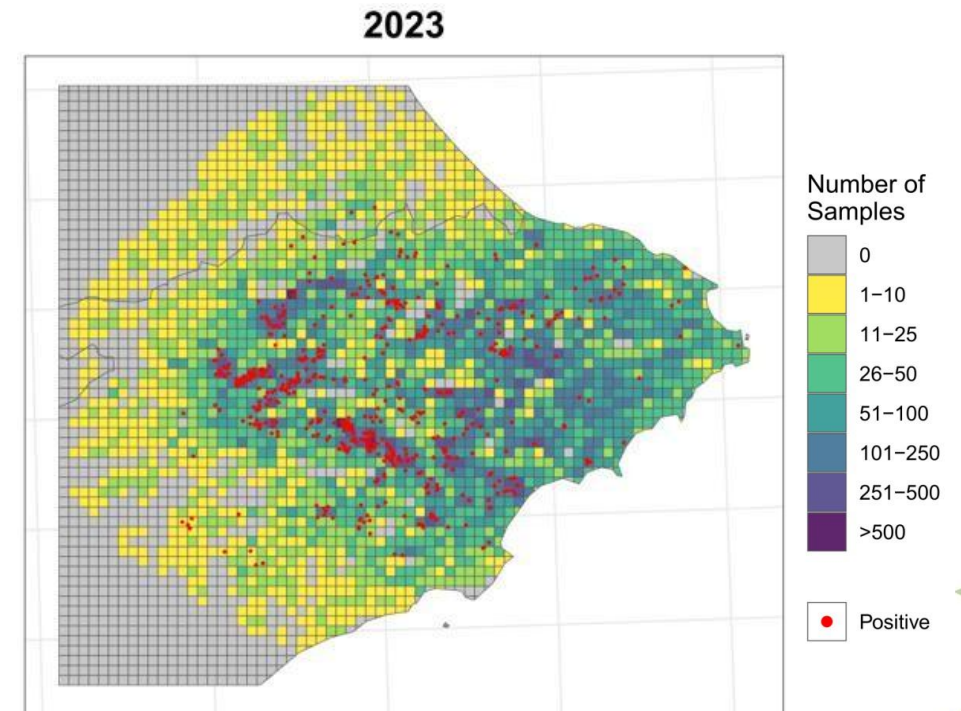
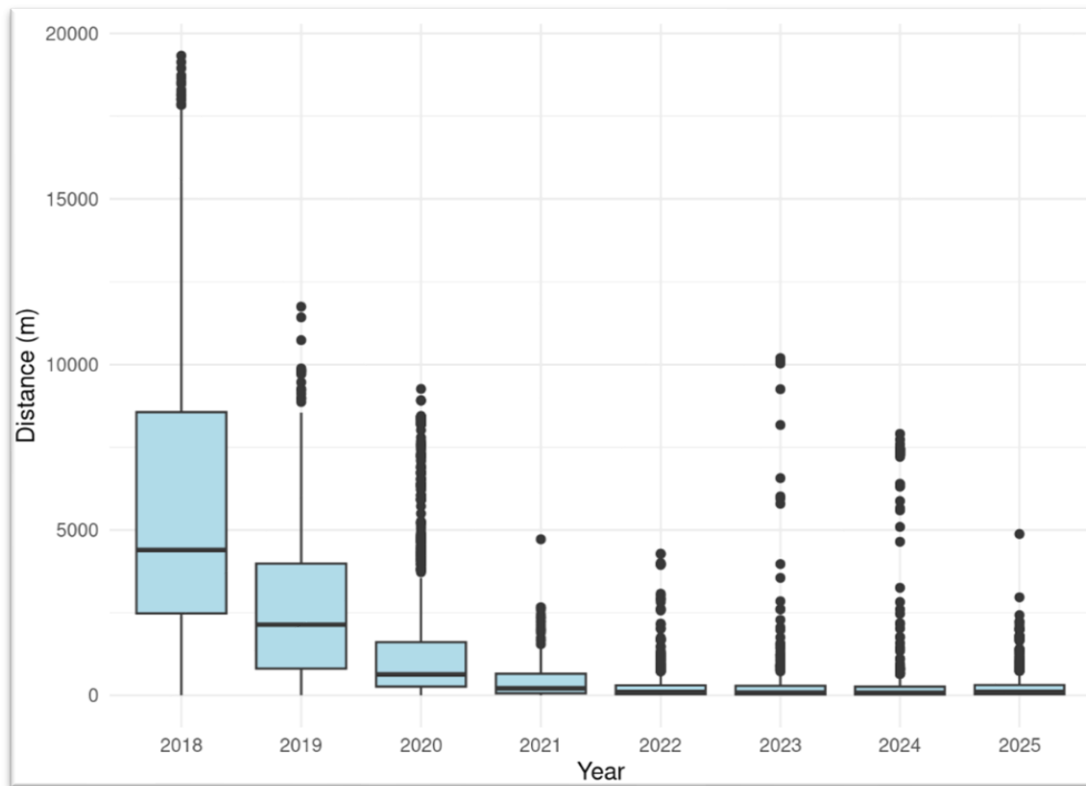


1x1 km grid cells



New detections and eradication areas

- Overall decrease in the distance between new positives and the nearest eradicated area
- The observed increase is consistent with the expansion of surveillance into areas with previously low surveillance effort





Highlights for the outbreak of *Xf* subsp. *multiplex* in Alicante

- Strong effect of the spatial component on the *Xf* occurrence
 - Spatial analysis supported the extent of the surveillance area around the outbreak
 - Survey effort determines the performance of outbreak management plans
 - Outbreak delimitation in Alicante was achieved progressively over several years
 - Survey effort was heterogeneous over time, with greater survey effort around positives
 - New detections occurred progressively closer to eradicated areas
 - No substantial outbreak spread has been observed so far
- 

Grazie!

Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



Source: GVA



HORIZON project (European Union) No 101060593 BeXyl (Beyond Xylella, Integrated Management Strategies for Mitigating Xylella fastidiosa impact in Europe)



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



REGIONE PUGLIA



Detection of *Xylella fastidiosa* in important Mediterranean plants during dormancy

Anne-Laure Boutigny, Anses, France



Context

The multiplication of *Xylella fastidiosa* (Xf) within a host plant depends on:

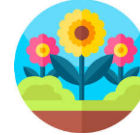
- environmental conditions,
- bacterial strains,
- host species/cultivars



For trade purposes => an analysis of the plants to be planted is required

To maximise Xf detection => sampling should preferably be performed during the **plants' active growth period**

late spring



autumn



Context

The multiplication of *Xylella fastidiosa* (Xf) within a host plant depends on:

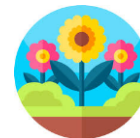
- environmental conditions,
- bacterial strains,
- host species/cultivars



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To maximise Xf detection => sampling should preferably be performed during the **plants' active growth period**

late spring



autumn



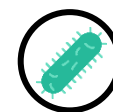
For deciduous plants =>

analysis often required during the dormant phase when bacterial activity is the lowest



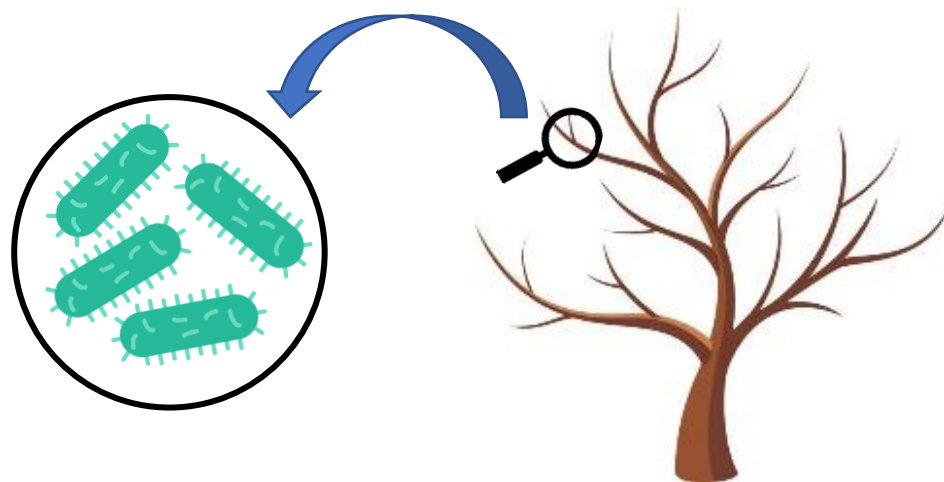
Experiments conducted within EU projects (POnTE, XF-ACTORS) have shown that **Xf could be detected in some deciduous plants, all year round, including during :**

- the asymptomatic phase
- **the dormancy**



Aim

Evaluate methods to detect *Xylella fastidiosa*
and identify subspecies
in dormant plants of interest
for Mediterranean countries



★ **Great interest in dormant wood analysis:** analyses during the winter, interesting for trade

Project Xf-DORM

Duration : 2023-2025

20 laboratories

33 scientists

15 countries



Department
of Industry



Netherlands Food and Consumer
Product Safety Authority
Ministry of Agriculture,
Nature and Food Quality



NACIONALNI INŠTITUT ZA BIOLOGIJO
NATIONAL INSTITUTE OF BIOLOGY



Department
for Environment
Food & Rural Affairs



المعهد الوطني للبحث الزراعي
Institut National de la Recherche Agronomique



An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Tasks

Task 1: Inventory

- Dormant plants that could be infected with *Xf*
- Methods (sampling, DNA extraction, diagnostic tests) to detect *Xf* and identify *subspecies* in dormant plants

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(CREA, Italy)



Tasks

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(CREA, Italy)



Task 3: Evaluation of methods and *Xf* dynamics on dormant samples naturally infected with *Xf*

(ANSES, France ; IPSP, Italy ; IVIA, Spain ; PPIS, Israel)

- **Collect and share dormant plant materials** (naturally infected with *Xf*)
- **Organize a Test Performance Study (TPS)** on naturally infected samples
- **Evaluate the distribution dynamics of *Xf*** in woody stems naturally infected throughout the year including dormancy



Tasks

Task 1: Inventory

- **Dormant plants** that could be infected with *Xf*
- **Methods (sampling, DNA extraction, diagnostic tests)** to detect *Xf* and identify *subspecies* in dormant plants

Task 2: Evaluation of methods on dormant samples spiked with *Xf*

(CREA, Italy)



Task 3: Evaluation of methods and *Xf* dynamics on dormant samples naturally infected with *Xf*

(ANSES, France ; IPSP, Italy ; IVIA, Spain ; PPIS, Israel)

- 
- **Collect and share dormant plant materials** (naturally infected with *Xf*)
 - **Organize a Test Performance Study (TPS)** on naturally infected samples



Collection of samples naturally infected with Xf for the TPS

- ✓ Dormant twigs naturally infected with *X. fastidiosa* and healthy twigs (ANSES, France ; IPSP, Italy ; IVIA, Spain ; PPIS, Israel)
- ✓ Collected in France, Italy, Spain and Israel
- ✓ Twigs from almond tree, plum tree and grapevine
- ✓ Infected with different subspecies (*multiplex*, *fastidiosa* and *pauca*) and Sequence Types (ST6, ST53, ST1)



twigs (~ 50 cm)

=> Good diversity of naturally infected samples

Panels sent to participating laboratories

- ✓ 10 samples in each panel : **8 infected samples** and **2 healthy samples**, including **1 lure** different for each laboratory, **blind testing**
- ✓ **Homogeneity** and **stability** of samples verified
- ✓ Sent to **14 participating laboratories**

Preparation of samples



twigs of about 50 cm



cutting into 2 cm pieces and homogenization



10 pieces randomly selected to form a sample

Tested protocols

Various protocols for *Xf* detection and *subsp.* identification were tested, at the choice of the participating laboratories

DNA extraction:	<i>Xf</i> detection:	<i>Subsp.</i> identification:
CTAB, Quickpick, Dneasy plant mini Kit, Dneasy Mericon Food Kit, 2 Maxwell Kits	Harper PCR simplex, Harper PCR duplex, Bonants PCR, NSWDPI endpoint PCR, Agdia AmplifyXRT	Hodgetts PCR simplex, Dupas PCR simplex, triplex and tetraplex

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A common protocol for Xf detection (CTAB DNA extraction/Harper real-time PCR) was proposed by the organizer and tested by 10 laboratories
=> Analysis of the results of all methods for all participants

Results

Performance criteria calculated for protocols tested by 3 or more than 3 laboratories

DNA extraction	Molecular test	Number of laboratories
CTAB	Harper simplex	10
QuickPick		7
Dneasy		5
CTAB	Hodgetts simplex	4
QuickPick		3
QuickPick	Dupas tetraplex	3

3 *Xf* detection protocols

3 *subsp.* identification protocols

★ diagnostic sensitivity
diagnostic specificity
accuracy
repeatability
reproducibility

Results: summary

DNA extraction	Molecular test	Performance criteria					Number of laboratories
		Diagnostic sensitivity	Diagnostic specificity	Accuracy	Repeatability	Reproducibility	
CTAB	Harper simplex	98,6	100	98,9	97,1	97,8	10
Dneasy		91,4	100	93,3	97,5	89,3*	5
QuickPick		98,0	100	98,4	98,8	97,3	7

* 100% if exclusion of 1 laboratory which has no experience in this extraction method

=> Xf detection protocols validated

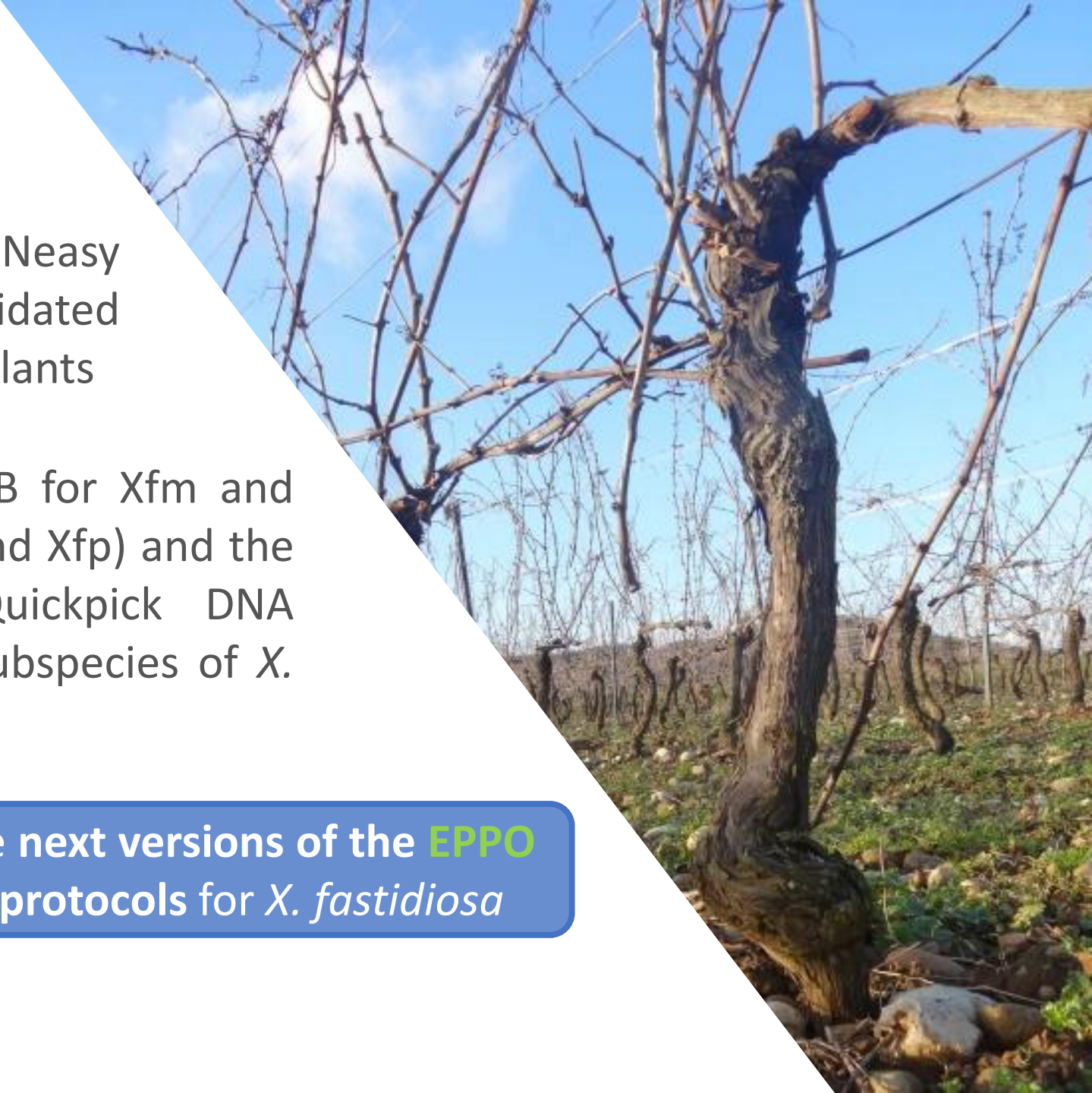
CTAB	Hodgetts simplex (Xfm)	100	100	100	100	95,8	4
CTAB	Hodgetts simplex (Xff)	87,5	100	97,2	100	95,8	4
QuickPick		83,3	100	94,4	100	95,1	3
CTAB	Hodgetts simplex (Xfp)	100	100	100	100	95,8	4
QuickPick		100	100	100	100	95,1	3
QuickPick	Dupas tetraplex	100	100	100	96,8	100	3

=> *subsp.* identification protocols validated

Rq: Other protocols reliable to detect *Xf* or identify *subspecies* in dormant plants but only tested by 1 or 2 laboratories

Conclusions

- **Harper PCR simplex** (after CTAB, DNeasy and Quickpick DNA extractions) validated to detect *X. fastidiosa* in dormant plants
 - **Hodgetts PCR simplex** (after CTAB for Xfm and after CTAB and Quickpick for Xff and Xfp) and the **Dupas PCR tetraplex** (after Quickpick DNA extraction) validated to identify subspecies of *X. fastidiosa* in dormant plants
- The results will be proposed for the next versions of the **EPPO PM7/24** and **ICPP DP25** diagnostic protocols for *X. fastidiosa*



Acknowledgements

Anses, Plant Health Laboratory

- *Valérie Olivier*
- *Carène Rivoal*
- *Bruno Legendre*
- *Ugo Mouisse*
- *Aurélie Forveille*
- *Christelle Dousset*



Collection of samples, spiked samples, Xylella dynamics

- ***Maria Saponari***, CNR, Italy
- ***Ester Marco-Noales***, IVIA, Spain
- ***Yael Meller***, PPIS, Israel
- ***Julie Beguet***, DRAAF Occitanie, France
- ***Stefania Loreti***, CREA, Italy

And all the participants





LARGE SCALE VALIDATION OF qPCR ASSAY FOR SIMULTANEOUS IDENTIFICATION OF XYLELLA FASTIDIOSA SUBSPECIES IN A WIDE RANGE OF PLANT SPECIES NATURALLY INFECTED

Loconsole G., Ligorio A.M., Amoia S.S., Cavalieri V., D'Attoma G., Boscia D., Saponari M.

Institute for Sustainable Plant Protection (IPSP)—National Research Council, Bari 70126, Italy

Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



Genetic characterization
of *Xylella fastidiosa* (Xf)
isolates in EU

LEGISLATIVE
REQUIREMENT!!!

Official Journal of the European Union

COMMISSION IMPLEMENTING REGULATION (EU) 2020/1201

of 14 August 2020

as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells *et al.*)

Annex II:

Lists host plant
species/genera
according to
their
susceptibility
to the different
subspecies

ANNEX III

Outbreak
areas
demarcated
according to
the
subspecies of
the reported
bacterial
isolates

Annex IV

Test for
identification
of *X.
fastidiosa*
and its
subspecies

A. Tests for the screening and identification of the presence of Xf

1. Real time PCR based on Harper *et al.*, 2010
2. LAMPbased on primers developed by Harper *et al.* 2010
3. Real time PCR based on Ouyang *et al.*, 2013
4. Conventional PCR based on Minsavage *et al.* 1994

B. Molecular tests for the identification of the subspecies of Xf

1. Multi Locus Sequence Typing (MLST) based on Yuan *et al.*, 2010 determining all subspecies;
2. PCR based on Hernandez-Martinez *et al.*, 2006 determining the subspecies *fastidiosa*, *multiplex* and *sandyi*;
3. PCR based on Pooler & Hartung 1995 determining the subspecies *pauca*.

2024/2507

COMMISSION IMPLEMENTING REGULATION (EU) 2024/2507

of 26 September 2024

4. Real time PCR based on Dupas *et al.* 2019, determining all subspecies

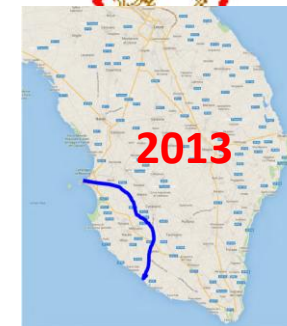
5. Real time PCR based on Hodgetts *et al.* 2021, determining all subspecies

COMPLEX PHYTOSANITARY SCENARIO emerged in Apulia region (southern Italy) in 2024-2025



Symptoms resembling those of
Pierce's disease

No specific ALS symptoms
recorded

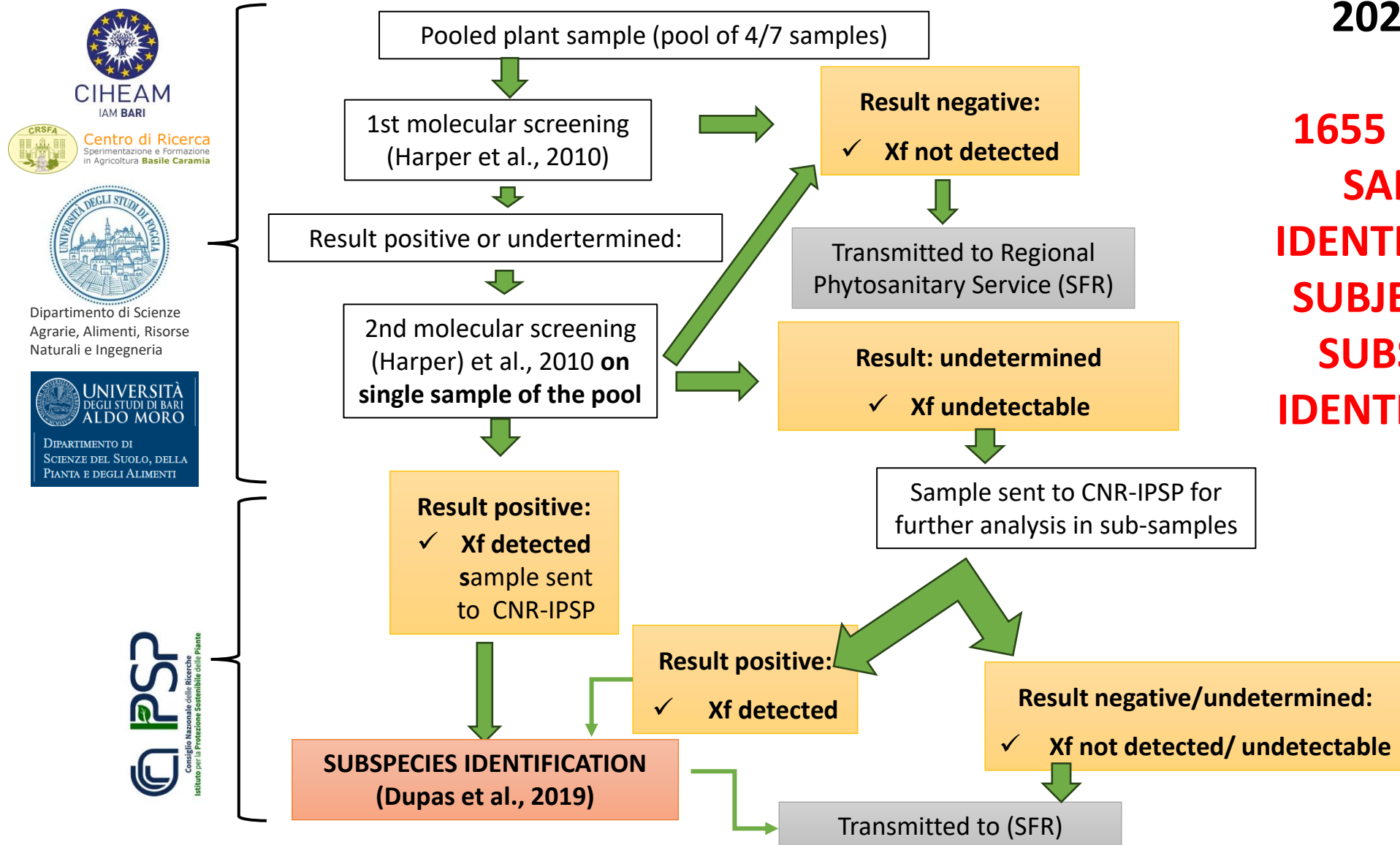


Olive Quick Decline Syndrome

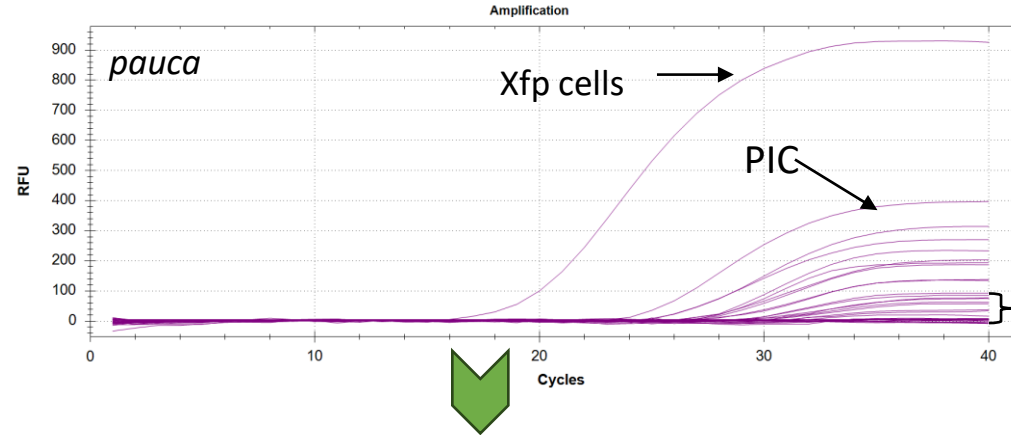
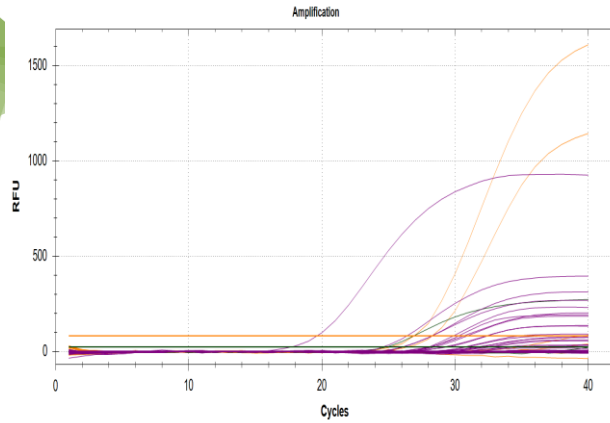
WORKFLOW OF THE DIAGNOSTIC PROCEDURES ADOPTED IN APULIA REGION FOR THE MONITORING PROGRAM for Xf (analysis in accordance with the standard EN ISO/IEC 17025)

2024-2025

**1655 POSITIVE
SAMPLES
IDENTIFIED AND
SUBJECTED TO
SUBSPECIES
IDENTIFICATION**



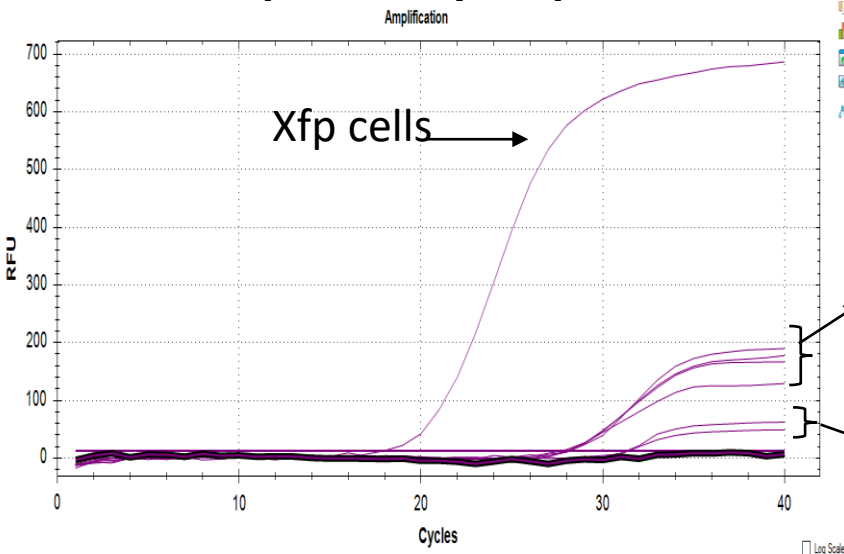
Reaction conditions slightly modified to improve amplification signals for *pauca*: **from 60°C to 56°C for annealing-**



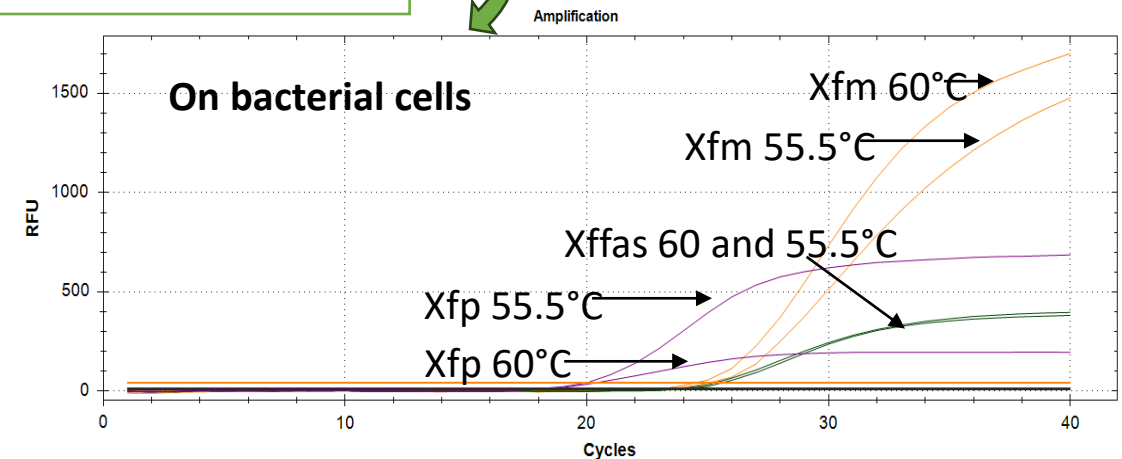
Critical results at 60°C: generated very low RFU for *pauca* with lack of detection in some positive samples at low concentration

Annealing-extension gradient: range 63-55 °C

On 2 naturally infected olive samples with Cq values by Harper ~30



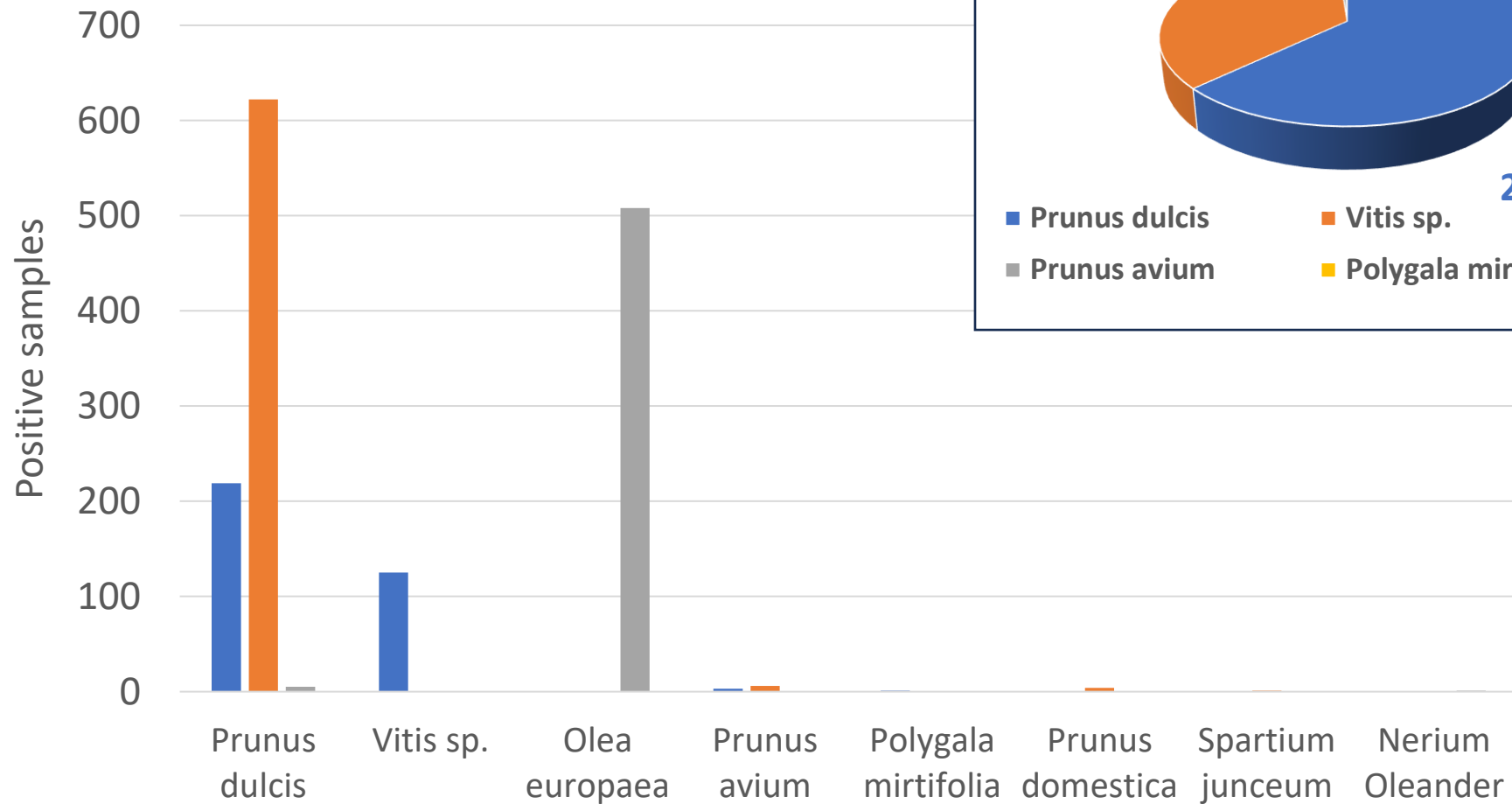
S1 at 55-56.6 °C;
at 60°C = N/A!!
S2 at 55-56°C
over 56°C = N/A!



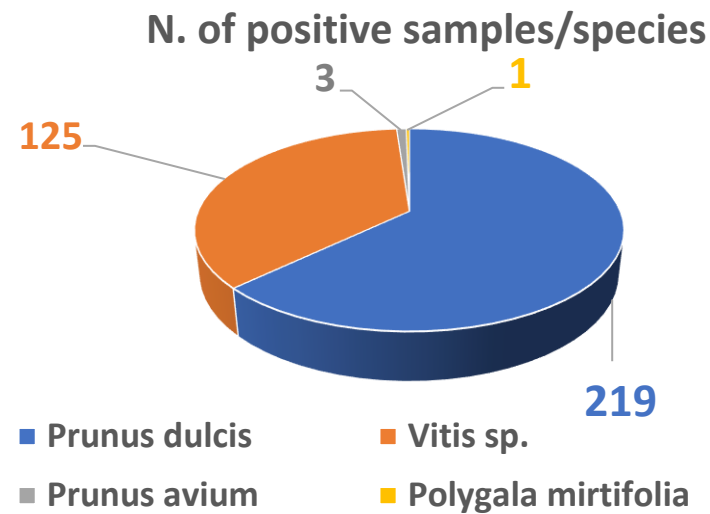
annealing-extension at 56°C - Effects on RFU and Cq values:

- No difference for *Xf fastidiosa*;
- No significant difference for *Xf multiplex*;
- Δ RFU ~200-400 (higher at 55-56°C) for *Xf pauca*; 60°C can impact on the results for samples with a low concentration of *Xfpauca*

Risultati analisi 2024+2025

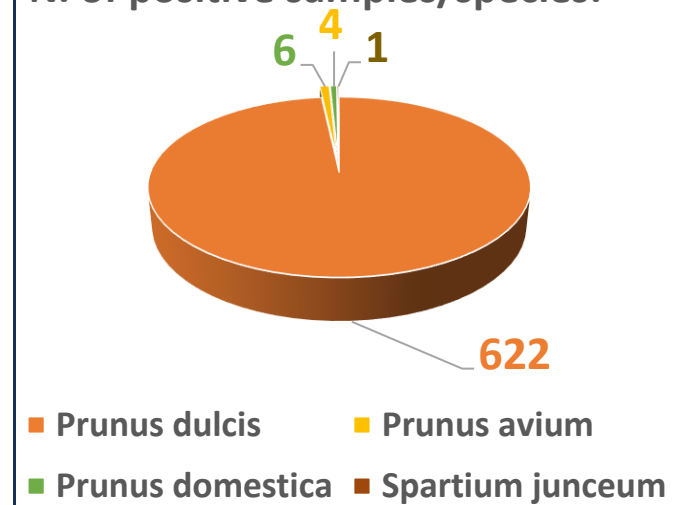


subsp. fastidiosa ST-1



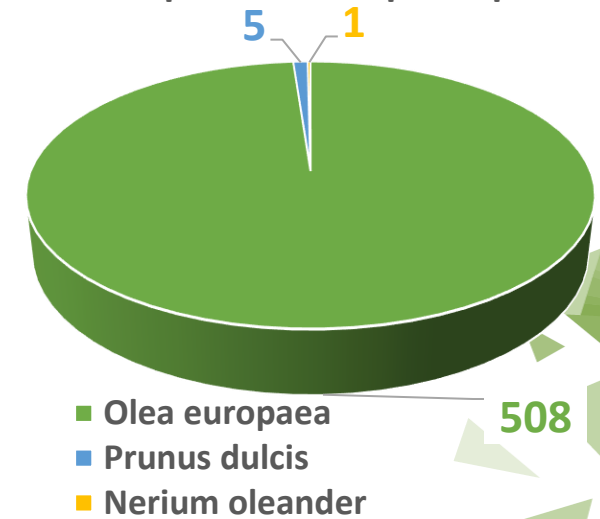
subsp. multiplex ST-26

N. of positive samples/species:

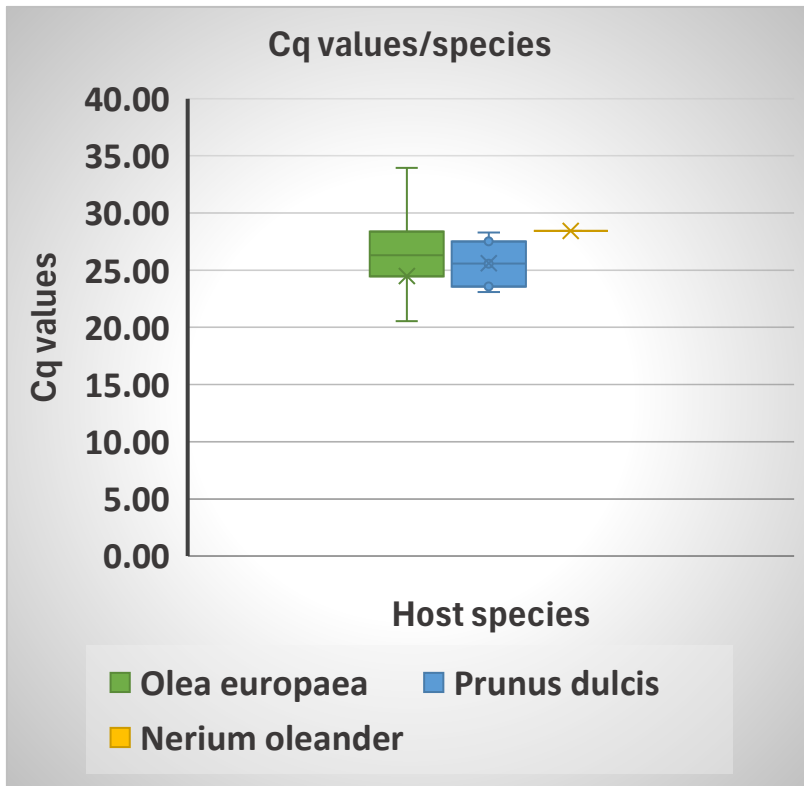


subsp. pauca ST-53

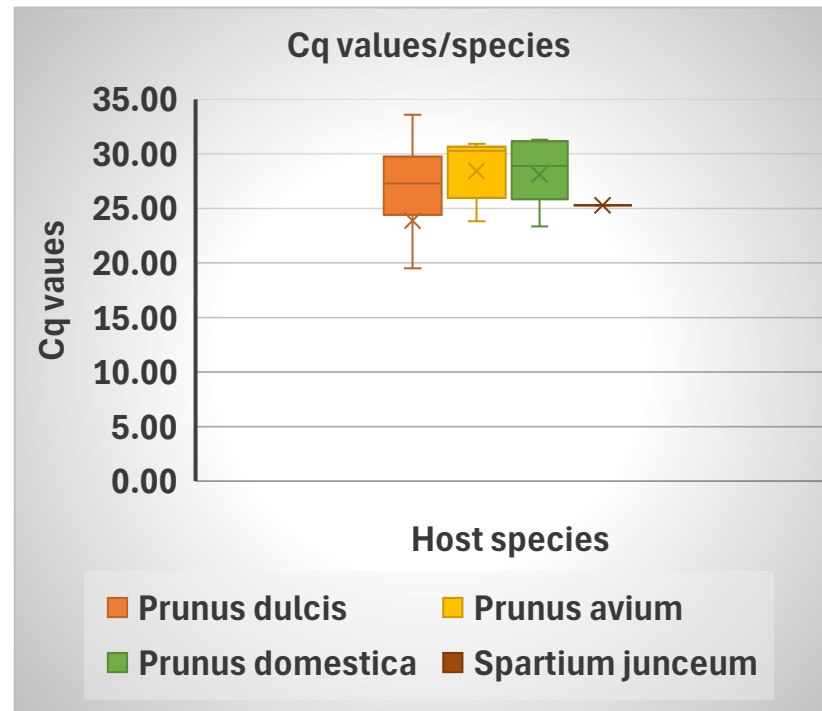
N. of positive samples/species



subsp. *pauca* ST-53



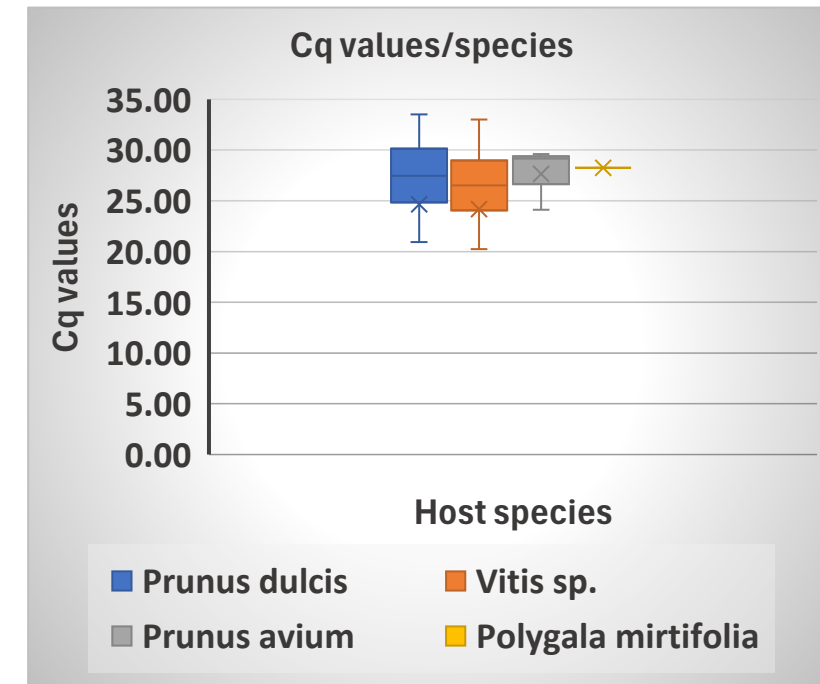
subsp. *multiplex* ST-26



Range Cq values:

- 19.53-33 in almond (622 plants);
- 23.80-30.92 in cherry (6 plants)
- 23.36-31.29 in plum (4 plants)
- 25.57 in spartium junceum

subsp. *fastidiosa* ST-1



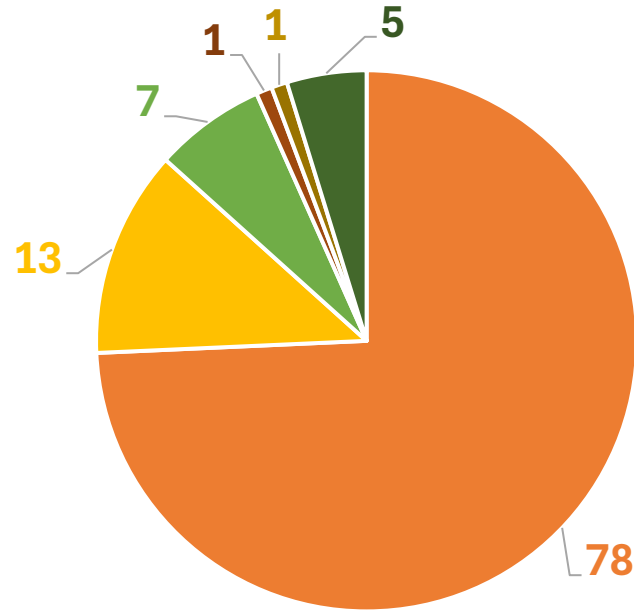
Range Cq values:

- 20.92-33.00 in almond (125 plants)
- 20.24-33.00 in grapevine (219 plants)
- 24.11-29.61 in cherry (3 plants)
- 28.25 in P. myrtifolia (1 plant)

Range Cq values:

- 20.50-33 in olive, where bacterial colonization is well-established
- 23.08-28.29 in almond (5 plants)
- 28.44 in oleander (1 plant)

105 undetermined results = Cq>33



- Prunus dulcis
- Olea europaea
- Vitis spp.
- Prunus armeniaca
- Prunus avium
- Ficus spp.

Samples reanalyzed

69 pos	32 neg	4 undetermined
61 <i>Prunus dulcis</i> (multiplex)	13 <i>Prunus dulcis</i>	4 <i>Prunus dulcis</i>
5 <i>Olea europaea</i> (pauca)	8 <i>Olea europaea</i>	
3 <i>Vitis</i> spp. (<i>fastidiosa</i>)	4 <i>Vitis</i> spp.	
	5 <i>Ficus</i> spp.	
	1 <i>Prunus avium</i>	
	1 <i>Prunus armeniaca</i>	

Monitoring programm 2025 for Xf in insects in accordance with the standard EN ISO/IEC 17025 by Harper et al. 2010 + loos et al. 2009 (Xf + 18S)

Tested 610 insect samples

11 pos

Dupas et al., 2019

- 3 with fastidiosa
- 4 with mutiplex
- 4 with pauca

CONCLUSION

- The optimized Dupas et al. (2019) triplex qPCR assay is **reliable for simultaneous detection and subspecies identification** of *Xylella fastidiosa*.
 - Lowering the annealing temperature to **56°C improved sensitivity for subsp. *pauca*** without affecting other subspecies.
 - The assay proved **reliable also in insect samples**, supporting vector monitoring
-
- Large-scale application confirmed **clear host–subspecies associations** and supported effective surveillance.
 - The **robustness** of the triplex qPCR assay to differentiate *fastidiosa*, *multiplex* and *pauca* was also **confirmed by MLST analysis** performed on new outbreaks and newly identified host species.
 - Although in the province of Bari all three subspecies coexist, **positive samples did not show any mixed infections so far**, including in **almond** which is susceptible to multiple subspecies.
 - Overall, it represents a **rapid and robust tool for phytosanitary management** alternative to MLST analysis for a rapid and large-scale characterization of the Xf positive findings.

Thank you for your attention



giuliana.loconsole@cnr.it



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

utad

 **ICNF**
Instituto da Conservação
da Natureza e das Florestas

 **EFSA**
EUROPEAN FOOD SAFETY AUTHORITY

 **BEXYL**
BEYOND XYLELLA

 **EUPHRESKO III**

 **CIHEAM
BARI**

 **PSP**
Consiglio Nazionale delle Ricerche
Istituto per la Protezione Sostenibile delle Piant

 **REGIONE PUGLIA**



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

Xylella fastidiosa: from eradication to containment, the Porto metropolitan area (Portugal) case study

utad

ICNF
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BEXYL
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REGIONE PUGLIA



OVERVIEW



FIRST DETECTION IN
PORTUGAL ^[1]
January 2019



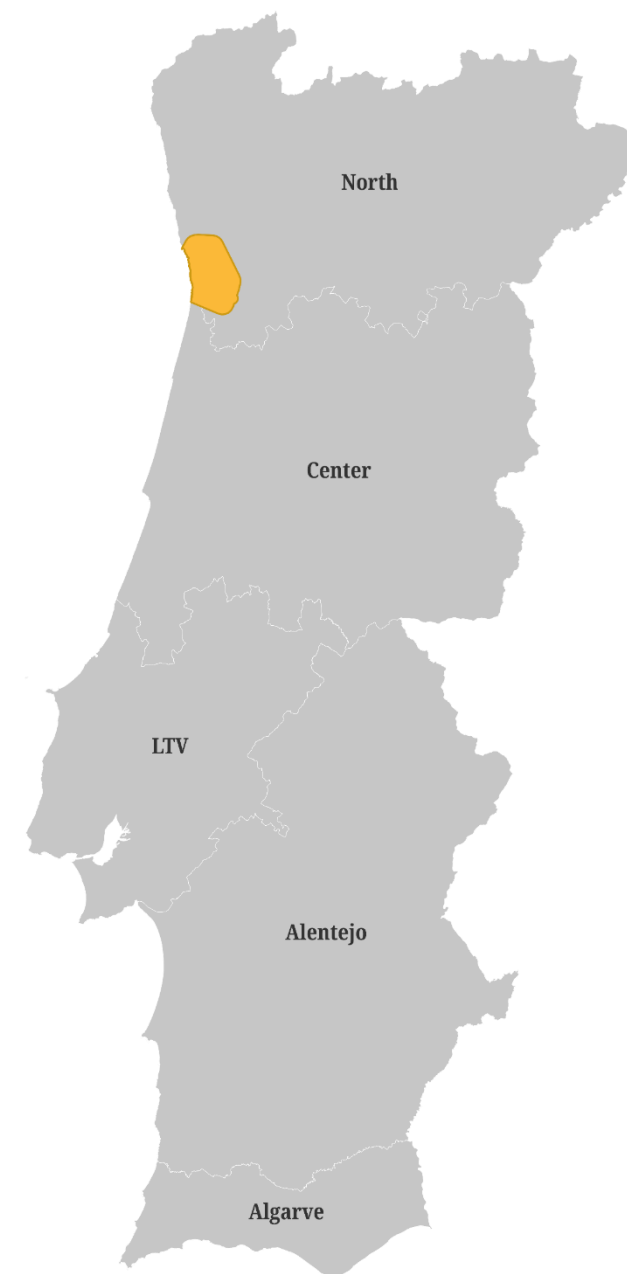
INITIAL LOCATION
**Vila Nova de Gaia,
Porto**



STUDY AREA
**Porto Metropolitan
Area (AMP)**



BIOCLIMATIC ZONE
Atlantic-influenced



REGULATORY FRAMEWORK

2019

EU DECISION 2015/789

First detection protocols and immediate eradication strategy framework implemented nationwide.

2020

EU REGULATION 2020/1201

Consolidated European approach updating buffer zones and phytosanitary inspections.

2024

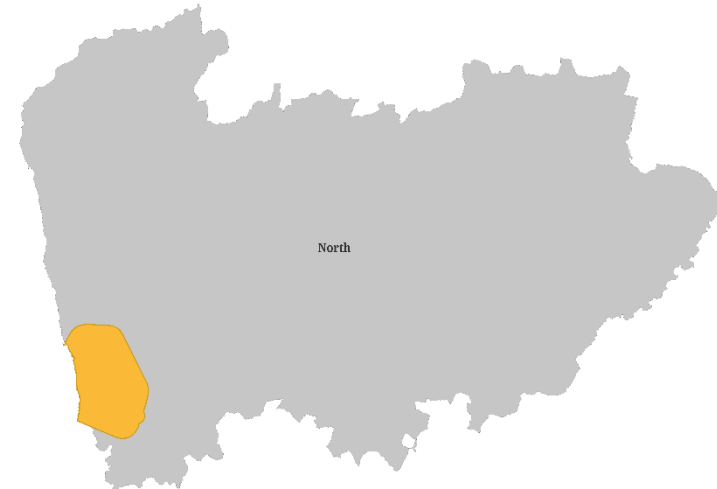
EU REGULATION 2024/2507

Regulatory transition to structured long-term containment strategies in the Porto area.

JAN 2019



2024





Key Ecological Driver: The Meadow Spittlebug (*Philaenus spumarius*), primary vector of *Xylella fastidiosa*, highly adapted to Porto's Atlantic climate.*

OBJECTIVES

1. Spatiotemporal Analysis

Characterize temporal trends in pathogen detection and model spatial patterns across the territory.

2. Ecological Drivers

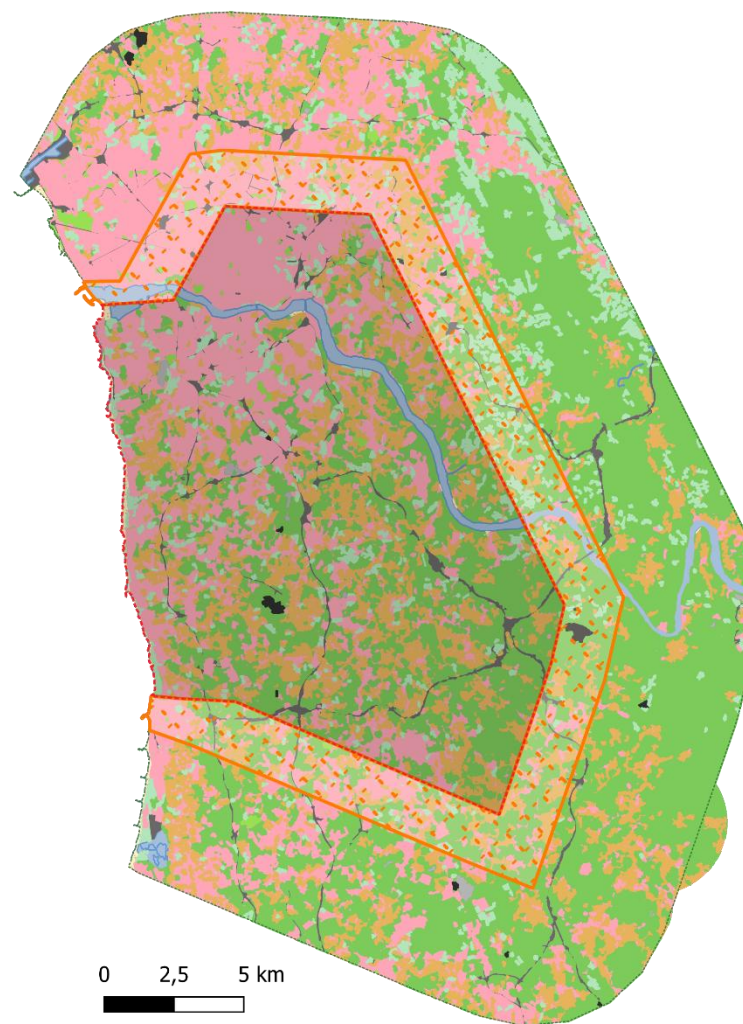
Evaluate infection dynamics across different host plants and land-use regimes.

3. Applied Outcomes

Provide a robust, evidence-based epidemiological framework to support regulatory management and containment strategies.

METHODOLOGY

- ❖ **Study area:**
Complex land use mosaic
- ❖ **Temporal scope:**
2019 – 2025
- ❖ **Technical & Regulatory Framework**
 - DGAV Action Plan for Eradication of *X. fastidiosa* and its vectors [2]
 - EU Regulations [3,4,5]
 - EFSA guidance [6]
 - ISPM 27 (DP) 25 for *X. fastidiosa* [7]



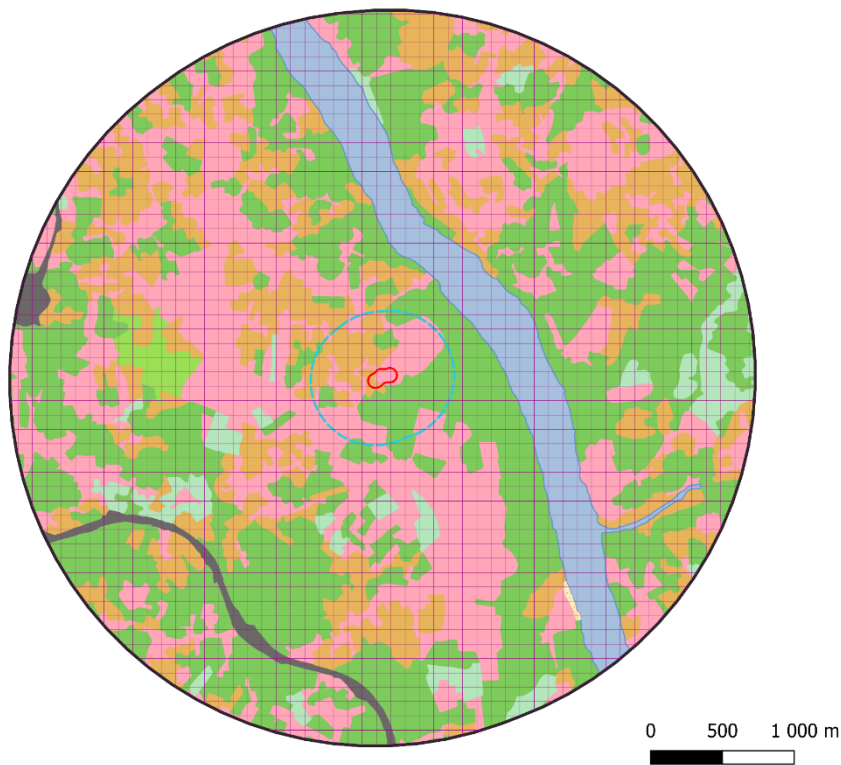
Containment zones

- Infected zone- interior
- Infected zone- containment: IZ-C
- Buffer zone- containment: BZ-C

Land Use

CLC 2018 version 1.3.0

- Agricultural
- Dump sites
- Water bodies
- Public garden
- Forest
- Beaches, dunes, sands
- Semi-natural
- Urban
- Transport network
- Bare rock



Georeferenced survey grid

- Coded 100 x 100 m grid

Eradication zones

- IZ
- BZ1
- BZ2

Land Use (CLC 2018 version 1.3.0)

- Agricultural
- Water bodies
- Public garden
- Forest
- Beaches, dunes, sands
- Semi-natural
- Urban
- Transport network

METHODOLOGY

❖ Survey Preparation

Systematic survey grid; RIBESS+ statistical tool (EFSA).

❖ Ecosystems

Agricultural, urban, forest & semi-natural.

❖ Vegetation Strata

Arboreal, shrub, and herbaceous layers.

❖ Host Condition

Symptomatic & asymptomatic plants.



METHODOLOGY

Spatial Analysis

- ❖ **Software & Datum**
 - Python & QGIS 3.44
 - WGS84 coordinates
- ❖ **Haversine Formula**

Calculate kilometer-scale distances and expansion profiles
- ❖ **Convex-Hulls**

To track the outbreak's annual maximum spatial envelope

Statistical Framework

- ❖ **Data Harmonization**

Standard land-use categories and botanical taxa ($n > 100$), with Wilson 95% Confidence Intervals (CI)
- ❖ **Symptom Performance**

2×2 contingency framework comparing symptomatic vs. asymptomatic plants:

 - Odds Ratio (OR) with 95% CI to evaluate diagnostic performance based on symptoms

RESULTS

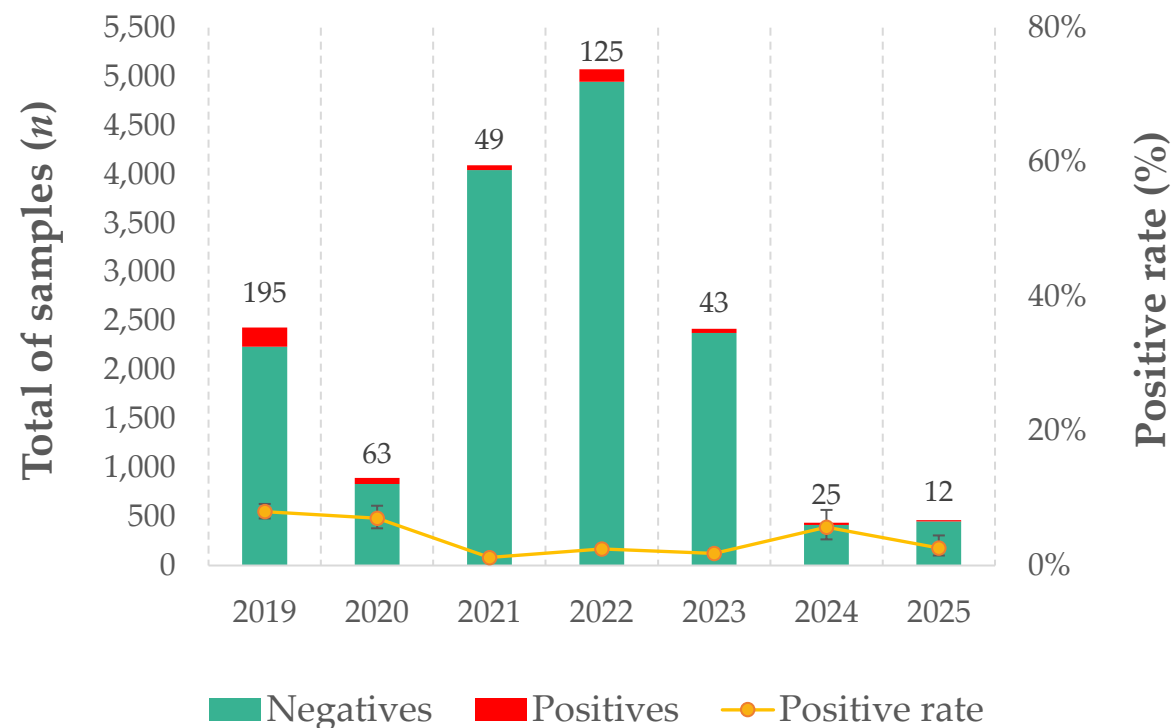
Dataset:

- ✓ 15,820 samples
- ✓ 512 positive detections of *Xf*:
 - subsp. *multiplex* ST7 (108)
 - subsp. *fastidiosa* ST2 (4)
- ✓ 3.24% overall positivity rate

□ **Trends:** Higher positivity between 2019–2020 (reactive sampling) and in 2024 (5.69%; risk-based sampling); diluted between 2021–2023.

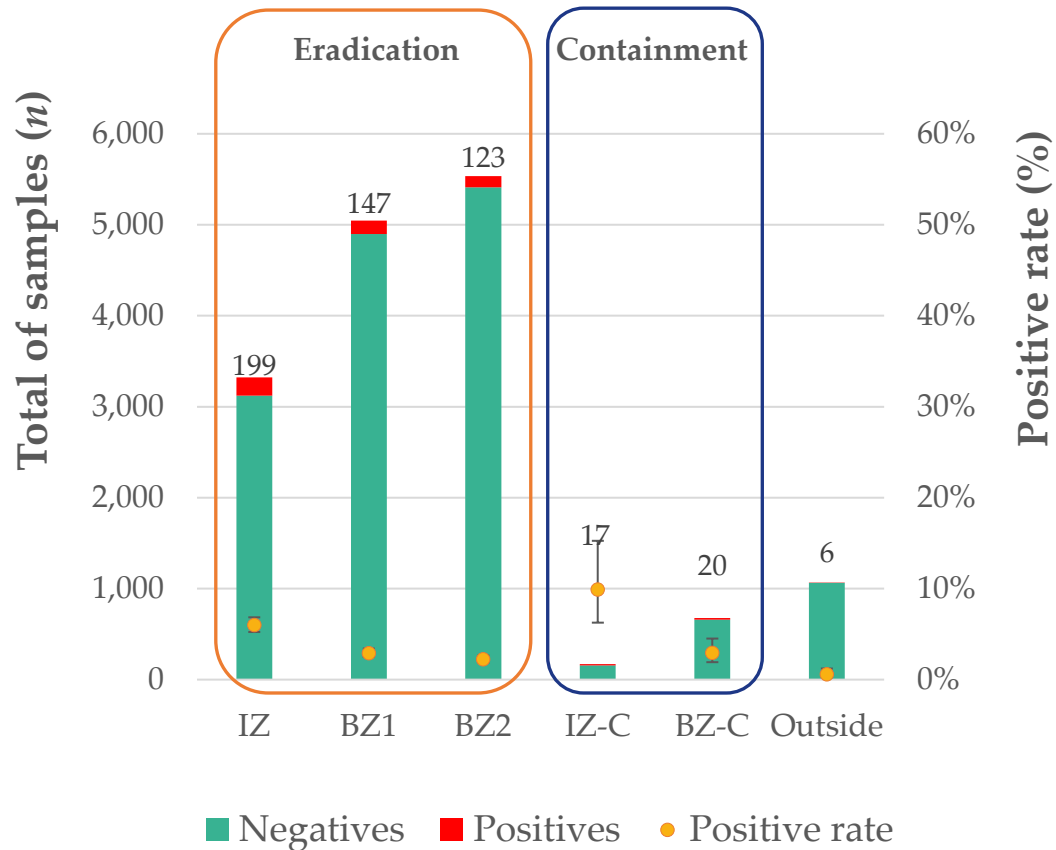
□ **Data Nature:** Annual trajectories reflect surveillance-driven detection rates rather than direct estimates of true landscape prevalence.

Plant samples by Year



Whiskers: 95% Wilson CI

Plant samples by Regulatory-zone



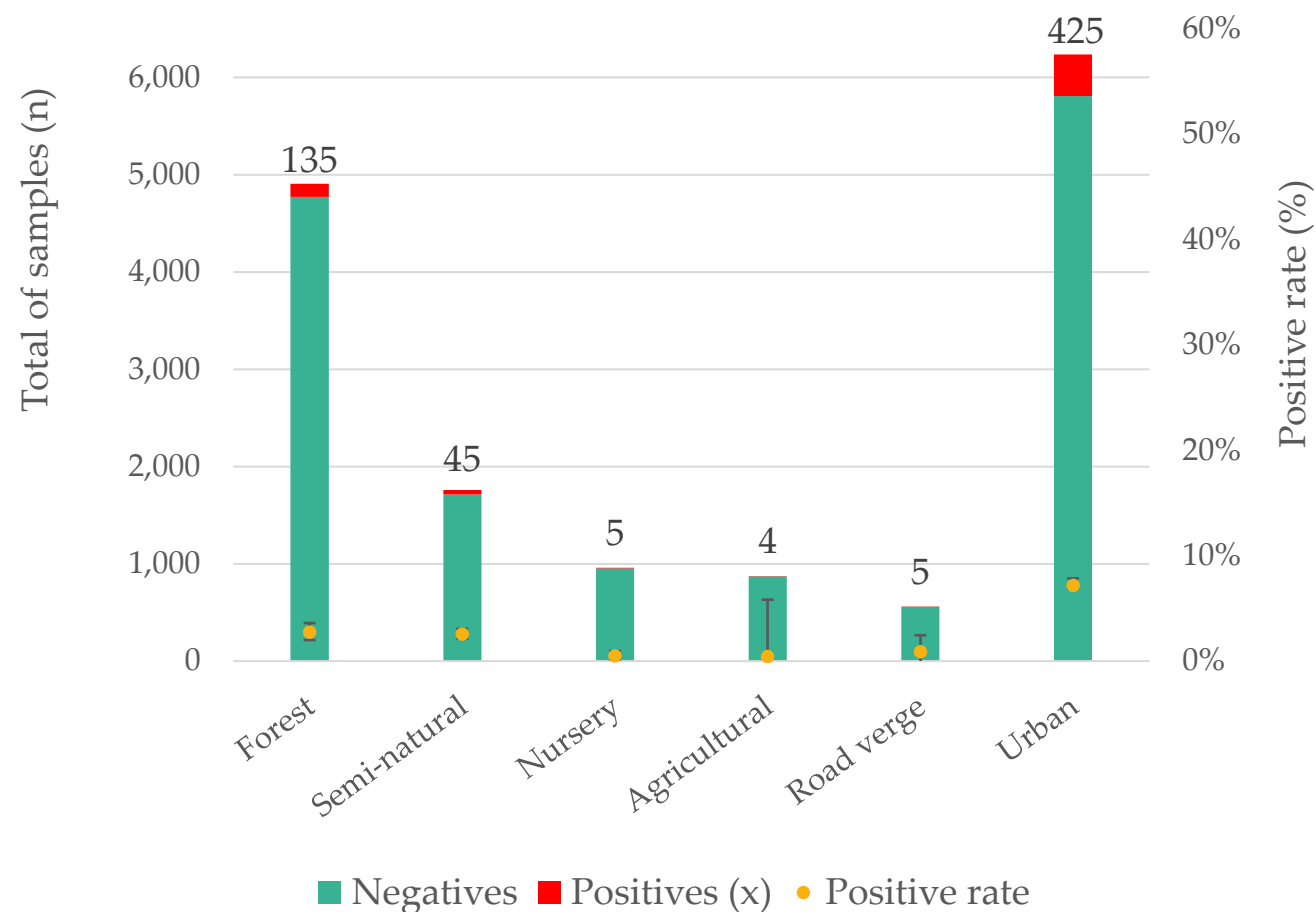
RESULTS

- Spatial Gradient:** Sampling asymmetry drives CI widths (IZ-C with $n = 172$ vs. BZ2 with $n > 5000$).
- Core Intensity:** Strong confinement within IZ and IZ-C (9.88% peak rate).
- Recent Containment:** Wider CIs reflect limited sampling restricted to the post-2024 regulatory transition.
- External Status:** Near-zero positivity outside the demarcated area (0.56%), indicating rare spillover events or previous infections.

RESULTS

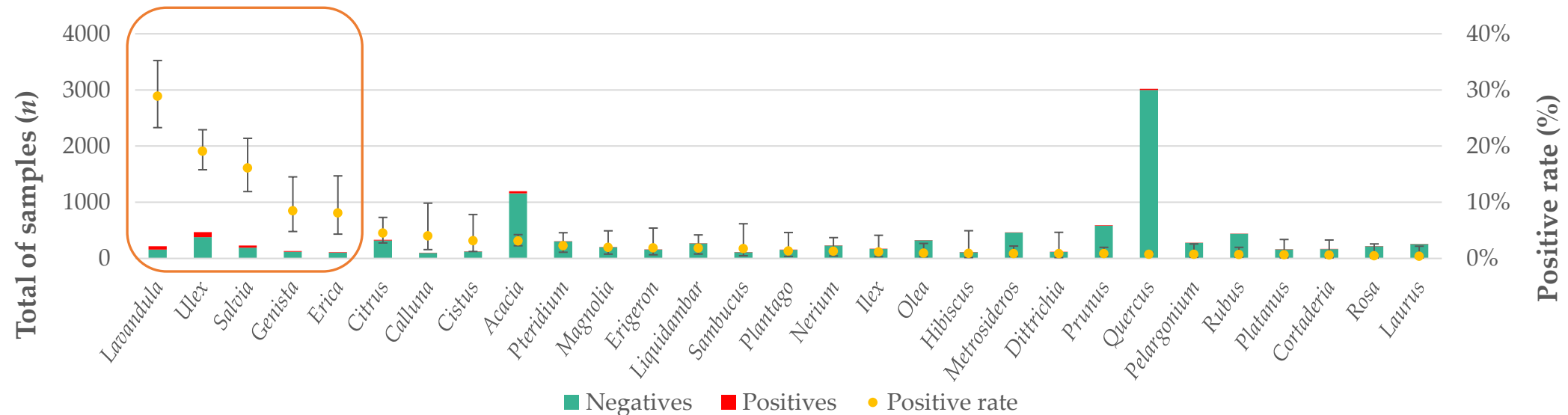
- ❑ **Wild Reservoirs:** Low but highly persistent infection levels in Forest (2.75%) and Semi-natural (2.56%) habitats.
- ❑ **Low Yield & Barriers:** Near-zero rates in agricultural areas (<0.5%).

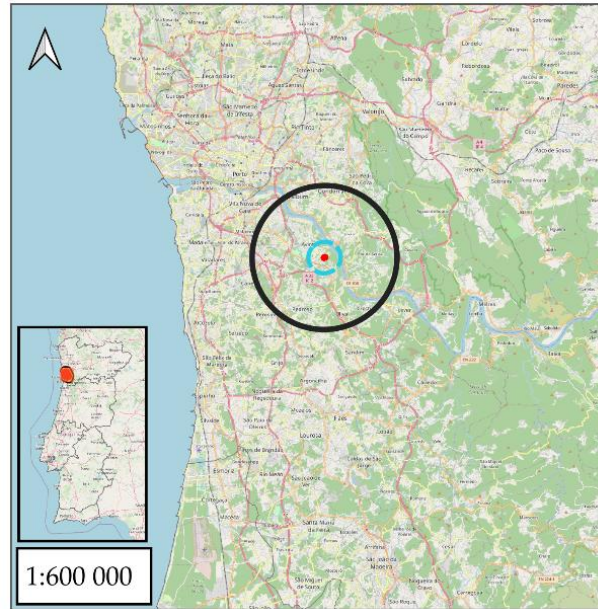
Plant samples by Land use



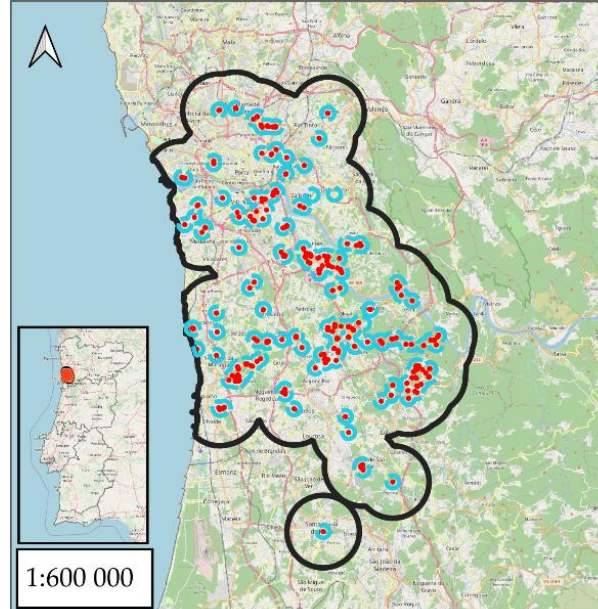
Plant samples by Host genera ($n > 100$)

- ❑ **Taxonomic Filter ($n > 100$):** Screened 29 robustly sampled species and genera to eliminate statistical noise from low-frequency hosts.
- ❑ **Ornamental Amplifiers:** *Lavandula* (~29%) emerges as the primary urban amplifier and main risk driver among heavily sampled ornamental targets.
- ❑ **Wild Shrub Reservoirs:** Wild Fabaceae genera [*Ulex* (~19%) and *Genista* (~9%)] act as widespread, persistent reservoirs for the bacterium in unmanaged and natural areas.
- ❑ **Low-Risk Production Crops:** Near-zero positivity rate in major agricultural and forestry cornerstones: *Olea* (0.92%), and *Quercus* (0.72%; $n > 3,000$).

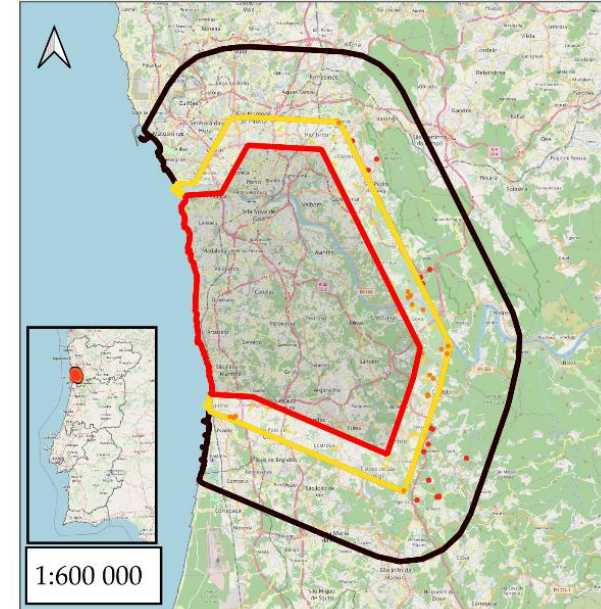




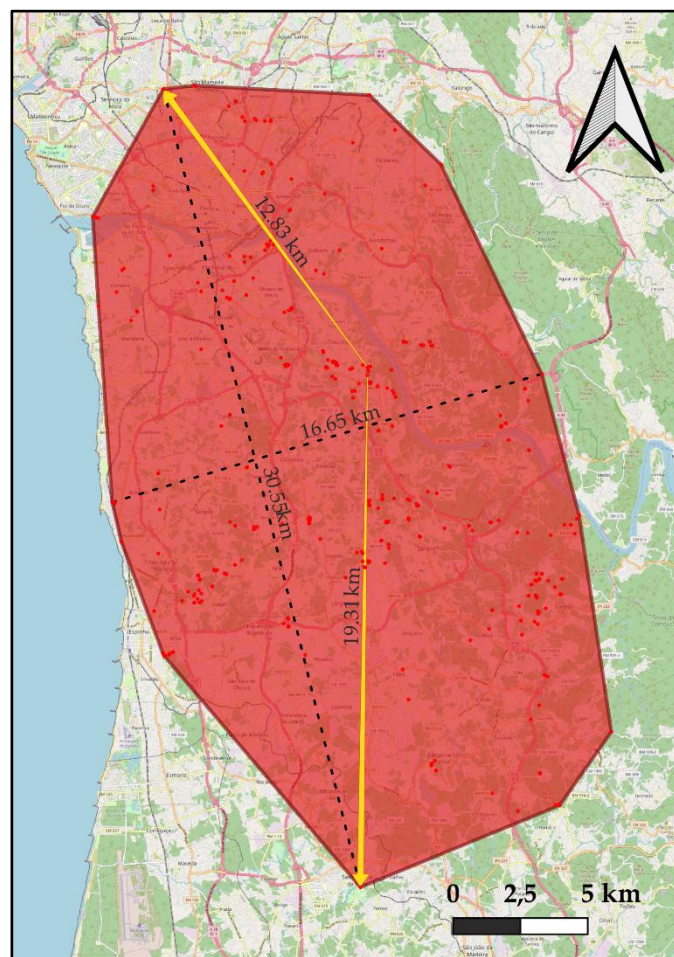
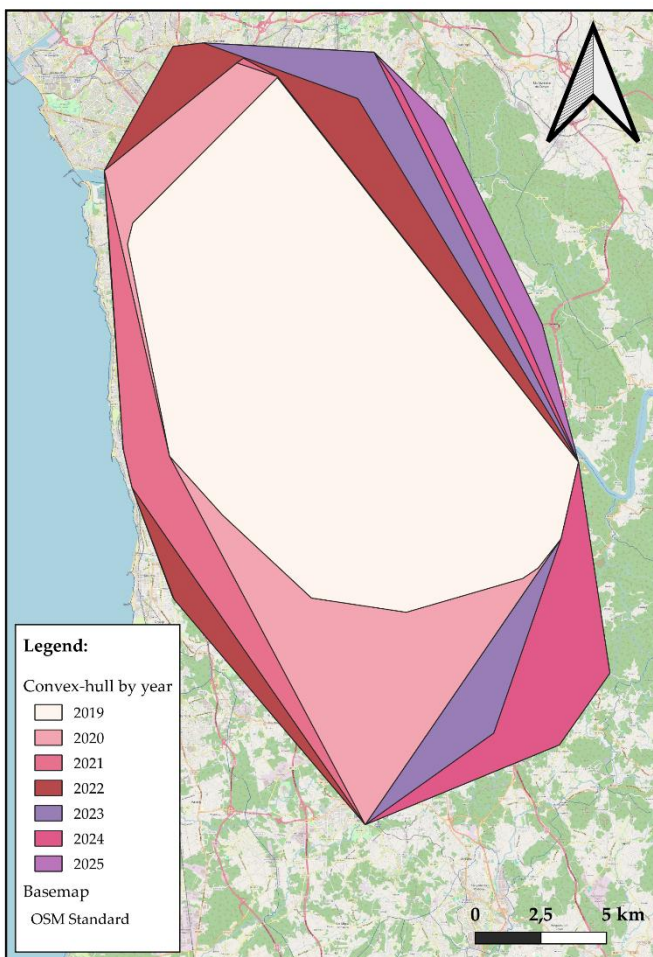
2019: Initial confirmed detection in 2019.



2023: Operational threshold of the eradication strategy.



2025: Current delimitation under containment.



RESULTS

- **Epidemic Scale:** Total demarcated area with 67,400 ha by 2025, with a 30.55 km maximum linear extent.
- **Spatial Asymmetry:** Clear directional bias from the index case, extending 12.83 km north versus 19.31 km south.
- **Early Radial Jump:** Sharpest increase in confirmed distance occurred in 2020 (19.34 km).
- **Metric Caveat:** Convex-hulls represent annual and cumulative maximum detection envelopes, heavily shaped by annual surveillance intensity.

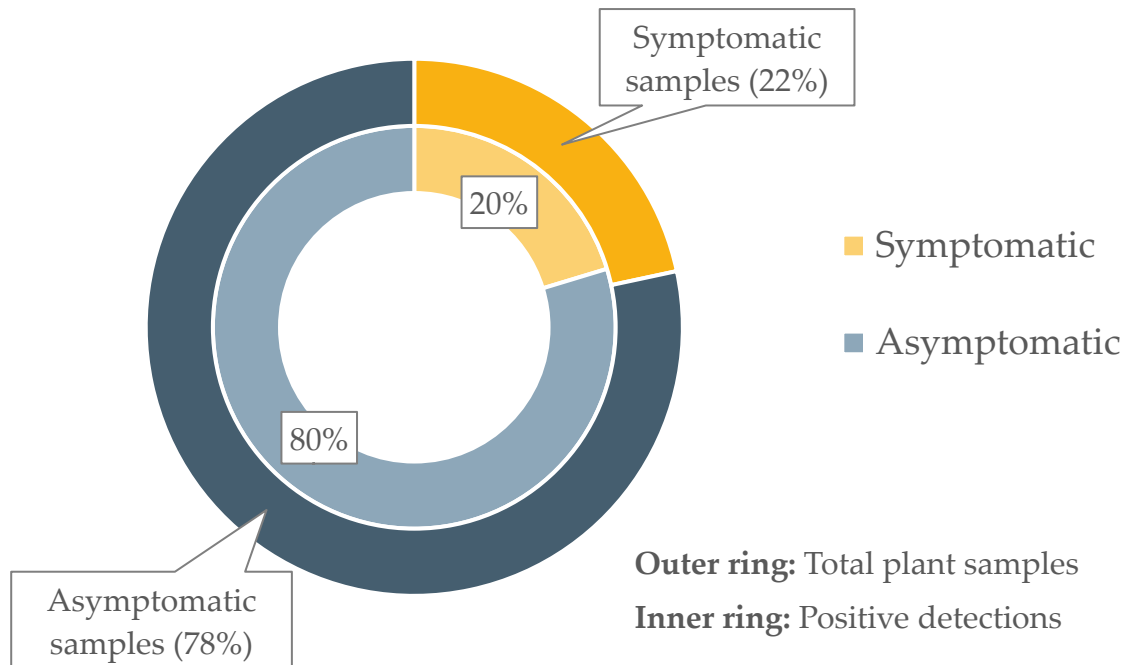
Year	2019	2020	2021	2022	2023	2024	2025
Max. dist. from first detection (km)	11.47	19.34	12.45	14.84	15.21	17.77	17.72



RESULTS

- ❖ **Genetic Predominance:** Overwhelming dominance of *X. fastidiosa* subsp. *multiplex* ST7 ($n = 119$), mirroring other Mediterranean epidemic fronts.
- ❖ **Complex Introduction:** Occasional detections of *X. fastidiosa* subsp. *fastidiosa* ST2 ($n = 11$) indicate different historical events, but exact trade pathways or timing could not be determined.
- ❖ **Proliferation Drivers:** Epidemic shaped by early anthropogenic/ornamental network movement, followed by localized vector transmission.
- ❖ **Surveillance Caveat:** Incomplete MLST coverage indicates lineage predominance within the typed subset rather than a full genetic stability reconstruction.

Plant samples by symptomatology (%)



RESULTS

- **Symptom Parity:** Evaluated 3,424 symptomatic and 12,396 asymptomatic plants; positivity rates were statistically identical (3.04% vs. 3.29%).
- **No Diagnostic Link:** An Odds Ratio of 0.92 (95% CI: 0.74–1.15) confirms that visual symptoms do not increase the likelihood of pathogen detection.
- **The Asymptomatic Burden:** 80% of all confirmed positive cases (408 out of 512) showed no visible symptoms, reflecting extended latency or weak host expression.
- **Surveillance Takeaway:** Visual inspection alone is entirely insufficient; robust, systematic molecular screening is mandatory for accurate delimitation.



CONCLUSIONS & STRATEGIC SHIFT

- ✓ **Beyond Eradication:** A stable 30.55 km asymmetric footprint and mixed dispersal (human + vector) demonstrates that the outbreak has already surpassed the scope of eradication.
- ✓ **Ecological Integration:** Pathogen is deeply embedded within a resilient landscape mosaic of ornamental and wild reservoirs, structurally driven by the *Xfm* ST7 lineage.
- ✓ **Regulatory Realignment:** Data directly supports the transition from Regulation (EU) 2020/1201 (Eradication) to the amended Regulation (EU) 2024/2507 (Containment).
- ✓ **Strategic Imperative:** Containment is a data-driven, proportionate response focused on slowing outward spread and protecting high-value agricultural sectors.

REFERENCES

- [1] Carvalho-Luis, C.; Rodrigues, J.M.; Martins, L.M. Dispersion of the Bacterium *Xylella fastidiosa* in Portugal. *J. Agric. Sci. Technol. A* **2022**, *12*, 35–41.
 - [2] DGAV Plano de Ação Para Erradicação de *Xylella fastidiosa* e Controlo Dos Seus Vetores [*Action Plan for Eradication of Xylella fastidiosa and Control of its Vectors*] **2020**.
 - [3] EU Commission Commission Implementing Decision (EU) 2015/789 of 18 May; **2015**.
 - [4] EU Commission Commission Implementing Regulation (EU) 2020/1201 of 14 August; **2020**.
 - [5] EU Commission Commission Implementing Regulation (EU) 2024/2507 of 26 September; **2024**.
 - [6] EFSA; Lázaro, E.; Parnell, S.; Vicent Civera, A.; Schans, J.; Schenk, M.; Schrader, G.; Cortiñas Abrahantes, J.; Zancanaro, G.; Vos, S. Guidelines for Statistically Sound and Risk-based Surveys of *Xylella fastidiosa*. *EFSA Supporting Publications* **2020**.
 - [7] IPPC ISPM 27 - Diagnostic Protocols for Regulated Pests DP 25: *Xylella fastidiosa* **2025**.
 - [8] Harper, S. J., Ward, L. I., and Clover, G. R. G. Development of LAMP and real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. *Phytopathology* **2010**, *100*, 1282-1288.
 - [9] EPPO PM 7/24 (5) *Xylella fastidiosa*. *EPPO Bulletin* **2023**, *53*, 205–276.
- * Image source: https://live.staticflickr.com/8163/7361567206_15a4d3a4a6_b.jpg

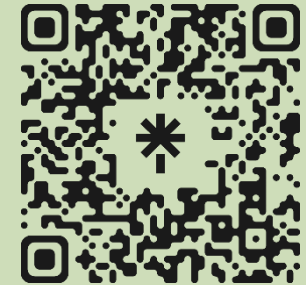
ACKNOWLEDGEMENTS: We gratefully acknowledge the official monitoring data kindly provided by the Directorate-General for Food and Veterinary (DGAV), NPPO of Portugal.

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Conservation and Forests
(ICNF)



National overview

Poster: 123



Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026



REGIONE PUGLIA

TEN YEARS OF XYLELLA FASTIDIOSA IN THE BALEARIC ISLANDS

IMPACTS ON CROPS AND LESSONS FROM A MULTI-SUBSPECIES EPIDEMIC

Olmo D.¹, Nieto A., Pedrosa A.¹, Adrover F.¹, Borràs D.¹, Aguiló M.², Montesinos M.², Moralejo E.², López M.³, Beidas O.³, Monterde A.⁴, Navarro I.⁴, Barbé S.⁴, Olivares C.⁵, Velasco-Amo M. P.⁵, Román-Écija M.⁵, Trapero-Casas J.L.⁵, Navas-Cortes J. A.⁵, Juan A.³, Marco-Noales E.⁴, Landa B.B.

¹IRFAP ²TRAGSA ³CONSELLERIA AGRICULTURA ⁴IVIA ⁵IAS-CSIC

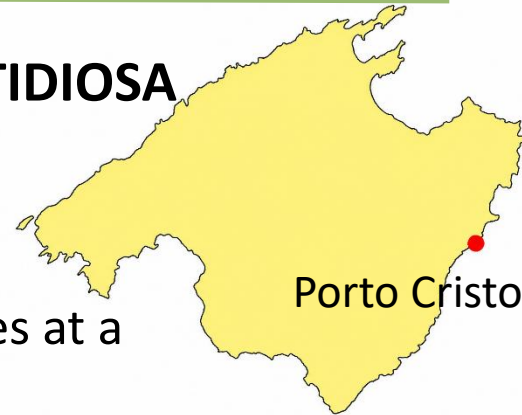


dolmo@irfap.es

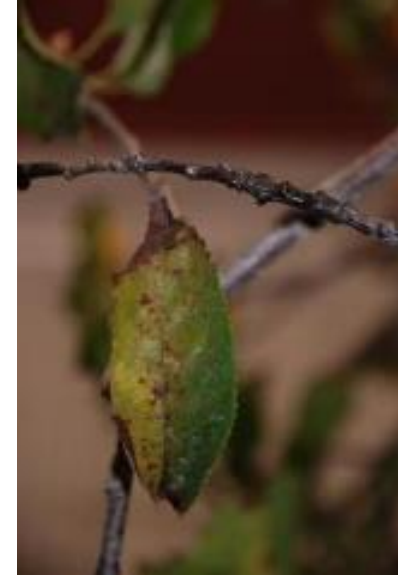


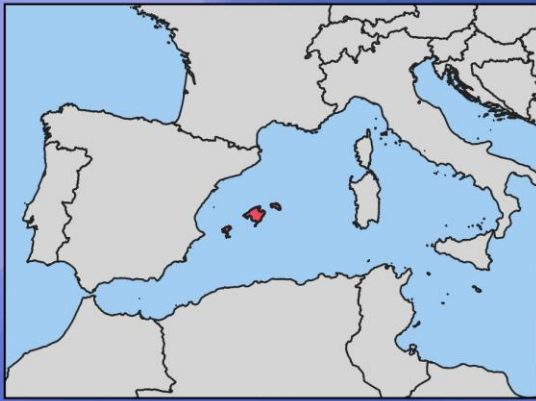
2016

FIRST DETECTIONS OF XYLELLA FASTIDIOSA IN THE BALEARIC ISLANDS

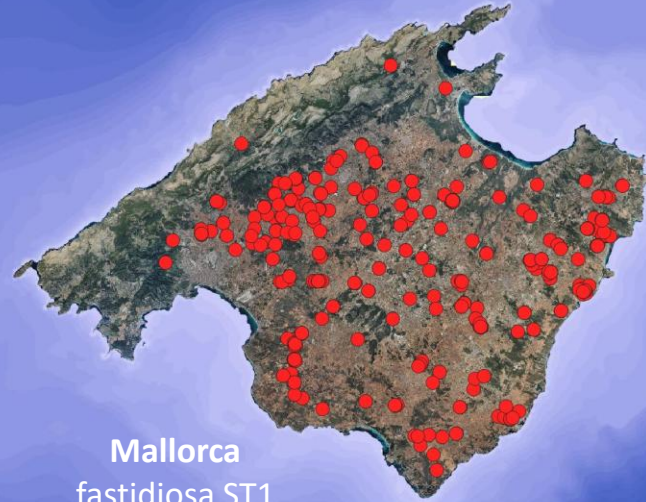


- First detection in October 2016 in three cherry trees at a garden center in Porto Cristo (Manacor).
 - Second detection in December 2016 in *Polygala myrtifolia* at the same garden center.
 - January 2017 to present: **numerous detections** across Mallorca, Ibiza and Menorca.
-





OUTBREAK EXTENT



Mallorca
fastidiosa ST1
multiplex ST7, ST81,



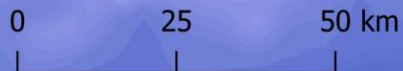
Menorca
multiplex ST81



Ibiza
pauca ST80

Formentera

CONTAINMENT
Commission Implementing Decision (EU) 2017/2352
of 14 December 2017



38 PLANT SPECIES *XYLELLA FASTIDIOSA* DETECTED (BI)

Acacia saligna (MA-ST81, IB-ST80)

Calicotome spinosa (MA-ST1)

Cistus albidus (MA-ST81, ME-ST81, IB-ST80)

Cistus monspeliensis (MA-ST1)

Clematis cirrhosa (ME-ST81)

Ficus carica (MA-ST81, ME-ST81)

Fraxinus angustifolia (MA-ST81)

Genista hirsuta (IB-ST80)

Genista lucida (MA-ST1)

Genista valdes-bermejoi (MA-ST81)

Helichrysum stoechas (MA-ST81, ME-ST81)

Juglans regia (MA-ST1)

Lavandula angustifolia (MA-ST81, IB-ST80)

Lavandula dentata (MA-ST81, MA-ST53, IB-ST80)

Nerium oleander (MA-ST81, MA-ST53, IB-ST80)

Olea europaea europaea (MA-ST81, MA-ST53, ME-ST81, IB-ST80)

Olea europaea sylvestris (MA-ST81, MA-ST53, ME-ST81, IB-ST80)

Phagnalon saxatile (MA-ST?)

Phillyrea angustifolia (MA-ST81)

Phlomis italica (ME-ST81)

Polygala myrtifolia (MA-ST1, 7, 81; ME-ST81, IB-ST80)

Prunus avium (MA-ST1)

Prunus domestica (MA-ST81)

Prunus dulcis (MA-ST1, 7, 81; ME-ST81, IB-ST80)

Rhamnus alaternus (MA-ST1,81; MA-ST53, ME-ST81)

Rosmarinus officinalis (MA-ST81, ME-ST81, IB-ST80)

Ruta chalepensis (MA-ST1)

Salvia officinalis (MA-ST81)

Santolina chamaecyparissus (MA-ST81, ME-ST81)

Santolina magonica (ME-ST81)

Spartium junceum (MA-ST81)

Teucrium capitatum (MA-ST1)

Ulex parviflorus (IB-ST80)

Vitex agnus-castus (MA-ST81)

Vitis vinifera (MA-ST1)

IN THE BALEARIC ISLANDS /MA:
MALLORCA, ME: MENORCA, IB: IBIZA,
/ ST?: ST NOT YET DETERMINED,
UNDER STUDY

MAIN HOSTS

- *Prunus dulcis*
- *Olea europaea* var. *europaea*
- *Vitis vinifera*
- *Olea europaea* var. *sylvestris*
- *Lavandula angustifolia*
- *Polygala myrtifolia*
- *Rhamnus alaternus*
- *Fraxinus angustifolia*



GRAPEVINE SYMPTOMS



ALMOND TREE SYMPTOMS



OLIVE TREE SYMPTOMS



MALLORCA & MENORCA (MULTIPLEX ST81)

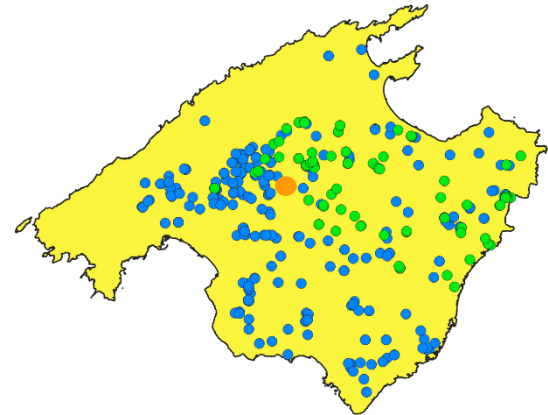


IBIZA (PAUCA ST80)

NEW OUTBREAK – PAUCA ST53 IN MALLORCA



Sencelles (January 2024)



- PAUCA
- FASTIDIOSA
- MULTIPLEX



PAUCA ST53

Inicio > Noticias > Noticias de actualidad > La cepa italiana de la Xylella aparece en Mallorca

Noticias de actualidad

La cepa italiana de la Xylella aparece en Mallorca

By Editorial febrero 2, 2024

4 0



Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/jpm



Citation: Moralejo, E., Quetglas, B., Montesinos, M., Adrover, F., Olmo, D., Nieto, A., Pedrosa, A., López, M., Juan, A., Marco-Noales, E., Navarro-Herrero, I., Barbé, S., Velasco-Amo, M.P., Olivares-García, C., & Landa, B.B. (2024). Outbreak of *Xylella fastidiosa* subsp. *pauca* ST53 affecting wild and cultivated olive trees on the island of Mallorca, Spain. *Phytopathologia Mediterranea* 63(3): 475-480. doi: 10.36253/phyto-15891

Accepted: December 27, 2024

Published: December 30, 2024

Short Notes

Outbreak of *Xylella fastidiosa* subsp. *pauca* ST53 affecting wild and cultivated olive trees on the island of Mallorca, Spain

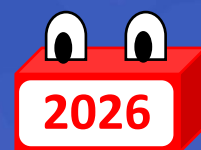
EDUARDO MORALEJO^{1,*}, BÁRBARA QUETGLAS¹, MARINA MONTESINOS¹, FRANCISCO ADROVER¹, DIEGO OLMO², ALICIA NIETO², ANA PEDROSA², MARTA LÓPEZ², ANDREU JUAN¹, ESTER MARCO-NOALES¹, INMACULADA NAVARRO-HERRERO¹, SILVIA BARBÉ¹, MARÍA PILAR VELASCO-AMO¹, CONCEPCIÓN OLIVARES-GARCÍA¹, BLANCA B. LANDA¹

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12 JUNE



Menorca
multiplex ST81



Ibiza
pauca ST80

Formentera

Mallorca
fastidiosa ST1
multiplex ST81, ST7
pauca ST53

(UE DECISION & RESEARCH SAMPLES)

No. detections	No. samples	No. plant species analyzed	No. plant species detected
4215	38118	490	38

- RESEARCH - MAIN FINDINGS

2018-2022



271.180 €

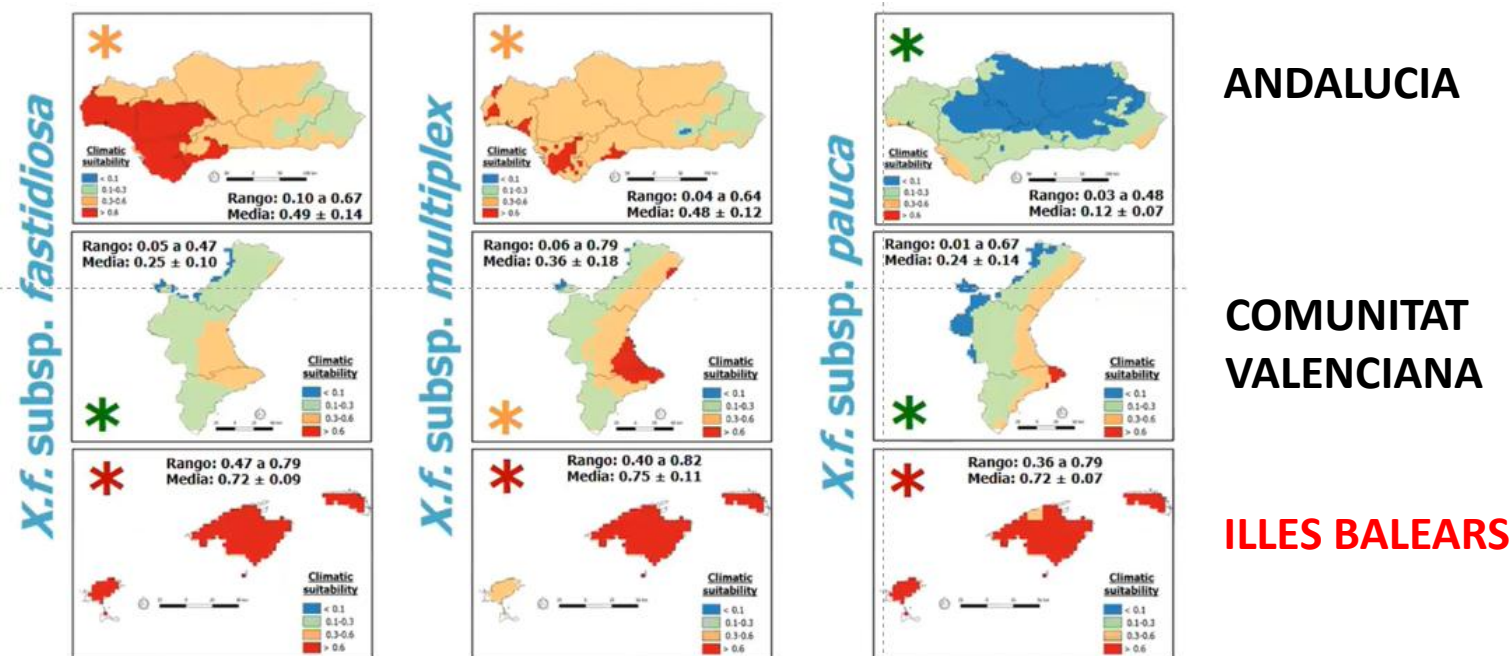


Desarrollo de estrategias de
erradicación, contención y
control de *Xylella fastidiosa*
en olivo en España



WP1: Risk Assessment

ivia



Balearic Islands

High risk for all three *Xylella fastidiosa* subspecies

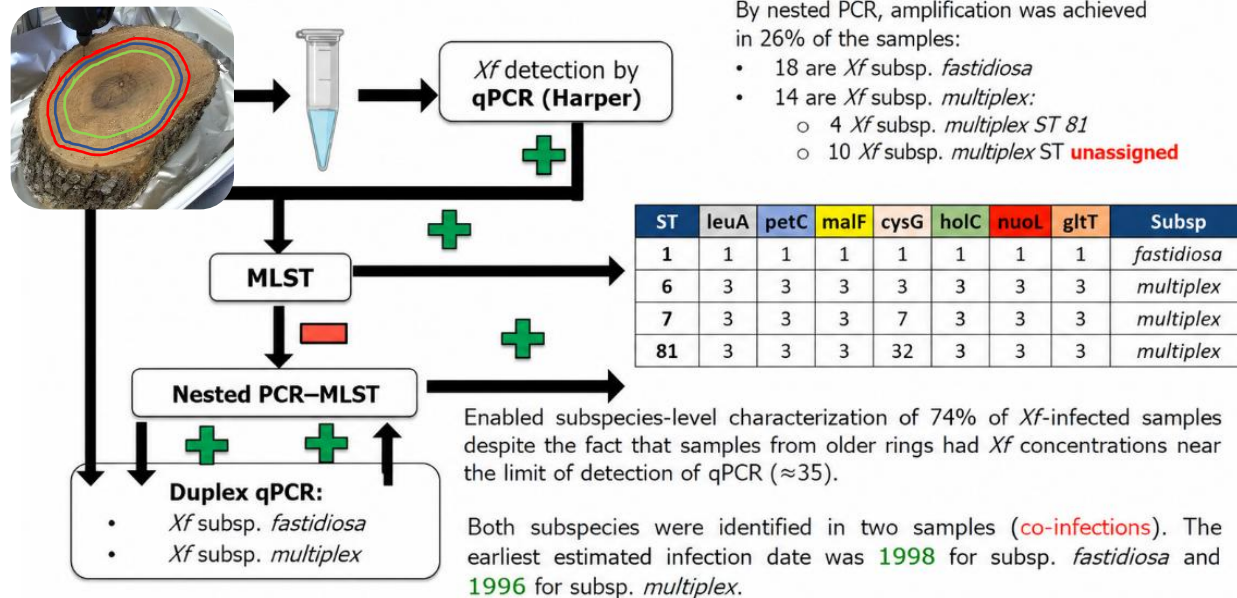
RESEARCH MAIN FINDINGS

WP2: Genetic Structure and Host Range



Validation of protocols for the identification of *Xf* subspecies

Methodology: DNA was extracted from dated rings of almond trunks infected by *Xf* from plots located on the island of Mallorca. The presence of *Xf* was analyzed by specific qPCR (Harper *et al.*, 2010).



Improvement and optimization of identification and characterization techniques

Subspecies-level detection from growth rings of *Xylella*-infected trees (Dendrochronology)

***Xylella fastidiosa* present in Balearics Isl. since 1990's**

RESEARCH MAIN FINDINGS

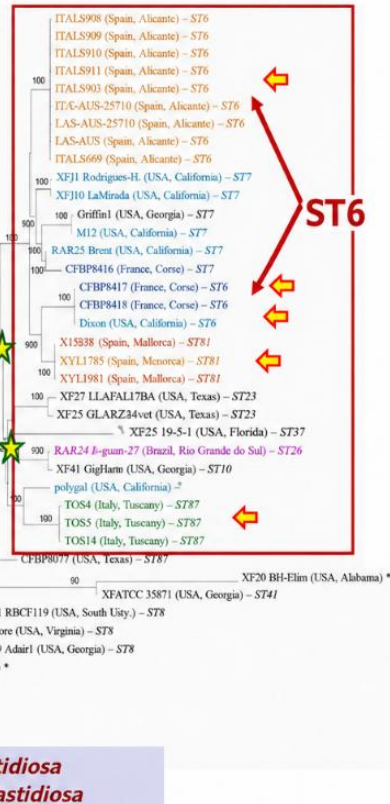
WP2: Genetic Structure and Host Range

Activity 2.3. Isolation, caracterización and sequencing of the genome of *Xf* type isolates



Evolutionary relationships between European and American isolates of *X. fastidiosa*

- ★ 92% - 97% likelihood of Southeastern USA origin at node
- 51% - 69% likelihood of Southeastern USA origin at node



Ancestral reconstruction based on geographic origin:

- *X. fastidiosa* subsp. *multiplex* originated in the Southeastern USA
- Isolates from California, Europe and Brazil are the result of introductions

Five introduction events in Europe:

- Italy (ST87)
- Corsica (ST6 and ST7)
- Spain-Balearic Islands (ST81)
- Spain-Alicante (ST6)

Bayesian phylogenetic inference analyses indicate probable introduction dates:

- *Xf* subsp. *fastidiosa* 1990-97
- *Xf* subsp. *multiplex* 1991-98

With the closest ancestors in *Xf* populations from California (USA) in grapevines and almonds

European introductions represent independent events

Xff and *Xfm* have been present in the Mallorca since the 1990's. It was introduced...

...from California (USA)

- Landa et al. 2020. AEM
- Moralejo et al. 2020. Com. Biol.

2019-2022



SÍNTESIS DEL PROYECTO
DISEÑO E IMPLEMENTACIÓN DE
ESTRATEGIAS DE CONTROL
FRENTE XYLELLA FASTIDIOSA
EN LAS ILLES BALEARS



1.050.000 €

Natural vegetation

- 13 novel hosts reported to science.

**Natural vegetation surveys uncover
hidden reservoirs of infection.**

Early detection

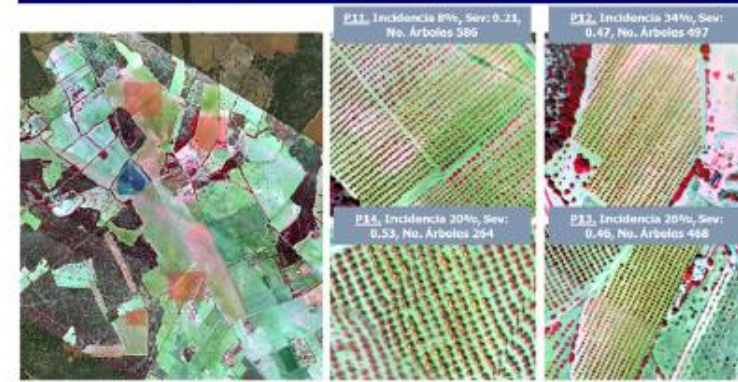
**Validation of an aerial detection system
for *Xf* in almond trees**



Santolina magonica killed by XF
(Balearic endemism)

B

Imagen Sensor hiperspectral SWIR, 166 cm/pixel, 60 bandas (900-1700 nm)



Vectors:

- Main vector: *Philaenus spumarius*.
- Drought-driven vector migration.
- Balearic climate favors the bacterium over its vector.

Climate change may reduce transmission.



Figura 3. De arriba abajo y de izquierda a derecha: *Philaenus spumarius*, *Neophilaenus campestris*, *Hyalesthes obsoletus* y *Aphrodes sp.*

Varieties:

- Almond genetic diversity has not limited the spread of the epidemic

> 80% Almond affected by ALSD in Mallorca

VARIEDADES NO AFECTADAS	
VARIEDADES AUTÓCTONAS	
Cabana	Mare de Deu
Cantaros	Pere Xina
Capirons	Progresos
Horrach	Verd
VARIEDADES FORÁNEAS	
Cristomorto	Lauranne
Ferragnes	Soleta
Marinada	Tarraco
Moncayo	Vairo

2022-2026



7.089.069 €



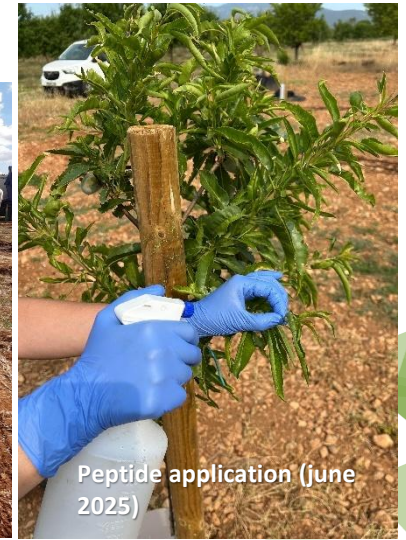
- Sentinel plots
- Germplasm banks / Variety collections
- Remote sensing
- Innovative control strategies (Syncoms, phages, peptides)
- Genomics



Phages application
(aug 2025)



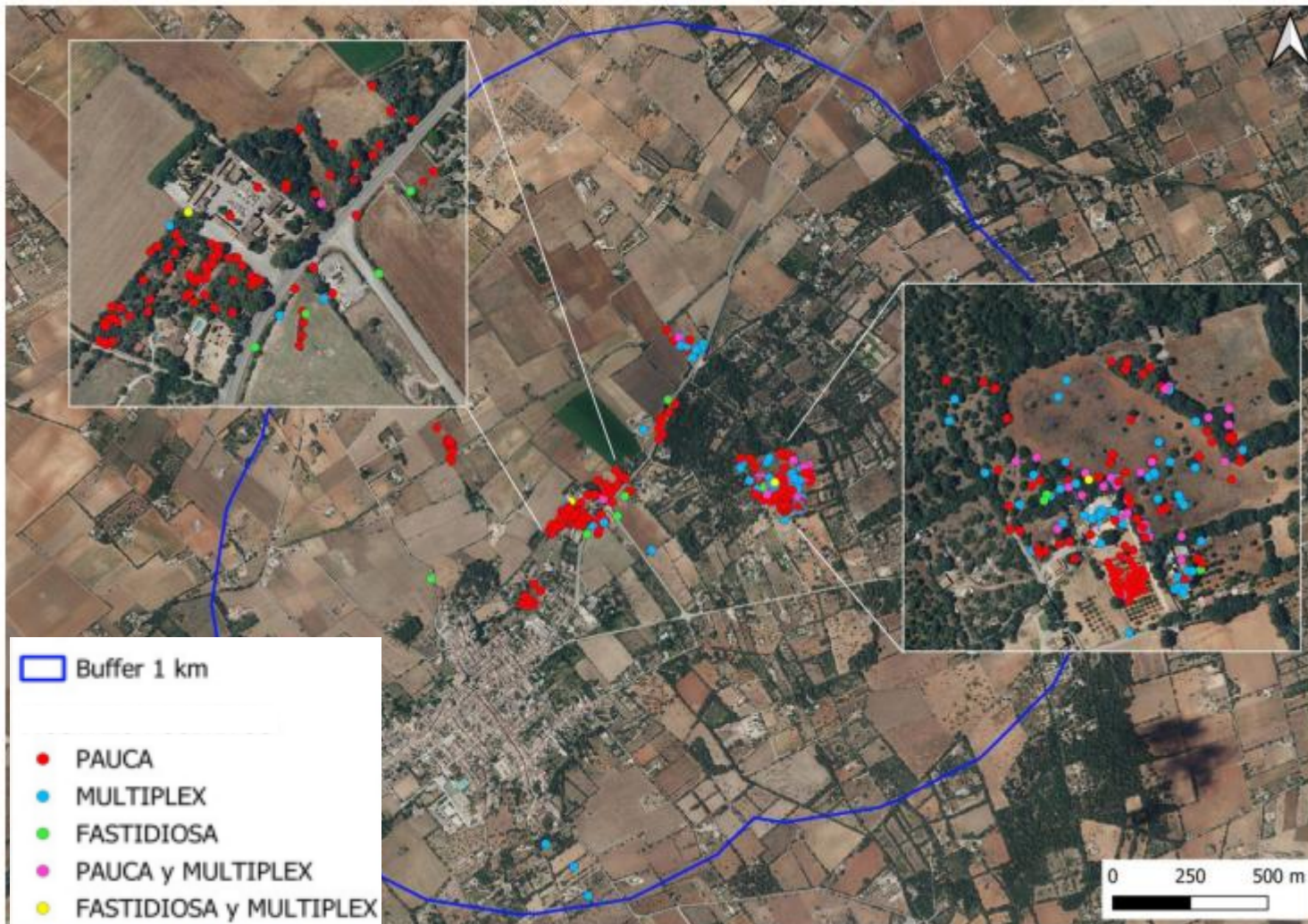
Syncom-primed Olive Trees
plantation (sept 2025)



Peptide application (june 2025)

NEW OUTBREAK – PAUCA ST53 IN MALLORCA

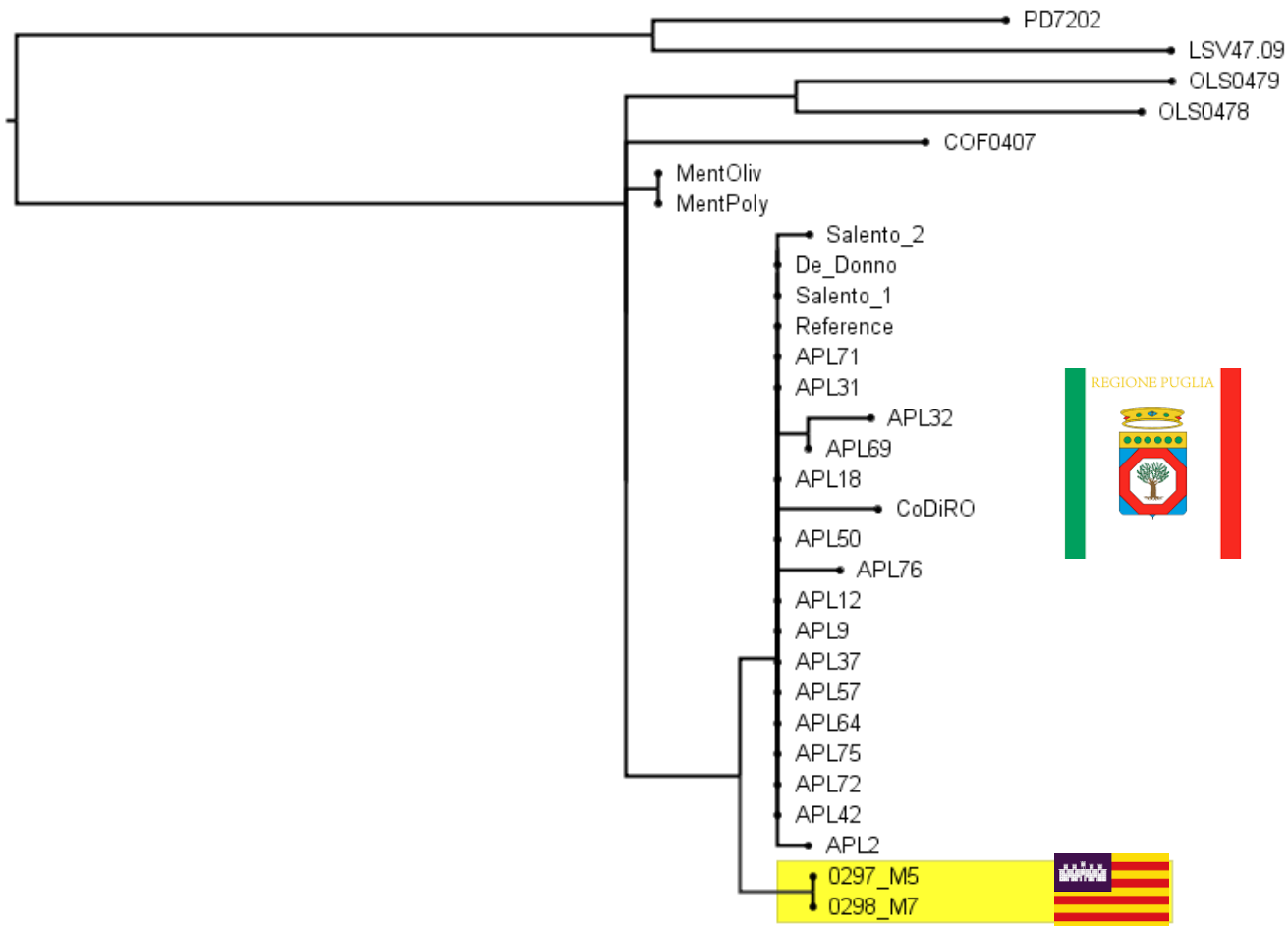
a particularly interesting case



- Detected in: Olea, Rhamnus, Lavandula, Nerium
- The *pauca* outbreak is **highly localized** (<2 km)
- 3 subsp. coexisting (**pauca, multiplex y fastidiosa**) in a small area.
- **Co-infections** have been detected. (two different *Xf* subsp. in same plant)

NEW OUTBREAK – PAUCA ST53 IN MALLORCA

a particularly interesting case



Mallorca and Apulian ST53 strains are virtually identical.

Hypothesis: It is a recent introduction (<10 years), likely via asymptomatic ornamental plants



Thank you for your attention

Olmo D.¹, Nieto A., Pedrosa A.¹, Adrover F.¹, Borràs D.¹, Aguiló M.², Montesinos M.², Moralejo E.², López M.³, Beidas O.³, Monterde A.⁴, Navarro I.⁴, Barbé S.⁴, Olivares C.⁵, Velasco-Amo M. P.⁵, Román-Écija M.⁵, Trapero-Casas J.L.⁵, Navas-Cortes J. A.⁵, Juan A.³, Marco-Noales E⁴, Landa B.B5.



**Conselleria d'Agricultura,
Pesca i Medi Natural**
Institut de Recerca i Formació
Agroalimentària i Pesquera



Grupo Tragsa



**Conselleria d'Agricultura,
Pesca i Medi Natural**
Direcció General d'Agricultura,
Ramaderia i Desenvolupament Rural

ivia
Instituto Valenciano
de Investigaciones Agr





Xylella fastidiosa

5th European conference

Bari, Italy | 22-26 June 2026

Rapid volatiliomic screening for Xylella fastidiosa: towards portable tools for phytosanitary surveillance



Dr. Elisabetta Poeta

National Research Council, Institute of Bioscience and BioResources
(CNR-IBBR), URT-Re, Via J.F. Kennedy, 17/i, 42124 Reggio Emilia, Italy.

E-mail: elisabetta.poeta@cnr.it

Team Supervisor

Dr. Veronica Sberveglieri

Team Member

Dr. Estefanía Núñez-Carmona



Five stages for sensor fabrication

1

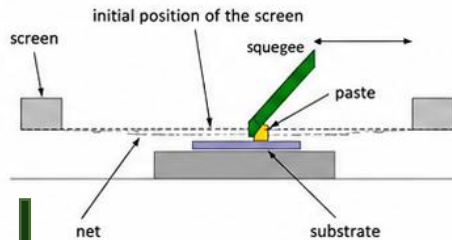
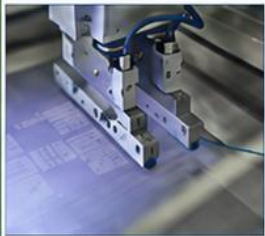
Preparation of the pasta



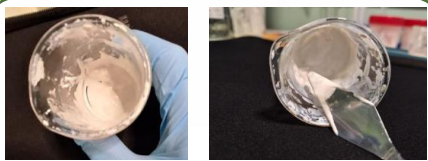
PROs of Screen-Printing technique

- ✓ Easy to implement: process adaptable to various materials and substrates.
- ✓ Low cost and scalable: suitable for industrial production
- ✓ High Reproducibility: uniform deposition of sensing layers
- ✓ Ease of automation: compatible with semi- or fully-automated production lines
- ✓ Versatility: allows multi-layer printing and integration of different materials

Paste Deposition Process



Prepared sensing paste



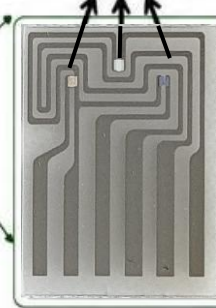
2

Sensor wafer production



Printed sensor wafer

Sensor's sensing elements



Single sensor

3

Firing in a muffle furnace



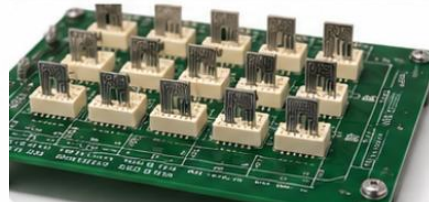
4

Annealing in a muffle furnace

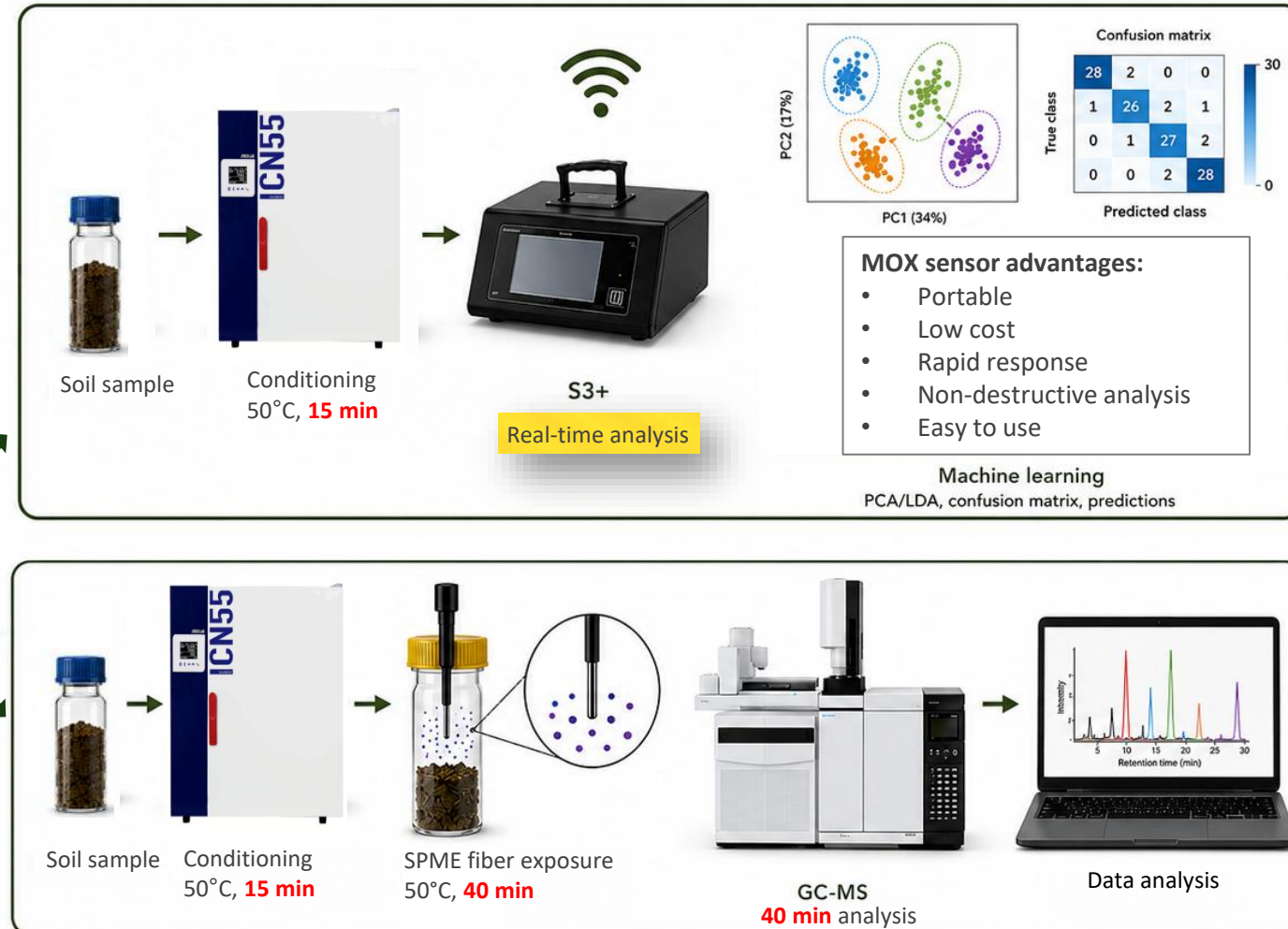


5

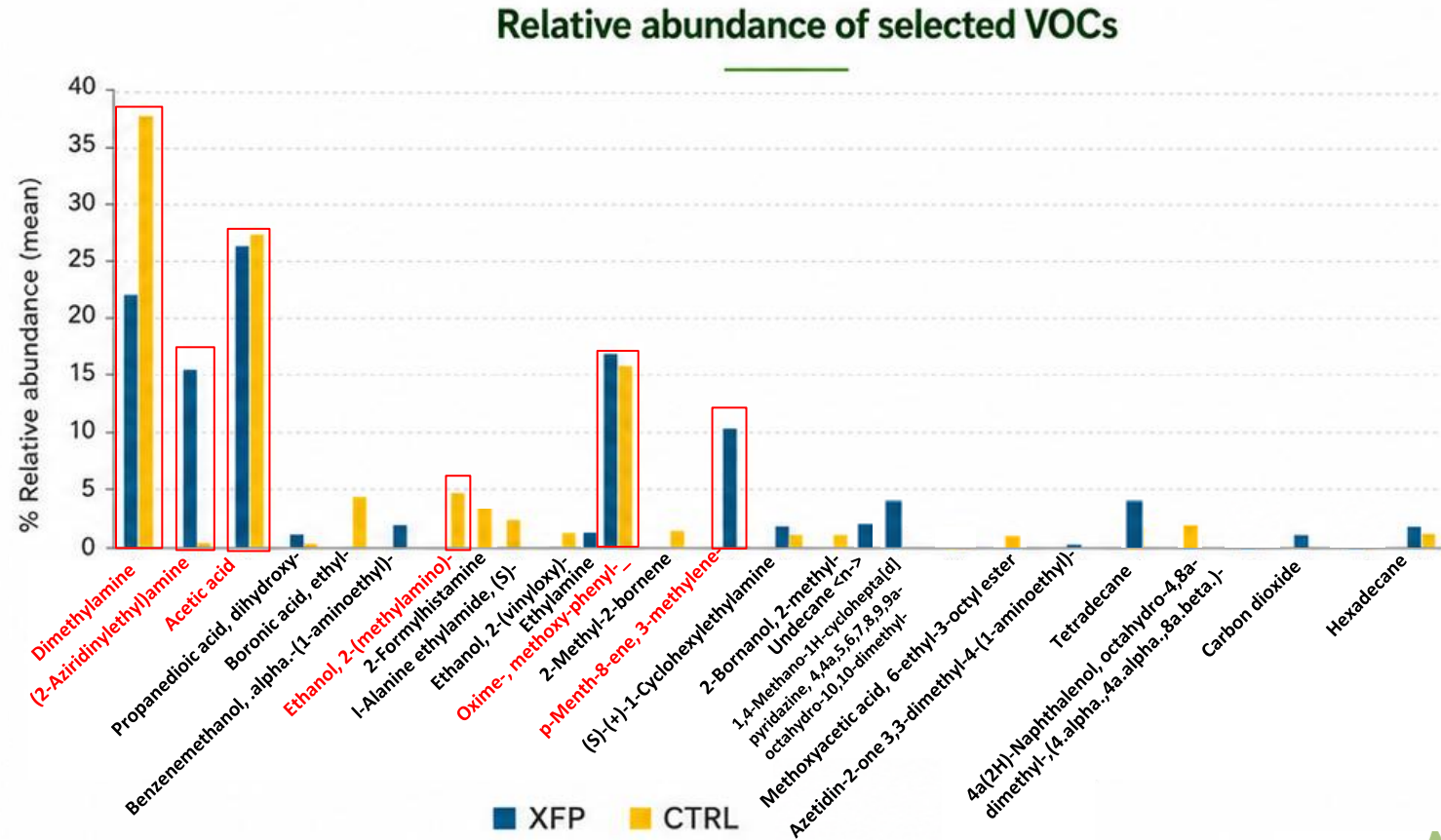
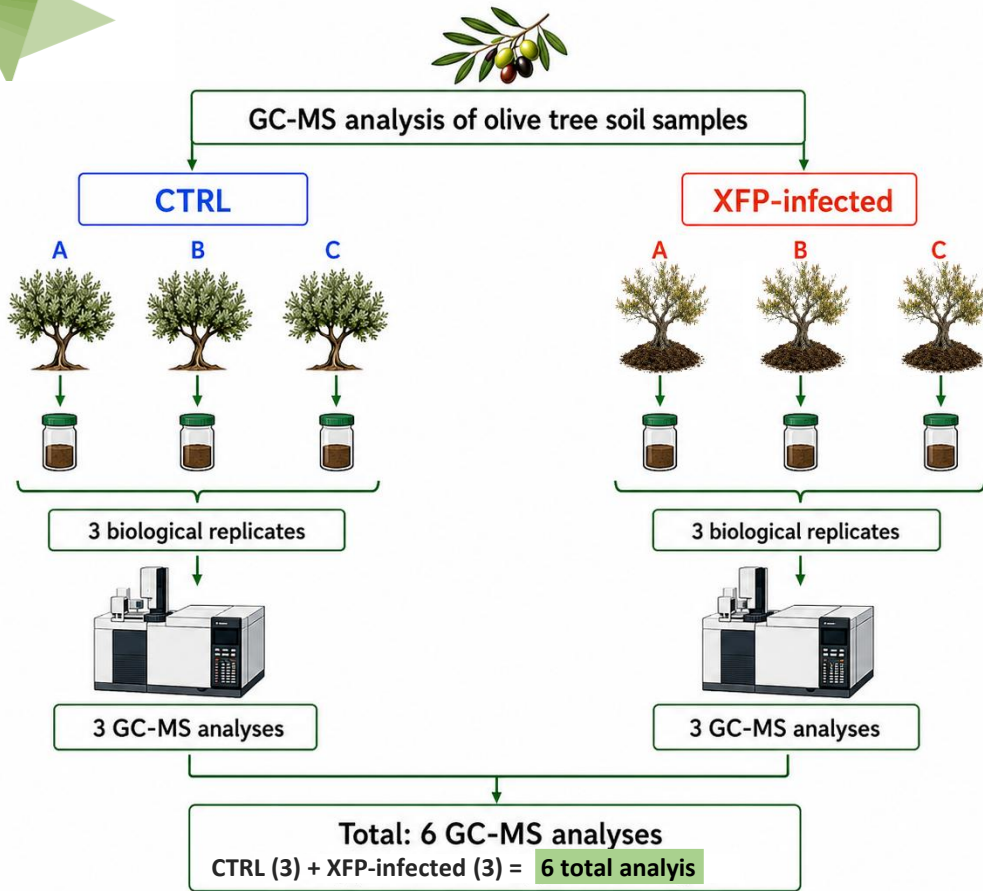
Aging



Integrated GC-MS and MOX Sensor-Based Approach for the Early Monitoring of *Xylella fastidiosa*



GC-MS RESULTS

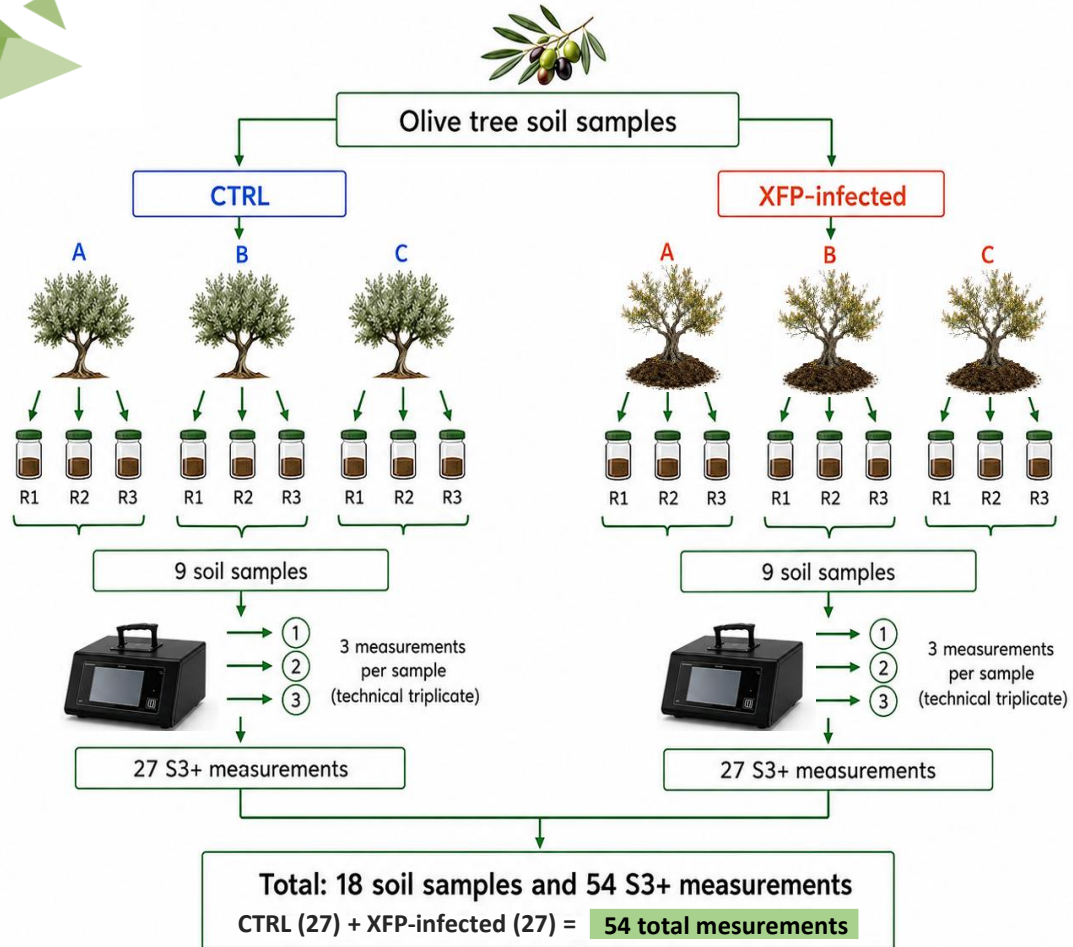


Key observations

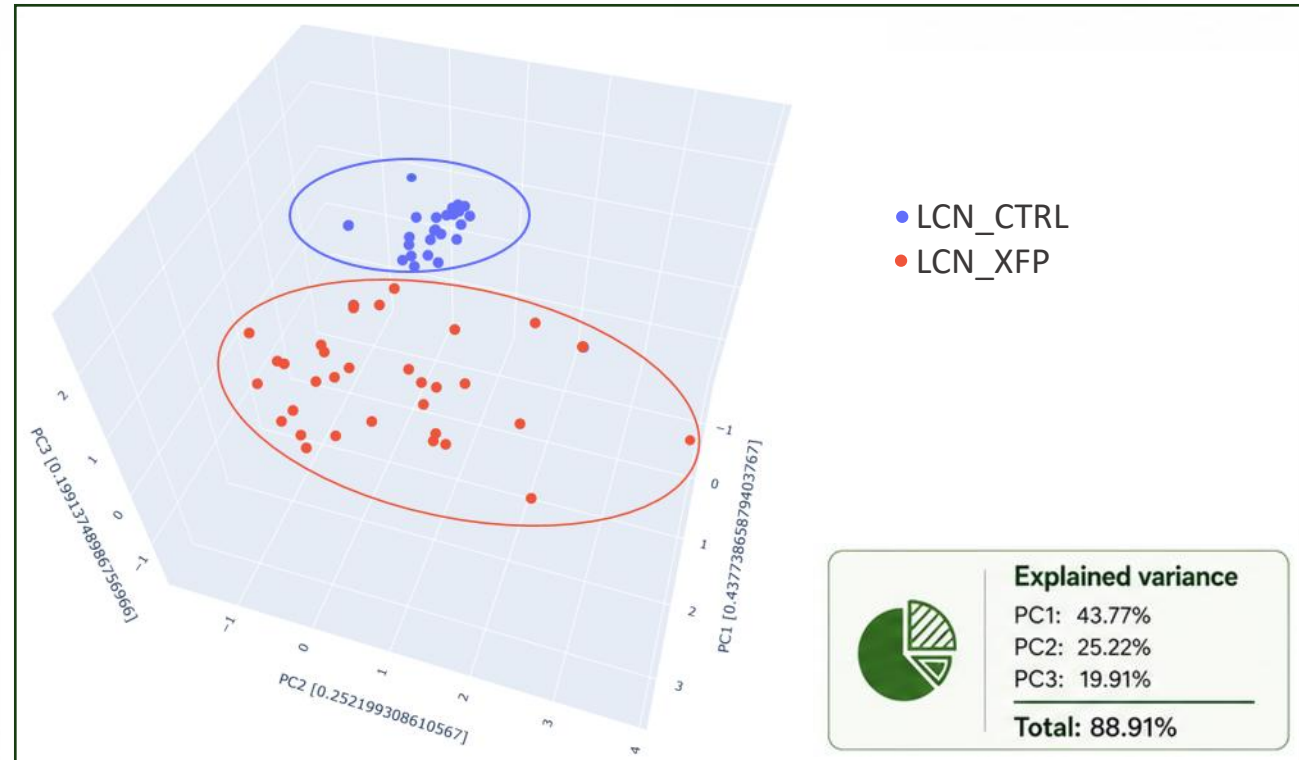
- CTRL and XFP-infected soils showed different volatile fingerprints.
- Several VOCs related to soil/rhizosphere activity showed changes in relative abundance.
- These variations may reflect differences in organic matter transformation and rhizosphere biochemical processes between the analyzed soil conditions.

MOX SENSORS RESULTS

Real time analysis



3D PCA



Key observations

- PCA showed a separation trend between CTRL and XFP-infected soils.
- CTRL samples were more compact, whereas XFP-infected samples showed droader variability.
- PC1-PC3 explained 88.91% of the total variance, supporting differences in the MOX sensor response patterns.

CONCLUSION AND FUTURE WORKS



Conclusions

- Customized MOX sensor arrays captured differences between CTRL and XFP-infected soil sample groups through global VOC fingerprinting.
- This approach may complement conventional diagnostic methods as a rapid and portable screening tool for early monitoring.



Future Perspective

Portable in-field screening before visible symptoms

Supporting early screening of potentially infected plants directly in nurseries.



Future Works

- Expand the dataset with additional samples and measurement sessions.
- Include more olive varieties and validate the approach under real field conditions.
- Strengthen machine learning models for robust in-field decision support.

Thank you for your attention

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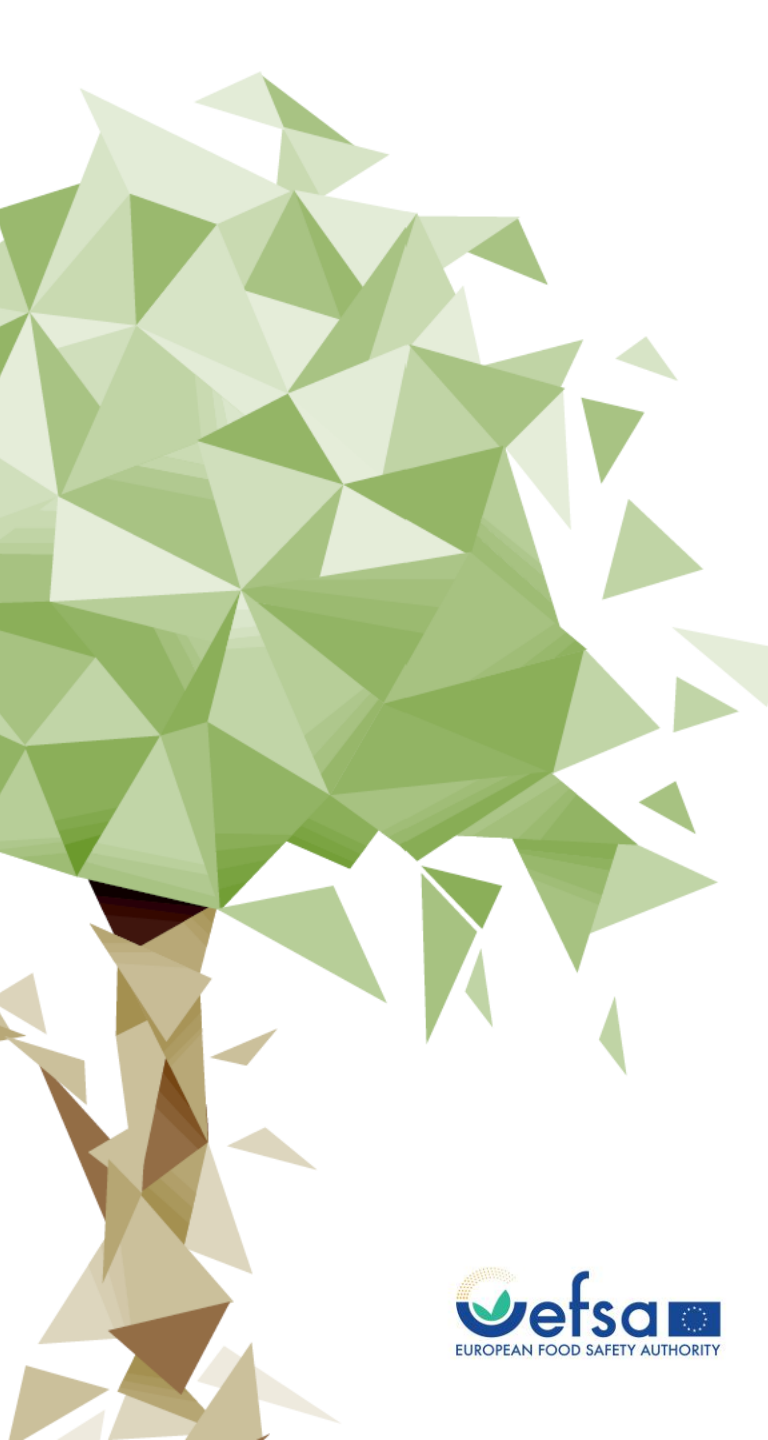
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(CNR-IBBR), URT-Re, Via J.F. Kennedy, 17/i, 42124 Reggio Emilia, Italy.

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HORIZON SCANNING FOR *XYLELLA FASTIDIOSA* MONITORING

Florian Kuntze (EFSA)

*Xylella
fastidiosa*

5th European conference

Bari, Italy | 22-26 June 2026

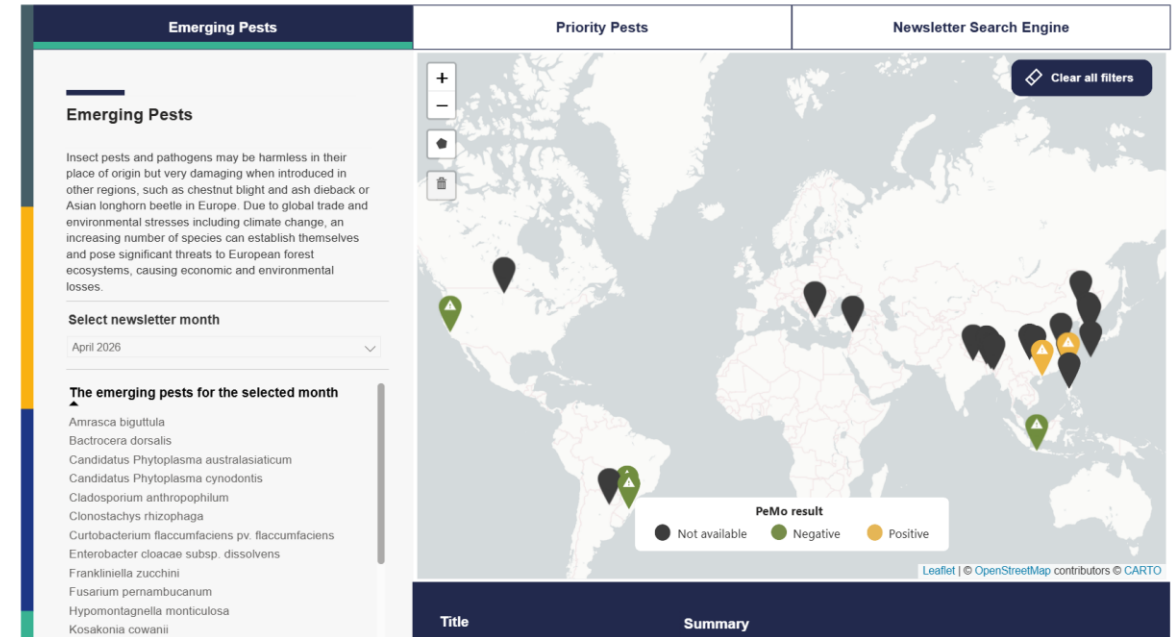


HORIZON SCANNING?



HORIZON SCANNING IN PLANT HEALTH

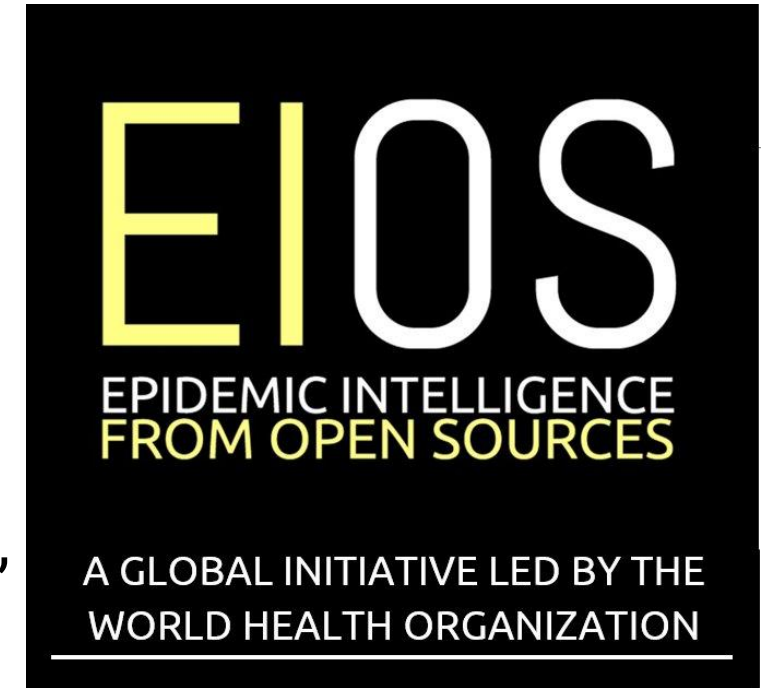
- Horizon Scanning since 2017
- Cooperation with the JRC and ANSES
- Publication of the monthly newsletter for Horizon Scanning on Plant Health
- Information via social media and our Horizon Scanning dashboard





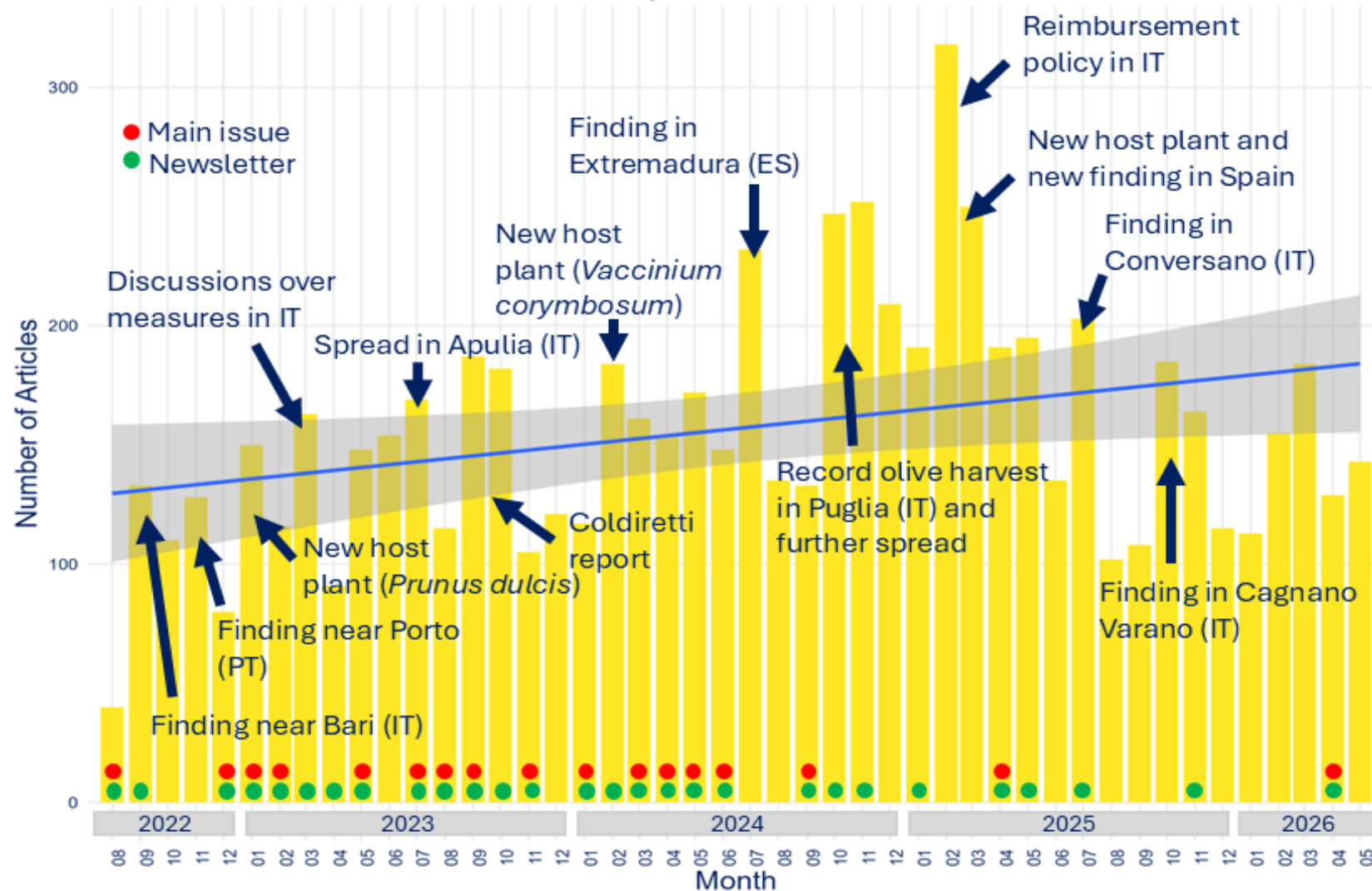
HORIZON SCANNING IN PLANT HEALTH

- A webscrapping platform for epidemic intelligence
- The basis of the Horizon Scanning activity at EFSA
- Identification and monitoring of signals for regulated, new and unknown pests
- 7000 - 10000 relevant articles per month



XYLELLA FASTIDIOSA – A TIMELINE IN HORIZON SCANNING

Fetches articles on *Xylella fastidiosa* per month



USE AND IMPACT

- Complementing procedures from EFSA and NPPOs since 2017
- Importance of considering **weak signals**, often the first signal of relevant issues
- **Global tracking** of *Xylella fastidiosa*
- Timely information on new outbreaks and spread
- **Supporting decisionmakers and risk managers** in their measures against *X. fastidiosa*
- Constant development and improvement



See you at Poster 115!

