Project title:	*Integrated decision support tools for management of downy mildew in onions*
Project number:	CP 184
Project leader:	David Gooch, Fera Science Ltd
Report:	Final report, December 2022
Previous report:	N/A
Key staff:	David Gooch Femke van den Berg Gilli Thorp Eleanor Wilson
Location of project:	Sand Hutton, York
7Industry Representative:	Emma Garfield, G's; Philip Langley, G's; James Howell, VCS
Date project commenced:	01-04-2020

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Paul Lansell

Operations Manager

Fera Science Ltd

Signature ..... Date .....

David Gooch

Project Manager

Fera Science ltd

Signature ..... Date .....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

CONTENTS

Grower Summary 5

Headline 5

Background 5

Summary 5

Financial Benefits 7

Science Section 8

Introduction 8

Materials and methods 9

Results 16

Discussion 32

Knowledge and Technology Transfer 35

References 35

Appendices 36

GROWER SUMMARY

Headline

Data collected during the project confirms that impaction spore samplers are as effective at trapping onion downy mildew spores as suction traps and could be used as a lower cost method for spore trapping, reducing equipment costs and time in kit maintenance and sample processing.

Evaluation of results derived during the study validate the use of LFD's to detect spore presence, and show the trend in spore numbers over time, however there are still concerns around the user friendliness of the LFD in its current form.

Results from the two years of sampling data indicate that where onions had previously been grown or were grown almost year-round, ODM spores were present at levels which could cause infection from early in the season.

Background

Downy mildew (*Peronospora destructor*) is a common disease that can result in major yield losses in bulb and salad onions and in onion seed production (Develash and Sugha, 1997) and crops may receive fungicide treatments as frequently as every 10 days. It is therefore crucial to have accurate and timely information around infection risk. Although the CropMonitor Pro (CMP) Platform by Fera Science Ltd on behalf of the Crop Health and Protection Centre now includes an onion downy mildew risk prediction module based on the MILLIONCAST algorithms (Gilles *et al*., 2004), this module cannot predict the onset of spore presence. This means that although CMP can help guide spray timings once spores are present, currently growers may use in-field spore sample detection methods to pinpoint the onset of the epidemic. This spore sampling is expensive and time intensive so any cost savings that can be made would enable increased uptake of these methods.

Summary

This project aimed to validate the performance of two rotation impact samplers as a low-cost alternative to the more sophisticated suction traps for use in conjunction with a lateral flow device for detection of onion downy mildew (*Peronospora destructor*) spores to aid early detection of the pathogen entering onion crops. In year one, the three types of traps were tested in onion trials at four locations provided by G's. In year two, the SporeStick rotation sampler was tested at three locations provided by G's, with an additional site located at Fera

trialling all three traps. Further validation was undertaken by growers evaluating the ease of use of the different samplers as decision aids in the field. Results show both types of rotation impact samplers are as effective at trapping ODM spores as the Burkard suction trap. Since the initiation of the project, the OptiSense SporeStick sampler is no longer commercially available, however the Rotorod and GRIPS impact samplers are still able to be purchased. There remains a concern that in some cases the difference in spore numbers found on the two replicate spore sticks from either the Rotorod or GRIPS was found to be larger than the differences seen between the Rotorod and the GRIPS sampler. This could be a result of either inconsistencies in the sample collection or inability of the LFD to provide a quantitative measure of spore presence.

Data collected from field samples was intended to be used in modelling work to define spore thresholds more clearly for disease development in the crop, however the data generated meant this was not possible. All trial sites were hosted on an updated version of the CropMonitor Onion downy mildew module and results updated in real-time for use in local disease management decisions. Results from the trials sites were used for knowledge transfer activities to the wider industry, including presenting at relevant industry events (e.g. BOPA meetings) across the 2022 season.

During the early stages of the project concerns were raised about the suitability of the Global Access Diagnostics (GAD) (formerly MOLOGIC) Lateral Flow Device (LFD) assay for detection of *P. destructor* spores collected using rotation impact samplers due to potential interference with the coatings (Vaseline or silicone grease) used on the spore collection sticks. This led to the project being extended to also validate the LFD for use in this setting whilst simultaneously developing Loop-Mediated Isothermal Amplification (LAMP) and real-time Polymerase Chain Reaction (PCR) assays, which could be used as alternative detection methods if the LFD is proven to not be fit for purpose.

Results derived during the study validate the use of LFD's to detect spore presence with the limit of detection somewhere between 10 and 50 spores, with no effect of the media into which the spores were spiked, however at lower spore concentrations readings are more variable. Some concerns remain around the spore numbers detected by the LFD's from field samples, in particular the high counts seen from the Fera site in 2022 where numbers were higher than would be expected from the beginning of sampling. However, the peaks of spores detected did align with incidences of infection and sporulation which gives confidence in the LFD in detecting trends in spore numbers, if not with accurate spore counts.

There are still concerns around the user friendliness of the LFD in its current form. The LFD is provided as a dipstick rather than a fully housed test kit which could lead to problems with

contamination. The differences seen in cube readings if the LFD is analysed before or after the specified 15 minutes is also of concern.

Validation of the PCR and LAMP assays has been completed with primer sets developed for both assays capable of detecting a minimum of 10 spores in spiked samples. Results from testing on samples extracted from different media were highly variable by both testing methods with LAMP results showing little differentiation in time to a positive result between spore counts. When field samples were compared by both LFD and TaqMan® PCR, the LFD consistently recorded much higher spore counts. Disease sightings suggest that the LFD results are accurate and the PCR results are lower than would be expected. This may be due to the DNA extraction method chosen and could possibly be remedied by trialling alternative methods.

Results from the two years of sampling data indicate that where onions have previously been grown or are grown almost year round, ODM spores seem to be present in relatively high numbers from early in the season, at levels possible to cause infection. The conditions required for infection may explain why several sites in 2021 and 2022 did not record any disease despite having numerous occasions when spore numbers were high enough to produce infection. Monitoring of spore counts can give an indication of when spore numbers start to increase and peak against background levels (which, as seen in 2022 may already be high), giving an indication of increasing risk of infection should environmental conditions be suitable for infection.

Financial Benefits

The exemplar sampler which is the Burkard cyclone spore trap has a price point of £2,547 excl. VAT (excluding batteries), whereas the alternative samplers have a much lower price point with the SporeStick coming in at £600 excl. VAT (excluding batteries) and the GRIPS-99M coming in at £525 (+ shipping & excluding batteries).

In addition to the alternative samplers being cheaper to purchase they are believed to be easier to handle given that the weekly changeover of eight Eppendorf tubes in the Burkhard cyclone would be replaced by changing over two matchsticks (or plastic sticks) coated in grease, resulting in a significant weekly time saving (approximately 20 minutes time saving per changeover event per trap).

SCIENCE SECTION

Introduction

Downy mildew (*Peronospora destructor*) is a common disease that can result in major yield losses in bulb and salad onions and in onion seed production (Develash and Sugha, 1997) and crops may receive fungicide treatments as frequently as every 10 days. It is therefore crucial to have accurate and timely information around infection risk. Although the CropMonitor Pro (CMP) Platform by Fera Science Ltd on behalf of the Crop Health and Protection Centre now includes an onion downy mildew risk prediction module based on the MILLIONCAST algorithms (Gilles *et al*., 2004), this module cannot predict the onset of spore presence. This means that although CMP can help guide spray timings once spores are present, currently in-field spore sample detection methods such as the Burkard sampler are not routinely used by growers to pinpoint the onset of the epidemic. This spore sampling is expensive and time intensive so any cost savings that can be made would enable increased uptake of these methods.

Here we investigated the potential to replace the exemplar Burkard cyclone spore trap with a lower cost alternative sampler. This project aimed to validate the performance of two rotation impact samplers as a low-cost alternative to the more sophisticated suction traps for use in conjunction with a lateral flow device for detection of onion downy mildew (*Peronospora destructor*) spores to aid early detection of the pathogen entering onion crops. The three types of traps were tested in onion trials at two locations provided by AHDB and/or G's. Further validation was undertaken by growers evaluating the ease of use of the different samplers as decision aids in the field. Data collected was to be used in modelling work to more clearly define spore thresholds for disease development in the crop. All trial sites were hosted on an updated version of the CropMonitor Onion downy mildew module and results updated in real-time for use in local disease management decisions. Results from the trials sites were used for knowledge transfer activities to the wider industry.

Main objectives

1. Validate rotation impact samplers for use as a more cost-effective alternative to suction samplers for robust detection of spore loads of *P. destructor* significant for increased risk to bulb and salad onion crops
2. Define spore thresholds for improved spray decisions for disease management in the crop
3. Deliver knowledge transfer to industry through demonstration of efficacy and value of an integrated decision support system for onion downy mildew.

During the early stages of the project concerns were raised about the suitability of the GAD Lateral Flow Device (LFD) assay for detection of *P. destructor* spores collected using rotation impact samplers due to potential interference with the coatings (Vaseline or silicone grease) used on the spore collection sticks. This led to the project being extended to also validate the LFD for use in this setting whilst simultaneously developing Loop-Mediated Isothermal Amplification (LAMP) and real-time Polymerase Chain Reaction (PCR) assays, which could be used as alternative detection methods if the LFD is proven to not be fit for purpose. . The LFD is not currently commercially available with the prototype supplied by GAD costing £23.03 (inc. VAT) per LFD in 2021.

Materials and methods

Validation of suitability of the Global Access Diagnostics (formerly Mologic) LFD assay for detection of *P. destructor* spores collected using rotation impact samplers

Inoculum bulking and spore sample preparation

Infected material was collected in person from Whitfields Eastwood Farm (CV32 6RA) in August 2021 and inoculation of clean plants attempted but this proved unsuccessful. Spore suspensions were prepared by washing spores off infected onion plants using distilled water and the spore concentration determined by counting all spores within 1 large square of a haemocytometer (1 x 1mm). The spore suspension was frozen for use in future validation and quantification work.

For LFD validation, the volume of stock spore suspension needed for the required number of spores was calculated. Due to the Global Access Diagnostics (GAD) (formerly MOLOGIC) protocol stipulating only a small amount (200µl) of buffer being added to the sample, we were concerned we would not be able to add large amounts of spore suspension for testing as this would overly dilute the buffer. This determined the maximum number of spores able to be tested. The spiking of matchsticks with predefined spore loads was done by dotting 10ul of spore suspension along the length of the stick and leaving them to dry overnight.

LFD performance validation

Dilution series

Dilution series, including a negative control, were prepared and tested using the LFD, PCR and LAMP method. The concentrations used were: 0, 10, 50, 100, 500, 1,000, 5,000 and 10,000 spores. Each test was performed in duplicate. Dilutions series were repeated for spores being added to water or with the inclusion of Vaseline. Initial experiments on LFD

spore detection carried out in year 1 of the project had shown no differences in cube reader scores from inoculated dilution series samples with added Vaseline or silicone. It was therefore decided that further validation would only include Vaseline as this would be the product used for coating spore sticks in the field.

The results for the dilution series will be compared against the calibrated GAD dose-response curve (see Appendix A).

Exposure experiment

An exposure experiment was performed testing spore samples using the LFD only. 20µl of stock spore suspension at a concentration of 6 x 10^5 spores per mL (12,000 spores total) was loaded on to Vaseline coated matchsticks and dried overnight before storing in the freezer prior to exposure. Spiked matchsticks were placed on an active SporeStick sampler for 1, 2, 3, 4, 5, 6 or 7 days before testing with the LFD.

This experiment will give an indication of how sensitive the spores are to degradation whilst being exposed to UV light under field conditions.

Design of real-time PCR and LAMP assays for the detection of *P. destructor*

Internal Transcriber Sequences (ITS) of *P. destructor* and sequences of closely related fungal plant pathogens were obtained from GenBank and alignments produced. Species specific real-time PCR and LAMP assays were designed for *P. destructor* covering highly conserved regions. Originally three real-time PCR and two LAMP assays were designed. Only one of the PCR and none of the LAMP assays resulted in detection therefore both the PCR and LAMP assays were redesigned (Table 1 and 2).

The newly designed assays were validated for sensitivity using the spore dilution series also used for LFD sensitivity testing. Assay specificity was tested using a range of fungal species known to infect onions or downy mildews most closely related to *P. destructor*. Pathogens tested for assay cross reactivity include *Botrytis allii*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Sclerotinia cepivorum*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium oxysporum*, *Phytophthora rubi* and *Peronospora viciae*.

Table 1. Real-time PCR assays designed for the detection of *P. destructor*

Sequence	Primer	Dye
ACTTGGCGGCTGCTGGTGGC	Pdes_ITS1_pr	FAM/TAM
ACGTGAACCGTATCAACCCAATT	Pdes_ITS1_F	
GCTCCAACCGAGGTCAGAACA	Pdes_ITS1_R	
TGAACGTTCTTCCTGCTATG	Pdes_ITS2_pr	FAM/MGB
CGGTATGATTGGCTTCGGCTA	Pdes_ITS2_F	
ATGAACTACGGTTCACCAGTTCG	Pdes_ITS2_R	

Table 2. LAMP assays designed for the detection of *P. destructor*

Sequence	Primer
CGTGAACCGTATCAACCCAATT	Pdes_ITS1_F3
GCCTAGACATCCACTGCTGA	Pdes_ITS1_B3
ACACTCGCCATGATAGGGTTCGTTTACTTGGCGGCTGCTGG	Pdes_ITS1_FIP
GCTTAAATTGTAAACCCATTCTTAAATACTGAAGTTGCTATCT AGTTAAAAGCAGA	Pdes_ITS1_BIP
AGTAGCCAGCCAGCAAAAAC	Pdes_ITS1_FL
TATACTGTGGGGACGAAAGTC	Pdes_ITS1_BL
CTTCTTTCCGTGTAGTCGGT	Pdes_ITS2_F3
GCCATAGCAGGAAGAACGTT	Pdes_ITS2_B3
CATACATTTCAAAGGACTCACAGCCGAGGATATGCCAGATGT GAAG	Pdes_ITS2_FIP
CTGCTGGTTGTGAAGGCTGTCACCTGTTTAGCCGAAGCCAAT	Pdes_ITS2_BIP
GATCCGAAAACCAGCCGCAA	Pdes_ITS2_FL
ACCGGTTTGTCTGCTATGGT	Pdes_ITS2_BL

Description of changes to original project

Because of the concerns around the suitability of the LFD for detection of *P. destructor* spores collected using rotation impact samplers and the delays with the LFD validation work until after the start of the 2021 field season, in 2021, only a limited number of samples were analysed. This allowed for i) an initial assessment of whether the LFD test results are affected by the spore trap type with which the samples were collected, ii) some within season information to be provided to the site managers to assist disease management and iii) initial testing of the usability of the data provided. At the same time the limited testing with the LFD meant that sufficient samples would be retained for retrospective testing with the method (LFD / LAMP / PCR) which proves most suitable for the validation of the spore traps.

Covid-19 also reduced opportunities for direct interaction with growers and the associated uncertainties around field access, potential job losses and other economic impacts meant Fera was unable to find two independent growers to volunteer for the in-field sample testing. The in-field testing was therefore cancelled for the 2021 growing season. However, G's kindly offered an additional site for sample collection, leading to five sites in total, rather than the original four sample collection and two in-field testing sites.

Field trial sites and sample collection

Year 2 (2022):

Sampling during the 2021 season showed no clear difference in performance between sampler types with the Burkard finding significantly higher spore numbers than impaction samplers in some instances, but lower numbers in others. The difference in spore numbers between replicates of the same impaction sampler type was also often larger than the between sampler type difference. It was therefore decided that for the 2022 sampling season only the simpler, less expensive SporeStick (Optisense, UK) would be tested at 3 of the sites, with the addition of a Rotorod impact sampler and a Burkard sampler at Fera, York to provide additional data on comparison between sampler types.

Experimental work to validate spore traps was undertaken at three sites provided and managed by G's with sites located at: 1) Croxton in Norfolk, 2) Wretham in Norfolk and 3) Hainey in Cambridgeshire along with a fourth site at Fera in York. At sites 1 to 3 only the SporeStick was tested. At the Fera site two types of rotation impact sampler were tested alongside a Burkard cyclone suction sampler to provide additional data for validation testing.

Sampling commenced during the beginning of April through to late July with spore samples collected twice a week until spores were detected with the LFD at which point samples were

collected weekly until symptoms were observed at the site at which point spore sampling ceased.

Spore samplers

Three types of spore traps were used:

Standard rotation impact samplers. A **Rotorod® Model 20** (Sampling Technologies INC., Minnetonka, MN, USA) or a **GRIPS-99M** (Aerobiology Research Laboratories, Ottawa, Ontario, Canada) was placed at the Fera site only in 2022. These are basic rotation impact samplers which consist of two non-retractable plastic rods (<2 mm wide) which are attached to a bar which spins at a constant 2400 rpm driven by a motor attached to a 12 V battery. In 2022, wooden sticks were utilised instead of the plastic rods due to supply issues. The rods were coated in an embedding grease (Vaseline) which results in spores being impacted onto the greased surface of the leading edge of the rod when the device spins. Sampling occurs independently of wind direction so the trap did not need specialist positioning. There is no timer incorporated, so the devices run consistently during the sampling period or have to be manually switched on and off by the user at the appropriate times. Experience with these devices shows that they should easily run for a week in the field without need to change the battery (12V DC). The price point for these traps is $900 CAD (+ shipping) for the GRIPS-99M (excluding batteries).

New generation impact sampler. Launched in March 2019, the **SporeStick** (Optisense, UK) has been developed in collaboration with aerobiology experts at Fera Science Limited to address the need for a versatile, low cost spore sampling device for detection of fungal spores. The SporeStick is similar in design to the two standard rotation impact samplers, with a rotating bar and two sampling rods coated in grease. These rods are also non-retracting, as research at Fera showed that retraction causes contamination of the device, which can be transferred when new rods are inserted. Instead of plastic rods the SporeStick uses wooden sticks, which are commercially available and more environmentally friendly. The wooden sticks have also been found to be more efficient in spore capture than the plastic rods (Chandelier et al., 2014) due to their rough surface. The SporeStick also incorporates a timer so that the device can be operated at specific periods of day or night or intermittently to prolong sampling efficiency (e.g. 1 min in every 10 minutes). The motor has a variable speed option and a forward and reverse function allowing the device to sample spores onto both the front and rear surfaces of the stick hence doubling the sampling surface and prolonging operational efficiency. Calibration equations used to calculate the equivalent number of spores per cubic metre of air have been adjusted accordingly to use data from this device. Finally, the device has an app https://appadvice.com/app/sporestick/1456794256). which

allows the user to programme the device speed of rotation and direction and set bespoke rotating periods. The price point for this trap is £600 excl. VAT (excluding batteries).

Suction sampler (Burkard cyclone spore trap). Previous AHDB-funded research on spore trapping of P. destructor has used a **Burkard** MTIST sampler or a Burkard cyclone sampler and so these two devices have already been validated for use in trapping spores of *P. destructor*. The Burkard cyclone sampler was used as the exemplar suction spore trap for this project as it is the most widely used and is available commercially. In addition, Fera has access to a significant number of these devices and considerable experience with operating them over many seasons. The Burkard cyclone trap samples at a flow rate of 16.6 L min^{-1} into a 1.5mL tube which is mounted in a rotating carousel. The dry cyclone impacts particles into the tube for a user-defined period of time (usually 24 hrs) after which the carousel turns to expose the next of up to seven tubes in total. The timing of capture/detection of the spores can therefore be more accurately determined using this device compared to the rotation impact sampler, but these devices are more labour intensive to set up and maintain and can be more prone to the effects of weather conditions. They are also more expensive with a price point of £2,547 excl. VAT (excluding batteries).

Quality assurance of trap performance prior to use. The specific set up and age of the devices can affect their trapping performance so all rotating impact samplers were serviced and set to the standard operating speed of 2400 rpm using a tachometer. The flow rate of the suction traps was also calibrated to 16.6 L per minute prior to deployment. This is not a universal standard procedure for spore traps, but routine maintenance schedules need to be implemented if these devices are to be reliably operated on-farm as a decision support device. Research shows that traps can deviate from their original settings over time (Frenz & Elander, 1996) and this would affect their performance in delivering consistent data for risk prediction.

Sample testing for spore presence

Following the defined exposure period, the rods or microfuge tubes located at the four sampler validation sites were removed from the traps and sent to Fera using pre-paid envelopes and stored in a freezer until testing occurred. The growers were also asked to record the first date of symptom expression at the trial sites. The rotation impact devices generated two replicate samples per sampling period whereas the suction sampler generated seven daily samples per week.

During the 2022 growing season one of the two replicate matchsticks from each sample timing and test site was tested by LFD. Only one of the two sample sticks were tested due to a limited number of LFD testing kits. The second replicate stick was tested from 10 sample timing/test sites to assess the differences in spore detection between replicates from the

same sample. Spore sticks were placed in transport tubes with three 5 mm ball bearings, five drops of buffer added and shaken by hand for 1 minute. Testing for spore presence was done using the GAD LFD following the protocol defined in Appendix A. All LFD tests were read using an electronic cube reader to capture a digital measure of the result. The cube readers were calibrated to read the specific LFD for onion downy mildew. Calibration curves, created using data from the dilution series validation testing, were used to calculate the equivalent number of spores in each sample.

In order to compare the sensitivity of the LFD to real time PCR on impaction sampler samples, the second matchstick from the Fera site was tested by DNA extraction followed by real time PCR. The matchstick was placed in a transport tube along with 0.5 g of 2.3mm zirconia silica beads and 150 µl of soil CTAB buffer and disrupted on a Vortex Genie 2 to thoroughly disrupt samples. The resultant sample was extracted using a Nucleospin™ Plant II kit (Macherey-Nagel™), following modified manufacturer's instructions.

To compare the performance of spore trap types and the sensitivity of testing methods between Burkard and SporeStick samples, Burkard samples from the Fera site were tested either by LFD or by DNA extraction followed by real time PCR, on alternate weeks (as all 7 samples were required for comparison against the SporeStick which ran for 7 days). For LFD testing five drops of the LFD buffer was added to the Burkard sample tube and vortexed. The content of the first tube was emptied into to next tube, vortexed and transferred into the next day's tube. This was repeated until all 7 tubes had been processed. All previous tubes were then centrifuged for 10 seconds to collect any remaining buffer which was pipetted into the final tube before testing by LFD using the GAD protocol. For real time PCR testing 150 µl of soil CTAB buffer was added to the first sample tube and transferred to all 7 tubes as described previously. The resultant sample was extracted using a Nucleospin™ Plant II kit (Macherey-Nagel™), following modified manufacturer's instructions.

In-season results reporting. All four trial crops were set up on the onion downy mildew module of CropMonitor Pro (www.cropmonitor.co.uk) which displayed daily updates on predicted risk of sporulation and infection by *P. destructor* at the location. Results of the diagnostic tests performed at Fera were added to the system with the option of first symptom reports to be added directly to the database and displayed on the downy mildew risk pages.

Deliver knowledge transfer to industry through demonstration of efficacy and value of an integrated decision support system for onion downy mildew

Website enhancements for onion downy mildew module on CropMonitor Pro (CMP)

The AHDB CMP onion downy mildew module featuring the Milioncast model was extended to capture results from LFD tests and disease observations. The site managers for the five

trial sites ran during the 2021 growing season were provided with access to the updated CMP services to support disease management. This was continued for the 2022 sampling season.

User feedback questionnaires

User feedback forms were created to capture the site manager's experience with:

1. Spore trap set-up
2. Spore trap maintenance
3. Sampling protocol and logistics
4. CMP data visualisation
5. Management and communications

See Appendix B for the full list of questions. The questionnaire results were used to improve site management and user experience during the 2022 growing season. A similar survey was performed after the second growing season to assess improvements made.

Other

See Knowledge and Technology Transfer section for further details.

Results

Validation of suitability of the Global Access Diagnostics (formerly Mologic) LFD for detection of *P. destructor* spores collected using rotation impact samplers

Further validation of the LFD on a wider range of spore numbers shows that detection is not affected by the media they were in (Vaseline or water) (Figure 1.). The lower the number of spores tested, the higher the variation in cube reader scores between repeats of the same media and between media. This may be due to accuracy of initial spore counts and the variation when carrying out a dilution series down to low numbers of spores.

There was very little difference in cube reader scores between the negative control and the samples containing 10 spores with one of the Vaseline samples recording a higher score at 10 spores than 0 (figures for negative control samples not shown on log scale graph). This suggests that the limit of detection for the LFD is somewhere between 10 and 50 spores Above 1000 spores, there was very little difference in readings.

The results of cube readings for the dilution series from both media types was averaged to produce a response curve from which spore counts from field samples could be read (Figure 2.).

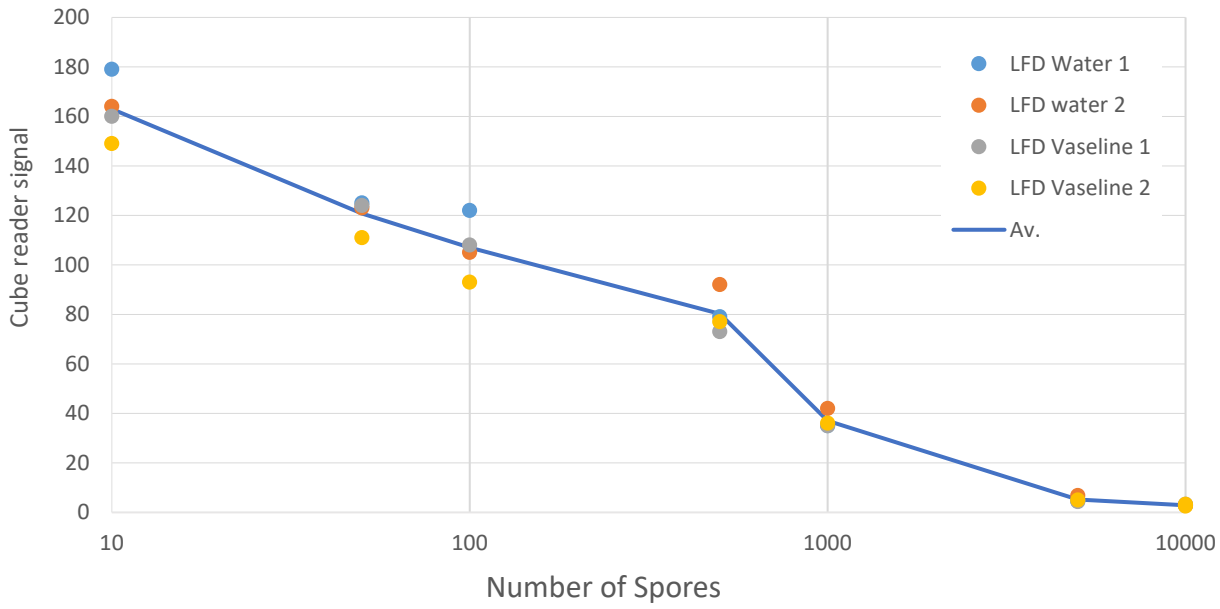


Figure 1. Cube signal versus spores per test for LFD tests performed on spore samples created in different media (Log scale)

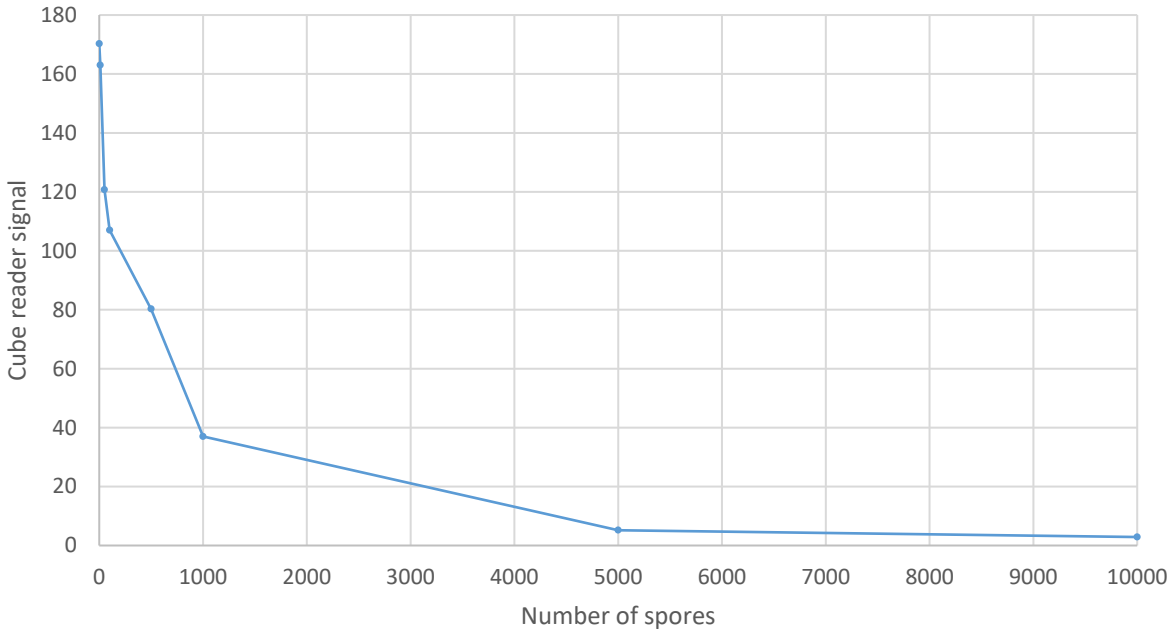


Figure 2. Average cube signal versus spores per test for LFD tests performed on spore samples created in different media

Exposure experiment

Results show there was no effect on the number of spores detected by LFD from samples exposed to environmental conditions for up to 7 days (Table 3.) The number of spores detected varied between replicates with no sample detecting the full 12,000 spores that had been loaded on to the sticks prior to exposure. Spore numbers varied even between the two replicate sticks from the same sampler however this variation is within a factor of 10. This level of variability would potentially not be considered as greatly different when evaluating spore counts.

Table 3. Number of spores, detected by LFD, from inoculated spore sticks exposed to environmental conditions for 1 – 7 days

Days exposure	Sample 1		Average Sample 1	Sample 2		Average Sample 2	Average
	Rep 1	Rep 2		Rep 1	Rep 2		
1	950	2910	**1930**	7200	6900	**7050**	**4490**
2	2630	4410	**3520**	9600	6850	**8225**	**5873**
3	4650	9900	**7275**	4150	N/A	**4150**	**5713**
4	10000	3900	**6950**	2880	4550	**3715**	**5333**
5	4530	4490	**4510**	4920	1500	**3210**	**3860**
6	4000	6200	**5100**	3780	640	**2210**	**3655**
7	3780	6850	**5315**	9400	4390	**6895**	**6105**

Validation of PCR and LAMP assays

The graph showing the sensitivity of the previously designed (Pdes3) and newly designed (Pdes_ITS1 and Pdes_ITS2) TaqMan PCR primers are shown in Figure 3. The lower the number of TaqMan cycles, the higher the amount of target DNA detected (40 cycles indicates a negative result). Results show all primers were able to detect 10 spores (the lowest number of spores tested). There was very little difference between the 3 different primer sets, therefore it was decided to use Pdes_ITS2 for sample testing as this showed one of the highest R squared value indicating the results were the closest to the fitted regression line.

Testing of all primers sets against DNA extracted from isolates of *Botrytis allii*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Sclerotinia cepivorum*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium oxysporum*, *Phytophthora rubi* and *Peronospora viciae* gave Ct values of 40 indicating a negative result.

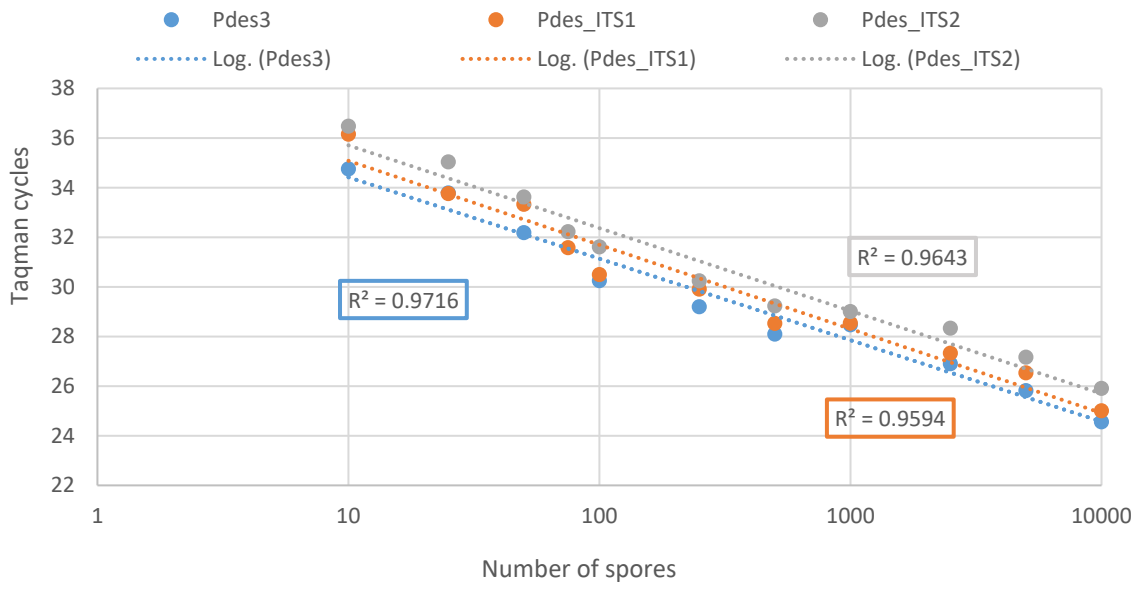


Figure 3. TaqMan cycle number (Ct value) versus spores for the 3 newly designed TaqMan primers (Log scale)

Results for the validation of PCR primer set Pdes_ITS2 when tested on the spore samples created in different media are shown in Figure 4. All negative control samples gave a value of 40 (not shown on the graph displaying log data). No differences were seen in detection between extracts indicating there was no inhibition from the Vaseline however variation can be seen between replicates of the same extract type. All samples of 10 spores gave a positive result confirming the limit of detection for real time PCR was 10 spores.

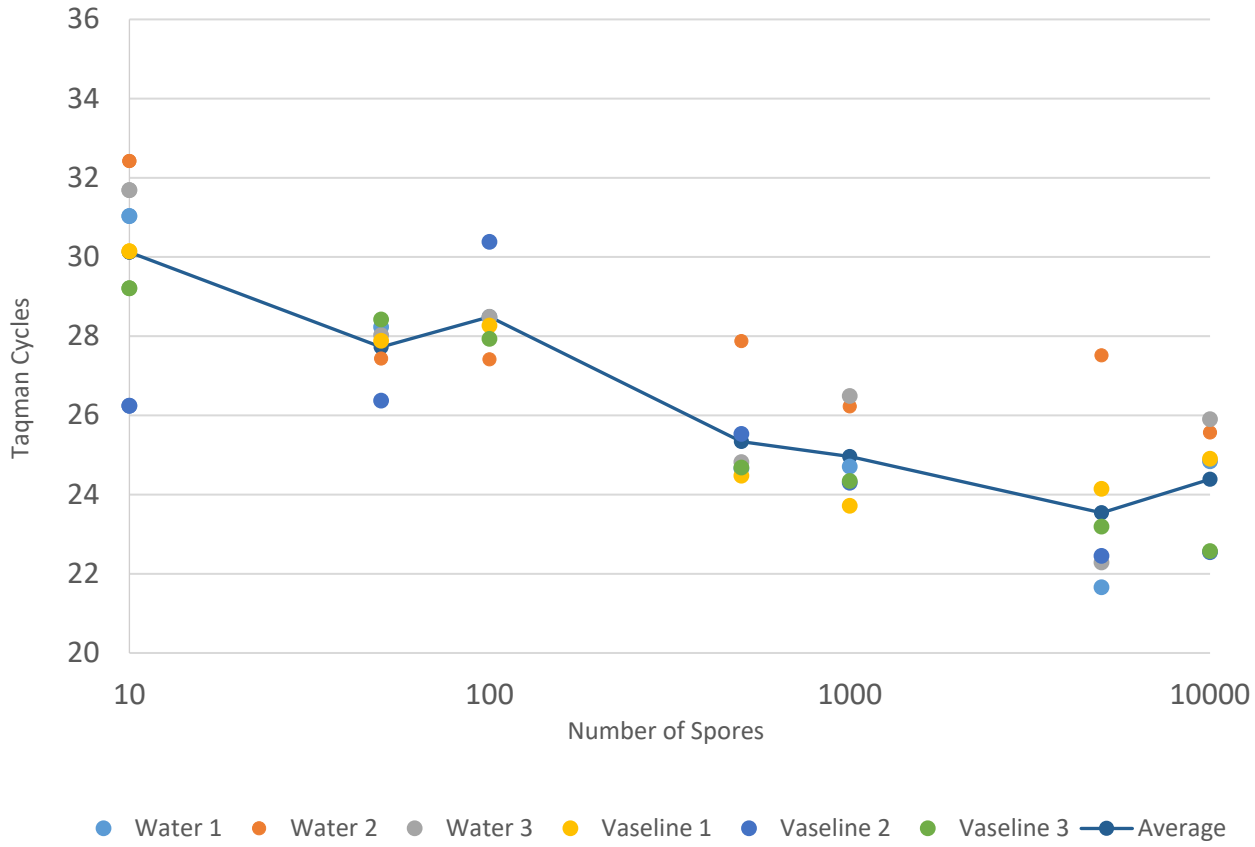


Figure 4. TaqMan cycle number (Ct value) versus spores per test for PCR testing performed on spore samples created in different media (Log scale)

Results for the newly designed LAMP primers are shown in Figure 5. The faster the time taken to amplification (indicating a positive result), the higher the amount of target DNA detected and therefore the higher the number of spores. Results show both sets of LAMP primers were able to detect down to 10 spores with very little difference in amplification timing between 100 and 10,000 spores. Primer set Pdes_ITS2 took the shorter amount of time to positive at all spore numbers tested therefore this set was chosen to take forward for further validation.

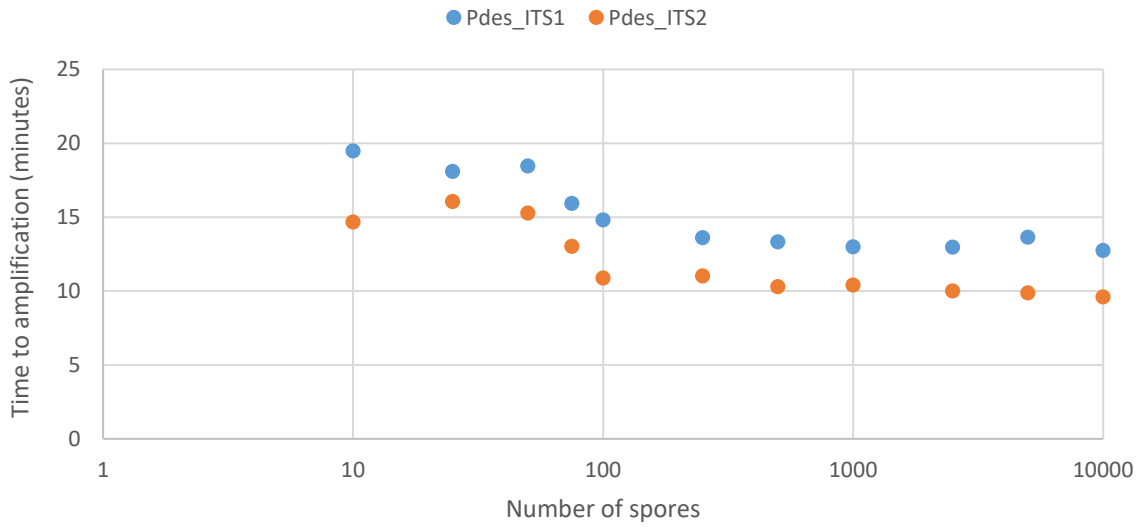


Figure 5. Time to amplification versus spore counts for the 2 newly designed LAMP primer sets (Log scale)

When the primers were tested against spore samples in different media, the results were highly variable with the time to positive very inconsistent (Table 4). The limit of detection in water samples was 100 spores although several replicates gave negative results at 500 and 1000 spores. The limit of detection in Vaseline samples with all replicates detecting DNA was 500 spores (with one replicate giving a positive result at both 50 and 10 spores).

Table 4. Time to positive result for LAMP testing performed on spore samples created in different media

No. spores	Water R1	Water R2	Water R3	Vaseline R1	Vaseline R2	Vaseline R3
10000	16.45	12.45	12.45	12.3	10.45	11
5000	12.3	15	11.3	10	10.45	11.15
1000	13.15	0	0	11.15	11.3	19
500	12.3	0	15.3	11.45	15.3	12.15
100	0	17.45	18.45	0	0	0
50	0	0	0	0	19.15	0
10	0	0	0	0	10.45	0
0	0	0	0	0	0	0

Results from the comparison of spore detection using either an LFD or DNA extraction followed by TaqMan® PCR show large differences between the methods with the LFD consistently recording much higher values (Table 5). Differences may be due to spores being removed from the sporestick for LFD analysis using large ball bearings with the tube shaken by hand for 1 minute (a method that would be suitable for use in the field), whereas for PCR analysis, spores were removed from the sporestick using smaller silicone beads and a disrupter Genie to rapidly vibrate the sample. The LFD method may be more effective at removing spores from the stick resulting in higher spore counts. Different extraction buffers and extraction methods were also used for each analysis. The PCR extraction involves numerous steps compared to the LFD method which may result in loss of DNA between steps. The LFD buffer may also be more efficient than the PCR buffer.

It is not known what pathogens were used in the validation of the GAD LFD therefore the higher counts could be due to cross reactivity with other pathogens found in field samples. Real time PCR primers developed by Fera were validated against a range of pathogens including downy mildews and those likely to be found in onions such as Botrytis, Sclerotinia and Fusarium so should not have produced any false positives due to cross reactivity.

Table 5. Number of spores detected from SporeStick samples from the Fera site analysed by either LFD or DNA extraction followed by TaqMan® PCR

Date	No. spores (LFD)	No. spores (TaqMan® PCR)
04 – 07/04/22	2150	5
07 – 11/04/22	970	0
11 – 14/04/22	790	485
14 – 19/04/22	4530	4
19 – 25/04/22	970	2
25 – 03/05/22	970	4
03 – 09/05/22	2150	39
09 – 16/05/22	940	2
16 – 23/05/22	5250	10
23 – 30/05/22	>10000	9
30 – 06/06/22	420	0
06 – 13/06/22	>10000	26

Validation of rotation impact samplers for use as a more cost-effective alternative to suction samplers

As seen in 2021, further testing in 2022 shows that there are differences between the spore numbers depending on which type of sampler the spores have been collected with (Table 6). Results from the Sporestick and GRIPS are from one of the two replicate sample sticks, results from the Burkard are from the combined samples correlating with the rotation impact sampler days. Once again there is no clear pattern in the data although in general it is the SporeStick impact sampler that records the greatest number of spores.

Table 6. Number of spores detected by LFD by spore sampler type.

Site	Date	SporeStick	GRIPS	Burkard
VCS Tunstall	13 – 20/08/2021	4160	2650	4930
VCS Tunstall	20 – 27/08/2021	>10000	3650	>10000
VCS Tunstall	01 – 08/09/2021	>10000	4270	>10000
Hainey	26 – 02/09/2021	>10000	8200	3650
Hainey	02 – 09/09/2021	9000	9100	5500
Fera	14 – 19/04/2022	4530	2790	92
Fera	25 – 03/05/2022	970	3500	>10000
Fera	09 – 16/05/2022	940	990	3500
Fera	23 – 30/05/2022	>10000	2280	4690
Fera	06 – 13/06/2022	>10000	3500	7200

Define spore thresholds for improved spray decisions for disease management in the crop

Spore monitoring

In total 69 samples collected with the SporeStick rotation sampler during the 2022 season were analysed using an LFD and the spore count calculated using the response curve. Spore counts for all sites are depicted in figures 6 – 9. High numbers of spores were detected from Wretham, Croxton and G's site from the start of sampling in early April with numbers averaging around 4000 spores. Spore numbers were lower at the York site with numbers generally staying around 1000 spores per week.

Spore counts at the Wretham site averaged around 4000 spores per week throughout the sampling period with peaks in spores at the beginning of May and again in early to mid June (Figure 6). Sampling ceased on the 17th of June 2022 when it was discovered infection and sporulation had already been noted in the crop before sampling was initiated at the beginning of April. The presence of disease before sampling was initiated meant the results aren't able to provide information about infection thresholds.

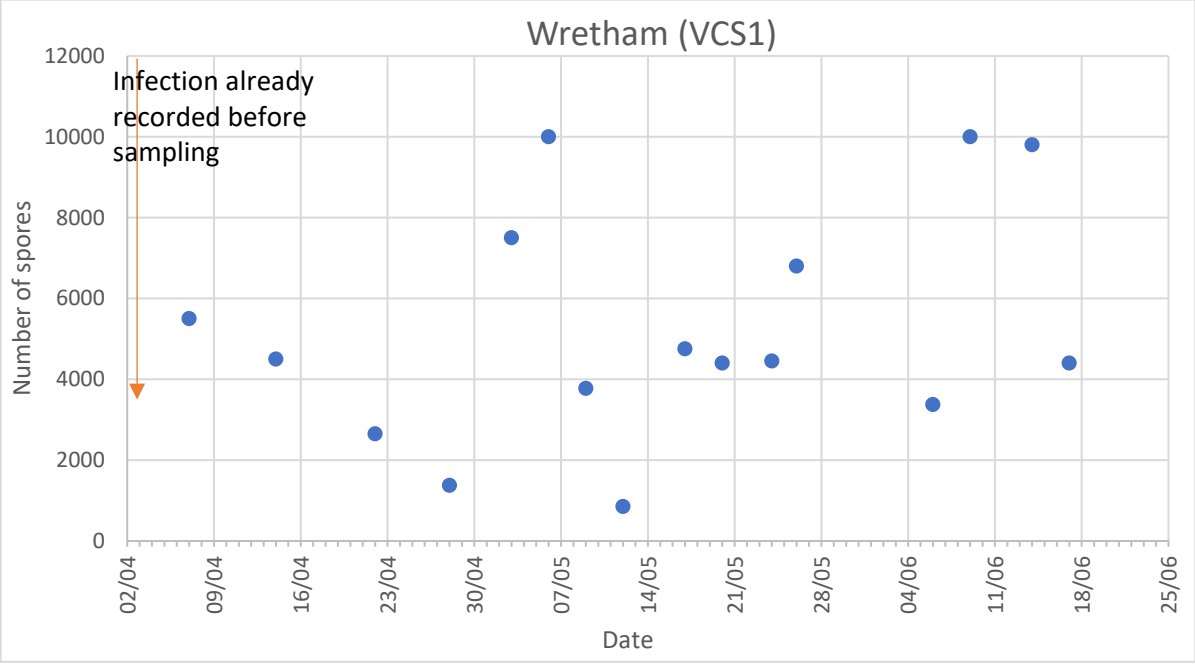


Figure 6. Number of spores detected by weekly SporeStick samples and LFD analysis at the Wretham (VCS1) site

Spore counts at the Croxton site were generally around 2000 - 4000 spores per week (Figure 7), similar to levels seen at the Wretham site, where disease was already present within the field. A peak in spore counts was seen in mid-May with numbers appearing to increase again in mid-June before the first infection was recorded on the 4th of July 2022. Unfortunately, several weeks sampling was missed between mid-June and the beginning of July, due to staffing issues. It was unknown if there was a further peak in spores during this time, which could account for resultant infection in the field, and give a clearer indication of number of spores required for infection.

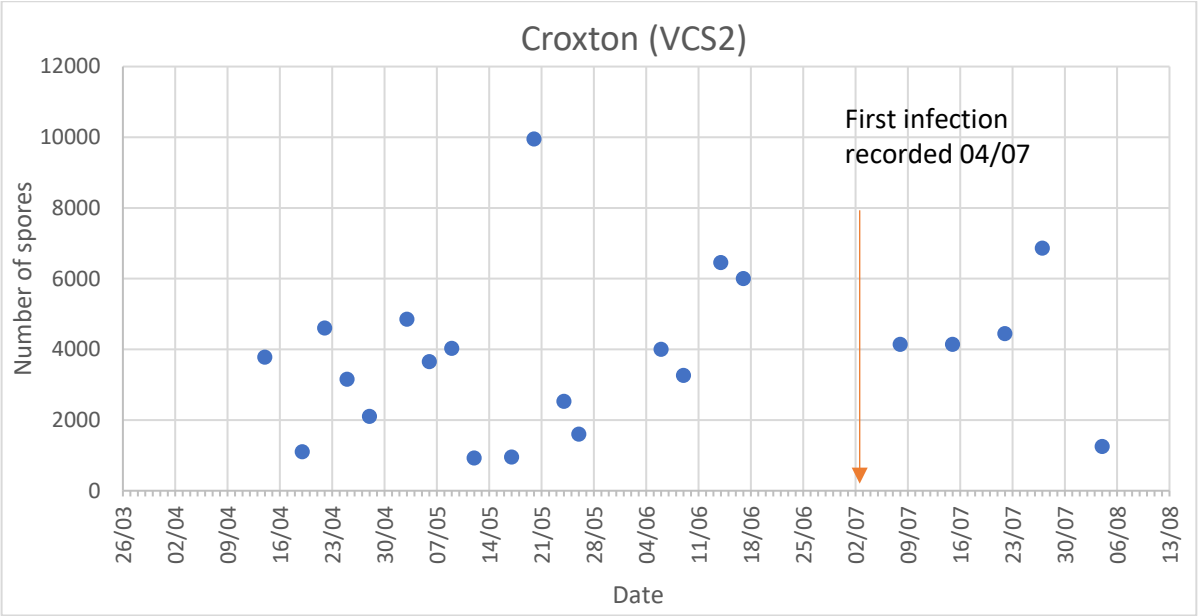


Figure 7. Number of spores detected by weekly SporeStick samples and LFD analysis at the Croxton (VCS2) site

Spore counts at the Hainey site were generally between 2000 and 4000 spores per week with peaks in spores detected at the beginning of May and again at the beginning of July (Figure 8). No samples were received between the 23rd of May 2022 and the 30th of June 2022 due to the malfunction of the SporeStick sampler. Despite spore numbers being similar to all other sites and having 2 peaks in spore counts, no infection was seen within the crop during the monitoring period. This may be due to environmental conditions not being suitable for infection or sporulation. Infection requires at least 2-3 hours of leaf wetness with spores only remaining infective for less than 24 hours, therefore they may not always remain infective until favourable conditions are encountered. Sporulation requires a temperature of less than 24°C on the previous day, followed by a relative humidity greater than 95% for at least 4 hours and an absence of rain during the night (*Hildebrand et al*. 1982).

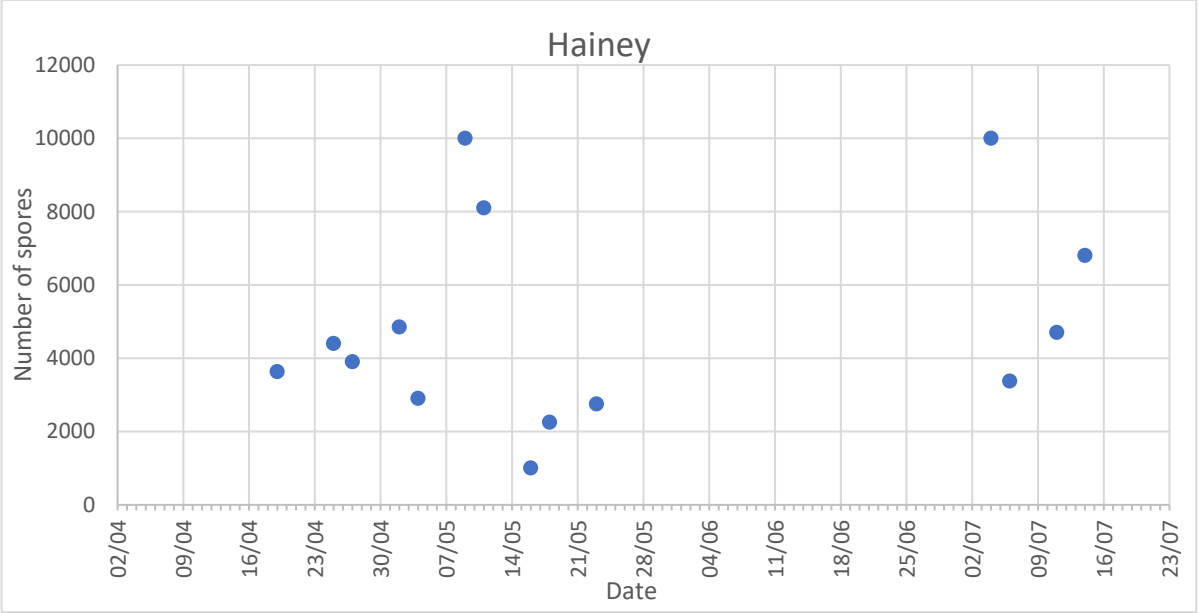


Figure 8. Number of spores detected by weekly SporeStick samples and LFD analysis at the Hainey site

Spore counts at the Fera site were lower than the VCS and Hainey sites with numbers averaging between 1000 and 2000 spores with a peak in spore counts in late May 2022 (Figure 9). The Fera site offered the opportunity to provide the most useful information regarding the correlation between (total) spore load and symptom onset date. A small plot was sown with onion sets with no onions previously cropped in the surrounding area. Infected onions which had been collected from a diseased field in 2021 and planted into pots, were placed adjacent to the newly sown plot as a potential inoculum source. Fresh sporulation was seen on the overwintered onions on 14th April 2022 (before the onion sets had germinated), the 9th of May 2022 and the 23rd of May 2022. This correlates with the peaks in the number of spores detected by the SporeStick sampler which occurred during 14th to 19th of April 2022, the 16th to 23rd of May 2022 and 23rd to 30th of May 2022. Infection was seen within the crop on the 7th of June, 15 days after the peak in spore detection. This correlates well with the 10 – 16 day latent period for onion downy mildew (Hildebrand *et al*., 1982) and disease development seen with other downy mildews where the period between infection and symptom development/sporulation is approximately 210 degree days (14 days at an average temperature of 15°C). After sporulation was seen in the crop, spore levels increased initially to over 10,000 spores before reducing and remaining relatively high at around 4000 spores, similar to the initial spore counts detected at the 3 other sample sites.

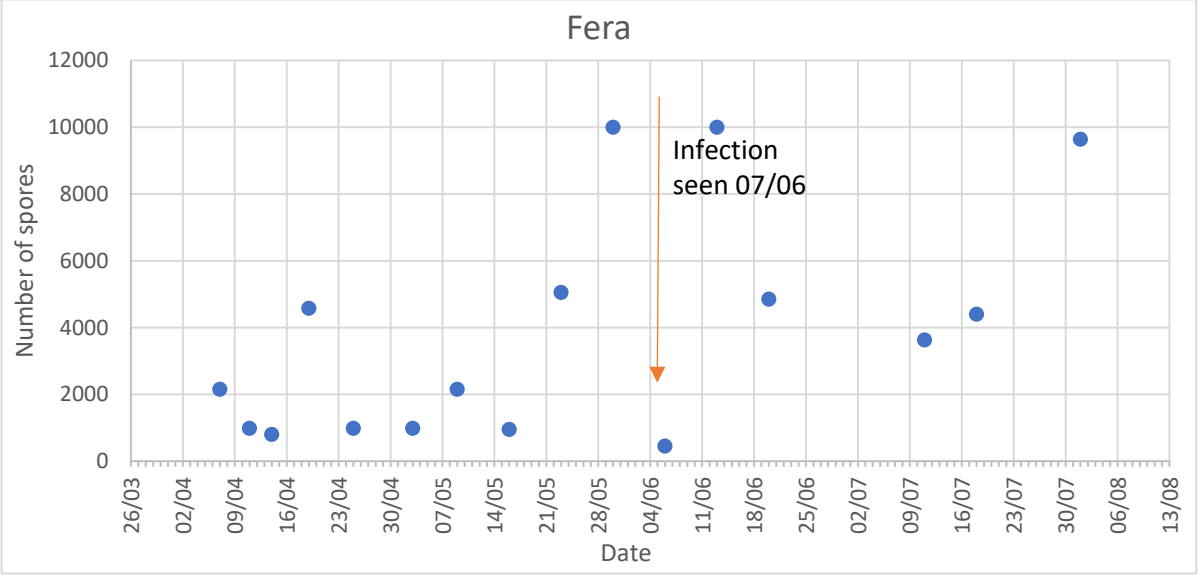


Figure 9. Number of spores detected by weekly SporeStick samples and LFD analysis at the Fera site

Results of testing both replicate sticks from an individual sampler from one sample date are shown in Table 7. Results between replicates from the same sampler in field are variable, however as seen with the exposure testing results using inoculated spore sticks, results between replicates are within a factor of 10 which would not be considered a large difference.

Table 7. Number of spores detected by LFD per replicate sporestick from the same sample date.

Site	Date	Rep 1	Rep 2
Wretham	12 – 15/05/2022	4750	>10000
Wretham	20 – 24/05/2022	4450	4700
Wretham	14 – 17/06/2022	4400	9950
Croxton	09 – 12/05/2022	925	4635
Croxton	24 – 26/05/2022	1600	>10000
Croxton	09 – 14/06/2022	6450	9640
Hainey	19 – 25/04/2022	4400	2875
Hainey	04 – 09/05/2022	>10000	>10000
Hainey	18 – 23/05/2022	2750	2650
Hainey	04 – 06/07/2022	3375	4275

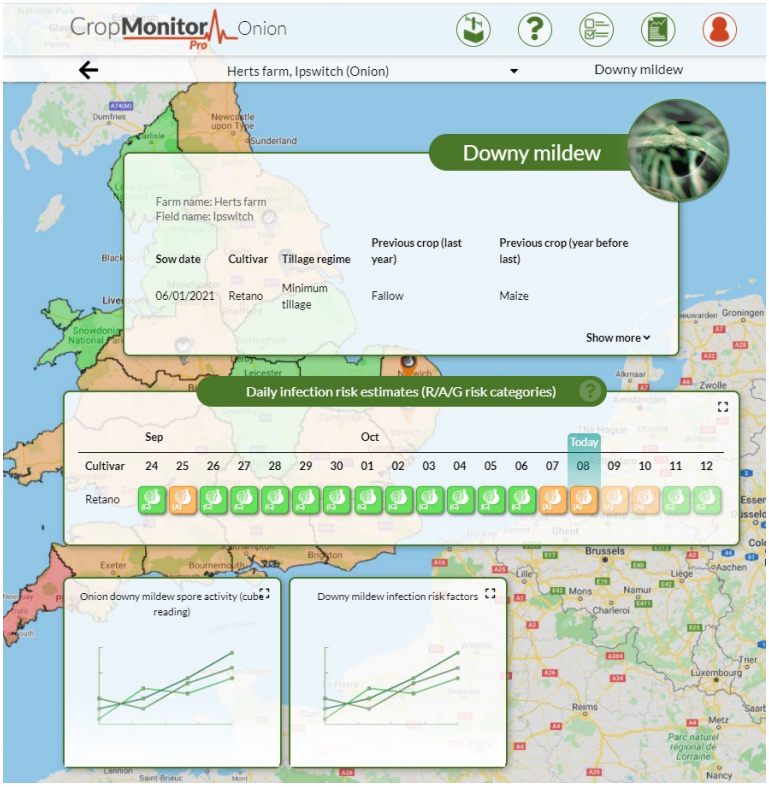
Deliver knowledge transfer to industry through demonstration of efficacy and value of an integrated decision support system for onion downy mildew

Website enhancements for onion downy mildew module on CMP

Two videos were generated and are now hosted on the AHDB horticulture website to 1) guide registration on CMP and add an onion field and 2) how to use the onion downy mildew risk prediction tool. Please see Onion downy mildew risk predictor | AHDB for the videos.

Figure 10a illustrates the updated site (field) specific page of the CMP onion downy mildew module. It now includes a plot illustrating the cube readings submitted for specific LFD testing dates (Fig. 10b) in addition to the downy mildew infection risk factors plot (Fig. 10c). The data reflected within this plot can either be entered directly into the database by the Fera project team or can be added by the site manager through the site specific 'My Sightings' page they have access to through their CMP registration. Currently, users can only enter either cube readings or GAD lateral flow score card readings, with the latter ranging between 0 and 10. In future the data entry options could be extended to allow for PCR or LAMP test result entries.

Figure 11 illustrates the new user data entry functionality available on the 'My Sightings' page. Users can submit both disease observation and spore presence test result data, with the spore presence test result data being visualised on the site-specific pages the day after data submission.



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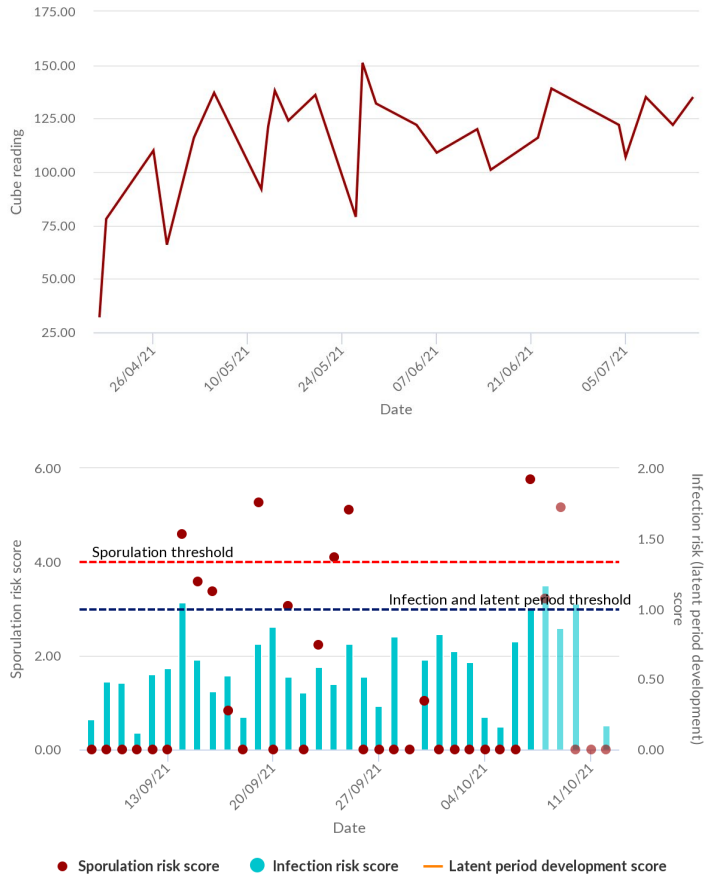


Figure 10. a) CMP onion downy mildew module site/field specific page, with expanded spore presence and infection risk factors plots in b) and c), respectively.

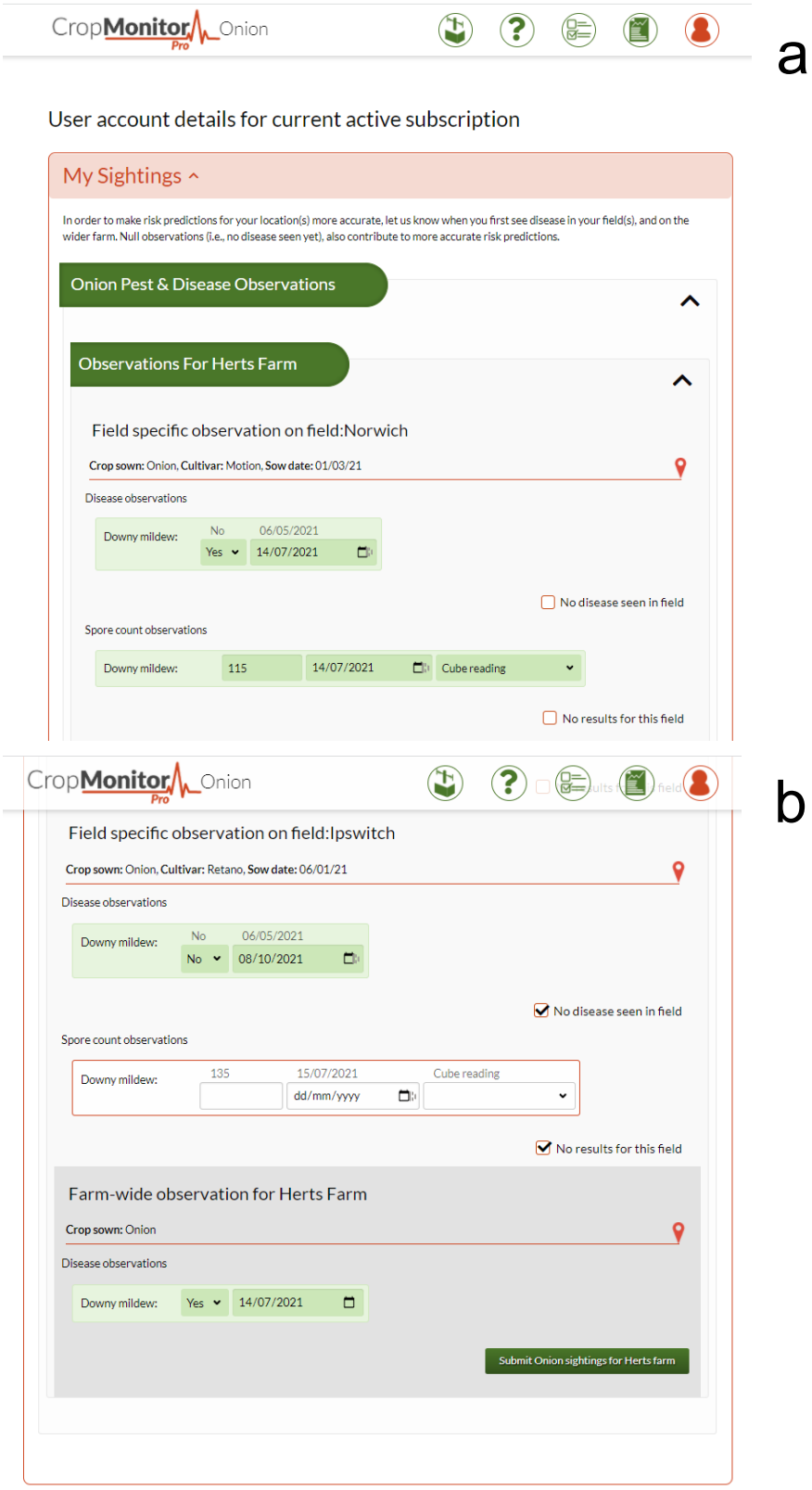


Figure 11. CMP data entry functionality for onion downy mildew as provided on the 'My Sightings' page. Data can be submitted per site (a) and/or for the whole farm (b).

User feedback questionnaires

Due to some issues with ease of the set-up, running and maintenance of the traps, in 2022 all growers were provided with an updated operating procedure, including better images to make instructions clearer. The SporeStick was the only sampler distributed to sites in 2022 and scored very negatively with regards to battery life. To overcome this problem, sites were provided with both the larger leisure battery and solar panel and the battery provided with the SporeStick so they could choose their preferred option. Testing at Fera also revealed charging of the SporeStick batteries was improved when the battery box was opened and the 2 individual batteries within charged separately using the leisure battery charger. This advice was passed on to the growers to implement. To improve Fera's management of sampling and troubleshooting, a single point of contact at Fera was provided in order to respond to any queries more rapidly.

All questions included in the 2022 user feedback questionnaire and the anonymised responses of the site managers are provided in Appendix B. Only one out of the two site managers completed the questionnaire. Results showed an improved response with regards equipment reliability and Fera's management with the site manager generally finding the whole sampling process good/easy with no issues encountered. Over the sampling period in 2022, there was some turnover of site managers however this did not seem to impact the receipt of samples.

Other

See Knowledge and Technology Transfer section for further details.

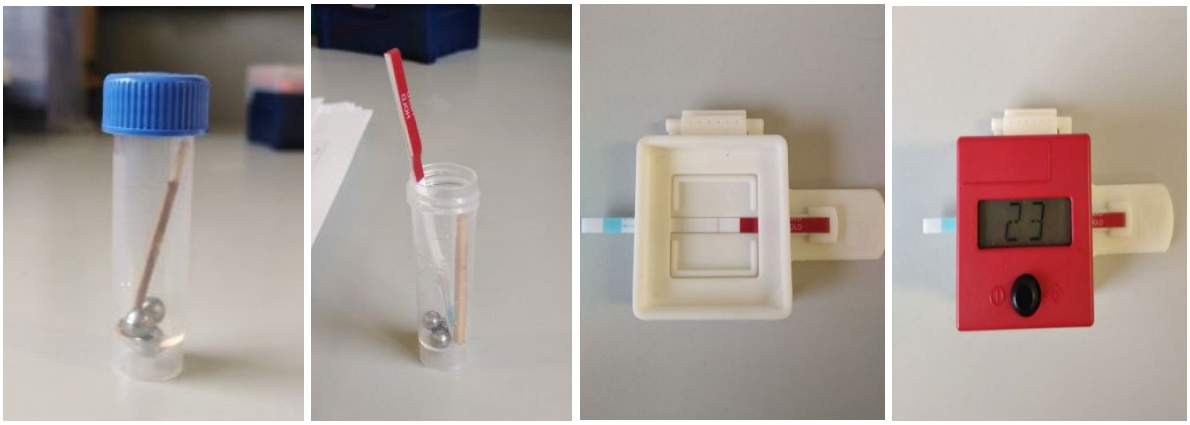
Discussion

The main aim of this study was to validate rotation impact samplers for use as a more cost-effective alternative to suction samplers for robust detection of spore loads of *P. destructor* significant for increased risk to bulb and salad onion crops. The 2022 sampling season provided additional sets of samples with which the sampler types could be directly compared. Numbers of spores detected from the different sampler types varied greatly during the same weekly sampling period. Overall the SporeStick impaction sampler detected the highest number of spores with only 2 instances (of 10) where the Burkard recorded larger spore counts, and 1 timing where the GRIPS detected the greater number. These results validate the use of rotation impact samplers as a detection method for ODM spores. Several problems were encountered with the SporeStick sampler over the course of the project. Initial problems with the battery pack provided by the supplier were overcome by changing to a larger leisure battery with an attached solar panel. Charging of the Optisense battery pack was also improved by individually charging the batteries inside the battery pack case with a standard battery charger, rather than the charger and cable provided by the supplier. There were also issues with the rotation arm from the SporeStick sampler becoming loose and detaching from the main unit. The arm could be reattached using a small allen key although this was awkward, and problems remained with several units. Advice from the manufacturer suggested supergluing the screw in to hold the rotation arm in place however this fix was not successful, and a new unit had to be sent. This led to the loss of over five weeks of data for the Hainey site whilst a solution to the problem was sought. Since the project was initiated the Optisense SporeStick sampler is no longer commercially available and can therefore not be recommended for future use for spore sampling. GRIPS and Rotorod rotation air samplers are still currently commercially available and use in this project has shown them to be a reliable alternative to the SporeStick.

Results derived during the study validate the use of LFD's to detect spore presence. Validation of the LFD at Fera using known spore numbers gave similar results to GAD with cube readings of 5.2 (Fera testing) and 15.5 (GAD testing) for 5000 spores, 80.3 and 82.5 for 500 spores and 120.8 and 130.5 for 50 spores. Readings at Fera gave a lower cube reading value at all spore counts therefore if sample results were only compared to the GAD calibration curve, a higher spore count would be recorded. The GAD curve was provided for the batch of LFD's sent in 2021 with the Fera validation calibration curve using LFD's provided in 2022. No new calibration was sent with the new batch of LFD's therefore it is unknown as to whether this difference may be due to the new batch of LFD's or to the accuracy of the LFD

to reliably quantify spore numbers. It must be assumed that new batches of LFD's are validated against a known number of spores by GAD as validation would likely not take place in the field were the LFD's commercially available. The GAD calibration curve suggests you cannot accurately identify spore loads <50 (potentially closer to <100 as the next point on calibration line is 500 spores). Testing of an increased range of spore counts at Fera confirms the limit of detection for the LFD is somewhere between 10 and 50 spores with no effect of the media into which the spores were spiked, however at lower spore concentrations readings are more variable. This could be important if you want to identify early low spore loads or if we need to accurately determine an accumulative spore load (and there are lots of days with low spore loads) which would then significantly change the estimate for the onset date of the epidemic. All samples from the 2022 sampling season recorded high spore numbers from the very start of sampling therefore this variation at low counts was not an issue. Results recorded at the Fera site by LFD analysis showed spore counts of approximately 1000 spores per week even from the start of sampling in April, when no onions in the vicinity were showing symptoms of ODM and no onions had previously been grown in the surrounding area. This value seems very high as no spores would be expected in this situation. However, the increase and decrease in spore counts detected by the LFD do correspond well with infection and sporulation seen on overwintered plants in pots and within the crop which gives confidence in the LFD in detecting trends in spore numbers, if not with accurate spore counts.

There are still concerns around the user friendliness of the LFD in its current form. The LFD is provided as a dipstick rather than a fully housed test kit. Ball bearings need to be added to the tube containing the sample stick and shaken for 1 minute (figure 12a). How well the tube was shaken could impact the disruption of any spores on the stick into the buffer, leading to variation and inaccuracies in cube readings and ultimately spore counts. A conjugate disc also needs to be added by the user which risks cross contamination, after which the stick needs to be dipped into the sample tube, which is difficult without having sample material sticking to the side of the LFD (figure 12b). In addition, the LFD needs to be read after 15 minutes, any readings taken before or after the specified time resulted in a different cube reading score and therefore spore count (figure 12c and d). These challenges raise concerns about the usability of the LFD in the field by growers, who may be unlikely to read the LFD after exactly 15 minutes and may find the testing process unwieldy.



a b c d

Figure 12 a) sporestick in tube with ball bearings, b) with LFD strip added after shaking, c) LFD strip in holder after 15 minutes incubation, d) cube reader with score after reading LFD strip

Validation of the PCR and LAMP assays has been completed, with primer sets developed for both assays capable of detecting a minimum of 10 spores in spiked samples. Results from testing on samples extracted from different media were highly variable by both testing methods. LAMP testing is a desirable PCR method as it provides results within 30 minutes and can potentially be used in the field. Results from extracted samples were highly variable and there was very little difference in time to detection between 10 and 50 spores and 100 to 10,000 spores on spiked dilution series samples. It is therefore not suitable as a method for quantification of spores and further validation and refinement would be needed for it to be used as a diagnostic method for field samples. When field samples were compared by both LFD and TaqMan® PCR, the LFD consistently recorded much higher spore counts. On 2 occasions when the LFD recorded spore numbers in excess of 10,000 spores, spore counts by TaqMan® PCR were just 9 and 26. Sporulation seen on onion plants in the area during this time would suggest that the LFD results were accurate and that the PCR results were lower than expected. This may be due to the DNA extraction method chosen and could be remedied by further work on different methods of extraction from the sample and disruption to remove the spores from the sample stick into the extraction buffer. The Nucleospin™ Plant II kit method was used in this project as this is a method that has been used previously to effectively extract from Burkard and Rotorod air samplers.

Monitoring at sites showed spore numbers in 2021 generally being low before mid-April with more frequent ‘high’ spore loads from then on whereas in 2022, high spore numbers were recorded from the start of monitoring in early to mid-April which continued thought out the monitoring period. Despite high spore numbers, infection was only seen at the Hainey site in 2021 whereas in 2022, infection was seen at all sites apart from Hainey. Unfortunately, the

additional data from the 2022 monitoring season does not increase the ability to clearly define spore thresholds for improved spray decisions for disease management. One site had infection within the crop before monitoring commenced and one site did not record any infection. Of the two sites that did identify infection, Croxton recorded between 4000 and 6000 spores from the multiple samples taken in the 2 to 3 weeks preceding disease being observed in the crop whereas at Fera, this number was between 5000 and over 10,000. Data from the Hainey site in 2021 indicates spore counts reached a peak of approximately 3500 in the 2 to 3 weeks preceding sporulation being observed in the field. This suggests that a minimum of 3500 spores are needed for infection although further research would be needed to validate this. Where environmental conditions are more favourable for infection, such as a longer period of humidity greater than 95% overnight, a smaller number of spores may result in higher levels of infection compared to when conditions are less suitable. Results from the two years of sampling data indicate that where onions had previously been grown or were grown almost year-round, ODM spores seemed to be present at levels likely to cause infection from early in the season. A lack of conditions required for infection may explain why several sites in 2021 and 2022 did not record any disease despite having numerous occasions when spore numbers were high enough to produce infection.

As downy mildew can only be controlled using protectant fungicides, it is important to know when spore numbers, and therefore risk of infection, are increasing. Although there may still be issues around the LFD detecting spores accurately, they are useful in showing the general trend of spore numbers, with numerous occasions where an increase in the number of spores above a background level has been followed by disease seen in the field. Further testing at an increased number of locations would help to validate this result. Daily monitoring of spore numbers by LFD may be required which, although relatively costly with the LFD costing just over £23 per unit, may be a price the industry would be willing to pay, particularly for crops such as salad onions where the leaf needs to be disease free for retail and infection by downy mildew can account for up to 10% crop loss costing approximately £1 million.

High levels of infection were seen in 2021 however the forecast (based on the Millioncast onion downy mildew model), used to provide the infection and sporulation risk plots within the CropMonitor Pro onion downy mildew module, did not always correlate to infection seen in the field. This may be due to the forecast using met office weather station data from the local area to predict the risk. However environmental conditions can very greatly within a local area, even within field, so it may be the weather data for the location wasn't precise enough for an accurate risk prediction. In order to give more accurate risk predictions, ideally a weather station should be located at each rotorod/trapping station to provide field specific conditions.

For future development, ideally an automated spore trap reporting daily readings would be located in each onion field, alongside a weather station to provide accurate data. If infection is known to start in a specific location within the field each year, the trap should be placed in that location to detect any increase in spore numbers at the earliest opportunity. The real time PCR and LAMP primers developed within this project have been validated to detect low numbers of *Plasmopara destructor* spores with high specificity and no cross reactivity with other downy mildews or pathogens likely to be found in onion crops. Further testing and development of extraction methods could enable these primers to be used within an automated trapping system in the future.

Knowledge and Technology Transfer

The main report describes website enhancements made to the onion downy mildew module on CMP and the user questionnaires developed to assess the grower's experience with using the different types of spore traps.

Fera attended CropTec 2021 during 24 and 25 November and spoke to attendees amongst other things about their decision support development, spore trapping and diagnostic testing expertise.

Project outcomes were disseminated at the October 2021 and February 2021 AHDB progress meetings as well as at the February 2022 and October 2022 BOPA update meetings.

No other events have taken place due to Covid-19 restrictions, which also meant that the on-farm demonstrations could not take place.

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Appendices

Appendix A. GLOBAL ACCESS DIAGNOSTICS (GAD) (FORMERLY MOLOGIC) Onion Downy Mildew Assay Method

- Invert dropper bottle and add 5 ‘hanging’ drops (200µL) of sample buffer to sample tube
- Leave for 30mins on the bench
- Add 1 conjugate disc to the sample tube and mix with sample buffer by flicking bottom of tube for 10 secs. Leave for 5 minutes and mix again for 5 secs
- Add test strip into sample with red ‘hold tab’ at the top
- Leave strip to run for 15 minutes
- Remove strip from sample and place in cube holder with base outside of reader and top butted at end of strip holder (refer to picture). Close holder and read (as per SOP197)
- Record T (test Line) reading and check for visible control spot/line

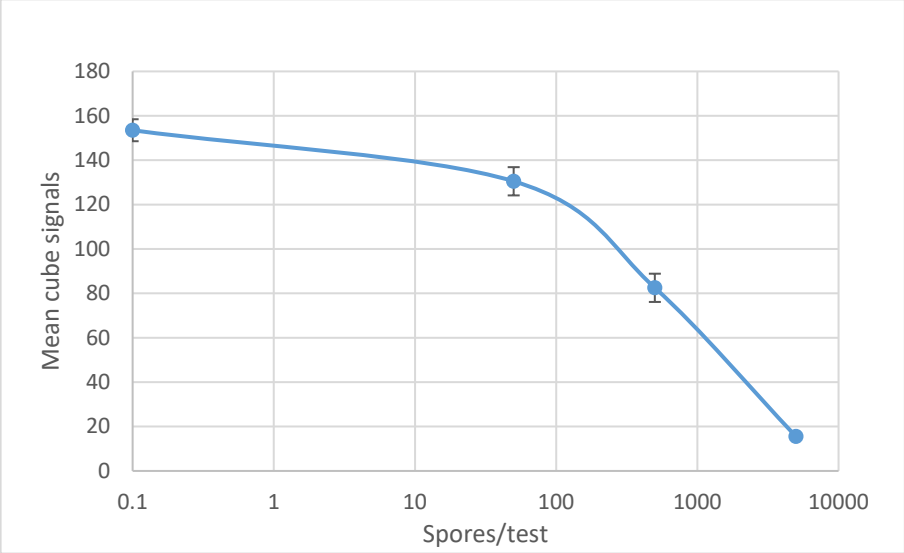

Figure A1. Typical GLOBAL ACCESS DIAGNOSTICS (GAD) (FORMERLY MOLOGIC) onion downy mildew LFD assay performance

Appendix B. Grower feedback

This Appendix list the questions included in the grower feedback questionnaire and the responses provided. The questionnaire was sent to the four growers running the five sites and the AHDB project officer. Only two responses were received.

1. Thinking about setting up the spore traps, please rate the ease of setting up
    a. SporeStick
        i. Easy: 1
2. Please use this space to provide any additional feedback/details on challenges faced setting up the spore traps
    -
3. Thinking about maintaining the SporeStick Spore Trap, please rate the device's performance against each category
    a. Lifespan of batteries:
        i. Good / Easy: 1
    b. Reliability of device
        i. Poor / Difficult: 1
    c. Sample changeover
        i. Very good / Very easy
    d. Ease of operation
        i. Good / Easy: 1
    e. Robustness of device
        i. Good / Easy: 1
4. We are keen to make improvements ahead of the next season, please use this space to provide thoughts/recommendations on alterations we could make to the running of the spore trap network for future use.
    -
5. Thinking about the sampling protocol, please rate the sampling process against each category
    a. Clarity of instructions
        i. Very Good / Very Easy: 1
    b. Complexity of protocol
        i. Very Good / Very Easy: 1
    c. Troubleshooting
        i. Good / Easy: 1
    d. Length of sampling season
        i. Good / Easy: 1

e. Sampling frequency and in-season changes
    i. Good / Easy: 1
f. Disease Observations
    i. Good / Easy: 1

6. So we can recommend improvements the process in the future, we'd like to better understand the challenges growers faced in-field in relation to the follow aspects of the protocol where issues were experienced at some sites/during some periods of the season
    a. Sampling twice per week until spores present at site – No issued encountered by site manager: 1
    b. Sampling weekly once spores detected at the site – No issues encountered by site manager: 1
    c. Sampling season concluding end of July - No issues encountered by site manager: 1
    d. Weekly disease observations - No issues encountered at site manager: 1
    e. Samples return in unlabelled sample bags / tubes - No issues encountered by site manager: 1
    f. Different number of samples returned vs. protocol - No issues encountered by site manager: 1
    g. Snapped sampling sticks not containing a full sample - No issues encountered at site manager: 1
7. We are keen to make improvements ahead of future sampling seasons. Please use this space to provide further feedback/comments on issues experienced with the sampling protocols and logistics process and where it could be improved.
    -
8. General feedback on CropMonitor Pro
    a. Ease of registration
        i. Good: 1
    b. Ease of navigation Good: 1
        i. Good: 1
    c. Reliability of service
        i. Poor: 1

9. Thinking about the data presented to you on ODM risk on CropMonitor pro - did you use this information for in-field decision making?
    a. Yes: 1

10. Thinking about the cube-reading score for samples tested for your site, did you use these results to make decisions in-field?
    a. No: 1
11. If yes, please describe how you used the cube reading score in your in-field decision making
    -
12. Thinking about Fera's coordination and management of 2022 sampling, please rate each category
    a. Support during the setup of traps
        i. Good: 1
    b. Support with troubleshooting trap issues
        i. Good: 1
    c. Support with sampling
        i. Good: 1
    d. Frequency of updates from project team
        i. Good: 1
    e. Awareness of delivery plan / next steps
        i. Good: 1
13. Please use this space to provide further comments/feedback on management and communications within the ODM project
    -