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Arable Crop Disease Alert System

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1. Abstract

This multi-disciplinary project aimed to produce an automated air sampling device, able to use a DNA-based method to detect airborne spores of key pathogens and text results for display on a website so that growers can react to smart spray applications. The system was designed for multiple devices to operate as a network to enhance information quality.

The high-volume devices, developed by the Burkard Manufacturing Co. Ltd, (Rickmansworth), sample 300 L/minute of air, and were found to detect spores affecting broad-acre crops in the proximity of a farm-yard, allowing the device to be mains-powered and located in a secure position. The device has excellent collection efficiency for spores as small as 4 µm (aerodynamic diameter) and is programmed by the user to sample for a set period each day. The collected sample is processed in liquid to burst all spore types to release DNA, a sub-sample is then transferred to a reaction tube where an isothermal DNA-based assay takes place. Two types of DNA-based assay can be used, LAMP or RPA assays, which differ in their reagents and the temperature of the reaction (65-72 or 37-40℃, respectively). The assay is measured by fluorescence and results are sent wirelessly by an internal 4G router to a server. Up to three different target pathogens can be tested from the same sample each day. Weather data, collected by an on-board met station, are also sent by text every 10 minutes. The data are automatically processed, collated and displayed on a web-portal. Simple rules applied to the data allow an automated calculation of the spore detection assay, indicating zero, low or high numbers of spores present and an estimation of occurrence of infection conditions can currently be made for yellow rust and Sclerotinia. Each device requires weekly attendance to replenish reagents and tubes and to perform simple maintenance.

The project has also developed new LAMP assays for the following pathogens: *Pyrenopeziza brassicae Sclerotinia sclerotiorum*, *Zymoseptoria tritici*, *Rhynchosporium* sp, *Oculimacula yallundae* & *O. acuformis* (joint assay), *Alternaria solani*, and for fungicide insensitivity in *Zymoseptoria tritici* (assays for reduced DMI sensitivity and separately an assay for multidrug resistance including reduced DMI and SDHI sensitivity). Other published assays were assessed for sensitivity and specificity to UK pathogens (*Phytophthora infestans*, *Puccinia striiformis* and *Fusarium graminearum*).

This novel device will lead to a new approach in precision agriculture by providing information in near real-time, on the presence of airborne spores and the weather conditions necessary for infection. The technology will in time, with use of appropriate reagents (DNA primers), be translated to improve disease control in other AHDB sectors and could be available for fungicide resistance monitoring in addition to disease forecasting.

2. Introduction

In the UK, arable crops essential for food security, suffer 15% yield loss to diseases, many of which are initiated by airborne spores (Hughes et al. 2011; West et al. 2008). Disease forecasts based only on weather conditions suitable for infection can lead to unnecessary fungicide use if disease inoculum is not present to cause infection. Farmers also have developed strategies to protect key leaves with fungicides but sporadic diseases (Fig. 1. e.g. Sclerotinia, and Fusarium) may infect at other growth stages. This leaves crops susceptible to disease or can lead to fungicides being applied unnecessarily. Two other sporadic diseases of wheat would also benefit from an inoculum-based risk system, these are yellow rust, caused by *Puccinia striiformis*, which has many new races threatening wheat crops and epidemics take-off after sunny weather in early spring; and the second disease is septoria leaf blotch, caused by *Zymoseptoria tritici*, for which even when there is a low risk of rain-splashed spores (conidia) reaching upper leaves from infections on over-wintered leaves low in the canopy, the fungus can produce a sporadic late phase of infection by airborne ascospores that can infect the flag leaf of wheat when an earlier fungicide spray has degraded. Current conventional air samplers are available and have helped scientists to understand the timing of these sporadic diseases to some extent. However, these air samplers cannot be used for real-time disease risk forecasting because of the delay taken to take the sample to a lab and process it. For a reliable airborne inoculum warning service to be effective, air samples need to be analysed and results disseminated within hours or a day at most. This project aimed to produce a new system to deliver a reliable disease forecast to alert farmers of imminent risk of these diseases in time for action using an innovative web-based risk alert system, taking data from a network of automated samplers, which could be operated at strategic sites, such as AHDB met sites, to provide an early warning to growers.

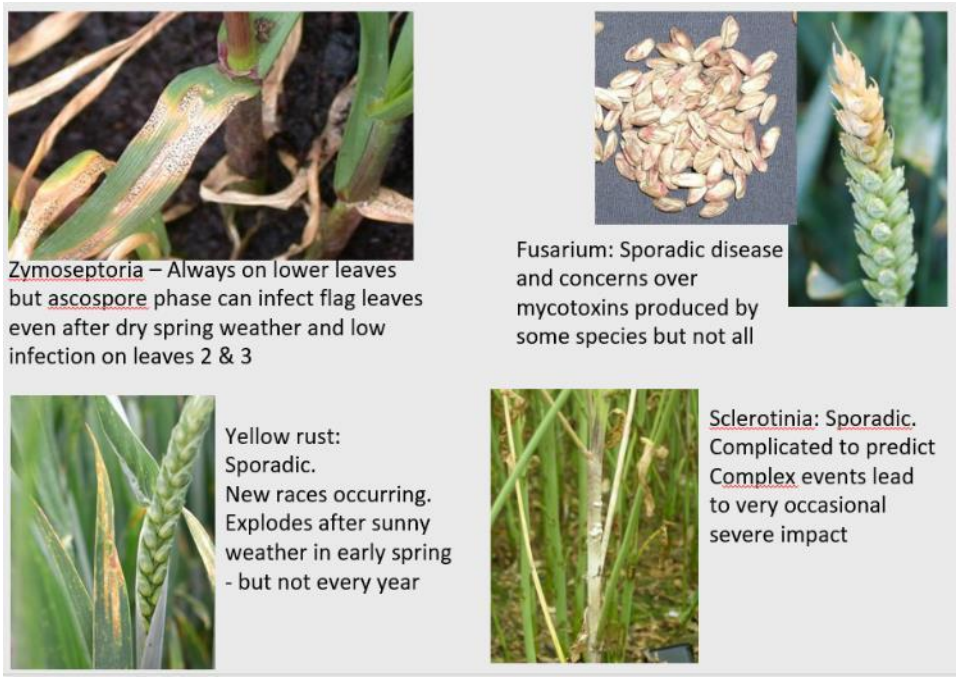

Figure 1. Target diseases in the UK that could be better controlled by direct detection of airborne inoculum.

3. Materials and methods

3.1. Designing and building the automated system

The system was loosely based on a previous prototype device developed under the SYield InnovateUK project in 2010-2013, which used a miniature virtual impactor as the air sampler, had no spore disruption method and the assay to detect *Sclerotinia* was based on incubating all spores in liquid culture media to allow detection of oxalic acid produced by growing *Sclerotinia* colonies, using a biosensor and potentiometer. Results were sent out by mobile phone text message. This was adapted to collect spores and other airborne particles into nylon pots approximately 10mm in diameter x 10mm in height, which were fed under an air sampler by a piston, driven by a small motor. The air sampler itself was completely replaced by a 3-D printed high-volume cyclone connected to a compact pump. The 3-D printing allowed for shapes to be created that could not previously be manufactured using traditional turning, cutting and boring methods in metal. After sampling, the collection pot was moved by a linear actuator, approximately 300 µL of liquid (usually water but a buffer is used if an RPA assay is being used) was added to the sample and the pot was moved again to a spore disrupting device, which mechanically disrupted the spores for three minutes. The pot was moved again to allow access to a pipette, mounted on a linear actuator perpendicular to the first axis and controlled by a servo-motor. The pipette was moved to collect a new filter pipette tip from a rack of tips and then moved to collect a set volume of liquid reagent (mastermix) from a rack of foil sealed vials. The liquid was added to a reaction tube, located in a carousel. The reaction tube contained dried reagents, comprising primers and dye, covered with a foil seal that could be pierced by the pipette. The pipette then moved to collect a set amount (18 µL) of the disrupted spore sample, which was transferred to the small reaction tube. The reaction tube was then heated using a small heater connected to a metal sleeve, which transferred the heat to the tube. The reaction tube was also illuminated from beneath by blue light at approximately 475 nm and emitted light at approximately 540 nm was measured by a fluorimeter. The fluorescence reading was recorded every 5 seconds and this measurement was sent as text using the on-board 4G router. All movements and processes were controlled by a control circuit board. The device was fed by mains power stepped down to 24 V. All components, boards or frames they were mounted on, brackets, attachments and wiring was visualised using 3D computer aided design software. The entire device was enclosed in a metal rain-proof cover on which was mounted the rotatable air intake of the air sampler, the antenna of the 4G router and the weather station. Outputs from the weather station were fed to the mother board of the device by cable and then relayed by the 4G router. Additional sensors were manufactured to augment the met station. This included a sensor to record solar intensity as a voltage from a small solar panel, which was adjusted by covering with an opaque cover to improve sensor range and was calibrated against a standard solar radiation sensor. The temperature sensor of the met station was found to be affected by direct sunlight when compared with a professional met station (Davis VanatagePro,

Davis instruments), so an additional vented aluminium cover was manufactured and fitted over the temperature sensor. This was found to eliminate the problem if heating by direct sunlight. Bearings of the anemometer were also upgraded to ensure a significantly greater lifespan.

3.2. Integration and testing the automated system

Each component of the system was designed and tested individually before assembly and again after assembly. The air sampler was tested in the wind tunnel at Rothamsted next to a standard seven-day (Hirst-type) spore trap at different wind speeds (2-6 m/s) with Lycopodium spores (diameter 35 µm) and Giant Puffball spores (diameter 4 µm) released down a shaft approximately 8 m upwind of the spore traps to ensure that the spores were evenly mixed in the airflow passing the spore traps. The final design of the spore disruptor was tested in the main device with known spore suspensions (see 3.4.1). The movements of the device were factory tested, initially using a prototype and all movements of the final design were tested in the factory and at field sites at Rothamsted.

3.3. Communications and web-portal development

The signals from the 4G router were sent by an AnyNet Secure SIM, which means it is not tied to any one network and will roam to the best available network in the area where the device is located. The communications system allows the device's location to be reported and allows reprogramming updates to be made as long as the device is powered up. Communications were tested in the factory and again between field-site locations. Data from the met stations and the spore assay result were arranged to be sent along with key text that identifies the device, provides a timestamp and location and can also include error messages and messages indicating the status of the device such as an "operator on site" message (sent whenever the command menu in the device is first operated), and also messages saying "sampling" or "waiting to sample", indicating the status of the air sampler itself. The times of day that sampling started and stopped is also recorded for each device. A system was coded to allow data in a predefined order or with predefined codes identifying what the data are to be collated for presentation as daily graphs on the web portal.

3.4. Development and testing of DNA release and LAMP assays

3.4.1. Lab-based assessment of DNA-release methods for automation

Different methods for in-tube release of DNA from spores of the target pathogens were assessed using standard suspensions of spores. The methods evaluated, that would be possible in a field-portable device, included: heating, sonication, addition of detergents, caustic reagents and acids, and mechanical disruption. The methods were assessed on different spore types taken from living

plants or cultures (*Puccinia striiformis* uredospores (previously freeze dried and rehydrated), *Sclerotinia sclerotiorum* ascospores (previously frozen), *Zymoseptoria tritici* (conidia from leaf washings were used experimentally to test the DNA-specific assay but the assay is intended ultimately to be used on air samples to detect ascospores) and *Fusarium graminearum* macroconidia (from culture), *Alternaria brassicae* conidia (from culture), and *Erysiphe betae* conidia (removed from plant leaves using the barrel of a miniature cyclone attached to a vacuum pump)). Spore suspensions were compared by microscopy before and after different periods of disruption to assess the percentage of spores that had been broken open to release their contents compared to the starting material. Suspensions of disrupted spores were tested directly in a lab-based (wet-reagent) LAMP assay for each pathogen. In addition, a known number of spores were spiked into a sample of air particles that were collected using a high-volume spore trap outdoors in March 2016. The air sample was known to be negative for the target organism but was tested when spiked with different target spores in order to assess whether any reduction in sensitivity occurred due to the presence of other DNA and products likely to be in the air.

3.4.2. Lab-based (wet reagent) assays

General methods for LAMP assay development are presented in the paper by Kaczmarek et al (2018) and in the new LAMP assay devised for detection of *Pyrenopeziza brassicae* (King *et al* 2017). The latter assay was developed in an associated AHDB-funded summer student bursary. LAMP assays were also developed for *Sclerotinia sclerotiorum*, *Zymoseptoria tritici*, *Rhynchosporium* sp, *Oculimacula yallundae* & *O. acuformis* (joint assay), *Alternaria solani*, and for fungicide insensitivity in *Zymoseptoria tritici* (assays for reduced DMI sensitivity and separately an assay for multidrug resistance including reduced DMI and SDHI sensitivity). Each assay was tested on a library of DNA purified from a wide range of plant pathogens and common airborne fungi. Assays were also tested on several isolates (or collected spores from an infected leaf for obligate biotrophs) of each specific pathogen.

In addition, existing assays, published by other researchers, were researched from the literature and the reagents for these assays were obtained and the method assessed for sensitivity and specificity to UK pathogens. These were: *Phytophthora infestans* (Hansen et al. (2016), *Puccinia striiformis* (Huang et al. 2011) and *Fusarium graminearum* (Niessen & Vogel, 2010).

3.4.3. Dried formats

The project originally planned for developed LAMP assays (that worked in lab conditions, using freshly prepared reagents that were taken from fridges or freezers, thawed if necessary, mixed, the sample added and the reaction performed in a real-time PCR machine) to be adapted into a dry format, i.e. freeze-dried reagents, in one tube so that a wet sample of disrupted spores could be added and the reaction measured. It was found that simply mixing the reagents, refreezing them and then freeze-drying them, was not a successful method. The dry format was preferred to

ensure long shelf-life, postage to the end-user site, ease of storage and ease of handling. Different methods were tested to mix the reagents in different orders, to freeze them either rapidly in liquid nitrogen (-80˚C) or more slowly in a conventional freezer (-20˚C) ahead of freeze-drying, addition of different stabilising chemicals such as sugars, and testing of different reagents or primers supplied by different companies. All methods attempted with arable crop pathogen assays did not work reliably when validated against freshly-made wet reagents in a lab, although there was some success with a *Phytophthora infestans* assay developed in a separate project. A compromise method was found to work for arable crop pathogens (below).

3.4.4. Compromise method

A review of the literature was made, including trade information from commercial reagent suppliers. It was noticed that, although reactions had to be performed once reagents had been mixed to prevent them from going off, it was possible to keep certain reagents separately in a wet format at room temperature and then mix them. Different reagents were prepared into tubes and then freeze-dried or kept as liquid reagent at room temperature. These were kept for a week at lab temperature (≈20˚C) and then mixed in various combinations (wet mastermix and dye with dried primers, wet mastermix with dried primers and dye, wet dye with dried mastermix and primers, wet primers added to dried mastermix and dye, wet primers and dye added to dried mastermix). A batch was also tested in which the liquid reagents were kept for a week at lab temperature and also warmed to 40˚C for two hours before use. The latter test was done to test for thermal stability of the wet reagents as the internal temperature of the device in conditions of strong sunshine could reach 40˚C. In addition, different mastermixes were tested in the wet format.

3.5. Validation of the system

Trials of the communications system and internal movements and processes of the autosampler were evaluated on two automated air samplers alongside Burkard seven-day (Hirst-type) air samplers at Rothamsted Research. The conventional air samplers were used to collect a sample each day that could be taken to a lab for retrospective analysis using the same assay as performed by the automated system to validate results. Originally, it was intended to test a greater number of devices but this was reduced due to budget constraints and the technical issue described above, which impacted on research time available for further validation. The Rothamsted air samplers were tested simultaneously at a ground-based site and a rooftop site (10 m above ground level), each site approximately 300 m apart.

3.6. Addition of Weather- based infection risk models

Past data sets were used to develop and partially assess simple weather-based models to indicate presence of infection conditions for two of the target pathogens, *Sclerotinia sclerotiorum* and *Puccinia striiformis*. These were compared against more complicated published models e.g. Dennis (1987). One method identified as necessary to be developed was an estimate of leaf wetness based on duration elapsed since last rainfall and/or presence of high humidity, as data from leaf wetness sensors was used in some models but not all met stations have this type of sensor available. Simple commands to process columns of weather data parameters were developed in Excel to produce a qualitative output, which was either '0' infection conditions not met, or '1' infection conditions met. This command line could be used to add infection risk to the dashboard presenting results of the spore trap.

4. Results

4.1. Designing and building the automated system

The sampler uses a high-volume cyclone (Fig. 2) operating at 300 litres of air per minute to sample airborne spores with high efficiency and deposit them into a collection vessel for downstream analysis.

Figure 2. Compact, high volume cyclone with rotating air intake, sampling at 300 L/min with excellent collection efficiency of particles down to at least 4 µm aerodynamic diameter.

At the end of the user-defined sampling period, the sample is moved through a series of different processes, which enables the instrument to detect the amount of spores of a target species that were in the air during the sampling period. To do this, the spores are broken open in order to release the DNA inside (see 4.4.1). Once broken open, a subsample of the disrupted spores is transferred to a tube of reagents within the device and heated to a specific temperature, in order to amplify and detect the DNA of a specific crop pathogen using a DNA-specific assay. This is a method to amplify a specific sequence of DNA so that the DNA can be detected. The DNA

sequence is known in advance to be unique to the pathogen of interest. If DNA is amplified, it means that spores of the species of interest were present in the sample. The DNA assay works by carefully designed primers binding to the target DNA and replicating a piece of DNA many times so that the amount of DNA in the reaction increases greatly. The reaction takes place at a constant temperature, which is set by the user according to the selected assay (the DNA assay could be either an RPA or LAMP assay, whenever stabilised reagents are available), and the resulting amount of DNA is quantified by a fluorescent dye that binds to DNA. Light is directed at the reaction tube to cause the fluorescence, which is measured frequently (currently every 5 seconds). Results are reported wirelessly (see 4.3).

Further assays are being developed to detect more plant pathogens. Without any changes, the instrument can be used in other crops that are affected by many other diseases. The instrument is set up to run multiple separate assays using the same sample, so it can monitor for several different pathogens every day. Therefore, it can help protect multiple crops on a farm throughout key times of the year. The instrument is powered by mains electricity so can be placed on a farm close to the crop ready to detect and report the first signs of spore release long before crop infection.

The instrument is extremely simple to operate and is designed to run without intervention by the user for periods up to 8 days. Through the WEB site, the instrument will inform the operators of its status, and when it will need refilling with the consumables. If the unit has an error or is running very low on consumables, it can get the WEB site to email the operators to get these issues resolved. The user can set up sampling periods to suit their requirements through a simple user menu in-built into the unit. The DNA test vials can be supplied prefilled with reagents. The user simply loads these into the instrument along with a small amount of liquid and new sample collection pots.

4.2. Integration and testing the automated system

Individual components were built and tested. The various components were connected into a prototype device using computer aided design to ensure physical connectivity of components. The entire system was factory tested, control programmes were updated to adjust movements. Timings and positions of components were adjusted over a period of several months and the prototype was tested in lab conditions with spore or DNA-samples added manually to tubes that were heated and fluorescence of the DNA-assay measured.

An improved final sampler was designed, made and factory tested. This had additional router and programming capabilities. The entire design was PAT tested and any high-voltage components were carefully protected. A user-manual and quick-start guide was produced giving step by step instructions on how to program the device for first use (Fig. 3). The device is controlled using a simple series of menus operated by a few buttons and a LCD screen showing basic commands and information.

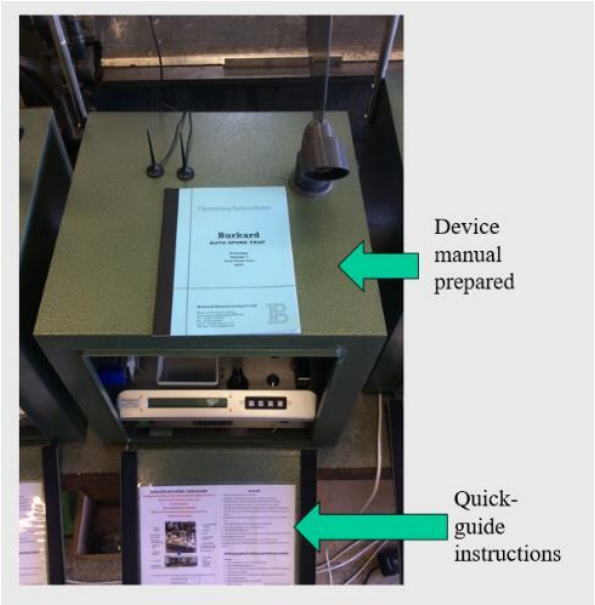

Figure 3. Final design of the device at the end of assembly and factory testing, with manual and quick-start/summary guide inside the door (shown open) of the device. Just inside the open door of the device, the picture shows the LCD display and four operating buttons used to programme commands using a simple hierarchical command menu.

4.3. Communications and web-portal development

Outputs of met data and the DNA assay are transmitted automatically over-the-air using the unit's internal 4G router fitted with an AnyNet Secure SIM, over a gsm network back to the AWS Cloud where it is analysed and reported on a web-portal in a matter of minutes. Met data are sent every 10 minutes, while results from the DNA assay are sent every 5 seconds during the reaction, which occurs only at the end of a sampling period. As the weather conditions and spore release have such a bearing on infection risk, it is possible for the weather data to be combined with risk models and processed to inform on infection risk. This is already available for yellow rust and Sclerotinia and more infection model capabilities will be added. The website portal address is: https://www.burkardportal.co.uk/

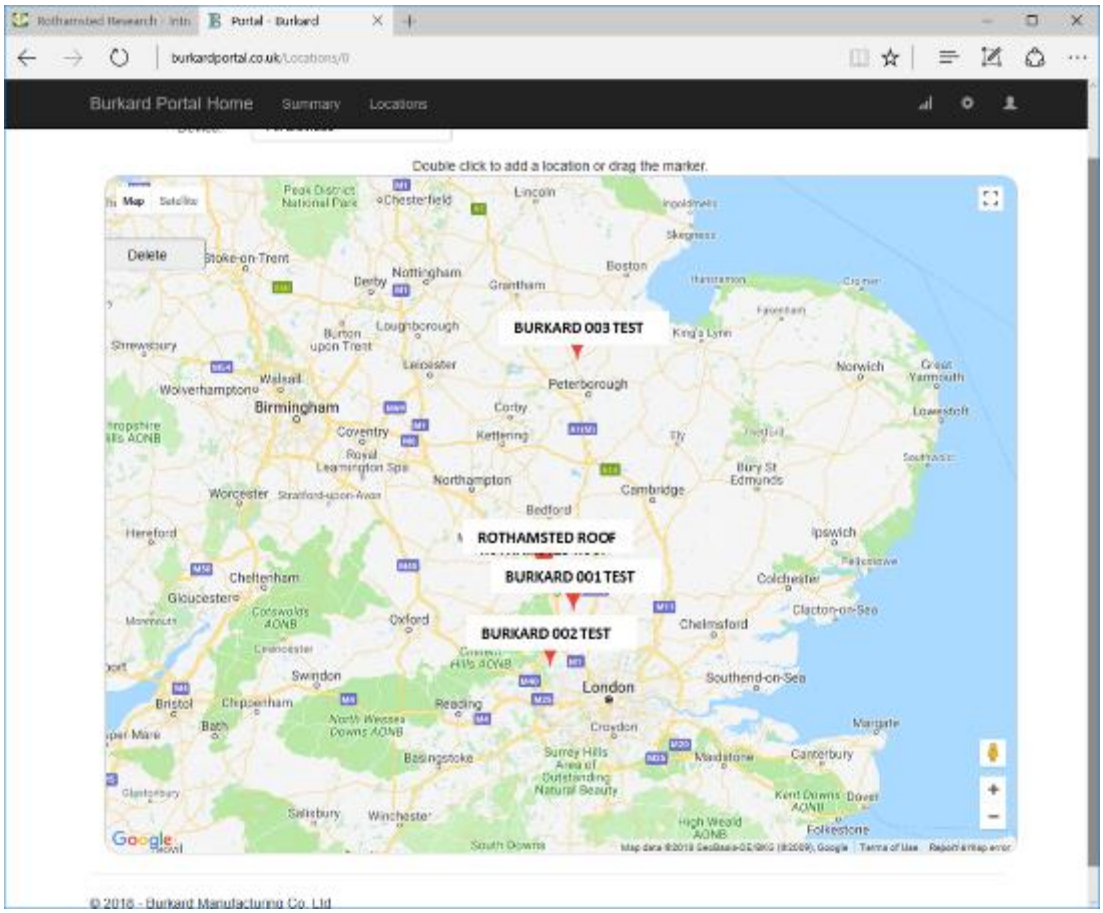

Figure 4. The opening web-portal screen.

Figure 4 shows the opening web-portal screen displaying locations of devices and their names that the user can click on to get the second screen.

Figure 5. Examples of positive (left) and negative (right) results on two consecutive days in August 2017 with Rothamsted's rooftop sampler.

Figure 5 shows two examples of the information displayed for a single device/location on two different days in which the result of the LAMP assay was positive (curve on the top left) and negative (curve on the top right) with various met data displayed beneath the LAMP assay result. The example shown were tests for *Phytophthora infestans* (potato late blight) as that assay was the only one working by completely dry reagents at the time before the compromise method (described in 4.4.4 below) was developed. Other possible results for the LAMP assay fluorescence measurement are shown in Figure 6.

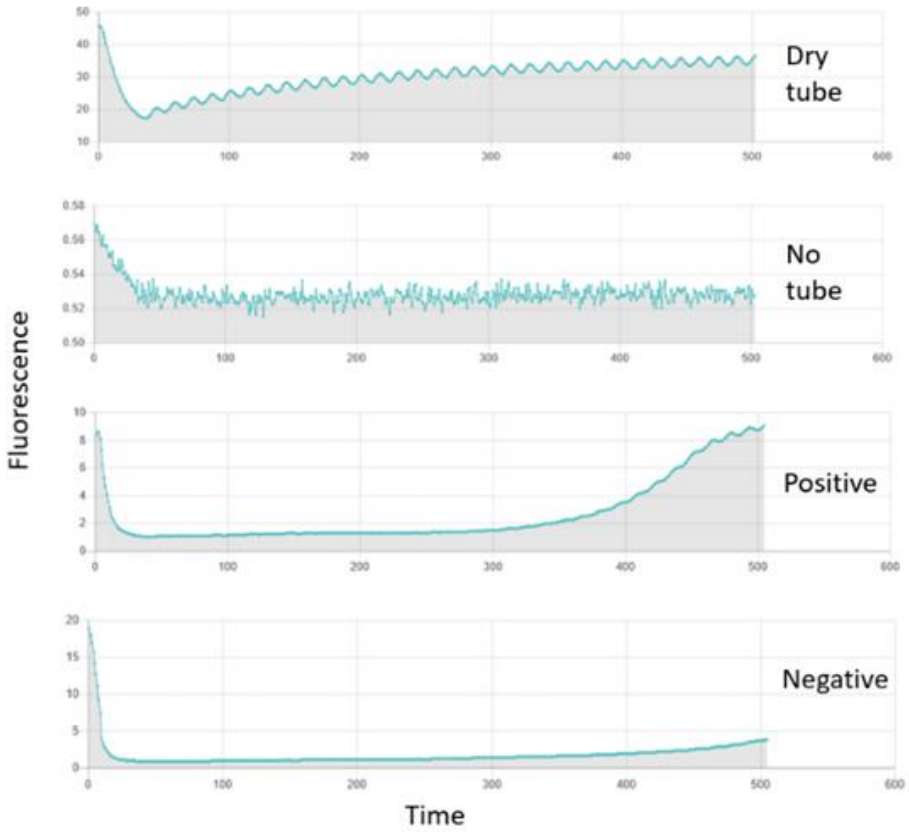

Figure 6. Outputs of different reaction results possible showing the ability to identify errors based on the type of fluorescence signal sent by the device. The positive reaction is automatically indicated if the signal at 500 time units is 5.5 times greater than that at 100 time units, otherwise the signal is indicated to be negative.

4.4. Development and testing of DNA release and LAMP assays

4.4.1. Lab-based assessment of DNA-release methods for automation

Certain spore types such as powdery mildews were ruptured simply by heating them in liquid to 65°C. Unfortunately, this did not work on tough spores such as rusts and Alternaria. Sonication with a low-power sonicator also did not work. A method that is currently commercially confidential was developed that allowed over 95% disruption of spores of all types in a period of only 3 minutes (e.g. Fig. 7). Crude suspensions of disrupted spores were successfully used directly in LAMP assays with no DNA purification step. However, when spores were spiked into 'dirty' air, i.e. samples of air particulates collected in March 2016, there was a slight reduction in sensitivity of the assay.

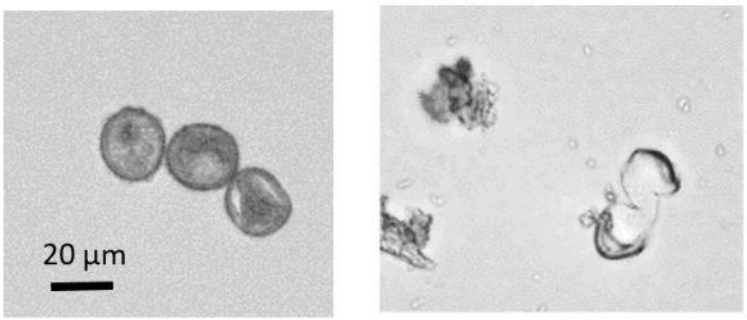

Figure 7. Example of intact rust spores before disruption (left) and disrupted spores (right).

4.4.2. Lab-based (wet reagent) assays

LAMP assays were generally found to be much less quantitative than qPCR assays. Results with crude disrupted spore suspensions could only be used to indicate presence or absence of the target organism without providing information on the relative amount of the target.

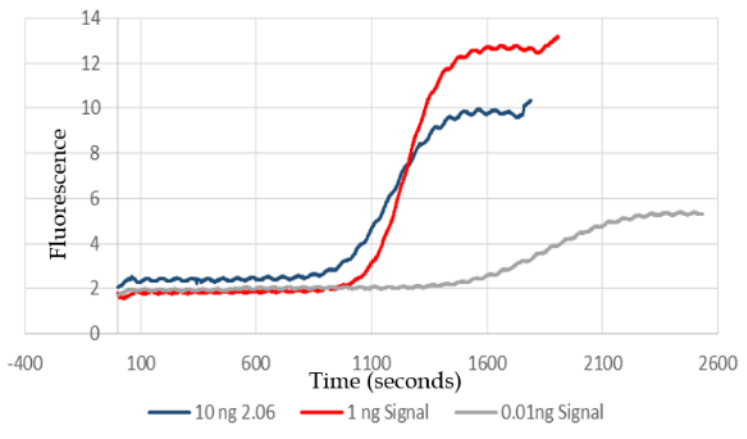

Figure 8. Example of a LAMP assay result for three different concentrations of target DNA (10, 1 and 0.01 ng), showing little difference between the reaction time taken for the 10 and 1 ng reaction to amplify, while the 0.01 ng reaction was late. The wavy lines are the result of the heater used in the prototype device, which did not maintain the correct temperature perfectly but fluctuated slightly.

Addition of disrupted spores or pure pathogen DNA to a sample of 'dirty' air particles was found to reduce sensitivity of the reaction slightly compared to testing the spores or pure DNA alone.

LAMP assays were developed or tested for the following pathogens or fungicide resistance alleles as shown in Tables 1 and 2.

Table 1. Loop mediated isothermal amplification for detection of economically important crop pathogens developed by / used at Rothamsted Research (RRes)

Target	**Disease caused**	**Developed by**	**Liquid (fresh) LAMP assay**			**Lyophilized LAMP assay**
			Specificity	**Sensitivity (pure pathogen culture DNA)**	**Screened against environmental (plant / air) samples**	**Current status**
Rhynchosporium sp.	Leaf blotch / scald of barley and rye	RRes (unpublished)	Confirmed	1 pg	-	Under development
Oculimacula spp*. (O. yallundae / O. acuformis)*	Eyespot of cereals	RRes (unpublished)	Confirmed (detects both *Oculimacula* sp.).	1 pg (spurious detection down to 0.1 pg)	In progress	Under development
Pyrenopeziza brassicae	Light leaf spot (LLS)	RRes; King et al. (2017)	Confirmed	1 pg	Applied to LLS symptomatic and asymptomatic *B. napus* leaf samples	Under development
Puccinia striiformis f. sp. *tritici*	Yellow rust	Huang et al. 2011	Confirmed (see published paper)	1 pg (see published paper); initial RRes results indicate much less sensitive and potential inhibitor issue. Other purportedly more sensitive assay recently developed (see Aggarwal et al. 2017)	Yes - see published paper	Tested but needs improvement

Alternaria solani	Early blight of potato and tomato	RRes, in progress	Preliminary*	No data	-	Under development
Zymoseptoria tritici	Septoria leaf blotch	RRes, manuscript in prep.	Confirmed (designed based on key gene thought to be involved in host specificity)	50 pg	Applied *in planta* to diseased pycnidial wheat leaf samples	Under development
Fusarium graminearum	Fusarium ear blight	Niessen et al. 2010	Wasn't as specific as suggested in the paper	<2 pg (see paper)	Applied previously by Niessen et al. (2010) to *F. graminearum*-infected barley and wheat grain samples	Tested but needs improvement
Phytophtora infestans	Late blight of tomato and potato	Hansen et al. (2016)	Confirmed (although also detects very closely related *Phytophthora nicotianae*)	2 pg (see published paper.	Yes - see published paper	Works but requires further optimization
Sclerotinia sclerotiorum	Sclerotinia stem rot	RRes (unpublished)	Confirmed (but late detection also of large amounts of pure *S. trifoliorum* + *S. minor* DNA). Note assay appears sensitive to inhibitors	20 pg	Applied *in planta* to infected oilseed rape petals and spore air tape samples (sensitivity down to 32 pg)	Tested but needs improvement

- *Validated against *A. solani* strains from the US and Russia. Also detects *A. tomatophila*, another extremely closely related species that falls in the same phylogenetic clade, with both species causing disease on tomato and potato. Does not, however, detect other more distantly related *Alternaria* species (e.g. *A. alternata*).

Table 2. Loop mediated isothermal amplification for detection of fungicide resistance mechanisms developed by / used at Rothamsted Research (RRes).

Target species (disease)	Resistance mechanism	Developed by	Specificity	Sensitivity (pure fungal culture DNA)	Applied against	Lyophilized LAMP assay
Zymoseptoria tritici (Septoria leaf blotch)	*MgCYP51* promoter insert (linked to reduced DMI sensitivity)	RRes, manuscript in prep.	Detects only *Z. tritici* isolates containing 120 bp MgCYP51 insert	100 pg	Applied *in planta* to diseased pycnidial wheat leaf samples	-not tested
Zymoseptoria tritici (Septoria leaf blotch)	*MgMFS1* promoter insert linked to multidrug resistance (reduced DMI and SDHI sensitivity)	RRes, manuscript in prep.	Detects only *Z. tritici* isolates containing 120 bp *MgMFS1* promoter insert	100 pg	Applied *in planta* to diseased pycnidial wheat leaf samples	-not tested

4.4.3. Dried formats

Various methods were tested using different mastermixes with primers and fluorescent dye. These were dried in different ways: frozen and freeze-dried, snap frozen in liquid nitrogen and freeze-dried, air-dried, vacuum dried. When a wet sample was added, the dried reagents rapidly dissolved and the reaction could be heated to the correct temperature and fluorescence measured. The snap-frozen followed by freeze drying method worked best. Addition of a polysaccharide stabilising agent also helped, although other potential stabilisers (sucrose, glucose, betaine) did not improve the assay. Use of higher quality HPLC-purified primers also improved the sensitivity of the assay and avoided false positives. Eva-green dye was found to be the most stable dye to use when dried. Despite these improvements, unfortunately it was also found that a completely dry format (dry mastermix with dried primers and dried dye in one tube) only worked well for one assay using primers by Hansen et al (2016), while those tested for other pathogens were either insensitive or produced false positives too frequently. A compromise method was developed (see 4.4.4. below).

4.4.4. Compromise method

A compromise method was developed based on LarvaLAMP mastermix supplied by Lucigen Products (http://astralscientific.com.au/lucigen-products/lavalamp-dna-mastermix.html) or Optigene LAMP mastermix (http://www.optigene.co.uk/gspssd-isothermal-mastermix-iso-001/).
These mastermixes were found to be stable in liquid form at room temperature for several weeks. However, if heated to 40˚C for two hours, they lost sensitivity. The mastermix was used as 15 µL at the standard concentration suggested by the supplier and was stored in a 0.2 mL PCR tube sealed with adhesive plastic film, which could be punctured be a pipette tip to take up the reagent. The PCR tubes used were cut from a 96-well 0.2 ml microtitre plate as each tube then had a horizontal flange at the top of the tube that gave more area for the adhesive film to bind to. This was stored on a cool-plate in the device to ensure good shelf-life. The mastermix was transferred to a similar, film-sealed tube containing freeze-dried HPLC-purified primers and Eva-green dye, to which was then added 10 µL of wet sample of freshly disrupted airborne particles (water with spores, pollens, other debris and non-biotic dust). The tube was then heated to the desired temperature (61-72˚C) depending on the assay and fluorescence was measured every five seconds (Fig. 9 below).

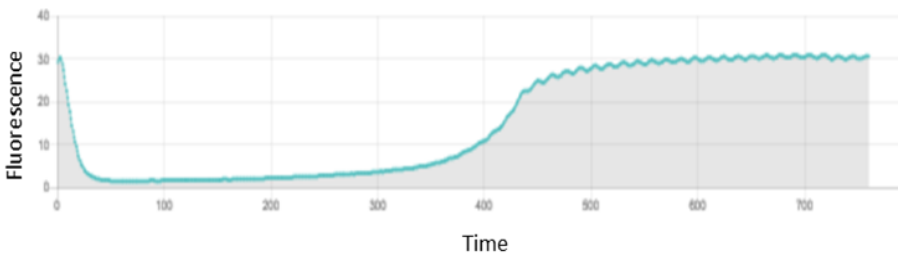

Figure 9. Result of a compromise LAMP assay method tested with the autosampler fluorimeter.

4.5. Validation of the system

Workings of the devices and communications methods were tested successfully outdoors at Rothamsted in summer 2017. Two devices were operated next to a conventional Burkard seven-day spore trap on a rooftop location and a field site (Fig.10). The programming of the device worked very well, with the met data collected continuously, the air sampler was timed to begin sampling at 7am and stop at 7pm each day. Over a period of two months, very few technical faults occurred. These included a jam in the collection pot loading – the loading system was modified to prevent collection pots from tilting which caused them to jam at the loading point. Another problem was that some pipette tips were not picked up. The cause of this was identified and a simple modification of the program solved the problem. Due to the technical problem with working dried LAMP assays, at the time of field-testing, only a dried LAMP assay for *P. infe*stans was tested. The compromise method was subsequently developed and was tested using the device in lab conditions with target spores added manually and with successful subsequent detection and communication of results to the website.

Figure 10. Automated spore traps operating at two locations at Rothamsted in summer 2017 next to seven-day spore traps.

4.6. Addition of Weather- based infection risk models

Previous unpublished work at Rothamsted tested infection conditions for *Puccinia striiformis* on wheat and found that no infection occurred with less than four hours' leaf wetness at any temperature (Fig. 11).

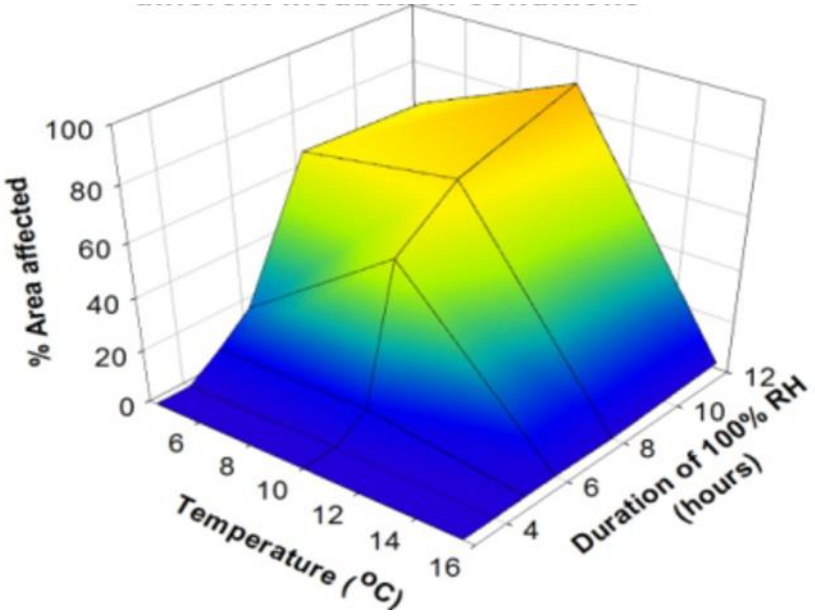

Figure 11. Amount of infection of wheat leaves inoculated with *Puccinia striiformis* spores and incubated in 100% RH for 0, 3, 4, 5, 6, 8 or 12 hours at three temperatures (4, 10 and 16°C), followed by incubation in a glasshouse in low humidity.

Two simple qualitative models were produced in Excel based on Fig. 11 and these were compared against a published quantitative infection model (Dennis, 1987) using met data from 2003 (Fig. 12).

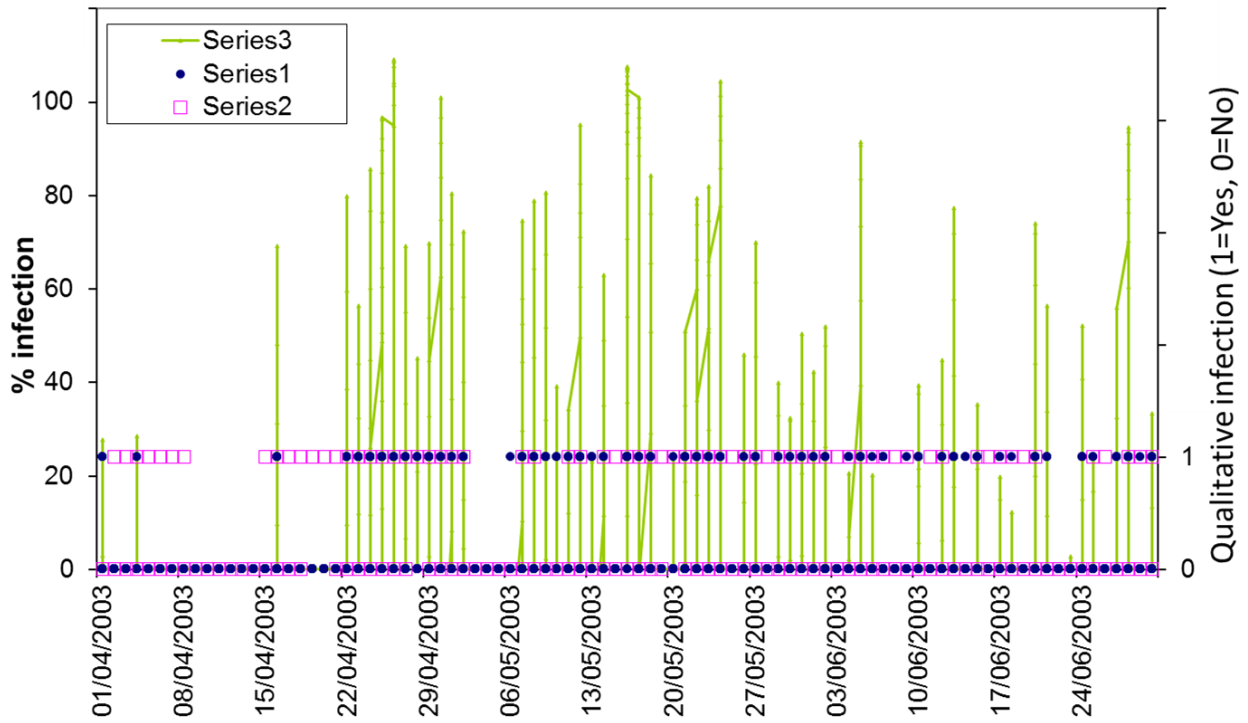

Figure 12. Yellow rust infection periods estimated using 2003 met data with a quantitative model (Dennis, 1987; series 3 - green line using the left-hand y-axis) and two qualitative models (series 1: minimum 4 h wetness and temperature between 2°C and 15°C, dark circles; and series 2: minimum

4 h wetness estimated from RH>92% or presence of rain (>0.2 mm per hour) in the past 4 hours, plus temperature between 2˚C and 15˚C, open purple squares, both using the right axis).

The qualitative model in which wetness was estimated from high RH or presence of recent rainfall (series 2, Fig. 12) was not validated using disease data from the field but was compared against results from the Denis (1987) model. It indicated several infection events on days when the published quantitative model did not, e.g. on 2-3/4/2003 and 5-8/4/2003 and 18-22/4/2003. The model that used leaf wetness data directly (series 1, Fig. 12) had only two days (7/5/2003 and 29/6/2003) when it was positive but the Dennis 1987 model was negative. These simplified models therefore may slightly over estimate the occurrence of infection conditions. The model that estimates wetness from high humidity or occurrence of recent rainfall can be modified to run on the met data supplied by the weather station on the automated spore trap.

A similar un-validated simple infection model was created for *Sclerotinia* based on Koch et al (2007) SkleroPro model. This was simplified in Excel by making a condition to register 0 or 1 per day if infection conditions are met. Infection conditions are: if minimum daily temp >7 ˚C and RH >80%.

For Fusarium head blight, an infection model was also produced based on wetness or high humidity >22h (RH>92%). Further infection condition models can be produced by simplifying published models but further work is required to validate them properly, which was out of scope for the current project.

5. Discussion

The novel device developed in this project has potential to allow a new approach in precision agriculture by providing information on exactly when and where growers should protect crops against disease, hence informing smart spray recommendations. It was intended that in addition to a potential AHDB-network, individual farmers, large farm organisations or extension companies would also be able to purchase and operate the devices to get better farm- or pathogen-specific information. It is expected that the devices will be available to purchase from the Burkard Manufacturing Company from autumn 2018 onwards. The project has produced new LAMP assays, which are rapid methods to detect specific DNA such as that of a species of a pathogen. Reagents for use with the DNAauto-spore trap will be available to be supplied to users via the Burkard company. One LAMP assay has been published and others are in preparation. Assays published by other groups were also tested and could be used by the system. Therefore, with further development of appropriate reagents (DNA primers), in time the technology will be translated to improve disease control in other AHDB sectors and could be available for fungicide resistance monitoring in addition to disease forecasting. The technology, through detection of *Fusarium graminearum*, will assist in control of mycotoxins and will optimise agrochemical performance as part of integrated crop management. It will improve control of sporadic diseases such as Sclerotinia in oilseed rape, yellow rust in wheat and late septoria leaf blotch infections. The information provided by the system could also give growers confidence to delay and even omit the application of pesticides when there is no risk, therefore reducing the amount of pesticides applied to crops and saving money.

The project suffered a major and unforeseen technical problem concerning translating effective lab-based assays using freshly prepared wet reagents into stable, dried formats with long shelf-life and good reliability that could be used in the autoDNA-sampler. Solutions to the problem involved different drying processes, addition of stabilising chemicals and testing of different reagents. A completely dry format was not found to work reliably for arable crop pathogen assays when validated against freshly-made wet reagents in a lab. Instead, the best working development by the end of the project was a compromise method involving some dried reagents and some separately-stored liquid reagents that have a moderate shelf life (several weeks at room temperature) and can be added successfully to additional dried reagents along with the spore sample with good sensitivity and specificity of results. Further research is needed to improve the shelf-life and sensitivity of the reagents currently available, ideally with a format of a completely dried set of reagents in one reaction tube to which a wet sample of disrupted spores could be added is the ideal method of choice.

Other aspects of the device were found to work very well with excellent collection efficiency of the air sampler proven when tested in a wind tunnel at Rothamsted, reliable movements of the downstream processing steps, including excellent disruption of spores to release DNA, successful

communication of data form the device to a server and collation of that data for presentation on a web portal operated by Burkard. The instrument is fitted with an AnyNet Secure SIM, which means it is not tied to any one network so it will use the best available network in the area wherever it is located.

Further research is recommended to optimise the system, further validate it against results from conventional air samplers for new target pathogens, to improve interpretation of results and to optimise sampler location when used for detection of specific pathogens. Currently we know that for some pathogens, a similar pattern of spore presence in air occurs each day over a season at different sites within a county scale but the absolute amounts of DNA, corresponding to concentration of spores in the air, varies greatly according to the height of the sampler above ground and its proximity to local sources of spores. The spatial variability of spores within a region is likely to vary from one pathogen to another due to difference in the density of cropping of the host plant and differences in the severity of each disease leading to differences in the strength of spore sources. Some buffering to smooth the spatial variability can be achieved by siting the spore sampler on a raised platform or flat-roof of a small building but this can also dilute the amount of spores that the sampler samples if the spores are coming from local sources but slightly improves the sampling of spores coming from more distant sources within the region (West & Kimber, 2015). The interpretation of disease risk also varies from pathogen species to species as some require a greater concentration of spores to establish a successful focus of disease, which may also be influenced by the coincidence of suitable infection conditions immediately or at some time after the spores were deposited. A degree of spore mortality therefore plays a part either in the air, during the dispersal process, or after deposition. The DNA-based diagnostic method chosen, by detecting DNA, does not distinguish between live or dead spores. This may lead to false alarms but in consultation with many industry personnel, particularly farmers and extension workers, it was often felt that presence of reasonable quantities of spores, even if dead, would usually be seen as an indicator of a significant source of inoculum. If spores were dead one day due to UV light or desiccation, a change of conditions might allow them to arrive in a viable state later. Therefore, it was felt that no distinction between dead or live spores was necessary. That may be reviewed for some spore types and in particular, light levels are measured by the on-board met station in case strong UV light could be taken to reduce the risk of a particular spore type such as many oomycetes, but not rusts or other pigmented spores that are known to survive long-distance transportation by wind. As the devices are powered by mains electricity, each one needs to be placed in a secure location on a farm where a power supply is available. This was preferred by consulted end-users over placing a battery-powered device in a remote field site, due to security concerns and because previous data from high volume samplers (as discussed above), suggests that airborne inoculum affecting broad-acre crops in the proximity of the farm scale can be detected in this way over a broad sampling area (West et al, 2017).

An additional improvement to the system might be to add a component of forecast weather for up to three days ahead of current time whenever the device has detected airborne spores or to add economics models based on crop growth stage and value of the expected yield to assist with spray decisions.

6. Knowledge Transfer

The project was presented as part of a seminar on Precision Agriculture for Enhanced Crop Protection, at Warwick Crop Centre, 23rd February 2017; also to the Rothamsted Research Association on 3rd March 2017; the Cereals event 14-15th June 17; Part of a seminar entitled "IoT and Data Analytics for increased crop production and food security" at the Smart Systems for Agriculture, at Queen Mary College, London 31st August 2017; part of a presentation entitled "Comparison of rooftop and field-based air samplers for early detection and population monitoring of plant pathogens" at the Science Protecting Plant Health, conference, Brisbane, 25-29th September 2017 (website: https://www.appsnet.org/publications/Brisbane_Presentations/West.pdf); and as part of a presentation entitled "Precision disease detection systems for agriculture and horticulture" at the AAB precision systems in Agriculture and Horticulture, Pershore College, 27th October 2017; it was featured in a presentation at the World Mycotoxin Forum, Amsterdam, 13th March 2018.

Presentations are also planned to be made at the Rothamsted public open weekend in June 2018 and at the International Congress of Plant Pathology, Boston, August 2018 and the International Congress on Aerobiology, Parma, September 2018.

Refereed scientific publications published so far include: West JS, Canning GGM, Perryman SA, Kaczmarek A, King KM (2017) Precision disease detection systems for agriculture and horticulture. Aspects of Applied Biology: Precision systems in agricultural and horticultural production v135, p1-8 (2017); and, King KM, Krivova V, Canning GGM, Hawkins NJ, Perryman SAM, Dyer PS, Fraaije BA, West JS. 2018. Loop-mediated isothermal amplification (LAMP) assays for rapid detection of *Pyrenopeziza brassicae* (light leaf spot of brassicas). Plant Pathology 67(1), 167-174. doi: 10.1111/ppa.12717.

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