Project title:	Development and testing of a lateral flow device for both gummy stem blight and powdery mildew in bio-aerosols during cucurbit production
Project number:	CP137
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Report:	Annual report, November 2016
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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.

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CONTENTS

GROWER SUMMARY

Headline

Air samplers have been used to monitor glasshouse air samples for spores which spread 'Myco' disease and cucumber powdery mildew. Laboratory and a 10 minute on-site test have been used to estimate low, moderate and high risk warnings of Myco inoculum in air samples. Time between infection and disease symptoms can be as short as two weeks. Control regimes for each risk warning are listed under Grower Action Points. Tests for powdery mildew will be assessed in 2017. Both species of cucumber powdery mildew have been found on UK grown cucurbits. In commercial production *P. xanthii* seems dominant.

Background

Disease spread: In the airborne environment many plant diseases are able to spread between and within cropping systems. By laboratory analysis or a field based lateral flow test, AHDB Horticulture (previously HDC) funded research has provided development of systems to monitor field inoculum (plant pathogenic spores) in bio-aerosols either on a daily or weekly basis. Tests have been developed to monitor a range of vegetable plant pathogens: *Peronospora destructor* (onion downy mildew), *Mycosphaerella brassicicola* (ringspot), *Alternaria brassicae* (dark leaf spot), *Pyrenopeziza brassicae* (light leaf spot) and *Albugo candida* (white blister). By identifying inoculum in air samples, growers are able to time sprays more effectively and make informed decisions as to which type of fungicide application to make. Project PE 001 Cucumber: Improving Control of Gummy Stem Blight, developed a laboratory test to monitor glasshouse air samples for *Mycosphaerella melonis* (Myco) spore presence. With knowledge of 'Myco' inoculum, this current project aims to add to work carried out in PE 001 and provide improved fungicide efficacy by their timed application. Diagnostic probes to *Podosphaera xanthii* and *Golovinomyces orontii* have also been developed for use within an integrated disease management system for the effective control of powdery mildew on cucumber.

Myco (Black stem rot or Gummy stem blight):

The causative agent of 'Myco' on cucumber is *M. melonis* (syn. *Didymella bryoniae).* The disease is of worldwide importance, with significant economic damage of cucurbits crops. The pathogen causes extensive stem & leaf infections which when severe can debilitate or even kill plants. As with the powdery mildew pathogen, airborne spores are produced and involved in the spread of the disease. The infection of flowers and developing fruit leads to fruit rot, though in many cases often disease symptoms are not visible until the fruit is marketed. This

leads to rejection and reduced retailer and consumer confidence in the product. Fungicides are used routinely in an attempt to suppress the disease and prevent plant and fruit losses. However, these had been found to provide only a partial suppression or reduction of the disease. No resistant cultivars are available and there is a suggestion that mildew tolerant cultivars are more susceptible to *Myco*.

Cucumber Powdery Mildew:

Numerous vegetable crops are susceptible to powdery mildew, but cucurbits are one group that are severely affected, and are a crop where fungicides are used routinely for control. It is probably the most common, widespread and easily recognizable disease of cucurbits. Like other powdery mildew diseases, its symptoms are characterized by the talcum-like, powdery fungal growth that develops on top and bottom leaf surfaces, petioles and stems but rarely on fruits. *Podosphaera xanthii* (also known as *P. fusca*) and *Golovinomyces orontii* are the main agents of cucurbit powdery mildew. The disease provides one of the most important limiting factors for cucurbit production worldwide, and in the absence of chemical, biological control or the use of tolerant/resistant varieties, can result in yield reductions as high as 40% . Poor ventilation, reduced light intensity i.e. partial shade and succulent plant tissue promote disease development, with it being spread via spores (conidia) to other plants on air currents. Although favouring dry conditions, spore release (disease dissemination) can occur at a range of high humidities and infection can occur without the necessity of a water film on the plant surface. On mainland Europe, *G. orontii* has been reported during early season cropping preferring a dry climate, whilst *P. xanthii* dominates during the summer months as humidity is increased.

The pathogen is unable to survive for more than a few days in the absence of a living host. The length of time between infection of the host plant by the spore and symptom appearance can be as short as 7 days but can take longer than this if conditions are below optimum for the infection process. At present, growers only know that powdery mildew is present once symptom development is observed and the pathogen is established within the crop. The application of fungicides is the principle practice in cucumber cropping for mildew control. However, powdery mildew pathogens have a high potential for fungicide resistance and there is a need for control programmes to be less reliant on blanket spray applications. There are new developments with commercially available bio-control products though in general their level of efficacy is not yet up to the standard required by growers for effective control.

Environment: Information on plant pathogen spore concentration (inoculum load) in air samples should be evaluated with local environmental conditions. The environment will influence infection and disease latent period, which is the occurrence between infection and visual symptom of disease. AHDB report FV 053a ~~reports~~ gives a latent period of up to one month for *Mycosphaerella brassicicola*, the causative agent of ringspot on Brassicas. The environmental conditions, spore concentration, the age of the host and crop variety are all important factors that will influence whether disease will occur and at what level. *M. brassicicola* spores are capable of remaining viable for a period until conditions became favourable for germination. A 48 hr time course experiment at temperatures between 10 and 25°C recorded 100% germination of *M. brassicicola* ascospores. For germination a relative humidity of >93% or surface wetness was required. *M. melonis* ascospores, which have a mucilage coating like *M. brassicicola,* will likely be able to survive for a time on the host surface, whilst ascospores of *Sclerotinia sclerotiorum* survived and retained pathogenicity after exposure to low relative humidity at 25°C for periods up to 12 months.

In Holland, an environmental model is under evaluation for control of Myco in cucumber crops (A. Dijk, pers. comm.). If successful, future work should look to integrate the environmental disease forecast with Myco spore concentration in air samples. This would provide information on when airborne pathogens are present at the necessary concentration required for infection of the crop and whether the environmental conditions are conducive for infection to occur. In this way, an informed decision can be made on when to apply the appropriate control measure. This could be done in an effective and targeted way in advance of infection occurring in the crop. This approach may however not be appropriate for powdery mildew where the environmental conditions during the growing season tend not to be limiting. Nevertheless, monitoring disease could help chemicals be applied in an informed manner to delay the initial onset of powdery mildew infection and perhaps reduce the total number of sprays, minimising the risk of resistance developing in the pathogen population.

Summary

During 2015 and 2016 at protected cucumber production sites in the UK, tests have been used to estimate Myco spores in daily and weekly collected air samples. In year 3 of the project (2017), collected air samples will be assessed for spore types which cause powdery mildew. Two air samplers look suitable for monitoring Myco spore epidemics and are reported below.

A Microtitre immunospore trap (MTIST), available from Burkard Manufacturing (http://www.burkard.co.uk) costs in the region of £1500 plus VAT. This includes the ELISA microtitre well adapter plate (figure 1a). The sampler runs directly off the mains and during trials has operated continuously across the cucumber growing period. Spores in the air are collected into microtitre wells. The air sample (4x8 well microtitre wells) is changed weekly and sent by post to a laboratory for assessment of Myco spore numbers. In a commercial environment, next day courier service (as used by vets) has potential to return results within same day of sample receipt.

Figure 1a. MTIST air sampler with base plate containing 4x8 well microtitre strips.

The ELISA process takes less than 4 hours to generate a Myco disease risk report of low (MTIST ELISA <0.2), moderate (>0.2<0.5) and high (>0.5). Using this technology, Myco spores have been identified in UK glasshouse air samples from March / April onwards. Myco spore levels peak in cucumber production at different times. For example, in 2015 spore levels at site 1 were at their highest in May. At site 2 in July. Site 3, which from the outset recorded the highest concentration of Myco spores in the air, produced the largest spore peak in June (MTIST ELISA > 2.3). All three sites remained at high risk of Myco throughout May to September 2015.

For the 2016 season, a request was made by AHDB to include additional information of Myco spore concentration by time-consuming microscopic examination of the microtitre wells. Reliability of data generated in this way is questionable as visually Myco spores are similar to other ascosporic species and at times the overall spore load is high (Fig 1b). Counts were however made and compared with MTIST ELISA results. At the beginning of the season there

was good correlation but towards the end of the season this relationship drifted. As in the previous year, Myco spores were observed in March with increasing concentration during April. In 2016, spore peaks were generally followed by periods of low spore pressure. As an example an account of site 2 is described below.

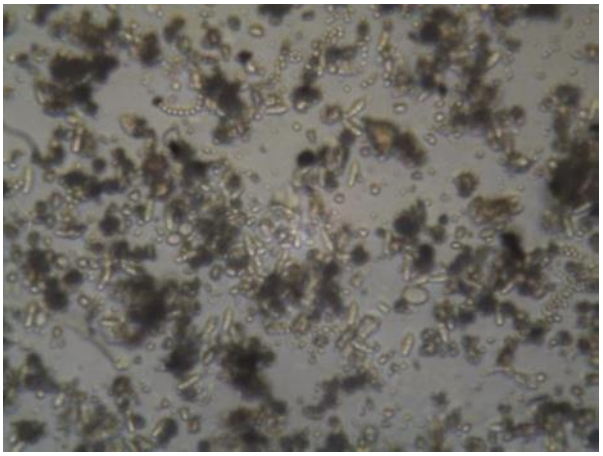


Figure 1b. Glasshouse bio-aerosol sample deposition on the base of a microtitre well as viewed by bright field microscopy (400x)

At site 2, low risk of Myco infection was observed until 15th March (<0.2 MTIST ELISA). Between the 15th and up until the 29th March the threshold between low and moderate risk was reached. Thereafter, Myco spores were identified both by microscopic examination and by ELISA at an increasing concentration. By the end of March the MTIST ELISA results recorded a high risk of Myco in air samples (ELISA > 0.5) and this continued during April. By 3rd May a low risk of Myco spores was reported in air samples. Myco disease was first observed on external plant tissue week commencing 5th April and in harvested cucumbers from the 27th April. If we accept potential for Myco infection from the 15th March (spore availability) a latent period of approx. 21 days is reasonable (i.e. Myco disease observed on 5th April). An application of Reflect was made week commencing 19th April. Production of spores was not affected until beginning of May. With a latent period of 14 to 21 days (dependent on environmental conditions) the next cycle of Myco lesions (disease symptoms) would be expected from the middle to end of May. The black lesions provide fruiting bodies and potential for spore release. Increasing Myco spores were observed from 17th May in air samples and a 'moderate' risk warning issued (MTIST ELISA between 0.25 and 0.5). By the 31st May a 'high' risk of Myco spores was recorded. From these results, it is possible to understand the polycyclic nature of the disease and how spore release can become continuous. By the middle of May the number of cucumbers displaying Myco had increased by over 600% when compared to the initial numbers observed from the 27th April.

The application in 2016 of recently approved fungicides (Talius and Reflect) may have reduced the overall Myco spore load and distinct populations of inoculum observed. Periods of high risk were observed between May and middle of June, 12th July to 15th August, 23rd August to 12th September. The crop was replanted at the beginning of July. Infected fruit were observed on the 19th July. Throughout August disease was observed on a susceptible variety (Snack) whilst the main crop was identified by the grower as 'relatively' disease free. Increasing numbers of infected fruit were observed from the 3rd week in August and an application of Reflect was made. Spore concentrations in air samples fell markedly week commencing 13th September. Conversely, infected fruit increased in concentration. The initial latent period for the second crop was less than 14 days and disease symptoms this time first were observed on the harvested fruit. It is likely that the warmer conditions shortened the disease latent period.

During the cucumber growing season, other air samplers were also operated in the glasshouses. A single tube and multi-vial cyclone air sampler (Figure 1c), available with a timer from Burkard Manufacturing at an approx. cost of £2500 plus VAT, were used to evaluate the potential of an on-site test for daily risk of Myco. The cyclone samplers were run directly off the mains and continuously for the growing period. The samplers were loaded weekly with either one tube (provides a record of what is in the air over a seven day period) or seven tubes (each tube represents a single day over a seven day period. An eight tube samples during the changeover). At the end of each week the tubes were collected and using a lateral flow tested for Myco spores. Air samples collected in a single tube over a 7 day period often contained debris. This compromised the test and the approach could not be used for reliable measurement of Myco spores. Air samples collected over a 24hour period into a tube were not visibly affected by debris accumulation. When these air samples were tested by lateral flow for Myco, spore periods were identified across each cucumber planting. Following each high risk period, Myco symptoms were observed in the crop two to six weeks later.

Figure 1c. Multi-vial air sampler with eight collection tubes.

To conclude, different air sampler and test formats have been used to predict when Myco spores are at a concentration in air samples to cause gummy stem blight on cucumber crops. It is estimated that the time from risk of Myco in air samples and symptom development on a cucumber crop is between two to six weeks. The time period will vary depending on the environment and if control treatments are applied.

Assessment of the different air sampling formats for measurement of Myco spores also showed that during early and mid-season there was good agreement between the different test types. However, in the latter part of the growing season this relationship broke down. A study in America has shown three different species causing gummy stem blight on cucumber (also other cucurbits). Only two of which look very similar by microscopic analysis. The potential exists for more than one species to occur in the UK and at different times during cucumber production season. This could account for the seasonal variation observed between the morphological test and the biomarker test (MTIST ELISA and lateral flow) in measurement of the Myco spore type (*M. melonis* but also known as *Didymella bryoniae*) commonly associated with gummy stem blight disease.

In Year 3 of this program of work studies will extend to the measurement of the two spore types (*G. oronti* and *P. xanthii*) which cause cucumber powdery mildew disease. In this project we have by DNA analysis, established that both of these spore types occur on cucurbits grown in the UK. Although, results to date indicate that *P. xanthii* is dominant in commercial cucumber production.

Financial Benefits

The main financial benefits will be in the use of these tests to reduce unnecessary crop protection inputs or to apply timelier crop sprays to cucumber cropping systems. Fungicide usage is costly and can be one of the major inputs in crop production after fuel and labour. Using the lateral flow device the grower/consultant will be able to check for Myco spores in the air and better time the first fungicide application. Targeted application of control measures will help delay the onset of pathogen resistance to fungicides, thus prolong their useable life. The cost of these tests must be compared with a typical spend of £200 per hectare for materials and labour for a single fungicide treatment. In high risk years it is possible to spend in excess of £4,200 per hectare on fungicide applications. However savings will be variable between years and depend on the number of spray applications made to the crop

Action Points

During 'inoculum' low risk periods

Rigorous hygiene

- Between crops remove all crop debris, clean thoroughly and sterilise (to include wires). Jet 5, chlorine bleach and Unifect G effective on four surface types. Fam30 less effective on concrete, Menno Florades less effective on aluminium and concrete (AHDB PE001a).
- Wash hands in soap and water followed by alcohol gel or foam. Soak cutting knives regularly. Disease can be spread between plants on infected knives.
- Remove all dropped fruit and all diseased fruit and plants as soon as they are seen.

Environmental

- Spore release is significantly greater between 16:00 and 07:00 hrs. This coincides with optimum conditions for infection (vents may be shut and RH levels are likely to be higher).
- Avoid reaching dew point (when vapour in air converts to water on plants) by partially opening vents.
- Apply heat boosts together with ventilation to keep foliage dry (particularly early in the morning). Avoid early morning irrigation.

Moderate risk

- Continue with low risk measures. According to manufacturer's guidelines and legislative regulations apply Signum, Talius or Reflect to the stem base.

High risk

- Continue with low risk measures. According to manufacturer's guidelines and legislative regulations apply Signum, Talius or Reflect to protect the whole crop. Note one application per crop Talius and two applications of Signum **or** Reflect.

SCIENCE SECTION

Introduction

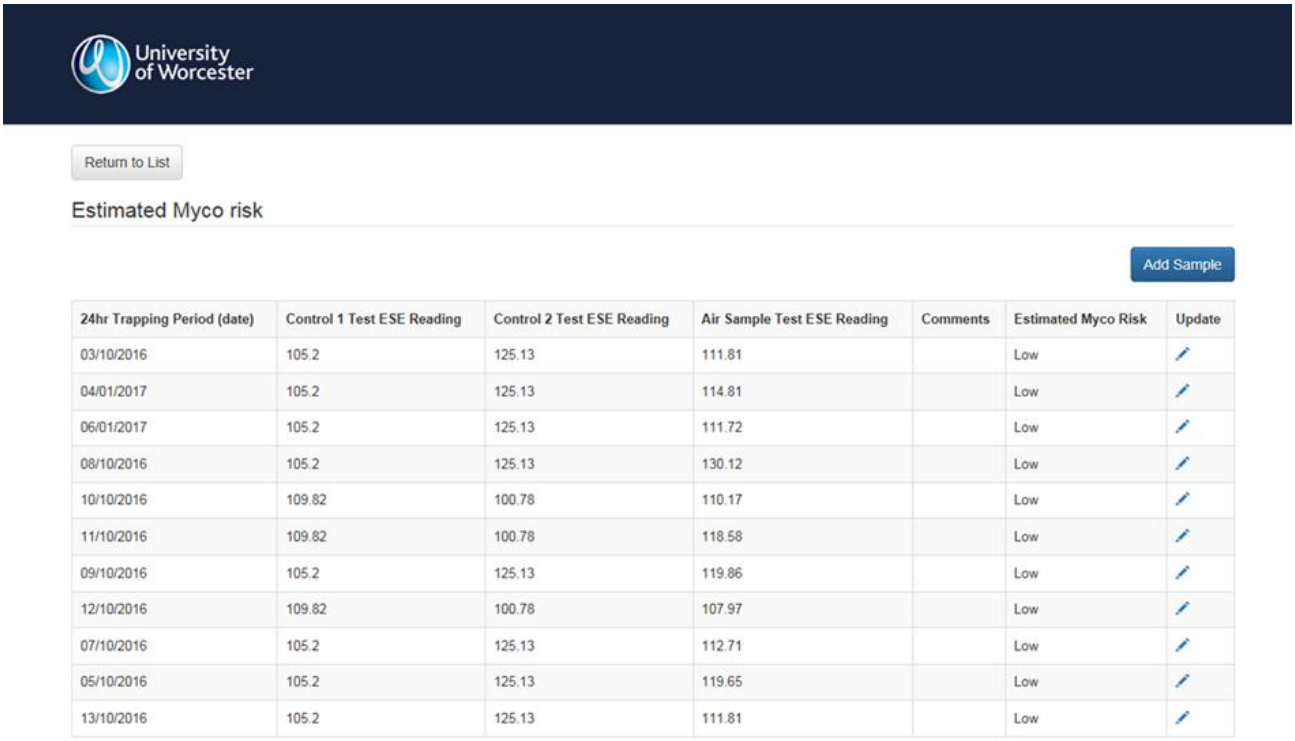
CP 137 aims to develop diagnostic assay systems which can be used to monitor glasshouse air samples for causative agents of gummy stem blight and cucumber powdery mildew. It is expected that these systems will form part of an integrated disease management system and determine when and what type of fungicide application should be made to prevent infection and arrest disease development. Monoclonal antibody cell lines have been developed to *Mycosphaerella melonis* (Myco), *Podosphaera xanthii* and *Golovinomyces orontii* (causative agents of cucumber powdery mildew). During this project the diagnostic probes will be utilised within assay formats and assessed for capability to monitor glasshouse air samples for target disease across the growing season. In this report, we describe Year 2 project activities where between March and November 2016, air sampling equipment was operated at selected UK commercial cucumber holdings.

Materials and methods

Environmental monitoring studies

At two cucumber commercial holdings, air samplers were operated as described in CP137 AHDB Year 1 annual report (2015). As in 2015, the microtitre well strips (4 x 8 wells) of the Microtitre immunospore air samplers (MTIST) were removed after each seven day exposure period and two of these processed by enzyme-linked immunosorbent assay (ELISA) to determine risk of Myco inoculum (*M. melonis* ascospores). The remaining two strips were stored at -20°C. In Year 3 of the project, these strips will be used to assess potential of an ELISA test to quantify trapped cucumber powdery mildew spores (*P. xanthii* and G. *orontii*). 2016 results of Myco inoculum load in the MTIST trapped air samples were relayed to participating growers within 3 to 7 days of sample receipt. The results were provided in the form of absorbance values which were plotted on a graph. A standard Myco positive control was run on each occasion to correct for variation in weekly assay conditions. As determined in year 1 of the project, the absorbance values provided an estimate of Myco risk: low (MTIST ELISA <0.2), moderate (>0.2<0.5) or high risk (>0.5). Environmental conditions or cultivar were not assessed. Disease observation and fungicide crop application records were maintained by the participating grower. In addition to analysis by ELISA, Myco spore concentration was estimated by microscopic examination of the microtitre wells (x400). Well counts were made blind and compared with MTIST ELISA results.

Air samples collected using cyclone traps (multi-vial and single vial cyclone, as described in Year 1 AHDB CP137 report) were sent weekly to the University of Worcester. The weekly and daily tubes were either assessed by lateral flow for Myco or stored at -20°C for later assessment of powdery mildew risk. From 20th September, Myco lateral flow devices were trialled on-site by growers to directly assess daily risk of Myco (multi-vial cyclone air sampler). A web interface was developed for growers to submit test and control results (Fig 2). Based on the control values, variation in weekly assay conditions (e.g. temperature, test batch and storage) was considered. The test and control results were compared with a laboratory Myco standard curve, the results adjusted accordingly and electronically reported to the grower.



24hr Trapping Period (date)	Control 1 Test ESE Reading	Control 2 Test ESE Reading	Air Sample Test ESE Reading	Comments	Estimated Myco Risk	Update
03/10/2016	105.2	125.13	111.81		Low	
04/01/2017	105.2	125.13	114.81		Low	
06/01/2017	105.2	125.13	111.72		Low	
08/10/2016	105.2	125.13	130.12		Low	
10/10/2016	109.82	100.78	110.17		Low	
11/10/2016	109.82	100.78	118.58		Low	
09/10/2016	105.2	125.13	119.86		Low	
12/10/2016	109.82	100.78	107.97		Low	
07/10/2016	105.2	125.13	112.71		Low	
05/10/2016	105.2	125.13	119.65		Low	
13/10/2016	105.2	125.13	111.81		Low	

Figure 2. Screen save of the Myco lateral flow test result interactive web interface (Site 1)

Myco lateral flow protocol and standard curve

Multiple prototypes were assessed for measurement of *M. melonis* ascosporic inoculum (Myco spores). The method described below is the optimised format used in Year 2 studies.

The lateral flow test framework was constructed using a Millipore 180 HiFlow™ cellulose ester membrane direct cast on to a Mylar backing (Cat No. HF180MC100, Millipore Corp., USA) attached at either end to an absorbent pad (Cat No. CF6 (Cat No.8116-2250, Whatman), and a sample pad (Glass Fibre pad Cat no. GFCP203000, Millipore). A pre-filter VF2 pad (Cat no.

8124-6621, Schleicher and Schnell, Whatman) was also incorporated between the sample pad and the membrane. This is to prevent particulate material and spores from travelling into the membrane, disrupting assay flow and blocking membrane pores. A test line of anti-mouse IgM (µ-chain specific) antibody produced in goat (Sigma M8644) at (0.5mg/ml) was applied to the cellulose ester membrane surface using a flat-bed air jet dispenser (BioDot Ltd, The Kingley Centre, West Sussex, UK) operating at a line travel speed of 15m s^{-1}. The sprayed membranes were air dried overnight at room temperature (18 - 20ºC) and cut in to 5 mm width strips. A volume of UW 325 in conjugate buffer was mixed with 5µl of an anti-mouse IgM 40nm gold conjugate (Code BA GAMM 40, British Biocell International, UK). 30µl of the antibody/ gold conjugate solution was pipeted on to individual competitive lateral flow device conjugate sample pads, air dried at 37ºC for 35 min and each pad was attached to the test strip The lateral flow devices were mounted within a plastic housing device (European Veterinary Laboratory, Netherlands : www.evlonline.nl) as shown in Figure 3a. Test and control line development was recorded using an ESE portable reader (Fig.3b)

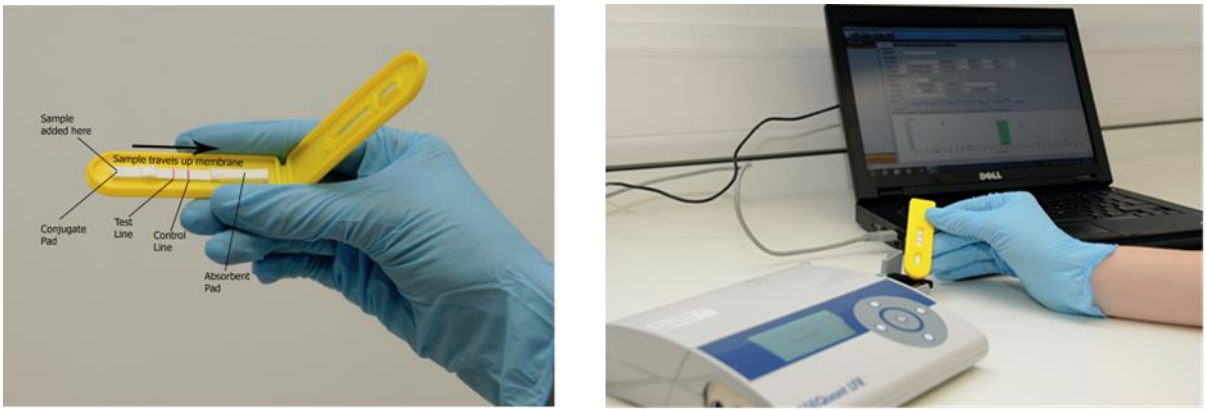


Figure 3. A lateral flow with test and control development (a) and quantitative measurement using an ESE Quant digital test reader (b).

Standard curve: Ascosporic inoculum of *M. melonis* was collected according to the methods of Kennedy (1999) and a soluble spore antigen preparation made (Kennedy and Wakeham, 2008). A doubling dilution series was prepared and dropwise 100µl aliquots of each dilution was aliquoted to the lateral flow devices. At 10 minutes, test line formation was measured using an ESE lateral flow reader.

Myco environmental daily cyclone grower lateral flow test protocol:

Growers were supplied with Myco lateral flow devices, antibody conjugate pad in a vial, extraction buffer, disposable micro pipettes and an instruction sheet (Figure 4). Daily cyclone collected air samples sent to the University of Worcester for testing were assessed for 'Myco inoculum' in the same way. Debris concentrated in weekly air samples were removed prior to

assessment by a wash of phosphate buffered saline 0.05% Tween 20 (PBST). The rationale for this is explained in the conclusions section and corresponds to a pre-wash stage that is used during the MTIST ELISA assay.

Grower lateral flow protocol

- Remove tube from air sampler and place in holder. This is known as the air sample collection tube.
- To Tube A (contains conjugate pad) add contents of Tube B using plastic pipette dispenser. Ensure that contents of Tube B are drawn slowly into the plastic pipette and slowly released into Tube B. Avoid creation of air bubbles. Press the conjugate pad firmly down into the liquid using the pipette device. Fold or squash the pad as necessary to ensure it is submerged. Firmly close the lid of Tube A and flick the bottom of the tube 5 times. Using the pipette device slowly draw the liquid up and down three times over the conjugate pad. Avoid creating air bubbles. The liquid will slowly go pink in colouration. Allow the tube to stand for 2 minutes.
- Holding the plastic pipette firmly down on the conjugate pad and being careful to avoid creation of air bubbles draw all the pink liquid from Tube A and transfer to the air sample tube. Flick the contents of the tube five times to allow liquid phase coverage of the tube side wall. Then let the tube stand for five minutes. This incubation period allows the probe to bind with Myco spores if they are present. Myco spores are sticky and adhere to the inner tube wall.
- Using the plastic pipette carefully transfer all of the liquid to the sample pad window of the lateral flow (Fig 3a). Do this dropwise so that the window does not flood. After the final drop set your timer for a 10 min period. At 10 min. Read the test using the ESE reader (Fig 3b).
- Repeat process with standard control tubes.

Figure 4. Lateral flow environmental grower kit with instruction sheet.

Assessment of cucumber powdery mildew species in UK production by molecular analysis

Infected cucumber leaves were provided by three commercial producers on four occasions between June and August 2015. In October, samples were received from 6 commercial producers and one 'non' commercial propagator. To determine whether infection was caused by *Podosphaera xanthii* or *Golovinomyces orontii*, leaf discs of 2.5cm diameter were cut from three infected leaves from each of the nurseries. To remove any sampling bias the leaf discs were taken as shown in Figure 5. Ten leaf discs were taken in total from each leaf, with two leaf discs making up one sample therefore giving five samples per leaf. DNA was then extracted from each sample using the Fast DNA Spin kit (MP Biomedicals) following the manufacturer's protocol prior to PCR amplification. The PCR reaction comprised 1x BioMix Red (BioLine and 0.16µM each primer, with 5µL DNA added to each 25µL reaction mix. The primer sequences and cycling parameters were as detailed in Chen *et al.* (2008) and the products were run on a 1.5% agarose gel in 1xTBE containing 1xGelRed (Biotium Inc. USA) and the products visualised in a Bio-Spectrum imaging system (UVP, LLC, USA). The occurrence and severity of infection by each species could then be assessed on the leaves throughout the season.

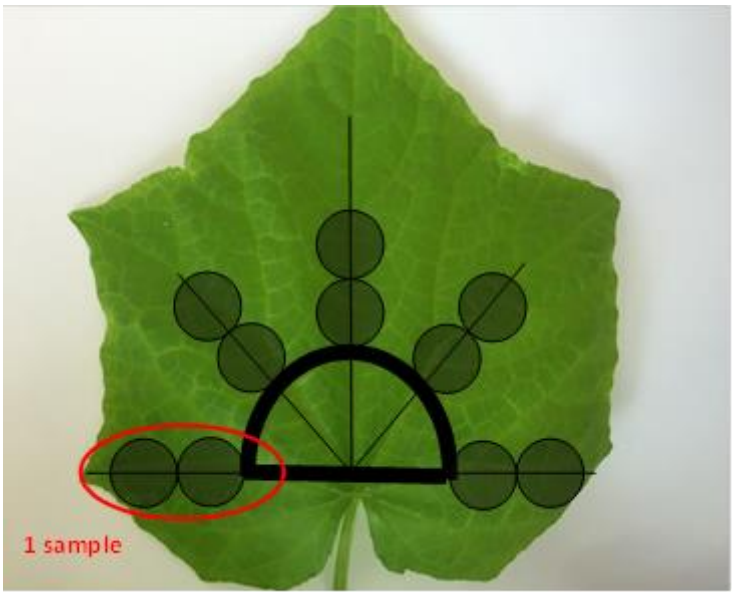


Figure 5. Sampling of 2.5cm leaf discs from cucumber leaves infected with powdery mildew; two adjacent leaf discs formed one sample.

Results

Environmental monitoring studies

Estimation of M. melonis *in air samples by MTIST ELISA and microscopic counts*

At sites 1 and 2, the weekly concentration of *M. melonis* spores trapped on the base of microtitre well strips was estimated by microscopic examination. On occasion, when spore load (all spore types trapped) was high, it was not possible to estimate. Of those counts made, when compared with ELISA results for the corresponding sampling period, an association of *M. melonis* spore concentration was at times observed at each of the sites. At the beginning of the season this relationship was highly correlative (r^2=0.8409) however as the season progressed a decline was observed (Figure 6 a,b,c and d). During the final weeks of the monitoring season there was divergence between microscopic estimation and measurement of *M. melonis* concentration by MTIST ELISA (Figure 7 a, b). At site1, an increasing concentration of *M. melonis* spore concentration were estimated by light microscopy from the 17th October and at site 2 from the 26th September 2016. The MTIST results for this period were low.

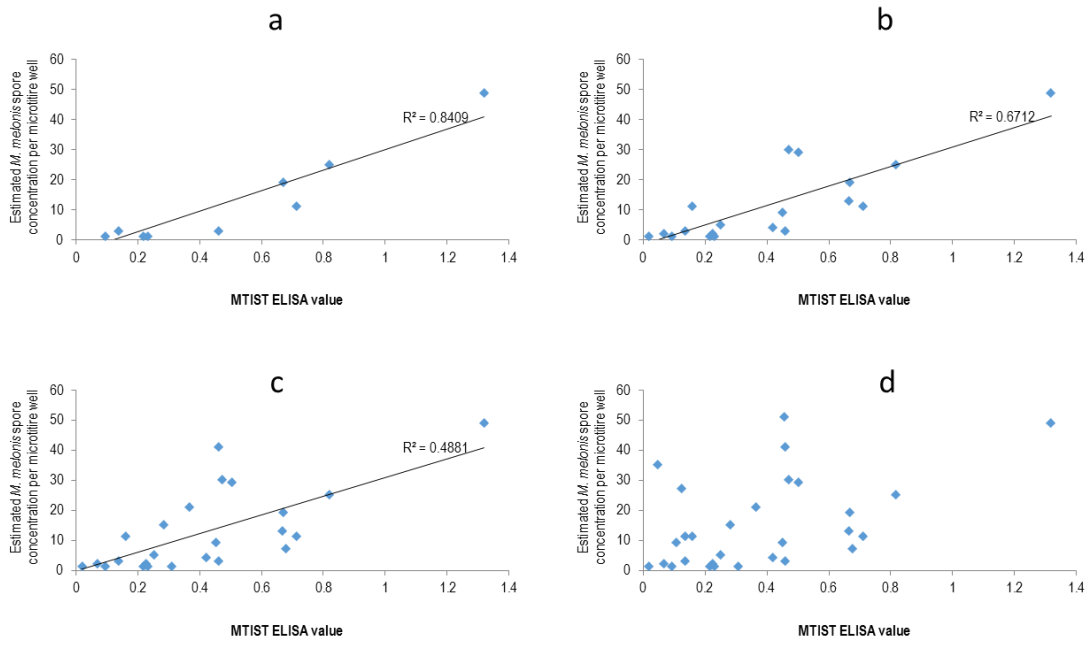


Figure 6. Relationship of MTIST ELISA and microscopic analysis of MTIST wells for estimate *of M. melonis* ascospore concentration. (a) weeks 1 -10, (b.) weeks 1-20, (c) 1-25 and (d) weeks 1-33

Estimation of disease risk, control strategies applied and disease observation

In year 1 of the project, the absorbance values generated by the MTIST ELISA were characterised as an estimate of *M. melonis* risk: low (MTIST ELISA <0.2), moderate (0.2<0.5) or high risk (>0.5). This information was not integrated with environmental conditions or varietal crop information. Based on this guidance, at site 2, low risk of *M. melonis* inoculum in air samples was observed until 15th March 2016 (<0.2 ELISA, Fig. 7b). Between the 15th and up until the 29th March the threshold between low and moderate risk was broken. Thereafter, *M. melonis* ascospores were both by microscopic examination and by ELISA identified at an increasing concentration. By the end of March the MTIST ELISA results recorded a high risk of *M. melonis* in air samples (MTIST > 0.5) and this continued throughout April. By the 3rd May a 0 reading was recorded (no risk of *M. melonis* in air samples). Myco disease was first observed in the crop week commencing 5th April and on harvested fruit from the 27th April. By mid-May, harvested cucumbers with Myco symptoms had increased by over 600% compared to initial symptoms observed on 27th April. This level of infection remained until harvest. An application of Reflect (isopyrazam) was made week commencing 19th April. Spore concentrations were seen to rise and fall.

During May and mid-June, from 12th July to 15th August, 23rd August to 12th September high risk of *M. melonis* spores in air samples were reported (Fig. 7a). The cucumber crop was replanted at the beginning of July. Myco infected fruit were observed within 14 days of planting. The level of infection remained constant and described by the grower as at a 'relatively low' level until 3rd week in August. On a susceptible variety (Snack), which resided next to the main cucumber crop, disease was observed at a moderate to high level. During the 3rd week in August, harvested cucumbers of the main crop showed an increase in Myco infection. In response an application of Reflect (isopyrazam) was made on the 26th August. Spore concentration in air samples fell week commencing 13th September. However, the rate of Myco infection in harvested cucumbers continued to rise until final harvest in November. From the 13th September onwards increasing numbers of spores with morphological characteristics similar to *M. melonis* were observed on the base of microtitre wells BUT the ELISA signal remained at low risk until week commencing 24th October.

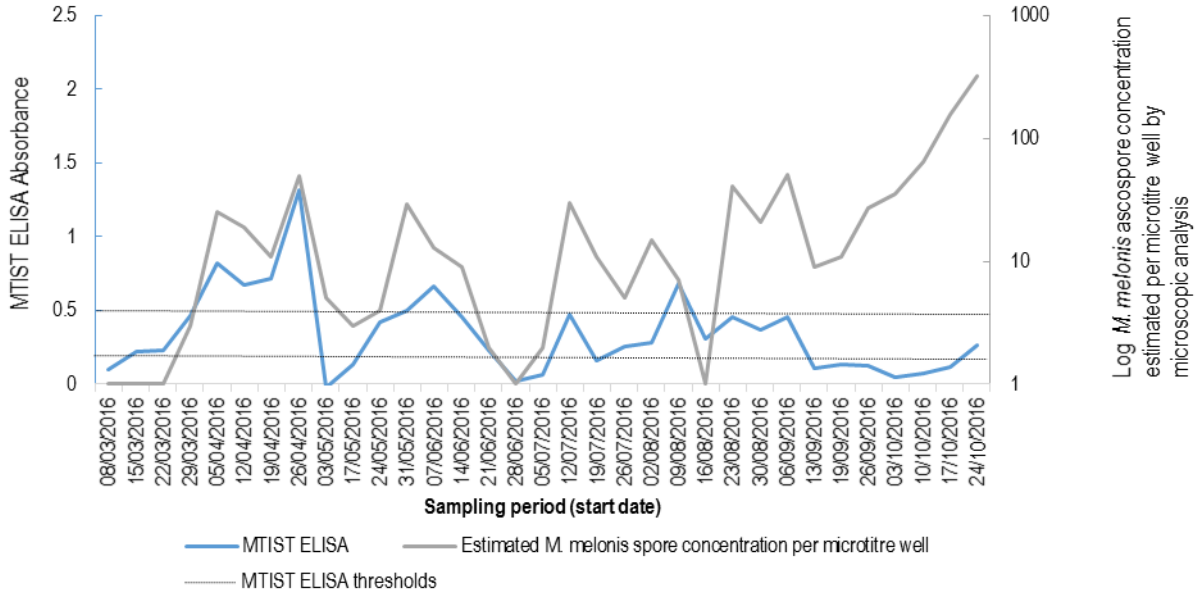


Figure 7a. *Mycosphaerella melonis* ascospore concentration in weekly MTIST air samples at site 2 as assessed by enzyme-linked immunosorbent assay (MTIST ELISA) and bright field microscopy.

Table 1. Treatments applied to the crop at Site 2 for control of Myco.

Chemical name	Application date	Crop Planting Period
Reflect (FRAC 7) (Isopyrazam (SDHI))	23/04/2016 26/08/2016	1 2
Switch (FRAC 9 +12) (Fludioxonil, Cyprodinil) and Chalk	04/05/2016 07/06/2016 19/07/2016 15/09/2016 05/10/2016	1 1 2 2 2
• Systhane (FRAC 3) (Myclobutanil, Cyclohexanome)	22/07/2016 26/07/2016 01/08/2016 07/08/2016 14/08/2016 31/08/2016 08/09/2016	2 2 2 2 2 2 2

- Grower reported that Systhane was applied for control of Powdery mildew. AHDB CP009a report (61-100%) reduction of Myco lesion development by *in vitro* tests

At the other commercial cucumber site (site 1), the *M. melonis* MTIST ELISA spore warning broke the low / moderate threshold on the 5th April 2016 (Fig. 7a). A high spore concentration in the air was identified for the week commencing 12th April. First Myco lesions were identified in the crop on the 16th April. An application of Signum was made shortly after. Increasing Myco lesions were identified in the crop on the 22nd April and an application of Reflect was made. Spore concentrations recorded in the crop remained high / moderate risk until week commencing 10th May. The second crop planting (23-26/05/2016) occurred during a period of moderate / high *M. melonis* inoculum risk by MTIST ELISA and remained at or near this until the week commencing 14th June. A similar pattern of spore concentration as measured by microscopic analysis was also observed for this period (Fig 7b). Thereafter, results of the MTIST ELISA placed the crop at low risk of *M. melonis* spores until 12th July. Microscopic analysis identified a steady increase in spore numbers from the end of June. Myco lesions

were not observed in the crop until the 13th July. An application of Switch was made at this time (Table 2). According to the MTIST ELISA, *M. melonis* spore concentration then peaked week commencing 19th July whilst microscopic counts continued to increase until week commencing 26th July. The crop was replanted 14th August during a period of high risk (MTIST ELISA and microscopic spore counts). The MTIST remained at moderate or high risk of *M. melonis* in air samples until 23rd August. Myco lesion development was first observed on cucumber plants on the 23rd September. The MTIST ELISA identified *M. melonis* spores in the air from around 13th September to 2nd October. By 3rd October a low MTIST reading was recorded. Between the 13th and 25th September the MTIST was near or just broke the high risk threshold. On the 23rd September an application of Reflect was made for control of Myco. Whilst, MTIST ELISA readings thereafter fell to a low level, spore observations by microscopic analysis identified *M. melonis* type spores in the crop (with the exception of week / commencing 10th October). The final crop cut was made on the 7th October 2016. Myco was not considered to be a major problem.

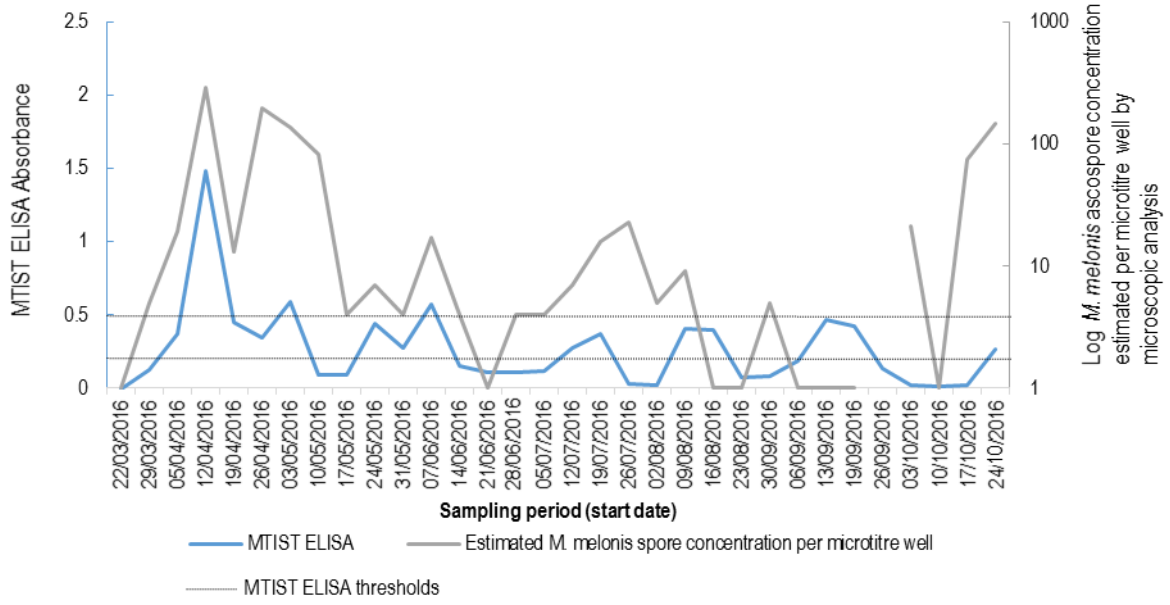


Figure 7b. *Mycosphaerella melonis* ascospore concentration in weekly MTIST air samples at site 1 as assessed by enzyme-linked immunosorbent assay (MTIST ELISA) and bright field microscopy.

Table 2. Treatments applied to the crop at Site 1 for control of Myco.

Chemical name	Application date	Crop Planting Period
Signum (FRAC 7+11) (Boscalid, Pyraclostrobin)	16/04/2016	1
Reflect (FRAC 7) (Isopyrazam (SDHI)	22/04/2016 23/06/2016	1 2
Switch (FRAC 9 + 12) (Fludioxonil, Cyprodinil) Switch & Chalk	03/06/2016 13/07/2016 27/08/2016	2 2 3
• Systhane (FRAC 3) (Myclobutanil, Cyclohexanome)	26/8/2016 02/09/2016 09/09/2016	3 3 3
Reflect (FRAC 7) (Isopyrazam (SDHI))	24/09/2016	3

- Grower reported that Systhane was applied for control of Powdery mildew. AHDB CP009a report (61-100%) reduction of Myco lesion development by *in vitro* tests

Use of an on-site lateral flow test as an estimation of disease risk

Weekly lateral flow test (single tube cyclone sampled): At site 1, there was some agreement of *M. melonis* inoculum potential in the weekly collected air samples with peaks and troughs of each test (MTIST ELISA and LFD) often overlapping (Fig 8). However, the positive correlation between the two variables (MTIST ELISA and Myco lateral flow) at either site was poor. Weekly samplers were often contaminated with particulate material (Fig. 9) which compromised sampler efficiency, altered dynamics of the cyclonic flow and restricted impaction of *M. melonis* to the tube side wall.

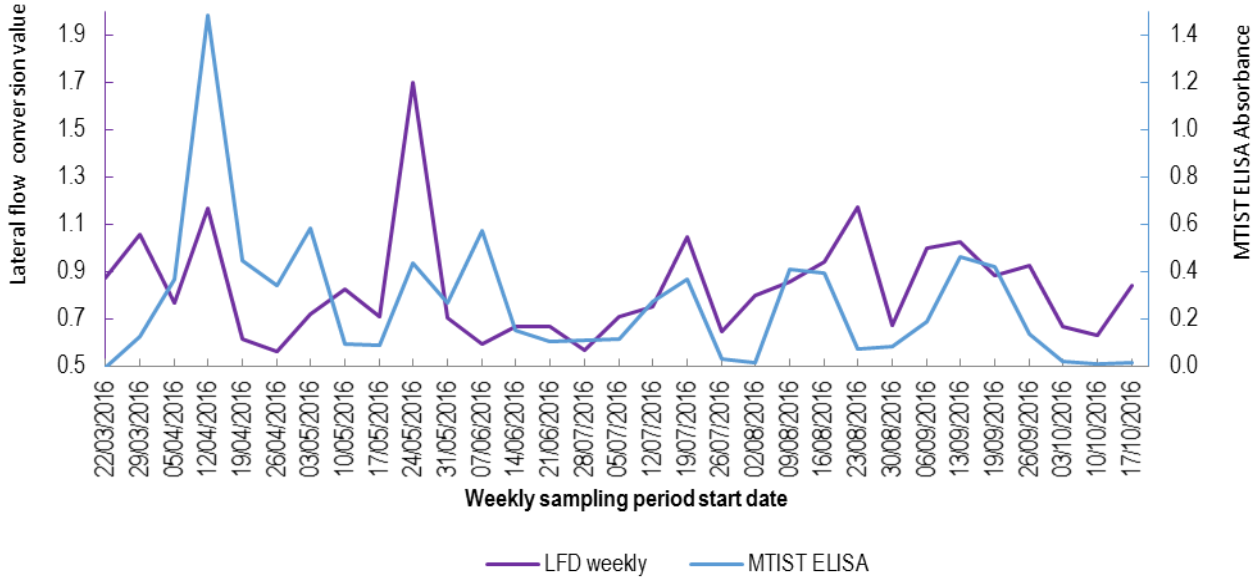


Figure 8. Site 1: Measurement of weekly air samples collected by a) an MTIST air sampler and processed by ELISA and b) a single cyclone air sampler and processed by competitive lateral flow.

Figure 9. Examples of single cyclone tube after sampling bio-aerosols in a commercial glasshouse over a week period.

Daily lateral flow test (seven single tubes positioned in a multi-vial cyclone sampled). Daily aerosols collected by the multi-vial air sampler were not affected with visual accumulation of debris. The tubes were tested at the University of Worcester for *M. melonis* spores and later in the season '*in-situ'* by growers. As environmental conditions such as temperature effect the lateral flow test dynamics, standard controls were used to correct for this both at the University of Worcester and *in-situ* at grower holdings. A standard negative (LFD extraction

buffer alone) and positive control results of a *M. melonis* antigen concentration range are shown in Figure 10.

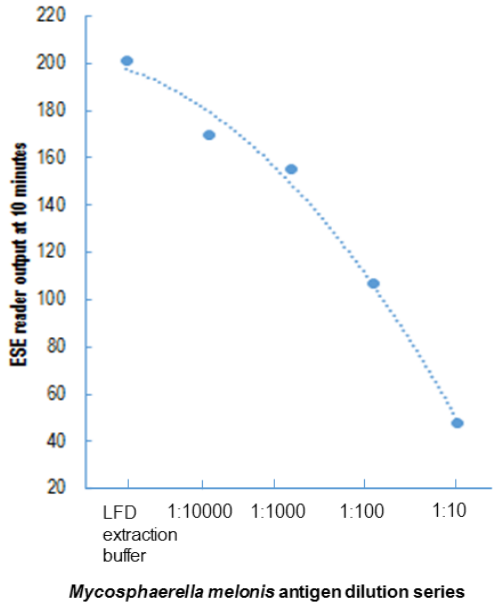


Figure 10. Across a 10 fold doubling dilution series quantitative measurement by lateral flow of *M. melonis in vitro* produced antigen.

Environmental sampling. The weekly absorbance values of the MTIST ELISA and the estimated microscopic *M. melonis* counts were referenced with the lateral flow daily values to determine potential for alignment of risk periods. At site 2, the results are shown in Table 3. A lateral flow daily ESE reader value of >140 was ascribed a 'Myco' low risk criteria, 140 > 125 moderate and <125 as at high risk of *M. melonis* spores. Table 4, describes this for Site 1.

Site 2: With the exception of eight days, the first eight week period recorded daily lateral flow readings at moderate or high risk of air samples with *M. melonis* spores (Table 3). When assessed by MTIST ELISA, seven out of the eight weeks were moderate or high risk of *M. melonis* in air samples. Visual estimate of *M. melonis* spore numbers by microscopy were relatively low for this period when compared with later in the season. Myco disease was first noted on plants during the 5th week of spore monitoring.

Between the 3rd May and the 24th October, daily lateral flow levels were mainly low. On occasion a moderate or high daily risk period was observed. The following dates were considered high risk:

- 3rd, 23rd May
- 6th, 15th, 21st and 28th June
- 6th, 10th July
- 19th September
- 31st October
- 4th, 5th and 6th November.

The MTIST ELISA for this period provided 11 weeks at moderate risk, three weeks at high risk and 11 weeks of low risk. A consistent relationship is not observed between the daily lateral flow and the MTIST or the microscopic counts (Table3).

Table 3. Ascribed daily lateral flow risk value compared with weekly MTIST risk and weekly *M. melonis* microscopic counts at Site 2.

Date (Week commencing)	LFD daily risk	MTIST weekly	Visually Estimated *M. melonis* in a MTIST microtitre well	Disease first observed in crop/ Controls applied
08/03/2016	H.H.M.M.H.L.M.	Low	0	
15/03/2016	M.H.H.H.H.H.H.	Moderate	0	
22/03/2016	M.H.H.H.H.H.L.	Moderate	0	
29/03/2016	H.H.H.H.M.M.H	Moderate	2	
05/04/2016	M.H.H.H.H.H.L	High	24	Myco lesions
12/04/2016	H.H.H.H.H.H.H	High	18	
19/04/2016	L.M.M.L.H.L.M.	High	10	Reflect
26/04/2016	H.H.H.M.L.L.H.	High	48	Myco first seen in fruit
03/05/2016	L.H.L.L.L.L.L.	Low	4	Switch
17/05/2016	L.M.M.L.L.L.H	Low	2	
24/05/2016	L.L.L.L.L.L.L.	Moderate	3	Big increase Myco fruit
31/05/2016	L.L.L.L.L.L.M.	High	28	

07/06/2016	L.L.L.L.L.L.L.	High	12	Switch
14/06/2016	L.H.L.L.L.L.L.	Moderate	8	
21/06/2016	L.M.L.L.L.L.L.	Moderate	1	
28/06/2016	H.L.L.L.L.L.L.	Low	0	
2nd Crop				
05/07/2016	L.H.L.L.L.H.L.	Low	1	
12/07/2016	L.L.M.L.L.L.L.	Moderate	29	
19/07/2016	L.L.L.L.L.L.L.	Low	10	Myco first seen on fruit Switch, Systhane
26/07/2016	L.L.L.M.L.L.M.	Moderate	4	Systhane
02/08/2016	L.M.L.L.L.L.L.	Moderate	14	Systhane
09/08/2016	M.L.L.L.L.L.L.	High	6	Systhane
16/08/2016	M.L.L.L.L.L.L.	Moderate	0	
23/08/2016	L.L.L.L.L.L.L.	Moderate	40	Big increase Myco fruit Reflect
30/08/2016	L.L.L.L.L.L.L.	Moderate	20	Systhane
06/09/2016	L.L.L.L.L.L.L.	Moderate	50	Systhane
13/09/2016	L.L.L.L.L.L.L.	Low	8	Switch
19/09/2016	H.L.L.L.L.L.L.	Low	10	
26/09/2016	L.L.L.M.L.L.L	Low	26	
03/10/2016	L.L.L.L.L.L.L	Low	34	Myco Switch
10/10/2016	L.L.L.L.L.L.L	Low	64	
17/10/2016	*.L.L.H.L.L.L	Low	156	
24/10/2016	M.L.L.L.L.L.L.	Moderate	318	
31/10/2016	H.L.L.L.H.M.H	High		

*Missing value

Site 1: In each of the first four weeks of sampling, daily periods of moderate and high risk were reported for *M. melonis* by the lateral flow device (Table 4). During the first two weeks of air sampling (24th March to the 4th April) the MTIST ELISA and spore microscopic counts remained low but thereafter two weeks of moderate or high risk were identified. Myco lesions were observed on plants during the 5th week after monitoring commenced.

In the second crop, daily lateral flow tests identified high risk periods on the 4th and 5th June, 12th and 20th June. Myco lesions were observed for the first time on the 12th July. A high risk by MTIST ELISA was identified week commencing 7th June 2016 and for this period spore concentration increased by microscopic count (Table 4).

In the third crop, the daily lateral flow recorded a high risk period on the 16th August. For this period the ELISA recorded moderate risk. Identification of spores resembling *M. melonis* remained at or near 0 until the 3rd October. Myco lesions were observed in the crop from the 19th September.

Table 4. Daily lateral flow risk value compared with weekly MTIST risk and weekly *M. melonis* microscopic count at Site 1.

Date (Week commencing)	LFD daily risk	MTIST weekly	Visually estimated *M. melonis* in a MTIST microtitre well	Disease first observed in crop
22/03/2016	L.L.M.H.L.H.H.	Low	0	
29/03/2016	M.M.M.L.H.H.L.	Low	4	
05/04/2016	H.L.L.L.L.L.L	Moderate	18	
12/04/2016	L.L.L.M.M.H.L.	High	290	Myco lesions Signum
19/04/2016	L.L.L.L.L.L.L.	Moderate	12	Reflect
26/04/2016	M.M.H.L.L.L.M.	Moderate	195	
03/05/2016	H.L.H.L.L.H.M	High	136	
10/05/2016	M.L.H.H.L.L.M.	Low	80	
17/05/2016	H.L.L.L.L.L.L.	Low	3	
24/05/2016	H.L.L.L.L.L.L.	Moderate	6	
31/05/2016	L.L.L.L.H.H.M.	Moderate	3	
07/06/2016	L.L.L.M.M.H.M.	High	16	
14/06/2016	M.L.L.L.M.M.H.	Low	3	
21/06/2016	H.L.L.L.L.H.M.	Low	0	Reflect
28/06/2016	M.L.L.L.L.L.L.	Low	3	
05/07/2016	L.L.L.M.L.L.L.	Low	3	

12/07/2016	L.L.L.L.L.L.L.	Moderate	6	Myco lesions Switch
19/07/2016	L.L.L.L.L.L.L.	Moderate	15	
26/07/2016	L.M.L.L.L.L.L.	Low	22	
02/08/2016	H.L.L.L.L.L.L	Low	4	
09/08/2016	L.L.L.M.L.L.L.	Moderate	8	
16/08/2016	H.L.L.L.L.L.L.	Moderate	0	
23/08/2016	L.L.L.M.L.L.M	Low	0	Systhane, Switch
30/08/2016	L.L.L.L.L.L.L.	Low	4	Systhane
06/09/2016	M.L.L.L.L.L.L	Low	0	Systhane
13/09/2016	L.L.L.L.L.L.L.	Moderate	0	
19/09/2016	L.L.L.L.L.L.L.	Moderate	0	Reflect Myco lesions
26/09/2016	L.L.L.L.L.L.L.	Low		
03/10/2016	L.L.L.L.L.L.L.	Low	20	
10/10/2016	L.L.L.L.L.M.M.	Low	0	
17/10/2016	L.L.L.L.H.L.L.	Low	74	
24/10/2016	L.L.L.L.L.L.H.	Moderate	146	

Assessment of cucumber powdery mildew species in UK production by molecular analysis

The results of the PCR amplification showed that all commercial leaf samples tested between June and August 2015 contained *P. xanthii* and with no *G. orontii* present (reported in Year 1). Similarly, samples collected during September and October 2015 identified only *P. xanthii* presence. However, *G. orontii* was confirmed on a dwarf cucumber (snack variety) from a non-commercial propagator.

Discussion

Microscopic counts versus MTIST ELISA : In Year 2 of the project, a request was made by AHDB to include additional information of Myco spore concentration by time-consuming microscopic examination of the MTIST microtitre wells. Reliability of data generated in this way is questionable as visually Myco spores are similar to many other fungal species. Also, at times the overall spore load is high and individual spores are difficult to identify.

To demonstrate the difficulty in identify species by microscopy a small selection of spore types are shown in Figures 11a-g. *Mycosphaerella iridis* is common July-October with pseudothecia found often on brown patches of Iris leaves. *Nectriella dacrymycella* common June – December. *Leptotrochila cerastiorum* is a pathogen on Mouse-ear chickweed (perennial weed common on lawns) and *Didmyella applanta* on raspberry (spur blight) common March – May. *Nectria punicea*, a saprophyte found on dead branches with spore production from February. *Mycosphaerella melonis* causative agent of gummy stem blight on cucurbits. *Didymella exigua*, is produced on dead stems of Campanula species (late summer/autumn). It is important to understand that once spore types enter the air stream they can travel considerable distances and enter protected cropping environments when vents are open.

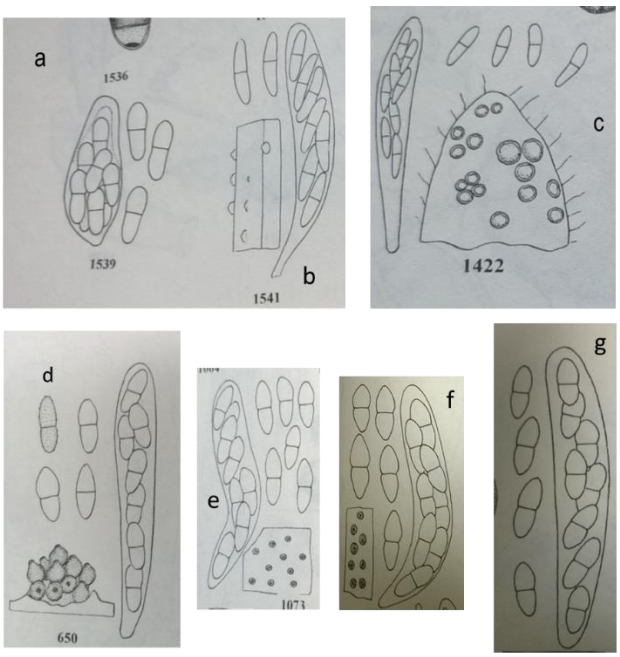


Figure 11 Spore types: a) *Mycosphaerella iridis*, b) *Nectriella dacrymycella*, c) Leptotrochila cerastiorum, d) *Didymella applanta, e) Nectria punicea, f) Mycosphaerella melonis (syn Didymella bryoniae) g) Didymella exigua*

At both commercial cucumber sites there was good correlation at the start of the season between microscopic estimation of *M. melonis* spores and MTIST ELISA values. Towards the end of the season this relationship declined, and in the final month of the study no correlation was observed. This may be due to a number of reasons:

- Seasonal variation provides different habitats with host and pathogen biodiversity. Cucumbers grown in a protected environment have a long growing season (January through to November in the UK) whilst outdoor crops follow a geographical and environmental pattern. In the UK this is primarily late spring, summer and autumn. The diversity of air-spora and potential for misidentification (by microscopy) will increase as

production intensifies. Although, not forgetting there are overwintered crops such as Brussels sprouts, cabbage, turnip that will be affected by fungal spores adapted for the colder months. Glasshouse venting regimes may also change depending on the season and restrict outside / inside air flow.

- The divergence between the two types of test (counting based on visual identification and recognition by a signature bioactive spore marker) could however relate directly to the pathogen (*M. melonis*). We know that powdery mildew on cucurbits can be caused by two different fungal species. On mainland Europe, *G. orontii* has been reported during early season cropping preferring a dry climate whilst *P. xanthii* dominates during the summer months as humidity is increased. Perhaps, there are different pathotypes of *M. melonis* or distinct species which occur in the UK at different times of the season. In the United States, a study has shown three genetically distinct *Stagonospora* species causing gummy stem blight (Stewart et al., 2015). *Stagonospora cucurbitacearum* (syn. *Didymella bryoniae* and *M. melonis*) is associated with gummy stem blight of cucurbits in the UK. If however, multiple species are present the signature bioactive spore marker may differ for each species involved. This could markedly effect test accuracy.
- Alternatively, during the season *M. melonis* spore characteristics may change. Perhaps as a result of spray applications or seasonal conditions. Like *Mycosphaerella brassicicola* (ringspot of Brassicas), *M. melonis* has a sticky adhesive mucilaginous sheath where, by immunofluorescence, the biomarker is located. This sheath provides protection, adhesion and it is thought a hydrophobic layer to prevent the spore from drying out and an aid in dispersal (Howard and Gow, 2007). It is possible that the spore coating may change during the season either as a result of fungicide action or for environmental reasons. A more hydrophilic spore could enhance germination potential more readily at a dew point. This may prove beneficial at specific times of the year.

Myco lateral flow development: In Year 1 AHDB CP137 study, laboratory analysis determined that a *M. melonis* diagnostic probe (UW 325), as used in ELISA, did not readily transfer to a lateral flow format. Different membrane types and buffers were assessed but found to be of limited value. During Year 2 of the project, diagnostic probes developed in AHDB PE001 were assessed in a lateral flow format as potential replacement bio-marker probes for *M. melonis.* UW 339 was found to measure *M. melonis* spore material by lateral flow and at a sensitivity suitable for environmental sampling. In Year 2 of the study, the lateral flow was used retrospectively to measure *M. melonis* inoculum potential in 2015 collected weekly and daily cucumber crop environmental air samples. No relationship was observed with the MTIST ELISA result and, when assessed for specificity, UW 339 was found to react with other spore

types. Debris collected in weekly exposed cyclone tubes was also identified as providing potential for inhibition of the assay system.

To overcome these problems two strategies were put in place:

- Further studies were made with UW 325 and after exhaustive attempts a lateral format was developed to provide a test with sensitivity suitable for assessment of *M. melonis* in environmental air samples. The test developed relies on a solid phase assay (similar to a plate trapped antigen ELISA) where the conjugated gold conjugate anti-*melonis* diagnostic probe (UW 325 monoclonal antibody) is incubated in the sample collection tube and, if present, will bind to *M. melonis* spores adhered to the wall of the collection tube. A five minute incubation period is required, with gentle agitation, prior to transfer of unbound tube contents to the lateral flow device. An anti-mouse (whole molecule) antibody is used at the lateral flow test line to measure any anti-*melonis* diagnostic probe not retained in the collection tube. When there are no *M. melonis* spores trapped in a tube a full complement of anti-*melonis* diagnostic probe will be returned to the lateral flow and a test line developed to full strength. The number of *M. melonis* spores impacted on the test tube wall will directly influence the concentration of anti-*melonis* probe returned to the lateral flow. The test is semi-quantitative.

- Weekly glasshouse cyclone samples collected into a single tube were often found to contain debris. A preliminary study with 2015 samples showed some improvement of the assay by removal of the debris over non-removal. A pre wash of phosphate buffered saline 0.05% Tween 20 (PBST) was used for the 2016 collected samples. This follows a similar process to that of the weekly MTIST ELISA test. The rationale being that some spore types, to include *Mycosphaerella* species, report a sticky adhesive mucilage coating which allows retention during the ELISA wash process (Wakeham et al., 2004 Kennedy and Wakeham, 2010). So, for cyclone tube collection, hydrophobic *M. melonis* spores adhered to the tube wall by impaction resist removal during a wash phase. However, there is vulnerability in this process. If the tube fills with debris (Fig 9) the trapping efficiency of the cyclone sampler is compromised and trapped spora thereafter are likely to impact within the debris and with a wash stage there is the potential for them to be removed prior to the immunoassay stage. Nevertheless, on multiple occasions there was good agreement during the season between the weekly MTIST ELISA and the weekly lateral flow device.

Monitoring environmental samples – disease latent period and which system? : **Planting 1, Site 1**. By lateral flow test, high daily levels of *M. melonis* spore antigen were identified in the first three weeks of sampling. Myco disease symptoms were observed in the crop at four weeks. *M. melonis* spores recorded by MTIST ELISA and microscopy were low in the first two weeks of sampling. The cyclone air sampler reports high efficiency over a wide range of captured particles and at 90% for those in the 1µm range (www.burkard.co.uk). It is possible that the peak of the epidemic occurred one to two weeks prior to commence of sampling. Conceivably, the cyclone sampler collected small particles retained in the atmosphere post deposition of the larger *M. melonis* spore. Equally, the cyclone sampler was more efficient in the collection of material associated with *M. melonis* at the start of inoculum dissemination. **Planting 2, Site 1**. All air samplers and associated assay systems identified *M. melonis* spore presence from the 24th May. The peak of the spore epidemic was around 7th June. Five weeks from this date (no control measures applied) Myco was observed in the crop. **Planting 3, Site 1**. A high daily lateral flow reading of Myco was recorded on the 16th August. The weekly lateral flow also recorded *M. melonis* presence for this period and week commencing 23rd August. The weekly ELISA predicted moderate risk for 16th August. Interestingly, few spores resembling *M. melonis* were recorded visually but six weeks later (Myco infection taken from 16th August) disease was observed in the crop. Systhane was applied on three occasions during this period so this may have extended the disease latent period.

Based on the findings at this site the latent period for the disease (time elapsed between exposure to pathogen and disease symptoms apparent) is between two and six weeks. The environmental conditions, spore concentration, the age of the host and crop variety are all important factors that will influence whether disease will occur, at what level and rate. Application of control measures will also influence disease progression. *M. melonis* may have a similar latent period to that of *M. brassicicola* (causative agent of ringspot on Brassicas). AHDB FV53a reports a latent period of up to 1 month for *M. brassicicola* spores and that they are capable of remaining viable for a period until conditions became favourable for germination .

Planting 1, Site 2. As at site 1, daily high *M. melonis* spore levels were identified in the crop by lateral flow from 8th March 2016 (start of air sampling). The weekly MTIST identified moderate risk from the 15th March. Spores resembling *M. melonis* were visible by microscopic examination from week commencing 29th March, albeit at a low level (two spores per microtitre well). Myco was observed in the crop on the fifth week of sampling (week commencing 5th April). This would complement observations made at site 1 where a latent

period of four to six weeks was estimated. **Planting 2, Site 2**. In the second crop, two days of high risk were identified by the lateral flow test on the 6th and 10th July. Thereafter, and until the 19th September most days were low risk with only occasional days observed at moderate risk. A low number of infected Myco infected fruit were observed in the first two weeks from planting and this confirms a latent period of two weeks (19th July, two fruit infected, 20th July 15 fruit infected). Regular control applications for Myco disease were made during this period and daily spore concentrations by the lateral flow test remained low. By the third week in August, the number of infected fruit within the main crop had increased (107 fruit infected). This may indicate that the initial infection period (6th and 10th July) provided a systemic pathway for the disease to progress *in planta*. A single daily high risk period recorded by the lateral flow on the 19th September would suggest the disease was able to cycle and release spores into the environment for dissemination. Within two weeks of this date infected fruit had risen significantly (448 fruit infected). These results would indicate that a daily lateral flow test may prove useful in determining risk periods of *M. melonis* spores in glasshouse air samples.

Interestingly, for this second planting there was poor agreement generally between the test formats. Spores which looked morphologically similar to *M. melonis* were seen throughout the planting period and at times to a very high concentration. Up until 6th September both the weekly MTIST and weekly lateral flow provided extended periods of moderate to high risk of *M. melonis*. Thereafter, whilst visual spore counts continued to increase, the weekly MTIST ELISA and weekly lateral flow assay fell predominantly to low risk. This decline in risk was approximately three weeks after a fungicide application of Reflect had been made (26th August). It is odd that given the application of Reflect to the crop, spore concentration as measured by microscopic analysis was little effected. Either, the application was ineffective or identification of the spore type is inaccurate. It is difficult to draw a conclusion from this data.

Conclusions

- A lateral flow has been developed and used at grower holdings to estimate daily and weekly bio-aerosol risk of *M. melonis* in cucurbit crops.
- Assessment of crops in Year 3 of the project will assist in determining the reliability of the different test formats.
- It is estimated that Myco in protected crops has an approximate latent period of two to six weeks depending on the environment and the control treatments applied.
- Although not in a commercial cropping system both *G. oronti* and *P. xanthii* have, by DNA analysis, been identified on cucurbits grown in the UK.

Knowledge and Technology Transfer

Wakeham, A.J., Keane, G., John, S., Townsend, J. and McPherson, M. (2016). AHDB CP137 Cucumber: Development and testing of diagnostic assays to monitor gummy stem blight and powdery mildew inoculum in air during cucurbit production. Cucumber and Pepper Growers' day at Waltham Abbey, UK on 12th October, 2016.

John, S. (2016). Hands on testing of the gummy stem blight lateral flow. Cucumber and Pepper Growers' day at Waltham Abbey, UK on 12th October, 2016.

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