

A remote field-based LAMP assay early warning system for fungal plant pathogens

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What do we do?

Assay Development:

- The design and development of antibody, enzyme and DNA tests.
- Optical tests and electrochemical biosensors.

Protein Stabilisation:

- Formulate assays that are stable at elevated temperatures for extended periods.
- Long term stability trials.
- Production of stabiliser formulations.

Manufacture of Plant Pathogen Tests:

- Optical LAMP DNA assays
- Lateral flow tests

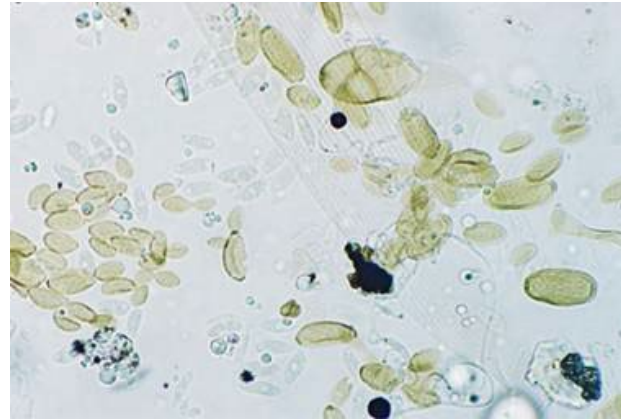
Air Samplers



- Epidemiology & forecasting
- Monitoring
- Real-time Detection

West & Kimber (2015)
Annals of Applied Biology
166: 4–17

Post-capture detection and quantification

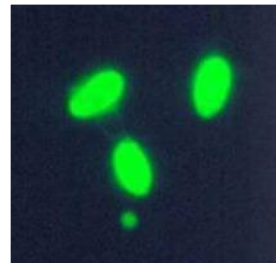


Microscopy

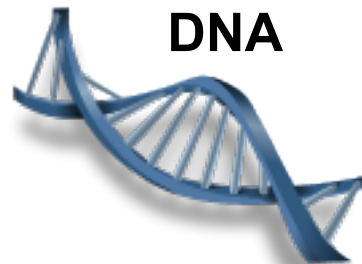
Originally lab-based and difficult to identify to species level. AI is being trialled. It is uncertain whether sub-species will be identifiable.

Immunological and Aptamer techniques

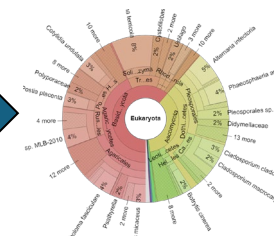
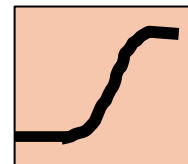
Rapid, on-site test but often difficult to design specific assays



Lab-based qPCR quantification of selected targets - also on-site rapid detection of target pathogens (LAMP and RPA methods)

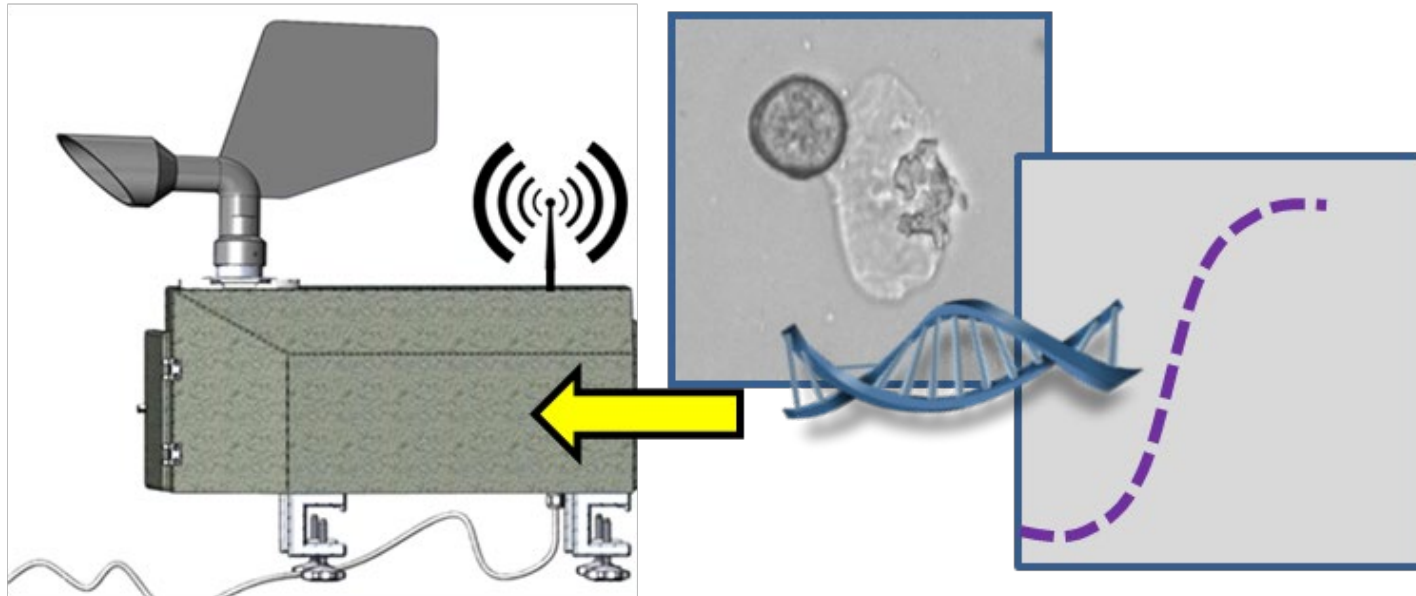


DNA



Metagenomic sequencing

The Idea



- Air sample capture
- Sampler to carry out DNA release, isothermal amplification & quantification, wireless reporting.
- Result integrated with infection models & risk prediction sent to end-users.



Spore Disruption

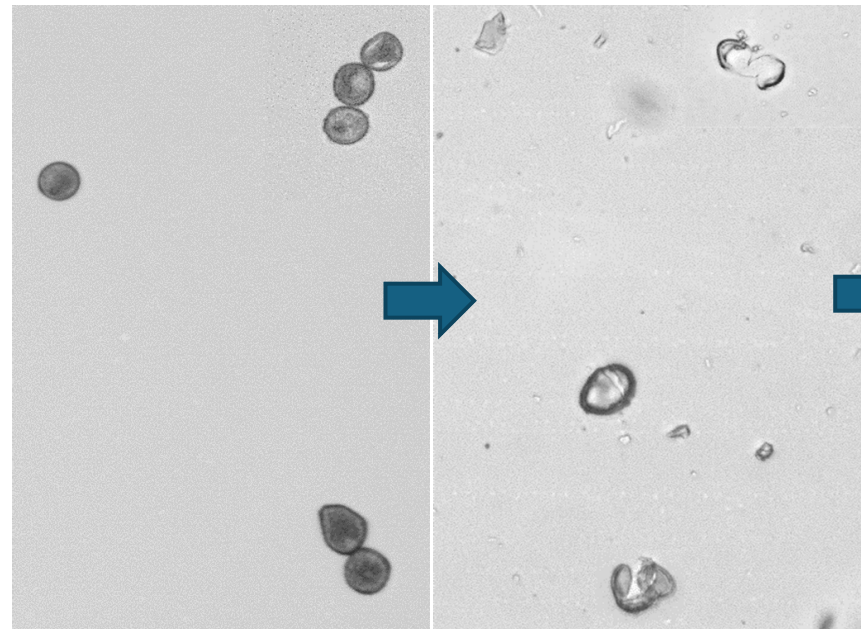
Spore Disruptor



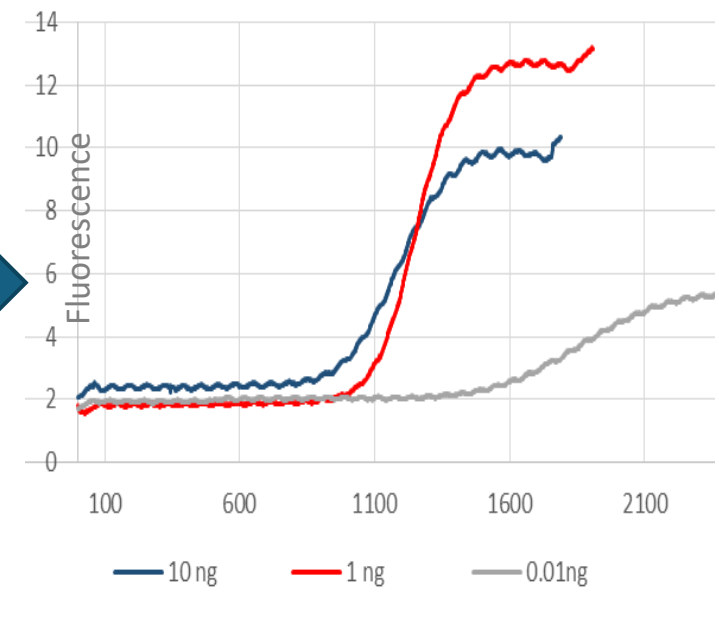
DNA release from collected spores

Original spore suspension

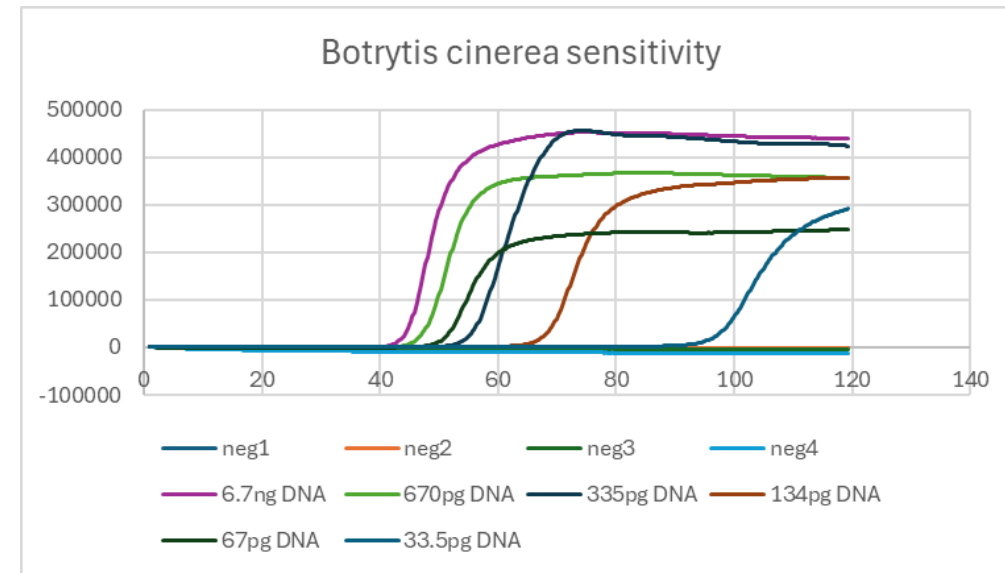
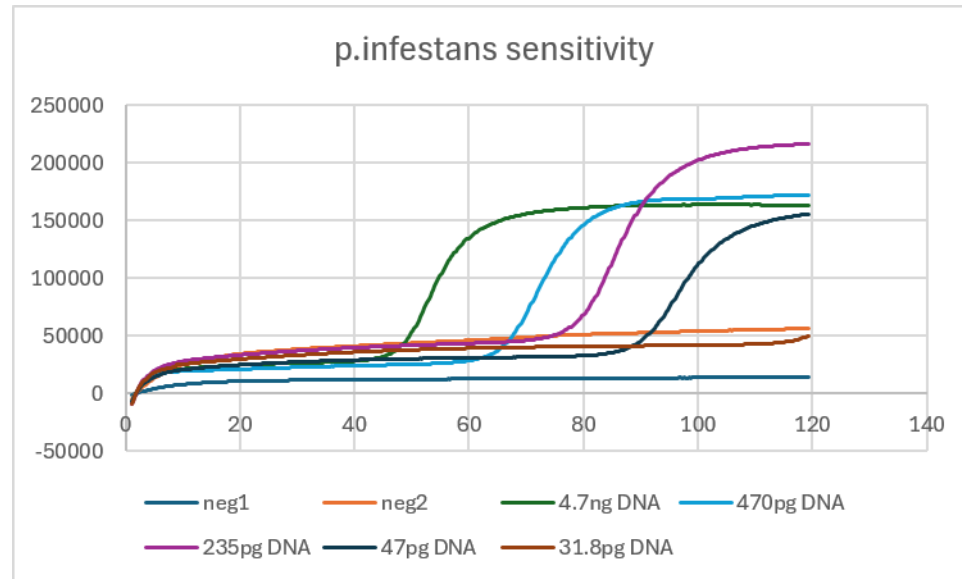
Disrupted spore suspension



LAMP assay results



Limit of Detection

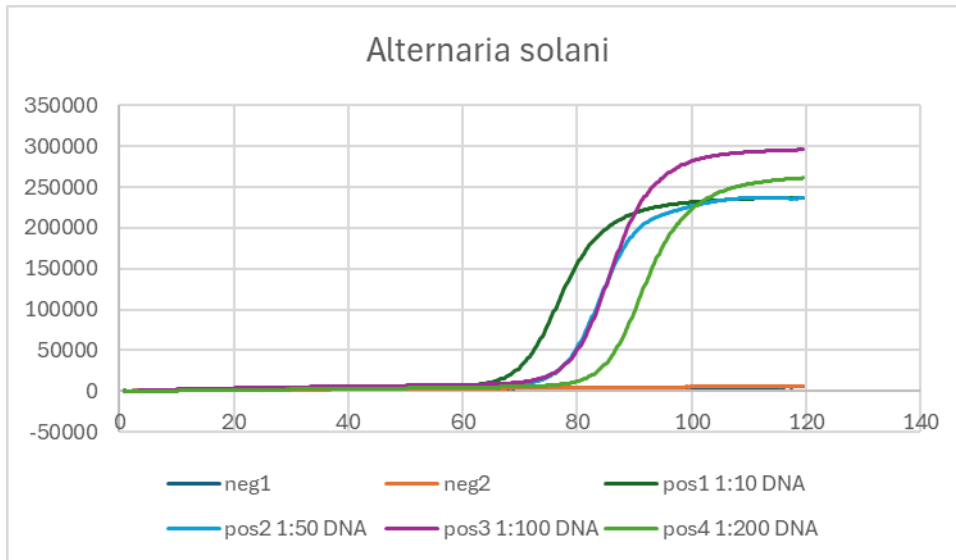


The limit of detection for *P. infestans* freeze-dried reagents is shown to be at least 31.8pg DNA (equivalent to the detection of 8-15 sporangia). Measured 4.7pg of DNA. This LOD is comparable to published data by Ristano et al (2020).

The limit of detection for *Botrytis cinerea* freeze-dried reagents is shown to be at least 33.5pg (equivalent to the detection of 180- 200 spores).

This matches the sensitivity of the LAMP assays in solution.

New Target



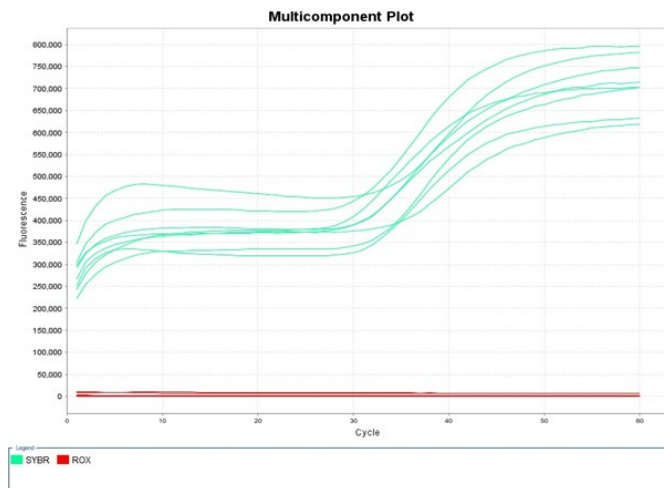
Current limit of detection 665pg

Expected to be in the same region of LOD as P.infestans

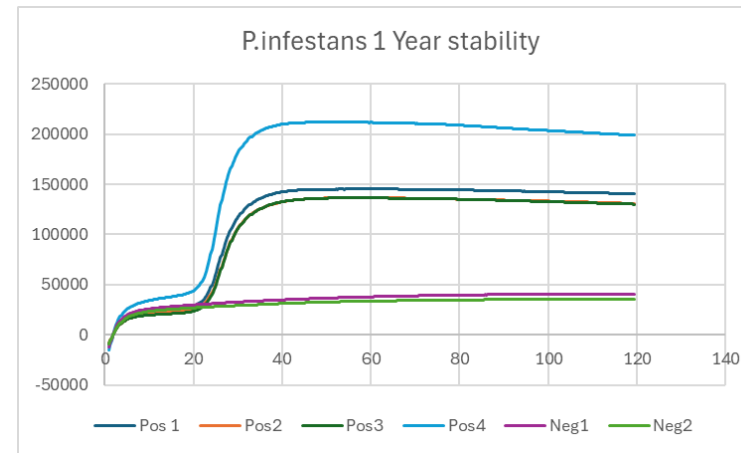
Long Term Stability

P. infestans (late potato blight)

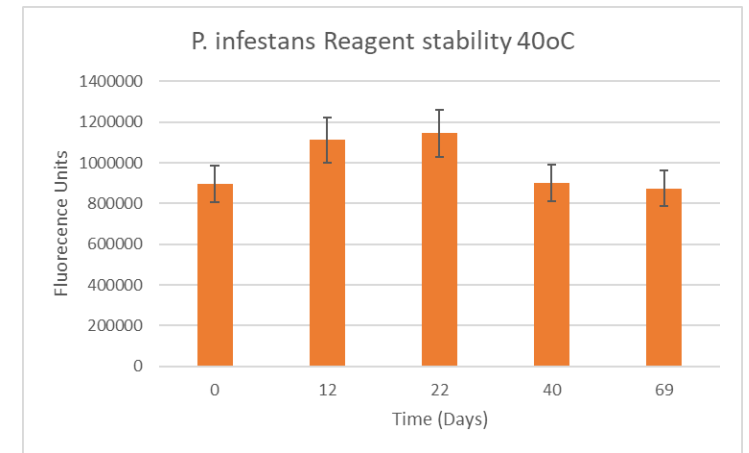
Day 0



Day 405

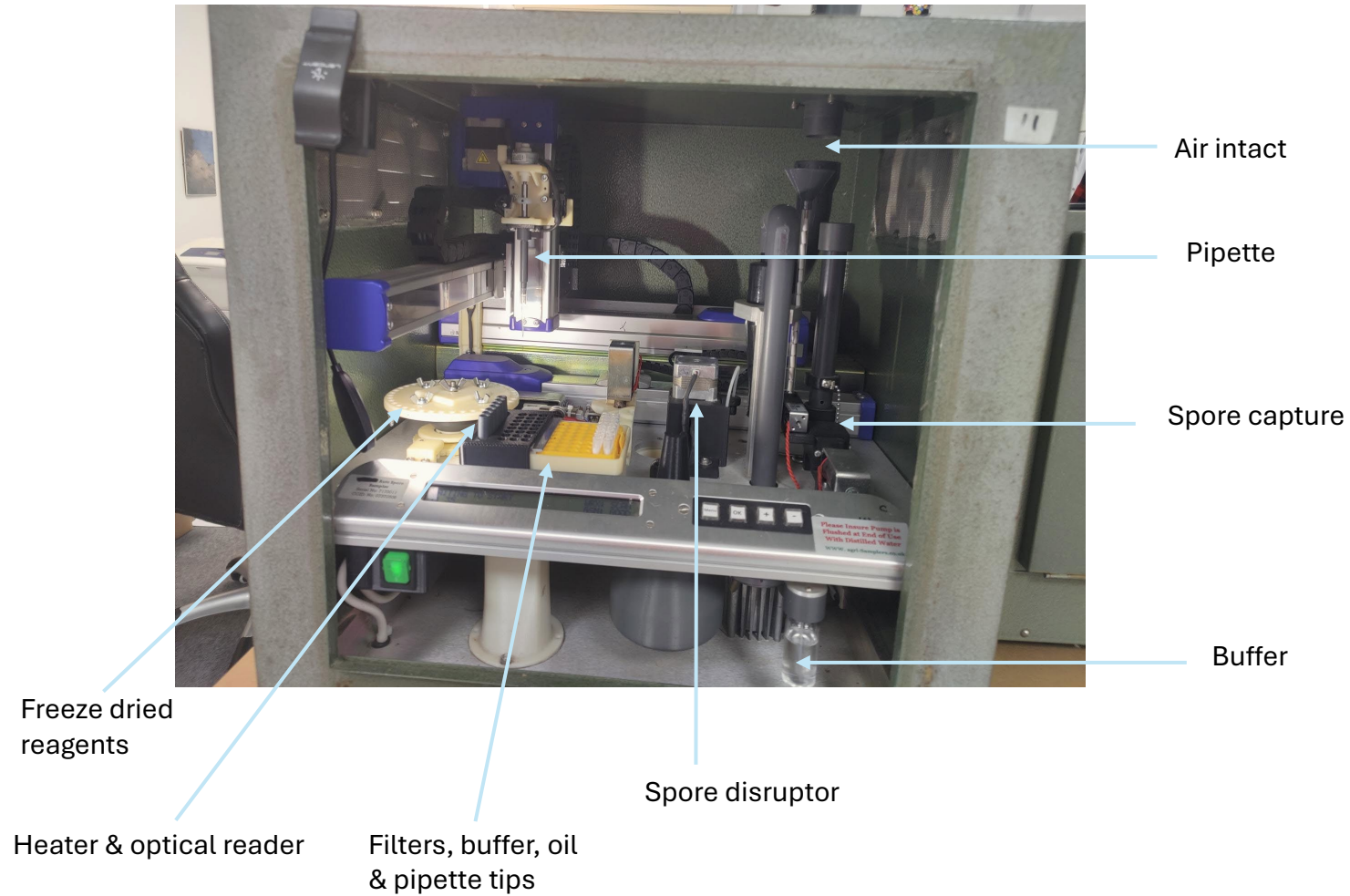


Elevated temperature stability



- Stability at room temperature 1 year.
- Stability at 40oC for 2 months.

DNA Auto Spore Analyser



DNA Auto Spore Analyser



DNA Auto Spore Analyser

- The DNA auto spore sampler is the world's first automated field analyser specifically designed as an early warning system for the detection of crop disease pathogens.
- One analyser can monitor around 100 Ha and can be configured to detect multiple crop disease pathogens.
- The auto spore trap samples 250 litres of air per minute, collecting airborne particles, such as fungal spores and bacteria into a sampling tube.
- The analyser then automatically analyses the collected sample for a number of preprogrammed plant pathogens.
- Results are transmitted via 4G mobile data to the cloud for analysis.
- Following 8 days of analysis the autospore trap is refilled with reagents.
- This technology was developed as a result of Innovate UK funding.
- Partners Polygenyn Ltd. Agri Samplers Ltd and Rothamsted Research.

Device: ROTHAMSTED ROOF

Router: HERA604_07370573

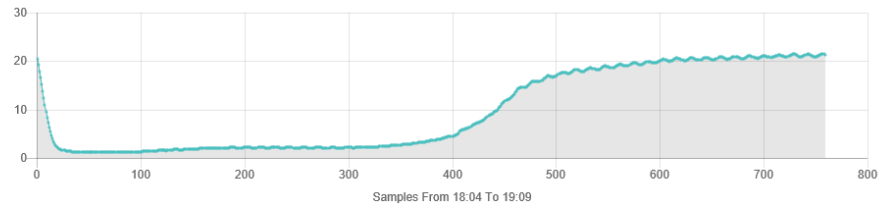
Date: 29/08/2017

Refresh

Messages

06:00 Air Sampling Started.
18:00 Air Sampling Completed.

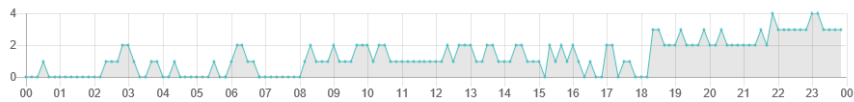
Fluorescence



Light Level



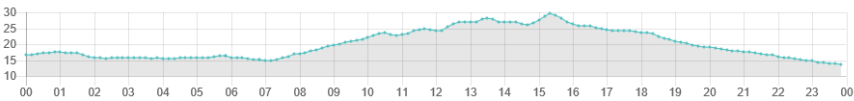
Wind Speed



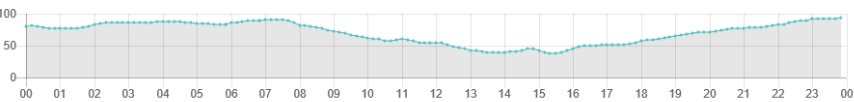
Wind Dir'n



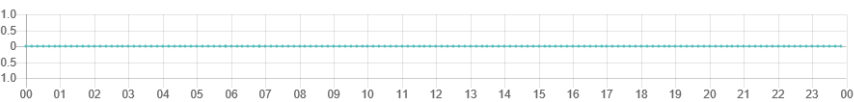
Temp



RH



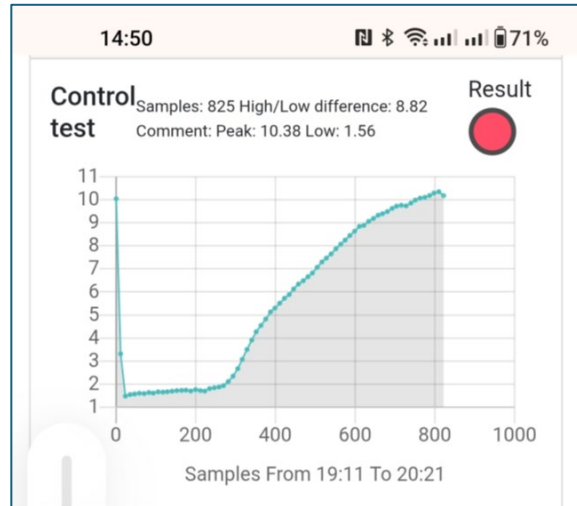
Rainfall



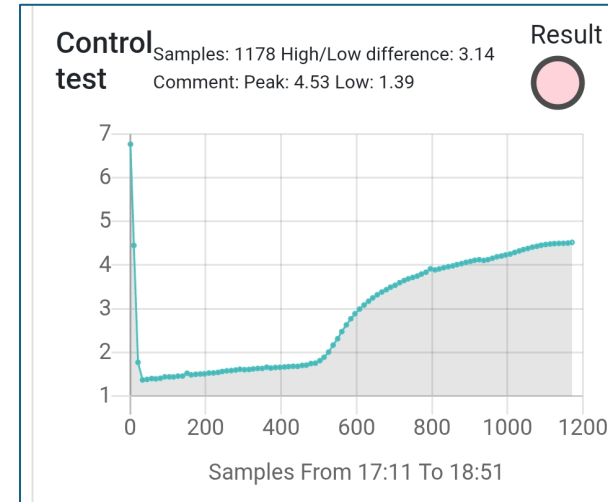
Screen-shot of the Agri Samplers Ltd web-portal

- User selects data from an individual site.
- This example shows a positive fluorescence result
- Up to 4 different pathogen assays can be made per sample
- Compatible with RPA or LAMP reagents
- Will email or text the user when reagents run low

Field Trials 2025



Botrytis detection in field summer 2025



Onion Downy Mildew detection in field summer 2025

Field Trials 2026

Current Field Trials running in 2026

- 3 instruments deployed in the UK by an agrichemical company to measure late potato blight, wheat yellow rust and Septoria
- 1 instrument deployed at Wageningen Plant Research in the Netherlands to measure early and late potato blight.
- Onion downy mildew and Cercospora leaf spot.
- 1 instrument deployed at a company in the UK to measure Onion downy mildew.

Future Field Trials:

- Further field trials with agrichemical company (2027-2028).
- Further trials with Wageningen Plant Research (2027-2028).
- Field trials for wheat yellow rust TBD.
- Field trials for Ergot detection in Rye TBD.
- Field trails in Vineyards TBD.

Plant Pathogen Tests Available

Plant pathogen assays available:

- **Late potato blight (*Phytophthora infestans*).**
- **Early potato blight (*Alternaria solani*)**
- **Cercospora leaf spot (*Cercospora beticola*)** (sugar beet).

- **Sclerotinia** which affect oilseed rape (canola), sunflowers and a range of vegetables, carrots. Lettuce etc

- **Brown Rust (*Puccinia triticina*)** wheat.
- **Yellow Rust (*Puccinia striiformis*)** wheat.
- **Septoria tritici**, wheat, rye, cucumbers, melons and tomatoes.

- **Botrytis cinerea**, grapes and soft fruits, strawberries, raspberries, blueberries.
- **Grape powdery mildew**, vineyards.

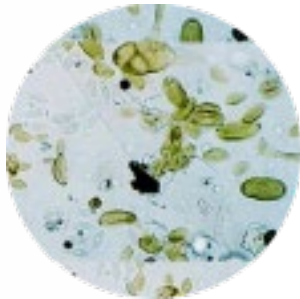
- We are currently developing a range of LAMP assays covering fungal resistant strains for wheat yellow rust YR15, septoria succinate-dehydrogenase inhibitors (SDHIs) and potato blight EU36 and EU43 strains.

Future



Rotorrod spore sampler

Captured spores



Spore disruptor



Fully integrated plant pathogen detection chip



Chip reader

Thanks



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