



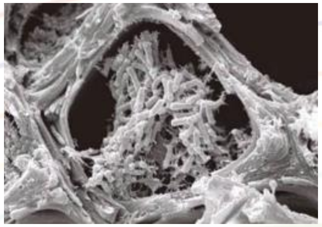

Diagnosis of *Xylella fastidiosa* in almond and grape spiked plant material, and in naturally infected olive trees with spontaneous canopy restoration

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EPPO Conference on Diagnostics of Plant Pests
Recent developments and future trends
2026-04-22/24, Vienna

Diagnosis of *Xylella fastidiosa* in almond and grape spiked plant material

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Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

- The EPPO Diagnostic Protocol PM 7/24 (5) *Xylella fastidiosa* gives some recommendations for sampling according to host plants, seasons and locations

- Sampling should preferably be carried out during the plant's active growth period (spring/summer), when the bacterium is more likely to be present in the tissues, to maximize the likelihood of detection

- Phytosanitary inspections are required for commercial activities prior to planting, specifically for deciduous species, during the autumn and winter months, when the plants are in dormancy face and thus less susceptible to transplant stress

- Although recent evidence indicates that *Xylella fastidiosa* can be detected in deciduous plants throughout the year in Mediterranean countries, the performance of diagnostic and detection tests depends on the plant species and geographical location.

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Goals

- Evaluate the performance of key molecular diagnostic protocols for the detection and identification of *Xylella fastidiosa* subspecies in spiked plant samples throughout the year, including during asymptomatic or dormant phase when bacterial concentrations are low
- Assess potential critical factors affecting detection, such as the presence of inhibitors in plant tissues that may vary with the season
- Identify the most effective diagnostic test for reliably detecting Xff and Xfm during the dormancy phase.

SPIKED samples preparation

Vitis vinifera

Prunus dulcis

February 2023-May 2025

- **autumn**
- **winter**
- **spring**
- **summer**

***Xf* subsp. fastidiosa ST1**

***Xf* subsp. *multiplex* ST87**

- **Leaf or wood tissue contamination**: ten-fold serial dilutions of *Xf* (10^5-10 CFU/mL)
- **DNA extraction:** automated KingFisher™ mL Purification System (QuickPick™ SML Plant DNA)
- **Molecolar analysis**: qPCR Harper *et al.*, 2010 erratum 2013, droplet digital PCR Dupas *et al.*, 2019a, tetraplex qPCR set 2 Dupas *et al.*, 2019b qPCR Hodgetts *et al.*, 2020

Analytical sensitivity (LOD)

XffST1

		February 2023	May 2023	July 2023	November 2023	February 2024	May 2024	July 2024	November 2024	February 2025	May 2025
Harper Ct values		10^3 33.2 ± 0.3	10^2 34.82 ± 1.3	10^3 33.10 ± 0.7	10^3 32.82 ± 0.5	10^3 33.49 ± 0.3	10^2 35.56 ± 0.5	10^3 34.95 ± 0.6	10^2 36.69 ± 0.7	10^2 36.10 ± 1.2	10^2 35.43 ± 1
ddPCR copies/uL		10^2 0.43 ± 0.11	10^2 0.72 ± 0.3	10^3 [illegible]5.23 ± 1.5	10^2 0.59 ± 0.18	10^2 0.38 ± 0.22	10^2 0.95 ± 0.31	10^3 1[illegible]30 ± 1.21	10^2 1.24 ± 0.6	10^2 1.33 ± 0.3	10^2 1.47 ± 0.1
Dupas	FAM	10^3 35.08 ± 0.9	10^3 34.96 ± 1.8	10^4 31.71 ± 0.1	10^3 35.02 ± 0.8	10^3 35.23 ± 0.7					
	HEX	10^3 33.15 ± 0.7	10^2 35.77 ± 0.9	10^3 33.62 ± 0.8	10^3 30 ± 0.2	10^3 33.44 ± 0.4	10^2 32.42 ± 0.4	10^3 34.39 ± 0.7	10^3 32.52 ± 0.6	10^3 30.81 ± 0.2	10^3 31.78 ± 0.2
Hodgetts Ct values		10^3 33.25 ± 0.5	10^2 35.06 ± 0.5	10^3 32.47 ± 0.6	10^2 36.44 ± 1.1	10^2 36.30 ± 0.7	10^2 35.40 ± 0.5	10^3 34.46 ± 0.5	10^2 36.60 ± 1	10^3 31.43 ± 0.3	10^2 34.92 ± 0.7

- **dormancy** seasons (February/November)→ ddPCR results to be the most sensitive test (LOD= 10^2 CFU/mL)
- **vegetative growth** seasons (May/July) → ddPCR, qPCR Harper and Hodgetts are comparable (LOD= 10^2 CFU/mL in May, 10^3 CFU/mL in July)
- **May** results to be the better period for the Xf detection
- the **tetraplex qPCR set 2** of Dupas et al., (2019b) appears to be less reproducible

Analytical sensitivity (LOD)

XfmST87

		May 2023	July 2023	November 2023	February 2024	May 2024	July 2024	November 2024	February 2025	May 2025
Harper ct values		10^2 35.60 ± 0.8	10^1 34.80 ± 1.2	10^2 35.47 ± 0.8	10^2 35.07 ± 0.9	10^2 34.41 ± 0.3	10^3 32.12 ± 0.6	10^3 35,76 ± 2.1	10^3 35.24 ± 0.3	10^2 34.11 ± 0.4
ddPCR copies/uL		10^2 0.43 ± 0.25	10^1 0.13 ± 0.44	10^2 1 ± 0.72	10^2 1.1 ± 0.50	10^2 2 ± 0.14	10^2 0.43 ± 0.33	10^2 2.59 ± 3.1	10^2 0.3 ± 0.13	10^2 0.19 ± 0.04
Dupas	FAM	10^3 34.22 ± 1.1	10^2 34.59 ± 1.1	10^3 34.07 ± 0.5	10^3 34.11 ± 0.5					
	ROX	10^3 31.12 ± 0.8	10^2 32.52 ± 1.9	10^3 33.34 ± 1	10^3 32.37 ± 0.4	10^2 33.31 ± 0.5	10^3 34.57 ± 0.7	10^3 35,76 ± 2.1	10^3 35.01 ± 1.1	10^2 29.96 ± 0.2
Hodgetts ct values		10^2 34.04 ± 0.4	10^1 33.25 ± 0.7	10^2 34.41 ± 0.7	10^2 33.95 ± 0.9	10^2 33,91 ± 0.3	10^3 35.49 ± 1.2	10^3 34.82 ± 1.54	10^3 34.47 ± 0.3	10^2 33,81 ± 0.7

- **dormancy** seasons (February/November)→ ddPCR results to be the most sensitive test (LOD= 10^2 CFU/mL)
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DNA extraction: CTAB versus KingFisher™ mL

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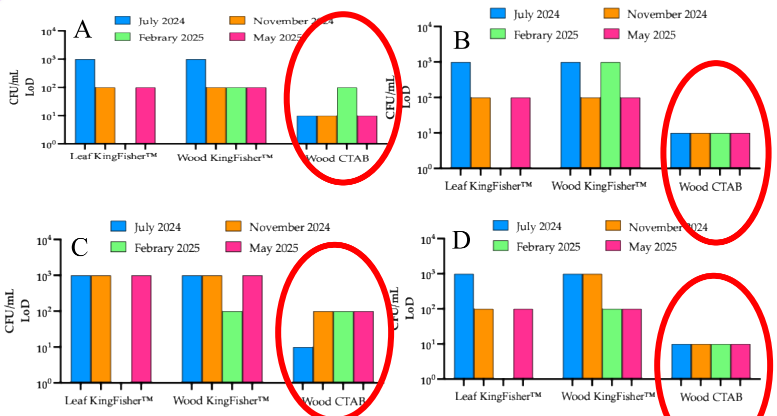
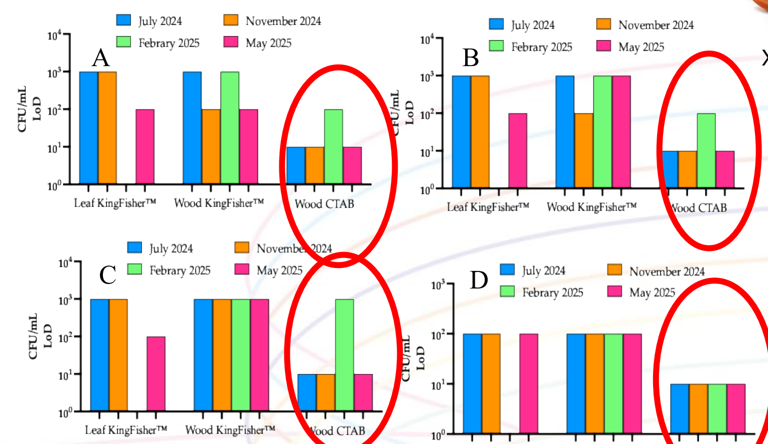
Extraction conditions assessed:
Leaf/KingFisher (QuickPickTM SML Plant DNA)
Wood/KingFisher (QuickPickTM SML Plant DNA)
Wood/CTAB

XffST1



XfmST87



A: qPCR Harper et al. 2010, erratum 2013 **B**: qPCR Hodgetts et al. 2021 **C**: qPCR Dupas et al. 2019 **D**: ddPCR Dupas et al. 2019

- **CTAB extraction** consistently provided **higher ASE** than KingFisher™; the LoD was generally **10-fold lower** than with KingFisher™.
- **CTAB** proved more reliable for **low bacterial load detection**
- **CTAB combined with ddPCR** maintained a **stable LoD of 10^1 CFU/mL**

Conclusions

- **ddPCR** proved to be the test with the highest analytical sensitivity regardless of season/matrix. Regarding qPCR of Harper et al. (2010, erratum 2013), the gold standard for diagnosing *Xylella*, analytical sensitivity declined in November and February

- **May** resulted the most favourable period for monitoring purposes, within all tested methods, showing minimal interference from matrix-derived inhibitors

- **wood-CTAB** combination consistently yielded the highest analytical sensitivity, with LOD values approximately 10-fold lower than the other methods

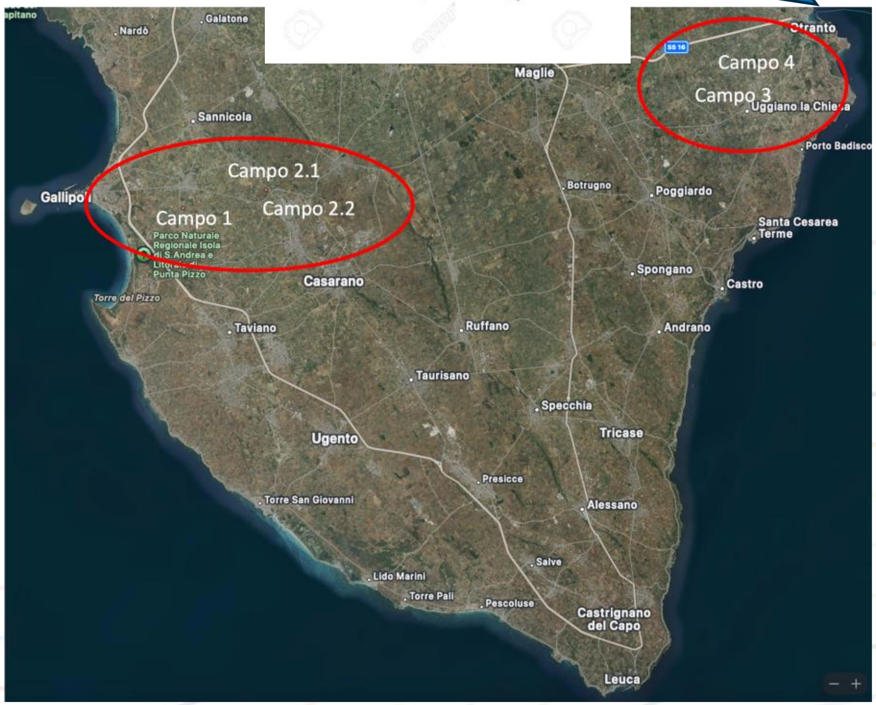
- Regarding ***Xf* identification** from plant matrices, Hodgetts et al. (2021) demonstrated greater sensitivity compared to Dupas et al. (2019b)

These findings highlight the importance of selecting, for grapevine and almond, optimal sampling periods, plant matrices, and extraction protocols as well as the appropriate molecular methods, to ensure accurate detection and timely containment.

Distribution of *Xylella fastidiosa* subsp. *pauca* in olive trees subjected to high natural inoculum pressure showing either signs of canopy vigour recovery or not.

Recovery of canopy vigour has been observed in olive trees in Salento in recent years, more than 10 years after Xfp first report.

Monitoring activities: from November 2023 to May 2025 (February, May, July, November)



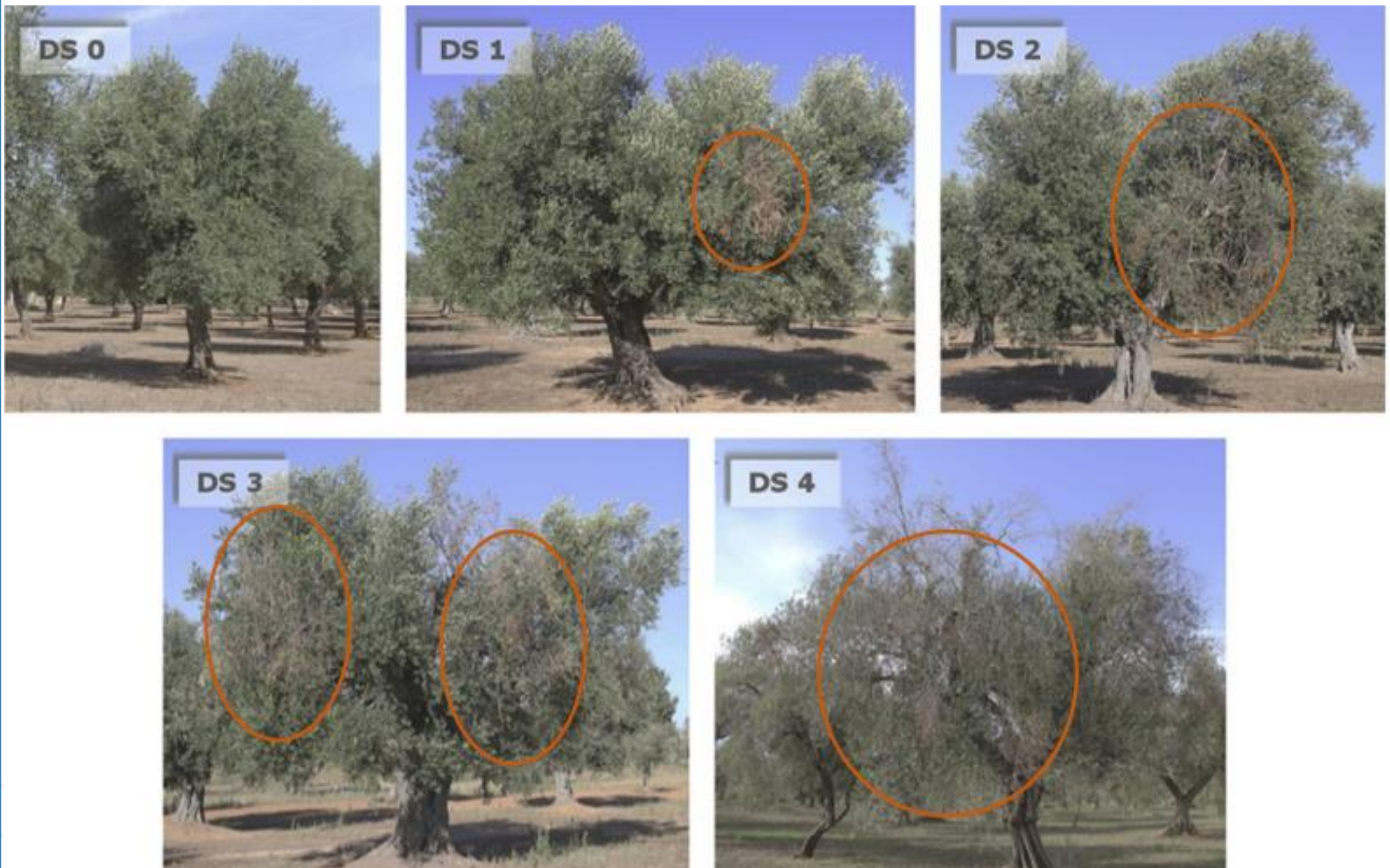
Low-symptoms:
Olive Grove 1, 2.1,3

High-symptoms:
Olive Grove 2.2, 4

Leaf DNA extraction:
automated KingFisher™mL Purification System (QuickPick™ SML Plant DNA)

Bacterial load: qPCR Harper et al., 2010; erratum 2013,

Disease Severity scale



0 No symptoms

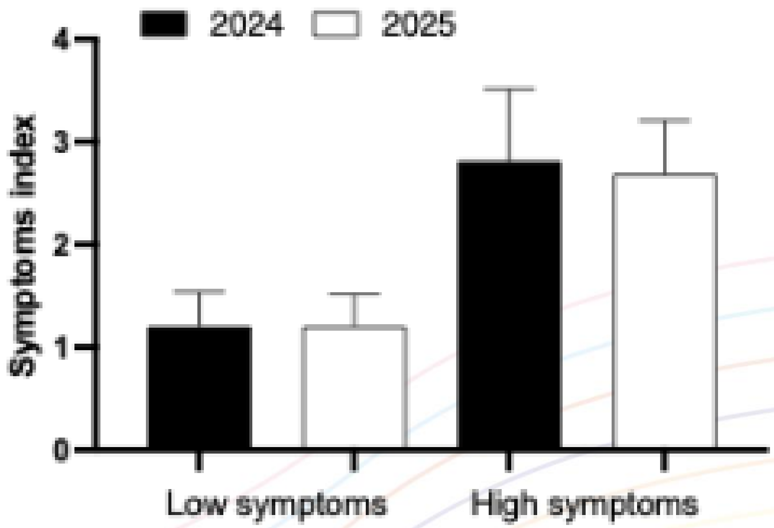
1 scorch on a few twigs

2 scorch affecting one or few branches

3 scorch affecting many branches or desiccation of a large portion of the canopy

4 totally desiccated canopy

Histogram comparing the symptom index of olive groves with low-symptoms vs. high-symptom levels for the years 2024 and 2025



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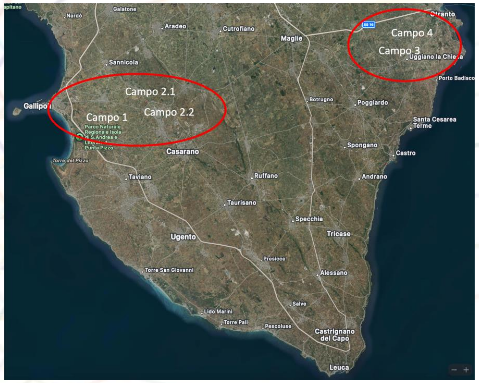
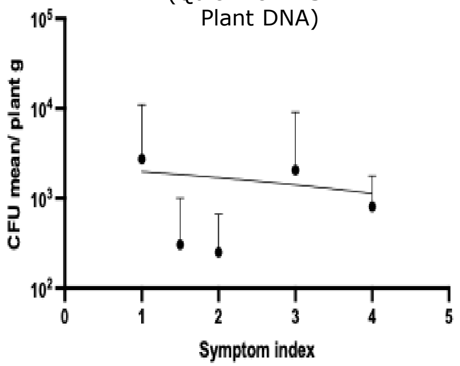


Significant differences in symptom index were observed between the two groups of olive groves in both years

Vegetation Indices Analysis
NDVI, NDWI, SAVI

- ✓All vegetation indices showed a general decline in both LS and HS olive orchards
- ✓The decrease was markedly stronger in HS orchards **(ΔNDWI=-0.087, ΔNDVI=-0.108)** compared to LS ones **(ΔNDWI=-0.026, ΔNDVI=-0.025**).
- ✓HS orchards experienced a greater reduction in foliage moisture (NDWI) and photosynthetic activity, canopy greenness (NDVI)
- ✓ LS orchards showed a more limited decline, consistent with canopy persistence/resilience

Automated KingFisher™ mL Purification System (QuickPick™ SML Plant DNA)



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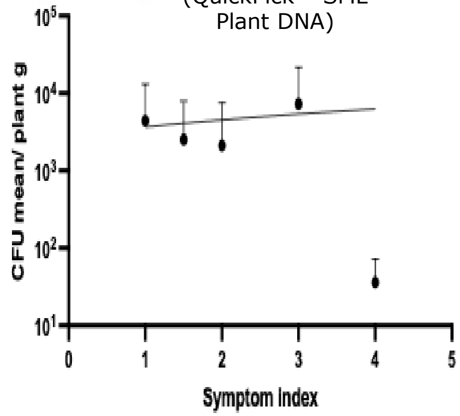
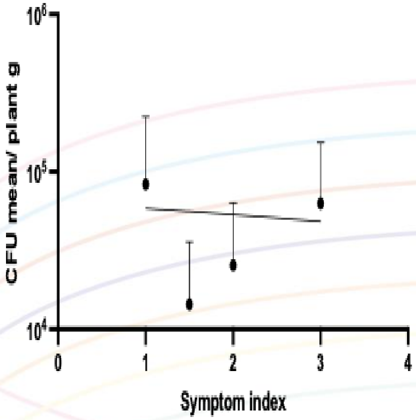


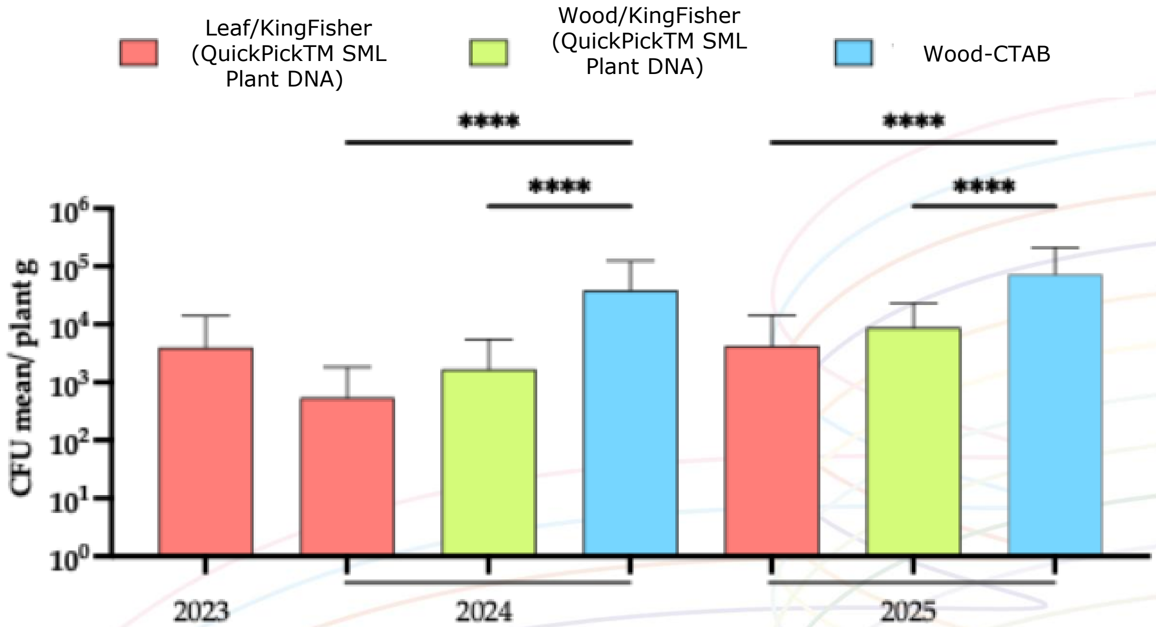
CTAB extraction



Linear correlation graph illustrating the relationship beetwen the detected DNA concentraction (y axis) and the symptoms index (x axis).

No direct correlation observed between symptoms severity and *Xfp* quantification

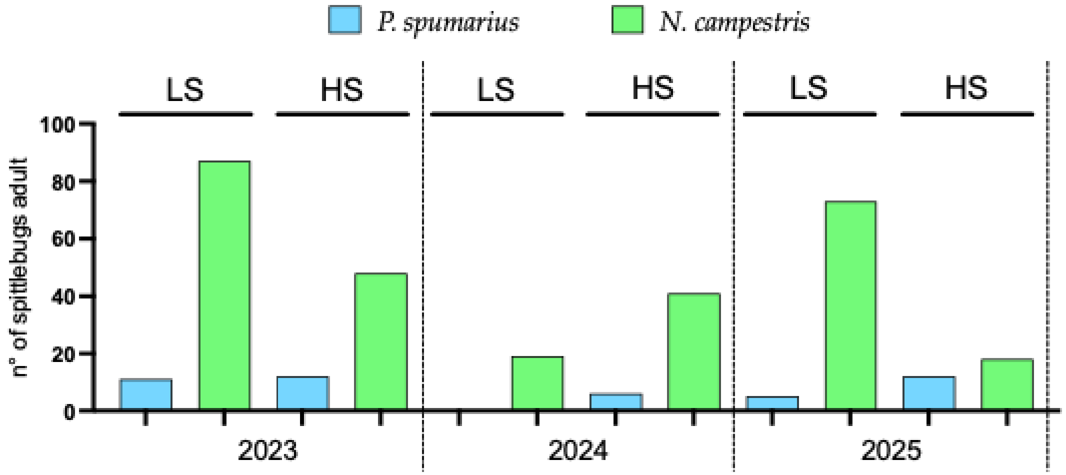
Analysis of the infection trend in the monitored area (2023–2025)



Within the same year, significant differences are observed depending on the matrix/extraction method combination.

No significant variation in bacterial load was observed over the three years of monitoring.

Vector sampling

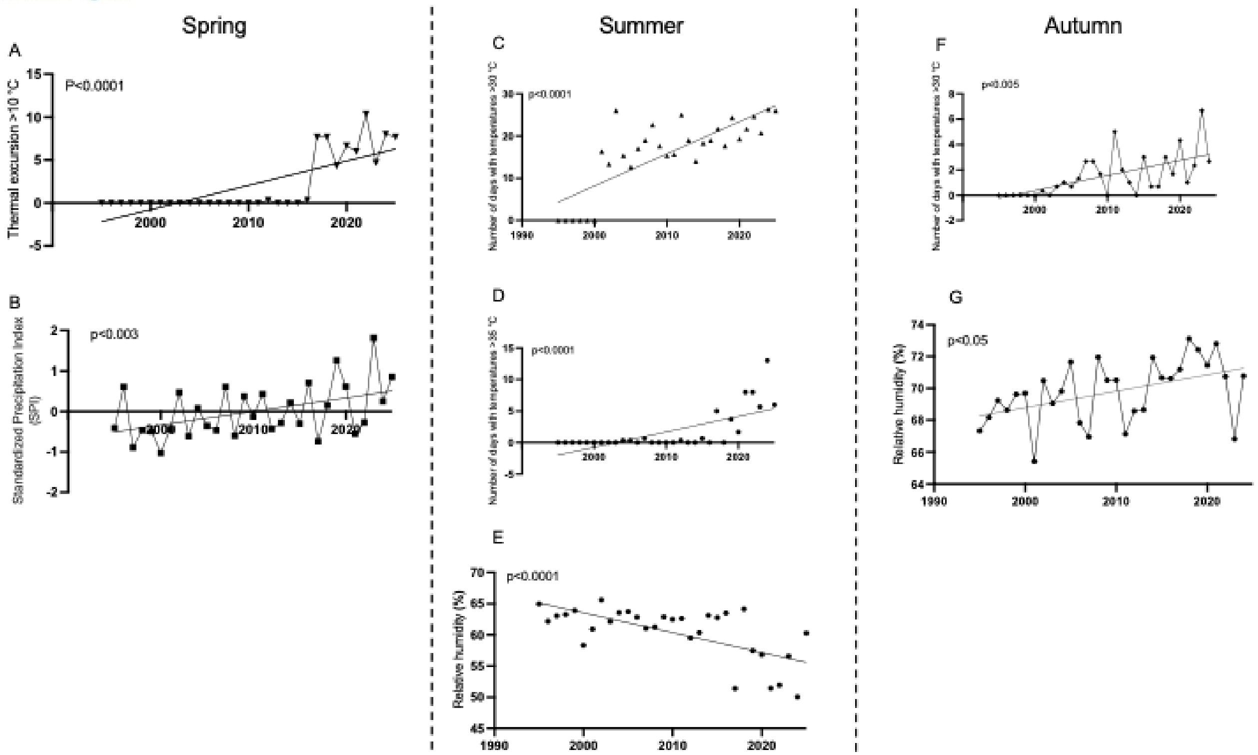


The vectors species *Philaenus spumarius* and *Neophilaenus campestris* were identified, with a clear predominance of *N. campestris*, consistently exhibited low bacterial loads.

Populations of *P. spumarius* were generally low, with bacterial loads around 10^2CFU/ml, corresponding to the limit of detection of the pathogen by RT-PCR.

In both LS and HS olive orchards, a reduction in the infectivity of *P. spumarius* was observed from 2023 to 2025.

Long-Term Climatic Dynamics (1995-2025)



A) Number of days with a diurnal temperature range >10 °C ($p<0.0001$) B) Standardized Precipitation Index (SPI) ($p<0.0001$) C) Number of days with daily maximum temperature >30 °C ($p<0.0001$) D) Number of days with daily maximum temperature >35 °C ($p<0.0001$) E) Daily relative humidity ($p<0.0001$) F) Number of days with daily maximum temperature >30 °C ($p<0.005$) G) Daily relative humidity ($p<0.05$).

Final remarks

- **Wood/CTAB** DNA extraction are confirmed as the best combination for detecting *Xf*, consistent with our previous findings on spiked samples
- No direct correlation between **disease index** and ***Xf*** in *planta* concen tration
- No statistically significant differences were observed in **the bacterial load** of the LS and HS infected plants
- In both LS and HS olive orchards, a **reduction in the incidence** of *P. spumarius* was observed

None of the factors investigated emerged as a primary driver of the observed phenomenon, emphasizing the need for further research to elucidate the physiological and molecular bases of canopy vigor recovery, its long-term persistence, and its interactions with plant-associated microbiota.

Thanks for your attention

Stefania Loreti, Valeria Scala, Giuseppe Tatulli, Erica Cesari, Alessandro Polito, Alessia L'Aurora, Riccardo Fiorani, Simone Lucchesi, Sabrina Bertin, Giorgia Bertinelli, Dario La Montagna

Founding Support Project: *"La diagnosi come strumento di prevenzione per il contenimento di Xylella fastidiosa: sviluppo ed ottimizzazione (DIACOX)" funded by the Ministry of Agriculture, Food Sovereignty, and Forests (MASAF)-DM n. 664531 28/12/2022*