Smart Diagnostics for Plant Health: From Electronic Noses to On-Site Molecular Detection

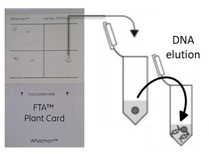
Inácio M.L., Rusinque L., Andrade E., Jorge M. S. Faria, & Camacho M.J.

***INIAV - National Institute for Agriculture and Veterinary Research**, Quinta do Marquês, Oeiras, Portugal*

***lurdes.inacio@iniav.pt**

Advances in pest diagnostics are transforming plant health surveillance by combining rapid field-based detection with laboratory-level precision. Isothermal amplification techniques, such as **LAMP** and **RPA**, enable on-site molecular detection of plant-parasitic nematodes and other pathogens, operating at constant temperature with minimal equipment and delivering results within minutes. In parallel, the European PurPest project is advancing **VOC-based** detection through electronic-nose systems, providing non-invasive, real-time monitoring and rapid field prescreening of pest activity. Together, these approaches form a new generation of integrated diagnostic platforms that enhance early warning, support precision pest management, and contribute to the goals of sustainable, low-impact agriculture.

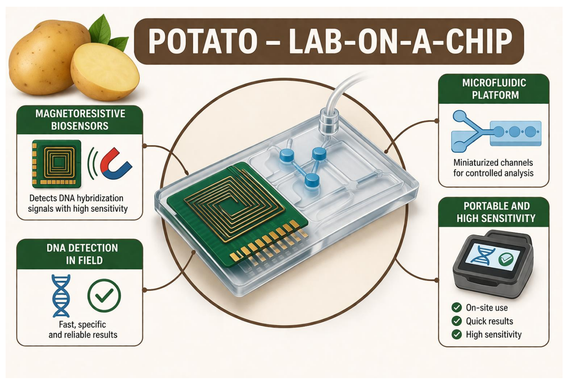
Lab-on-chip for the detection of pale potato cyst nematode *Globodera pallida*



GLOBOWARNING

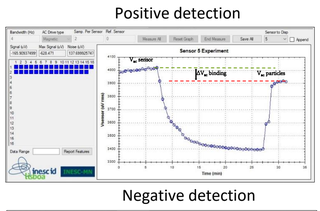
DNA extraction with FTA cards

- A microfluidic-based portable magnetoresistive (MR) device was adapted for *Globodera pallida* DNA detection.
- FTA cards were used for DNA extraction, due to its efficacy to field conditions.
- The detection of target biotinylated LAMP product involves it´s hybridization with the specific probe that has been previously immobilized on the chip sensing area.

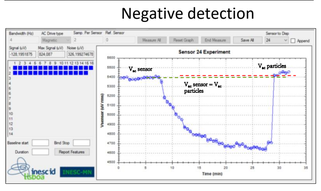


- The portable analytical device comprises an electronic reader and MR biochips, which includes six discrete sensing areas, framed by gold squares, with each area containing five MR-based sensors (Spin valves - SV), resulting in a total of 30 active sensors per biochip.
- Sensor's functionalization involves the immobilization of the specific probe and LAMP product hybridization.

Positive detection



Negative detection



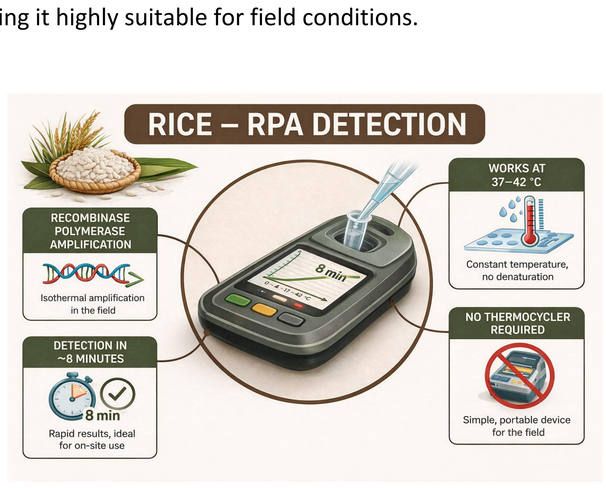
Detection relies on the binding of streptavidin-coated magnetic nanoparticles to biotinylated LAMP products, generating a change in sensor electrical resistance that is converted into an easy-to-read signal. The platform specifically distinguishes *Globodera pallida*, including FTA-extracted and mixed samples, from non-target cyst nematodes such as *G. rostochiensis*, *G. tabacum* and *Heterodera* spp.

Recombinase polymerase amplification (RPA) for the detection of *Meloidogyne graminicola*

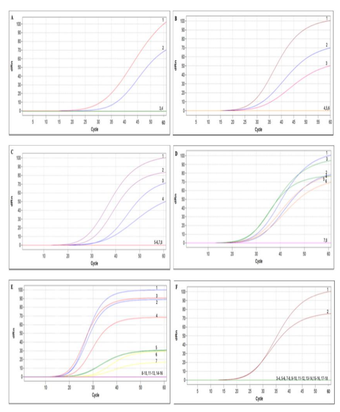
Euphresco | MeloRisk

RiceAlert

A recombinase polymerase amplification (RPA) assay was developed for rapid on-site detection of plant-parasitic nematodes. This isothermal technique operates at a constant temperature (37–42 °C), eliminating the need for thermal cycling and allowing the use of simple, portable equipment. The method can be applied directly to crude samples, avoiding time-consuming DNA extraction steps and making it highly suitable for field conditions.



The assay enables fast and reliable amplification of target DNA, with detection achieved in approximately 8 minutes. High sensitivity allows the detection of very low amounts of target material, while specificity is ensured through the use of species-specific primers, enabling accurate identification of targets such as *Meloidogyne graminicola*.



Real-time RPA amplification curves illustrating assay performance in what concerns
- **Rapidity and sensitivity** (~8 min detection of low target concentrations),
- **Specificity** (discrimination of target and non-target species),
- **Robustness** across different sample types and mixtures (crude extracts, dilutions, and mixed populations).

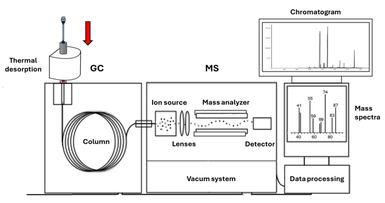
Portable in-field GC-system for the detection of pinewood nematode *Bursaphelenchus xylophilus*

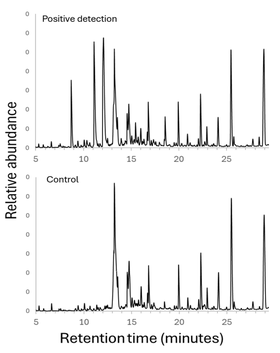
PurPest

www.purpest.eu

A portable headspace sampling system developed within the PurPest project enables VOC collection in the laboratory, greenhouse, and field under controlled conditions. VOC profiles are analysed by TD-GC-MS, and compound identification is supported by AMDIS and the NIST library. This approach supports the discovery of pest- and disease-specific volatile biomarkers for rapid, non-invasive plant health monitoring. Collected VOC samples are analysed by TD-GC-MS to identify compounds associated with pine wood nematode and pine wilt disease.

VOCs trapped on packed tubes are thermally desorbed and then analysed by GC-MS according to mass-to-charge ratio and relative abundance.





Compound identification is performed using AMDIS software and the NIST library, and positive samples are compared with controls to identify VOC biomarkers associated with pine wilt disease. This VOC database will support the development and optimization of sensor systems for pest-specific detection, enabling rapid, non-invasive detection in both laboratory and field conditions.

Conclusion

The integration of **lab-on-chip biosensors**, isothermal amplification (**RPA**), and **VOC-based e-nose** systems represents a new generation of plant health diagnostics, combining speed, sensitivity, and field deployability. These complementary approaches enable a tiered strategy, where rapid, non-invasive screening (VOCs) is coupled with highly specific molecular confirmation (RPA and lab-on-chip), significantly improving early detection and decision-making in pest management.

Beyond the specific case studies presented (**potato cyst nematodes**, **rice root-knot nematode**, and **pinewood nematode**), these technologies are highly adaptable and can be extended to a wide range of plant pathogens, including bacteria, fungi, viruses, and other nematodes. Their portability, minimal sample preparation, and rapid turnaround time make them particularly suitable for on-site diagnostics, surveillance programs, and biosecurity applications.

Overall, these innovative tools support a shift toward precision plant protection, reducing reliance on blanket treatments, enabling targeted interventions, and contributing to more sustainable and resilient agricultural systems aligned with global and European policy goals.

This work was supported by the EU under the PurPest project through grant agreement 101060634, and by FCT (*Fundação para a Ciência e a Tecnologia*) - the Portuguese Foundation for Science and Technology under PhD fellowships BD 138724/2018 and BD 2020.0554.1

REPÚBLICA PORTUGUESA | AGRICULTURA E MAR

NemaINIAV Plant Nematology Lab