Instituto Nacional de Investigação Agrária e Veterinária, I.P.

35th Symposium of the European Society of Nematologists
Cordoba, Spain
15-19 April, 2024

Smart technology for nematodes detection

Camacho M.J.[1,2*], Jorge M. S. Faria[1,2], Ana Fundurulic[1] & Inácio M.L.[1,2]

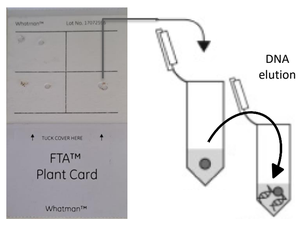
[1]***INIAV - National Institute for Agriculture and Veterinary Research***, *Quinta do Marquês, Oeiras, Portugal,* [2]***GREEN-IT*** *Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal*

***mjoao.camacho@iniav.pt**

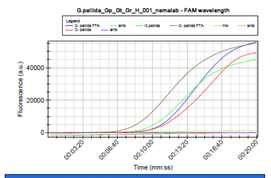
In the agricultural industry, different types of technologies are used as part of crop management to promote sustainability and increase crop productivity. Advances in sensing technologies are opening new opportunities for precision agriculture. Here, we present a **Lab-on-chip**, based on a magnetoresistive biosensor, developed for detection of the **pale potato cyst nematode - *Globodera pallida***; and a **portable in-field GC-system**, based on a gas chromatography (GC) coupled to mass spectrometry, developed for detection of Volatile Organic Compounds (VOCs) emitted by the **pinewood nematode – *Bursaphelenchus xylophilus***. This work provides insights for new strategies to develop smart devices for nematode in-field diagnostics.

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

DNA extraction and amplification



DNA extraction with FTA cards



Time to start (hh:mm:ss) - FAM		
G. pallida	(Sample)	0:12:33
G.pallida	(Sample)	0:10:47
G. pallida FTA	(Sample)	0:11:41
G. pallida FTA	(Sample)	0:09:14
amb	(NegCtrl)	N/A
amb	(NegCtrl)	N/A
mix	(NegCtrl)	N/A
amb	(NegCtrl)	N/A

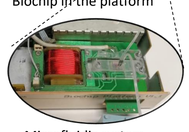
LAMP isothermal amplification

- 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.
- LAMP was the method developed for DNA amplification. The ITS rDNA region was used to design LAMP primers and the specific oligoprobe for *G. pallida* detection.

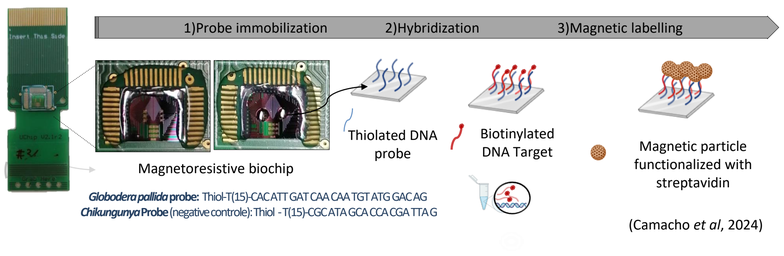
***LAMP primers:**
b-BIP: Biotin-TGG GGT GTA ACC GAT GTT GGT GAG CGA CCC GAC GAC AA;
b-B3: Biotin-CCC TGT GGG CGT GCC A
FIP: ACA CTC ATG TGC CCA CAG GGT GGG CTG GCA CAT TGA T;
F3: ACACATGCCCGCTATGTT
(Camacho *et al.*, 2024)

- 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.

Biochip in the platform



Microfluidic system

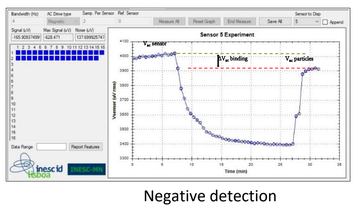


(Camacho *et al*, 2024)

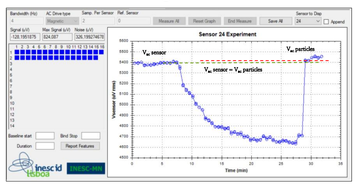
- The portable analytical device comprises an electronic reader and MR biochips, which includes six discrete sensing areas, framed by gold squares, with each area containing five MR-based sensors (Spin valves - SV), resulting in a total of 30 active sensors per biochip.
- Sensor's functionalization involves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

Data analysis and *Globodera pallida* detection

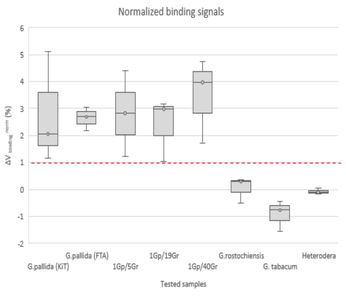
Positive detection



Negative detection



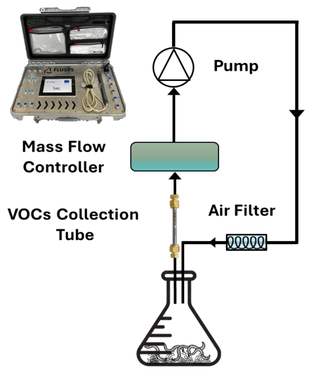
- Detection is achieved due to the interaction between the streptavidin-modified magnetic nanoparticles (MNPs) and the biotinylated target molecules, which induce a variation in the sensor's electrical resistance, being converted into an easy-to-read electrical signal.
- Normalized binding signals obtained from LAMP products of *G. pallida*, *G. pallida* extracted with FTA cards, and mixed samples of *G. pallida*/*G. rostochiensis* distinguish themselves from *G. rostochiensis*, *G. tabacum* and *Heterodera* sp. against the specific probe for *G. pallida* detection.



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

Collection of Volatile Organic Compounds (VOCs)

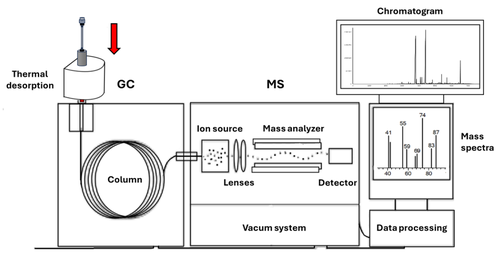
PurPest



Closed loop VOCs collection setting

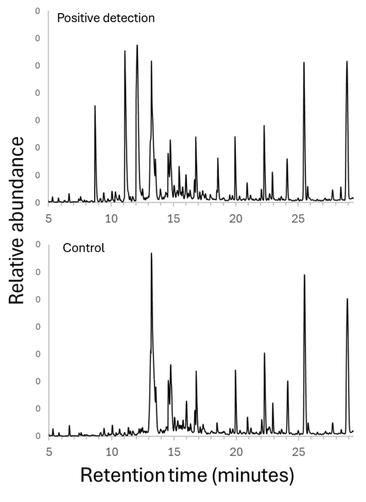
- Innovative headspace sampling device Fragrance Collector FSP-2246F was specifically manufactured for the needs of the PurPest project by FLUSYS GmbH – Fluid Control Systems.
- This technology was designed to draw in a predetermined amount of air at a controlled and adjustable flow rate, and samples are taken from the air to analyze fragrances.
- The device is robust, can be transported, and used in the laboratory, greenhouse and in the field.
- There are two different sampling modes: an open loop (OLS) and a closed loop sampling (CLS) mode that also allows sampling of volatiles in the field.
- Date and time, ambient temperature, and humidity are collected during every sampling, assuring the control of the testing environment and enabling reproducibility and assessment of variability due to abiotic factors.

Analysis of specific VOCs



- To define specific volatile organic compounds (VOCs) released by pine wood nematode (PWN) and pine trees affected by Pine wilt disease (PWD), volatile samples collected are analyed with Thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS).
- VOCs absorbed onto packed tubes are put into a desorption oven and heated to promote thermal desorption and analysis through GC-MS according to mass-to-charge ratio and relative abundance.

Identification of specific VOCs



- Identification of compounds present is done with AMDIS software and using NIST library.
- Samples with the positive detection are compared with the control to identify specific VOCs that can be used as a biomarker for PWD.
- A summarized VOC database will be exploited to optimize existing and develop new sensor concepts to detect pest-specific VOCs, starting from proof of concept to demonstration in field trials.
- With updated and tailor-made technological equipment, a sensor system prototype (SSP) will be developed and optimized to detect the VOCs and identify target pests in laboratory setting and in the field.
- This will allow a non-invasive and rapid detection of PWD.

CI-006

The combination of these three technologies allowed the detection of *G. pallida* with a detection limit of one juvenile, even when mixed with different ratios of other closely related species (diagnostic sensitivity) . The results reveal the suitability of the magnetic biosensor to detect the LAMP target product and the specificity of the probe, which consistently distinguishes *G. pallida* ($DV/V_{sensor} > 1\%$) from other cyst nematodes ($DV/V_{sensor} < 1\%$) regardless the extraction method used (analytical specificity).

The VOCs sampled from pure PWN cultures revealed the emission of three specific aliphatic compounds that characterize the mycophagous phase of the nematode and can be used for building portable nematode sensors. Future endeavors will analise emission of PWN infected material, greenhouse grown seedlings and field trees of *Pinus pinaster*, *P. nigra* and *P. sylvestris*.

REPÚBLICA PORTUGUESA | AGRICULTURA

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 COVID/BD/152764/20the23