AMPLIFICATION-FREE DETECTION OF *XYLELLA FASTIDIOSA* DNA IN XYLEM SAP FOR RAPID FIELD DIAGNOSTICS

Farjana Haque[1], Fatema Zerin Farhana[1], Sharmin Aktar[1], Milkiyas Toru Tantu[1], Md Akeruzzaman Shaon[1], Geoff M. Gurr[2], Muhammad J. A. Shiddiky[1]

[1]Rural Health Research Institute and [2]Gulbali Institute & School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Orange, NSW 2800, Australia

Background

THE PROBLEM: *Xylella fastidiosa* (*X. fastidiosa*) is one of the world's most devastating plant pathogens, threatening global agriculture and biodiversity. It infects over 350 plant species, including grapevine and citrus, and causes severe diseases such as Pierce's disease, stunted shoot syndrome, and citrus variegated chlorosis. This fast-spreading bacterium is responsible for over USD 220 billion in annual agricultural losses worldwide, moving rapidly from the Americas to Asia and Europe. Although Australia remains *Xylella*-free, the pathogen is considered the country's top-priority pest. Its potential introduction poses an imminent biosecurity threat, demanding vigilant, early, and field-ready detection strategies.

THE CHALLENGE: Current molecular diagnostics such as PCR, ELISA, and LAMP are sensitive and reliable, yet they remain impractical for on-site surveillance due to their: (*i*) High cost and technical complexity, (*ii*) Dependence on laboratory equipment and trained personnel, and (*iii*) Limited scalability for large-area or border screening.

THE NEED: To protect crops and ecosystems, *X. fastidiosa* must be detected early and anywhere it appears. What's urgently required is a scalable, portable, and precise point-of-care (POC) diagnostic platform that enables: (*i*) **Rapid, on-site pathogen detection** in the field or at borders, (*ii*) **Accurate results** without laboratory constraints, and (*iii*) **Widespread deployment** for proactive surveillance and biosecurity protection.

Aims

Develop a **rapid and reliable electrochemical (EC) platform** for detecting *X. fastidiosa* in field conditions by integrating multiple innovative components:

- Enhance EC sensor performance through magnetic particle isolation-based capture, isolation, and controlled heat release.
- Investigate gold–DNA affinity (adsorption) under direct current (DC) fields to improve the analytical performances (i.e., sensitivity, specificity, assay time, reproducibility) of the EC sensor.
- Design a low-cost, portable 3D-printed device for rapid xylem sap extraction and on-site testing.

Methods

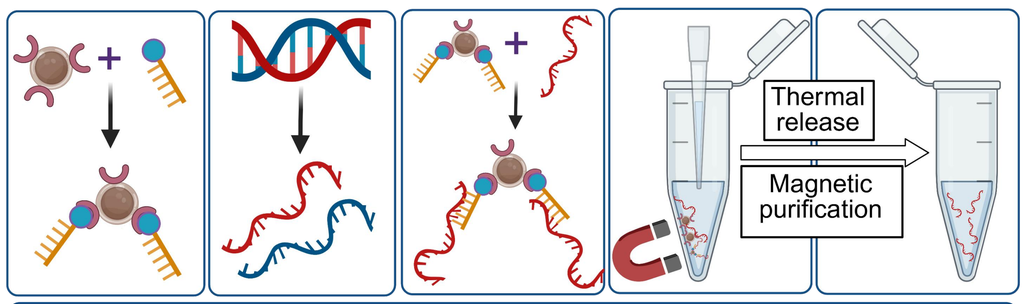
The EC assay workflow :

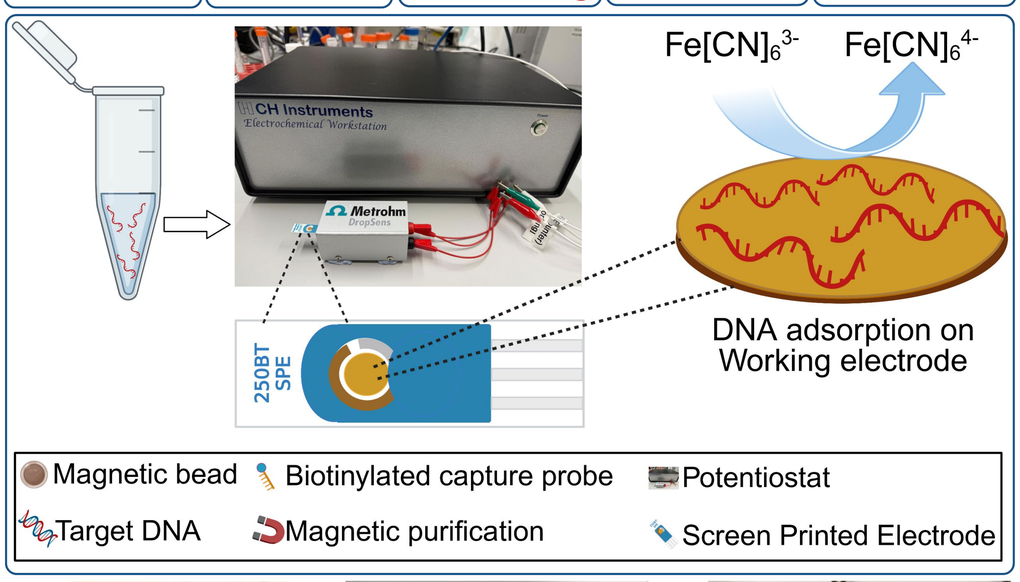
- **Magnetic DNA Capture**: *X. fastidiosa*-specific DNA is isolated using biotinylated probes bound to magnetic beads.
- **Thermal Release**: Captured DNA is released from beads by controlled heating.

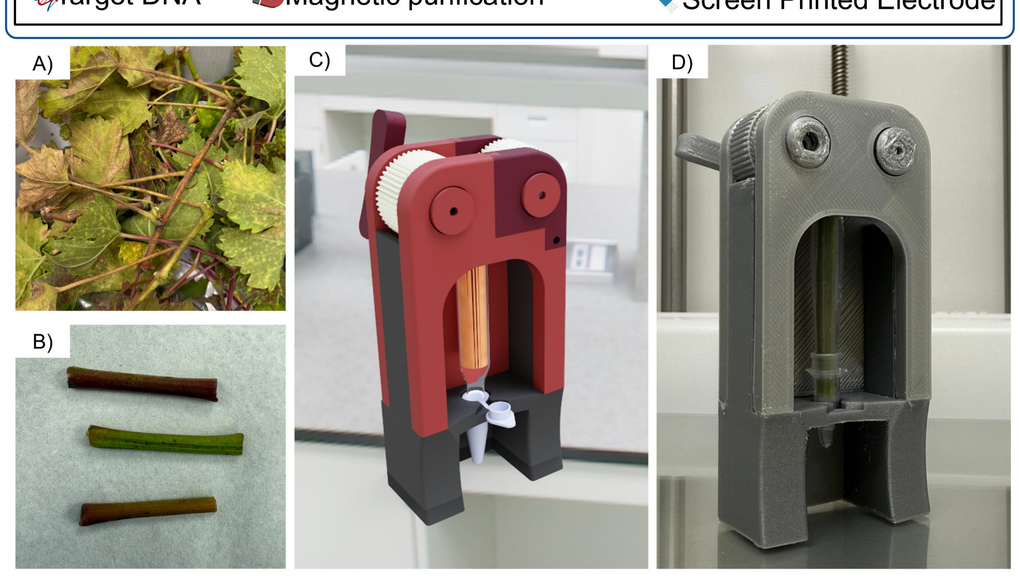
Magnetic Isolation Video

- **Electrode Adsorption**: Released DNA is adsorbed onto a screen-printed gold electrode under a 500 mV DC field for 30 s.
- **EC Detection**: Detection is performed via differential pulse voltammetry (DPV) using a ferricyanide redox system. The DPV signal correlates directly with the target DNA concentration.

VALIDATION STUDIES**:** The method was validated by PCR using grapevine xylem sap, extracted with a custom 3D-printed sap extractor and spiked with synthetic *X. fastidiosa* DNA.





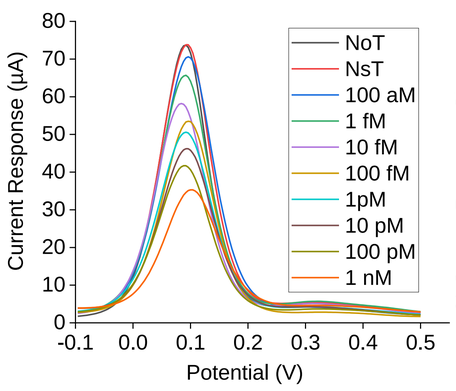


A) Collected grapevine for extracting sap. B) Clean stems prepared for extracting sap. C) Computational design for sap extracting device. D) 3D printed device.

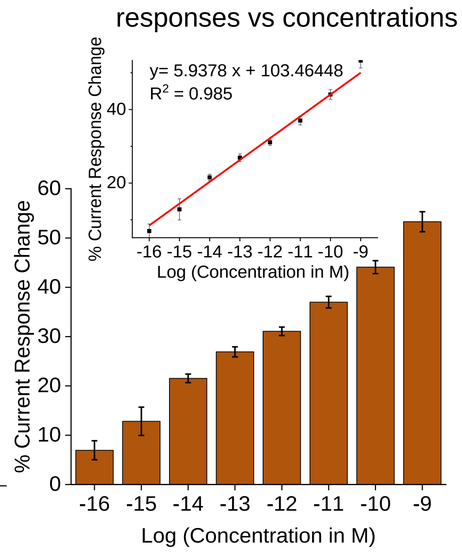
Results

Detection of *X. fastidiosa*-specific DNA

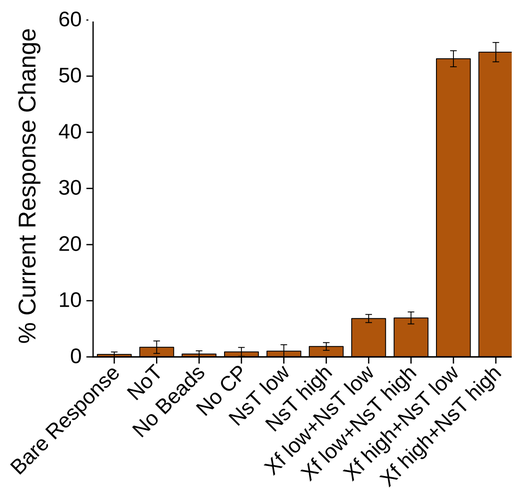
DPV responses with designated concentrations



Calibration plot: change of the current responses vs concentrations

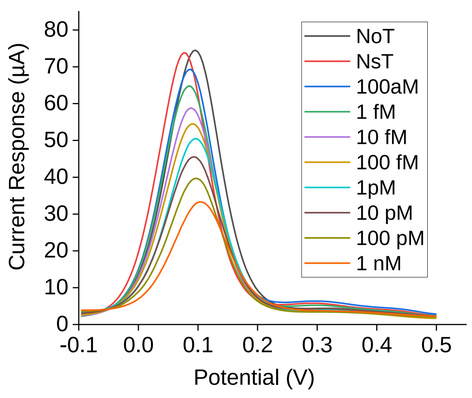


Series of Control Experiments



X. fastidiosa-specific DNA Spiked in Xylem Sap

DPV responses with designated concentrations



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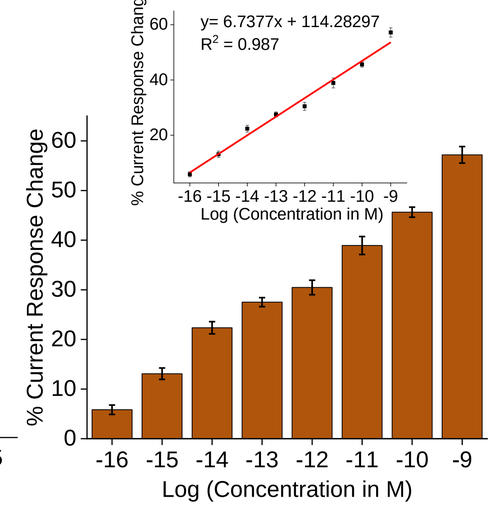

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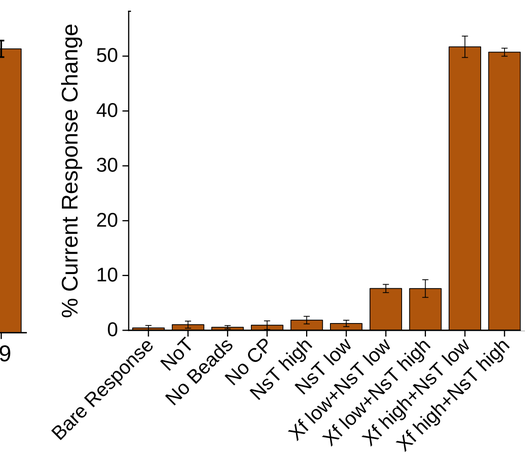
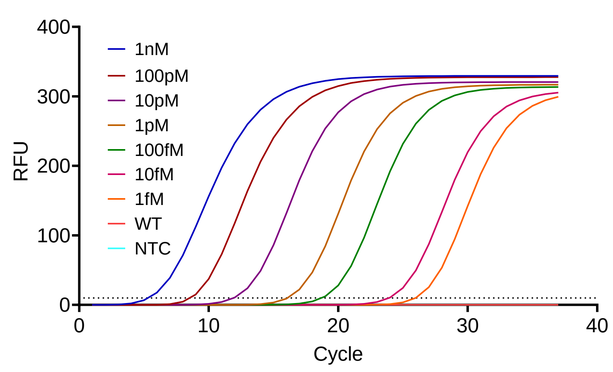
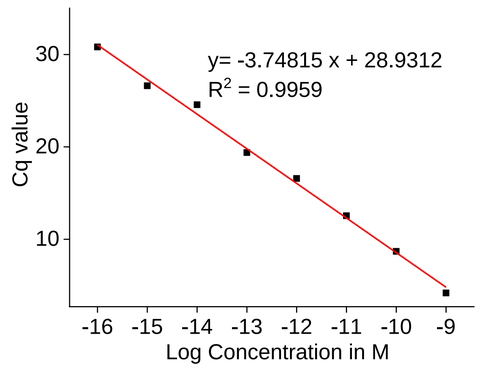


PCR Validation





Conclusion

This is the first amplification-free, gold-affinity-based electrochemical platform for *X. fastidiosa* detection, offering a simple, rapid, low-cost, and portable solution to enhance early pathogen surveillance and strengthen global biosecurity

Next Work

The method will be validated using *X. fastidiosa*-infected plant samples and further assessed under real field conditions

Reference

1. Zarco-Tejada et al., *Nature Plants*, 2018, 4, 432–439.
2. Chakraborty et al., *Advanced Sensor Research*, 2025, 4, 2400103.
3. Koo et al., *Analytical Chemistry*, 2016, **88**, 6781–6788.

Acknowledgement

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Contact