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:	CP 137
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Report:	Annual report, November 2015
Previous report:	Not Applicable
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Date project commenced:	01/11/2014
Expected completion date:	31/12/2017

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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.

[Name]
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GROWER SUMMARY

Headline

- Three types of air samplers have been used in UK cucumber cropping systems to collect weekly or daily bio-aerosols.
- Bio-aerosols have been tested by ELISA (laboratory immunoassay test) for *Mycosphaerella melonis* spore concentration (Myco disease). Disease risk alerts have been made available on a weekly basis.
- Daily bio-aerosol samples will be tested for disease using a lateral flow device (5 minute on-site test). The results will be evaluated against the laboratory ELISA test. The 5 minute test is at a prototype stage and will be available for trial to participating cucumber production sites in 2016.
- Antibody diagnostic probes are in development for the measurement of cucumber powdery mildew spores. The diagnostic probes will be tested for recognition of the two types of powdery mildew species that can occur on cucumbers in European production systems *(Podosphaera fusca* (also known as *Podosphaera xanthii*) and *Golovinomyces orontii).*
- Adopting measures to assess disease risk of cucumber production should allow professional producers to demonstrate an integrated pest and disease management system for compliance with the European Union sustainable pesticide use directive (2009/128/EC).

Background

In the airborne environment many plant diseases are able to spread between and within cropping systems. In the UK, using either laboratory based analysis or a field based pregnancy style test, AHDB Horticulture funded work has provided the development of systems to monitor field bioaerosols to target disease inoculum either on a daily or weekly basis. Air sampling systems and tests are available for the following 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 disease (spores) in air samples growers are able to time sprays more effectively and make informed decisions as to which type of fungicide application to make. Studies measuring *Mycosphaerella brassicicola* (ringspot on Brassicas) in bio-aerosol samples have shown that under ideal environmental conditions, high

concentrations of spores are required in the air for infection to occur (2000 spores per cubic metre). HDC PE001 (Cucumber: Improving Control of Gummy Stem Blight) developed monoclonal antisera and a laboratory based ELISA test to monitor glasshouse aerosols for *Mycosphaerella melonis* spore presence. To provide improved fungicide efficacy the timed application of control measures was made during periods of peak spore production (above 2000 spores per cubic metre).

Gummy stem blight (Black stem rot): The causative agent of gummy stem blight of cucumber is *Mycosphaerella melonis* syn. *Phoma cucurbitacearum* (syn. *Didymella bryoniae).* The disease is of worldwide importance, with significant economic damage in glasshouse cucumber & other cucurbits, including outdoor 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. 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. The fungicides that are available in the UK for use in cucumber production (primarily for powdery mildew control) 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 *Mycosphaerella*.

Recently, a range of alternative fungicides have been assessed in the UK for their efficacy in control of the disease (PE001) some of these materials will be available for the 2016 season. The timed application of control measures was made during periods of peak spore production. To provide this information in a more timely way the laboratory based immunoassay test (enzyme-linked immunosorbent assay (ELISA)) could be transferred to a lateral flow ‘on-site’ format for direct grower or consultant use.

Lateral flow immunoassays: These rapid on-site tests are used for qualitative and semi-quantitative detection of target analytes. The most well-known test of this type is the Unilever Clear Blue Pregnancy Test Kit. Lateral flows consist of a carrier material containing dry reagents that are activated by applying a liquid sample. Movement of this liquid allows passage across various zones where molecules have been attached and exert specific interactions with target analytes. Results are generated with 5 – 10 minutes by the formation of a control and test line as appropriate to the sample and the test type. They are designed for single use, can provide a multiplex test platform and, are available commercially for a wide range of applications. More recently they have become increasingly important in the diagnosis

of plant pathogens. The tests can be semi-quantitative and based upon test line depletion (visual or by electronic measurement), spore concentrations in the air can be estimated. A control line remains constant to show that the test has worked. The test is counter intuitive in that as spore concentration increases the test line decreases in colour intensity. At high spore concentrations no test line is visible (Fig. 1).

Figure 1. A competitive lateral flow assay with increasing spore numbers added

Information on plant pathogen spore concentration (inoculum load) in bio-aerosols can be utilised within an integrated disease management system. In Holland, an environmental model is under evaluation for control of *Mycosphaerella melonis* (Myco) in cucumber crops (A. Dijk, pers. comm.). If successful, future work should look to integrate the environmental disease forecast with disease load (Myco spore concentrations). This would provide information on when airborne pathogens present at a concentration required for infection of the crop and whether the environmental conditions are conducive for infection to occur. In this way an informed decision could 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,0 minimising the risk of resistance developing in the pathogen population.

Diagnostic probes developed in this project will be incorporated within a lateral flow test (for grower use) to discriminate and diagnose spore concentrations of both the gummy stem blight pathogen and powdery mildew in glasshouse growing crops. This would form part of an integrated disease management strategy to control disease from the outset and ahead of visible symptom expression.

Cucumber Powdery Mildew: Numerous vegetable crops are susceptible to powdery mildew, but cucurbits are one group that are severely affected and where fungicides are used routinely for control. It is probably the most common, conspicuous, 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 both leaf surfaces, petioles and stems but rarely on fruits. *Podosphaera fusca* (also known as *Podosphaera xanthii*) and *Golovinomyces orontii* are identified as the main agents of cucurbit powdery mildew. The disease provides one of the most important limiting factors for cucurbit production worldwide. In the absence of chemical, biological control or the use of tolerant/resistant varieties the disease can cause yield reduction (as much as 40%). Excessive ventilation, reduced light intensity i.e. partial shade and succulent plant tissue promote disease development. The disease is 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. fusca* 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.

Summary

Monoclonal antibodies have been developed to the two species of cucumber powdery mildew that are known to cause the disease in European cucumber production. These antibodies are now being assessed for their selective reactivity potential to *Podosphaera fusca* (also known as *Podosphaera xanthii*) and *Golovinomyces orontii.* The potential of these diagnostic probes will be evaluated in ELISA and lateral flow for quantitative measurement of the cucumber powdery mildew pathogen and later applied to commercial production for assessment.

Existing monoclonal antibodies, developed in HDC Project PE001 to the ascosporic stage of *M. melonis* (gummy stem blight), have been used to measure Myco disease pressure in cucumber glasshouse bio-aerosols. At three UK commercial cucumber production sites three types of air samplers were used in the study:

- A personal Microtitre immunospore trap (MTIST), available from Burkard Manufacturing (http://www.burkard.co.uk) is available at an approx. cost of £1500. This includes the ELISA microtitre well adapter plate. The sampler was run directly off the mains and operated continuously for the cucumber growing period. Spores in the air were collected into microtitre wells. The air sample (4x8 well microtitre wells) was changed weekly and sent by post to a laboratory at the University of Worcester for assessment of *Mycosphaerella melonis* (Myco) spore numbers. Results were relayed back to producers within 48hrs receipt of the air sample.
- A single tube cyclone air sampler (http://www.burkard.co.uk), available from Burkard Manufacturing (http://www.burkard.co.uk) at a cost of approx. £ 2500 was run directly off the mains and continuously for the period. Spores in the air were collected in to a single tube for a seven day period. The tube was changed once weekly and sent to a laboratory at the University of Worcester. The samples were stored at -20°C until assessment could be made for disease of Myco and / or Powdery mildew by lateral flow test.
- A multivial cyclone air sampler, available with a timer from Burkard Manufacturing can be purchased for an approx. cost of £2500. The sampler was run directly off the mains and continuously for the sampling period. Weekly, the sampler was loaded with seven tubes. At midnight by electro-mechanical control the tubes were rotated. This meant that for each 24 hour period a single tube was positioned to receive the glasshouse bio-aerosol sample. At the end of each week the seven tubes were changed and sent by post to the University of Worcester. As for the single cyclone air sampler the tubes were stored for assessment by lateral flow.

At two of the nurseries (Site 1 and Site 2) little disease (*Mycosphaerella melonis* ascospores) was observed on the base of the MTIST air sampler microtitre wells until April 2015. This microscopic observation was reflected in the weekly results of the MTIST laboratory ELISA test (Figure 2). Conversely, *M. melonis* ascospores were visible by microscopic analysis from the outset at Site 3 (Figure 3) and increased ELISA absorbance values were recorded. At each of the nurseries, M. *melonis* spore concentration was seen to rise steeply from the end of April into May. Email correspondence with the producer at site 3 identified that Myco was active and had spread through the crop by July 2015. Producers at Sites 1 and 2 reported only low level of infection in the first crop (Site 1, 2nd Crop planting 13/05/2015; Site 2, 2nd

Crop planting end of June). At each of the sites treatments were made for control of the disease.

The bio-aerosols, collected by the cyclone air samplers, will be used to evaluate a 5 minute prototype on-site test for measurement of gummy stem blight disease potential and powdery mildew. The gummy stem blight lateral flow test developed in Year 1 of this project, will be used in 2016 by participating growers to determine on-site usage potential. If successful the test would be expected to retail in the region of £7-10 but for quantitative measurement may require a digital lateral flow test. A reader currently retails at circa £1300 (ESE reader https://www.qiagen.com/gb/about-us/contact/oem-services/ese-instruments/esequant-lateral-flow-reader). Although smart phone readers with downloadable applications have recently been developed for clinical applications (www.novarumreader.com/novarum-mobile-reader). There is potential for this technology to be applied for disease assessment of plant pathogens.

Timely information on disease concentration in bio-aerosols should provide growers with capability to identify periods when crops are at risk and improve management of the disease through informed control strategies.

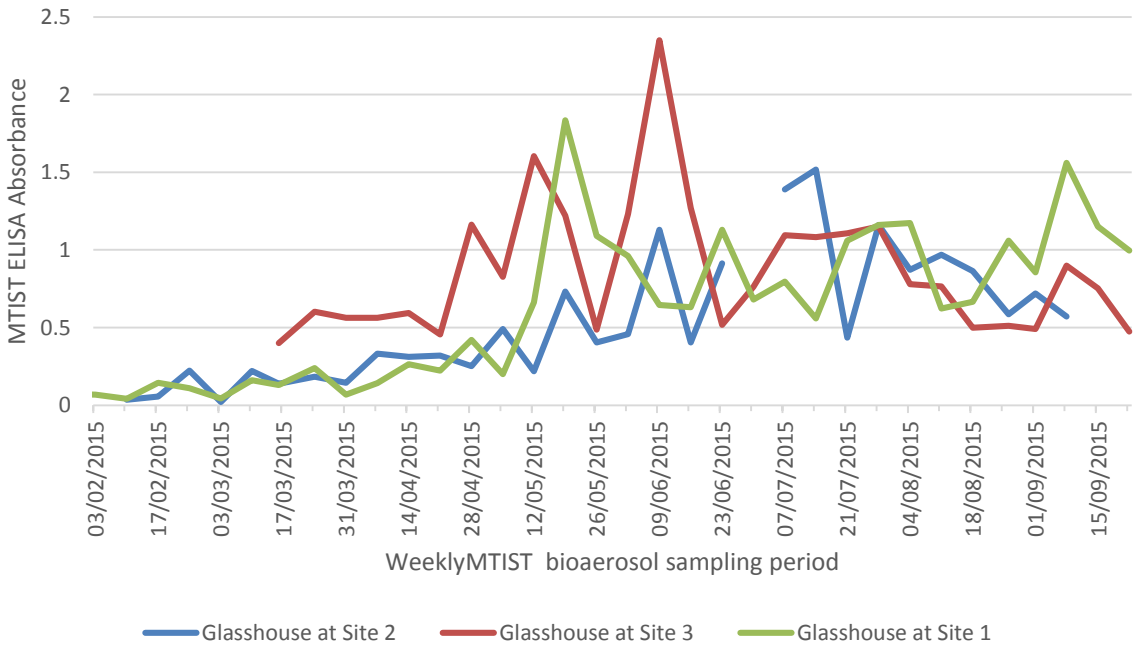

Figure 2. Measurement by ELISA of *Mycosphaerella melonis* ascospores collected in weekly bioaersols at three UK commercial cucumber propagation nurseries.

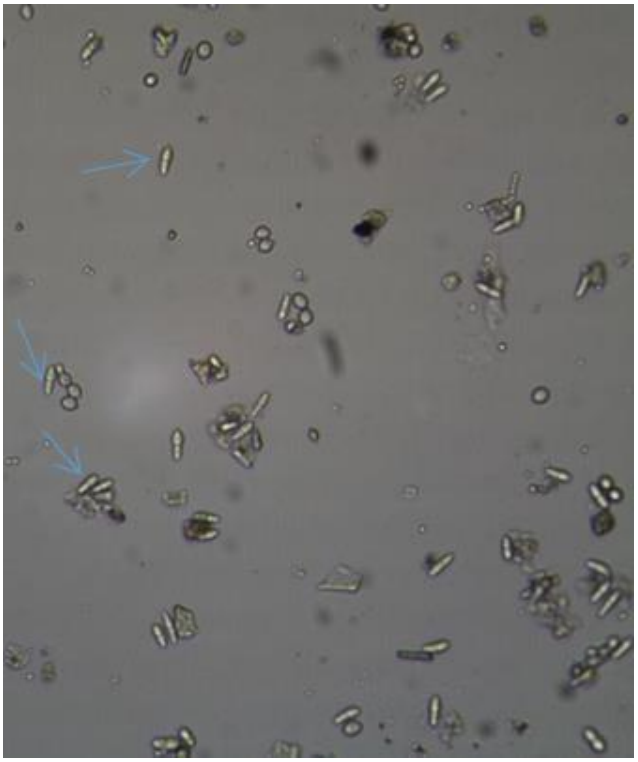

Figure 3. Base of a microtitre well viewed by bright field microscopy from glasshouse Site 3. Some examples of ascosporic inoculum resembling morphological characteristics of *M. melonis* are shown by blue arrows (there are many more on the plate).

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 the presence of gummy stem blight inoculum in the air and better time the first fungicide application. 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 overall reductions in sprays achieved.

SCIENCE SECTION

Introduction

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The aim of this project is to produce on-site tests which can be used to monitor glasshouse bio-aerosols for gummy stem blight and powdery mildew in glasshouse cucumbers. Intervention/control measures would be applied in response to disease risk from the on-site tests.

Studies measuring *Mycosphaerella brassicicola* (ringspot on Brassicas) in bio-aerosol samples has shown that under ideal environmental conditions, high concentrations of spores are required in the air for infection to occur (2000 spores per cubic metre). HDC PE001 (Cucumber: Improving Control of Gummy Stem Blight) developed monoclonal antisera and a laboratory based ELISA test to monitor glasshouse aerosols for *Mycosphaerella melonis* spore presence. To provide improved fungicide efficacy the timed application of control measures was made during periods of peak spore production (above 2000 spores per cubic metre).

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Materials and Methods

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

Infected cucumber leaves were provided by three commercial producers at four occasions between June and August 2015. 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 2. 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 BioSpectrum imaging system (UVP, LLC, USA). The occurrence and severity of infection by each species could then be assessed on the leaves throughout the season.

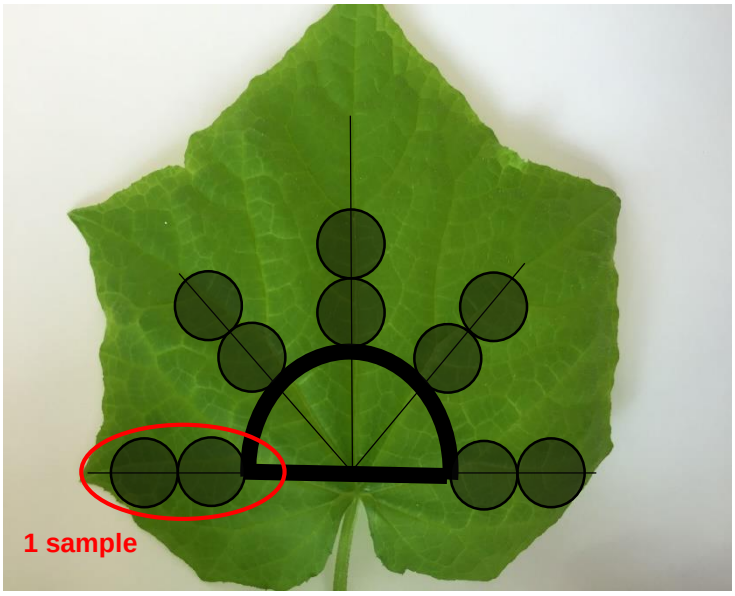


Figure 2. Sampling of 2.5cm leaf discs from cucumber leaves infected with powdery mildew; two adjacent leaf discs formed one sample.

Development of monoclonal antibodies for the measurement of cucumber powdery mildew (G. orontii and P. fusca) in glasshouse bioaerosols.

Collection of cucumber powdery mildew conidia.

A hand held Burkard surface cyclone sampler (Burkard Manufacturing Co., Rickmansworth, Herts., UK) was used to separately collect conidia of *G. orontii* and *P. fusca* from the leaf surface of sporulating infected cucumber leaf host material. One ml of chilled phosphate buffered saline ((PBS) 0.01M phosphate buffer, 0.0027M potassium chloride, 0.137M sodium chloride, pH 7.4) was added to the air sampler collection vessel. Using a spin mix (Gallenkamp Ltd, Cheshire) at high speed for five minutes the conidia were suspended in to the aqueous phase. The spore suspension was filtered through a polyester Spectra mesh membrane (47µm pore size; NBS Biologicals Ltd, Huntingdon, UK) to remove any large contaminating material. The liquid phase was collected and, by using a membrane of 10µm pore size, bacteria and other small leaf contaminants were removed after filtration. The filtrate was collected and resuspended in 2ml chilled PBS and, presence of powdery mildew conidia were confirmed by bright field microscopy (x 400). For each species (*G. orontii* and *P. fusca)* a conidial concentration of $1x10^7$ ml^{-1} was determined.

Immunogen preparation. Using a Fast Prep 120 instrument (www.qbiogene.com) at an operating speed of 5 for 25 seconds the conidia were disrupted. Each sample type was rested on ice for 3 minutes and the process repeated twice. After microfugation in an MSE Microcentaur (www.mseuk.co.uk), operated at 10,000 rpm for five minutes, the soluble phase of each disrupted conidial sample was retained and separately transferred to a YM30 microcon centrifugal unit (www.millipore.com). The sample was separated in to two fractions of > 30 KDa and < 30 KDa according to manufacturer's guidelines. The protein concentration of each was adjusted to 2 mg ml^{-1} and stored at -20°C.

Immunisation. Three Balb C mice were immunized, by intraperitoneal injection, each with 50µl of > 30 KDa conidial preparation mixed with an equal volume of Titermax adjuvant (Sigma T-2684). Three mice were immunized with the < 30 KDa conidial preparation. Finally, an additional three mice were immunized with a conidial concentration of $1x10^7$ ml^{-1}. The process was repeated monthly over an 8 week period. To determine the immune response of each mouse to the homologous immunogen preparation, tail bleeds (50µl blood sample / mouse) were taken 7 days after the third immunization.

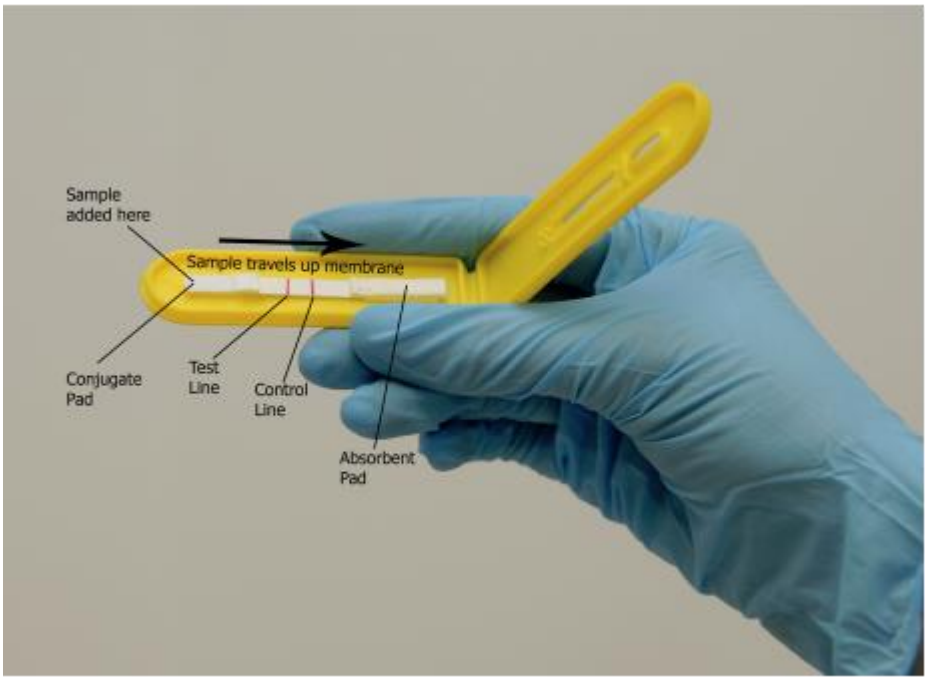
Tail bleeds. Using a standard plate-trapped antigen enzyme-linked ELISA (PTA-ELISA) polysorp microtitre wells (Catalogue No. 469957, Life Technologies Ltd, Paisley, Scotland) were each coated with 100µl of 10µg homologous antigen in PBS and incubated overnight at 4°C. The PTA-ELISA was carried as described by Wakeham (2004) but at the primary antibody stage doubling dilutions of each tail bleed were made in PBSTwC (PBS, 0.05% Tween 20 and 0.1% Casein [wt/vol]) to an end point of 1 in 128,000 and, each dilution probed against replicate homologous well-trapped antigen. A Strept ABC complex DAKO duet amplification system, (Cat.No. KO492; DAKO Ltd, Cambridge, UK) was used according to the manufacturers guidelines at the secondary Ab stage. Two weeks later a pre fusion immunogen boost was given, as described previously, to selected mice.

Fusion. The spleens of two mice were removed and, for each, a fusion was carried out according to a University of Worcester standard protocol using a modified method of Kennet (1978). Antibody producing B cells isolated from the spleen were fused in vitro with lymphoid tumour cells (myeloma). The cell hybrids (hybridomas) were supported in Dulbecco's Modified Eagles's Medium (Code No. D5976, www.sigmaaldrich.com) containing 10% Foetal calf serum (DME). On day 6 100µl DME fresh medium was added to each hybridoma and, the medium changed on day 10. Cell tissue (TCS) culture supernatants were screened by PTA ELISA and immunofluorescence (Kennedy et al., 1999) 14 days after cell fusion for the presence of antibodies which recognized conidial epitopes of *P. xanthii* or *G. orontii* were

selected. Cell lines which gave a positive results (> 3 times the negative control in PTA ELISA) were selected and twice cloned three times.

Develop a lateral flow device for measurement of M. melonis ascospores using existing monoclonal serum developed from PE001.

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 (CO83 Cellulose Fibre pad Cat no. CFSP223000, Millipore). A pre-filter VF2 pad (Cat no. 8124-6621, Schleicher and Schnell, Whatman) was also incorporated between the sample pad and the membrane. A test line of *Mycosphaerella melonis* spore antigen 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 strips. A volume of UW 325 of the antibody made up in conjugate buffer was mixed with 5µl of an anti-mouse IgM 40nm gold conjugate (Code BA GAMM 40, British Biocell International, UK). 30ul of the antibody/ gold conjugate solution was then pippeted on to individual clfd conjugate sample pads, air dried at 37ºC for 35 min and each pad was attached to the immunochromatographic test strip The clfd devices were mounted within a plastic housing device (European Veterinary Laboratory, Netherlands : www.evlonline.nl) as shown in Fig. 3a. Test and control line development was recorded using an ESE portable reader (Fig.3b)



a)

b)

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

A range of concentrations of UW325 and *M. melonis* spore antigen were assessed in this format for test line formation. The process was also repeated but using different membrane types:

- FF170HP (GE Healthcare)
- Nitrocellulose membrane (BioRad)
- Immunopore (GE Healthcare)
- High Flow Mebranes (Millipore)

Also, application of the *M. melonis* spore antigen to the test line was assessed in buffer combinations of sugar (1, 5 or 10%), Isopropanol (1, 5 or 10%) and pH (6, 7 or 8).

The above study was repeated but this time UW 325 was omitted and replaced with UW 339.

Commence bio-aerosol monitoring at a commercial cucumber operation with weekly testing for Mycosphaerella melonis spores.

Air samplers were set up at three commercial cucumber production sites at the beginning of the 2015 growing season (Fig. 4). A detailed description of the air samplers, operation and spore assessment is described below

Figure 4. Three air samplers (left to right - single cyclone, MTIST and multi-vial cyclone) set at a sampling height of 1 m in a UK commercial cucumber crop.

Microtitre Immunospore Trap (MTIST). A detailed description of the MTIST device can be found in Kennedy *et al.,* 2000. The sampler contains four microtitre strips each containing 8 wells (Fig. 5). The MTIST air sampler uses a suction system and particulates in the airstream are impacted on the base of each collection well of the four microtitre strips. The eight well microtitre strips were coated with a combination of 2 strips at 0.1mg ml-1 Poly-L-Lysine (Sigma P-1524) in distilled water and 0.05% sodium azide (Sigma P-1524) and 2 strips 5:1 mixture of petroleum jelly and paraffin wax (Wakeham *et al.,* 2004).

Figure 5. Personal MTIST air sampler with four, eight well microtitre strips

An MTIST spore trap was sited in a glasshouse at each cucumber production site and operated continuously through the season off a mains electricity supply (Fig 4). To provide a gummy stem blight risk estimate the 4x8 well coated microtitre strips were changed weekly and posted to the University of Worcester. On arrival at the University of Worcester two of the four 4x8 well microtitre strips were processed by PTA ELISA (plate trapped antigen enzyme-linked immunosorbent assay (Kennedy *et al.,* 2000)). The remaining strips were retained and stored at -20°C. These would be used later to assess potential to quantify Cucumber Powdery Mildew risk i.e. once a suitable Monoclonal Antibody had been produced. Often, as a result of postal delays, the microtitre wells were not processed by PTA ELISA until Friday. This may have resulted in reactive crop protection applications being carried out the following week. The recommended interval between spray applications for the experimental products was two weeks, so this also prevented reactive applications from being made if an application had been made the previous week.

Burkard cyclone air sampler (single vial). The characteristics of a cyclone air sampler are described by Ogawa & English (1955). Air is drawn through the sampler using a vacuum pump in the form of a cyclone. The height of the cyclone and air inlet, along with the width of the air inlet, air exhaust diameter and the diameter of the cyclone within the length of the exhaust pipe influence the relative efficiency of the trap. These characteristics have been drawn together and standardised within the Burkard cyclone sampler (Burkard Manufacturing Co.). The cyclone air sampler operates at an air flow rate of 10 to 15 L air / min, and air particulates are trapped in a 1.5ml microfuge tube (Fig. 6). At each of the sites the sample

tube was changed weekly, posted to the University of Worcester and on receipt stored at -20^{0}C. The tubes will be assessed in Year 2 of the project following development of a lateral flow testing device for *M. melonis*.

Figure 6. Single vial air sampler

Burkard cyclone air sampler (multi-vial). The sampling characteristics of the multivial sampler are as described for the single cyclone air sampler. However a difference being that the air sampler is loaded weekly with eight 1.5ml microfuge tubes (Fig. 7). By an integrated automated mechanism each tube is exposed once for a period of 24hrs to provide for the collection of air particulates. The sampling period for each 24hour period was activated at midnight. As for the single vial cyclone, seven tubes were collected once weekly after glasshouse exposure and posted to the University of Worcester. The eighth tube remained *in situ* so as to provide uninterrupted sampling for that 24hr period. On receipt the tubes were stored at -20^{0}C. The tubes will be assessed in Year 2 of the project following development of a lateral flow testing device for daily M*. melonis* ascospore concentration.

Figure 7. Multi-vial air sampler

RESULTS

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* with no *G. orontii* present. Earlier studies (cucumber plants grown at the University of Worcester, data not shown) had identified *G. orontii* in September/October 2014 while *P. xanthii* was detected throughout the growing season. Further leaf samples were obtained in October from growers and these samples are currently being evaluated.

Development of monoclonal antibodies for the measurement of cucumber powdery mildew (G. orontii and P. fusca) in glasshouse bioaerosols

An immune response by each mouse was observed by ELISA to the conidial preparation types administered. Six positive hybridoma cell lines (Coded 2F12, 4H1, 6C1, 6E9, 5C4 and 4E3) have been cloned from Fusions 1, 2 and 3. Additional fusions are in place for December 2015. The final set of mice (<30KDa) are to receive final immunogen boosts in January 2016.

Develop a lateral flow device for measurement of M. melonis ascospores using existing monoclonal serum developed from PE001.

No test line observation was observed by lateral flow with monoclonal antibody UW 325 using any of the combinations tested. When the test was repeated, but this time using UW339 as a probe for the selective measurement of *Mycosphaerella melonis*, a test line was observed with High Flow (Millipore) and Immunopore (GE Healthcare) membranes. An antigen test line application buffer of 10% sucrose, 10% Isopropanol and pH 7 was optimal for each. A lateral flow test sensitivity range for *M. melonis* ascospores is shown below in Figure 8.

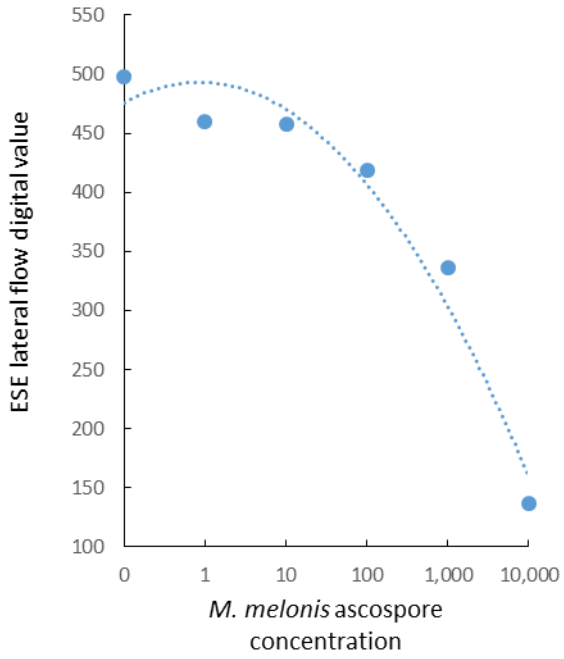


Figure 8. Measurement of *M. melonis* ascospore concentration range by lateral flow

Commence bio-aerosol monitoring at a commercial cucumber operation with weekly testing for Mycosphaerella melonis spores.

Site 1. *Mycosphaerella melonis* ascospores were recorded in bio-aerosols at Cucumber production Site 1 (Fig. 9). Spray application dates are also shown in Figure 9. The first crop was planted on the 6 January 2015. Low spore levels were recorded in the first weeks of the trial. The first application of fungicide (Amistar) was applied after significant numbers of spores were recorded during the week commencing 4 May 2015. This coincided with the removal of the first crop and the planting of the second crop on the 13 May 2015. A further application of Switch was applied during the week commencing the 18 May 2015. Numbers of *M. melonis* spores declined after the application of Switch (an effect which had been noted in previous experiments in cucumber crops). Spore levels declined but remained high and further peaks were observed on the 23 June 2015, and the 21 July 2015. The third crop was planted on the 22 July 2015. Successive weekly fungicide applications of Amistar (22 July 2015) followed by Switch (28 July 2015), Amistar (4 August 2015) look to have subdued a potential serious disease epidemic of *M. melonis* spores within the crop. Three weeks later, spores were observed at a lesser concentration in the bioaerosol sample tested. An application of Signum was applied at this time. Spore concentrations were seen to abate shortly after.

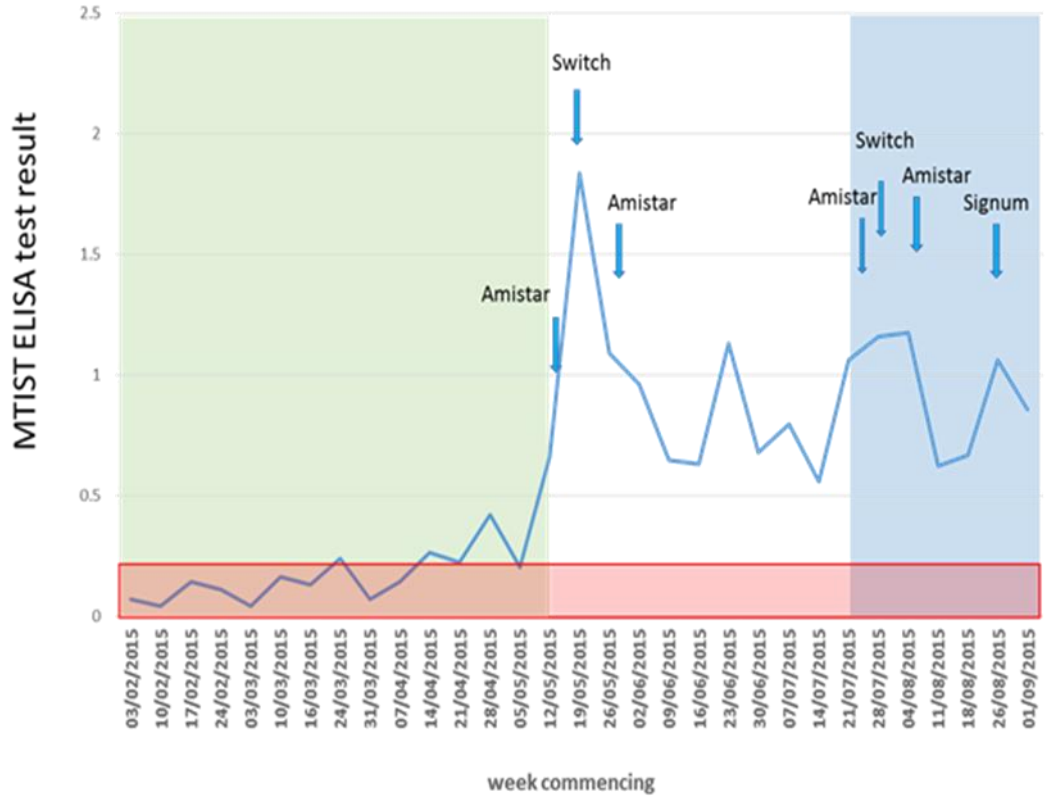


Figure 9. Monitoring glasshouse bio-aerosols for *M*. *melonis* ascosporic inoculum at Site 1 (three crops grown and indicated by coloured sections green, white and blue)

Site 2. The graph below shows the *M. melonis* ascospore levels recorded for site 2 with spray application dates (Fig. 10). The crop was removed from the glasshouse over a two week period. The first half of the glasshouse was removed on the 22 – 23 June 2015. The second half of the glasshouse was removed on the 6 and 7 July 2015. These were significant events however peaks in ascospore readings were observed before the glasshouses were cleared. Switch was applied in response to two peaks observed in February and early March 2015. Serenade ASO was applied in response to the ascospore readings rising above the 0.2 threshold at the end of April. It is unclear how specific the Serenade treatment would have been in controlling *M. melonis*. Further peaks in ascospore numbers were observed on the 18 May 2015 and 8 June 2015. Switch was applied in response to the latter peak. A further application of Switch was applied on the 13 July 2015 in response to a peak in *M. melonis*.

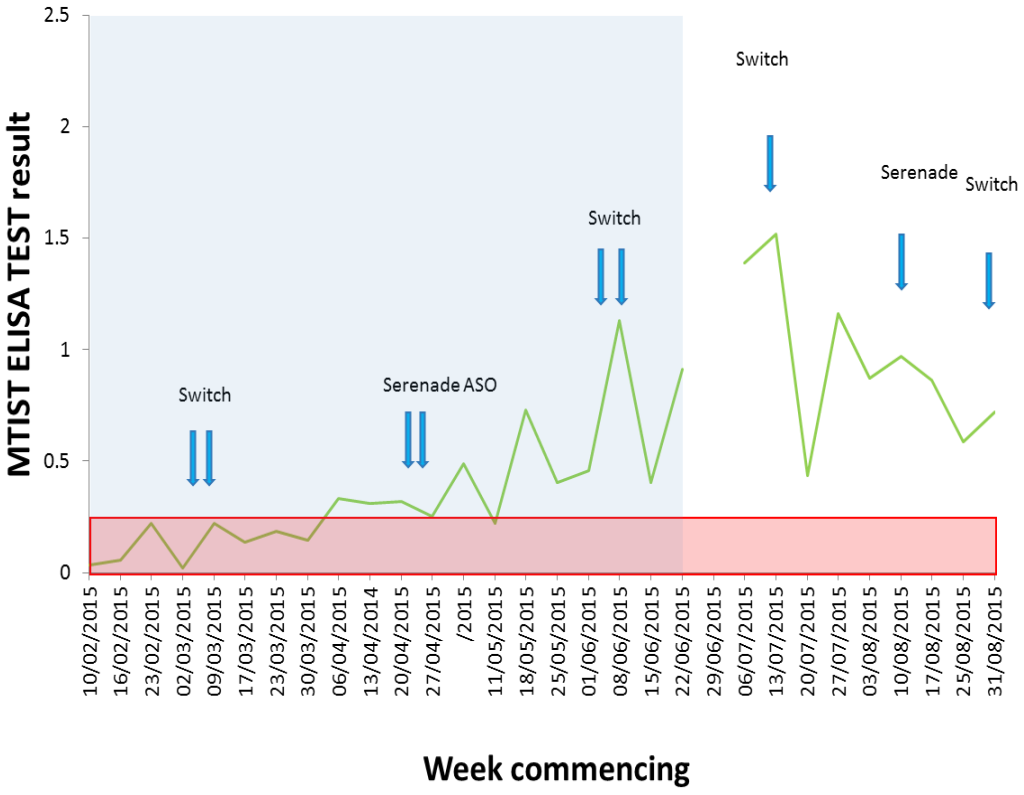


Figure 10. Monitoring glasshouse aerosols for *M*. *melonis* ascosporic inoculum at site 2 (two crops were grown and indicated by coloured sections blue and white)

Site 3 The third site did not supply fungicide application data. Only ascospore data is shown on the graph for the growing season (Fig. 11). The first crop was planted on the 6th January 2015. Significant ascospore concentration were observed in late March. Three increasing cycling periods of *M*. *melonis* ascospore numbers were observed around the 28 April 2015, 12 May 2015, and the 9 June 2015. Both Switch and Amistar were applied to the crop but the application date was not supplied. High levels of ascospores continued to be trapped over the period 7 July 2015 – 28 July 2015. Although, the cycling nature of the epidemic started in April it was contained during July with a plateau in spore concentration observed rather than a single increased larger peak (Fig. 11). At the end of July ascospore numbers in the air declined to much lower levels.

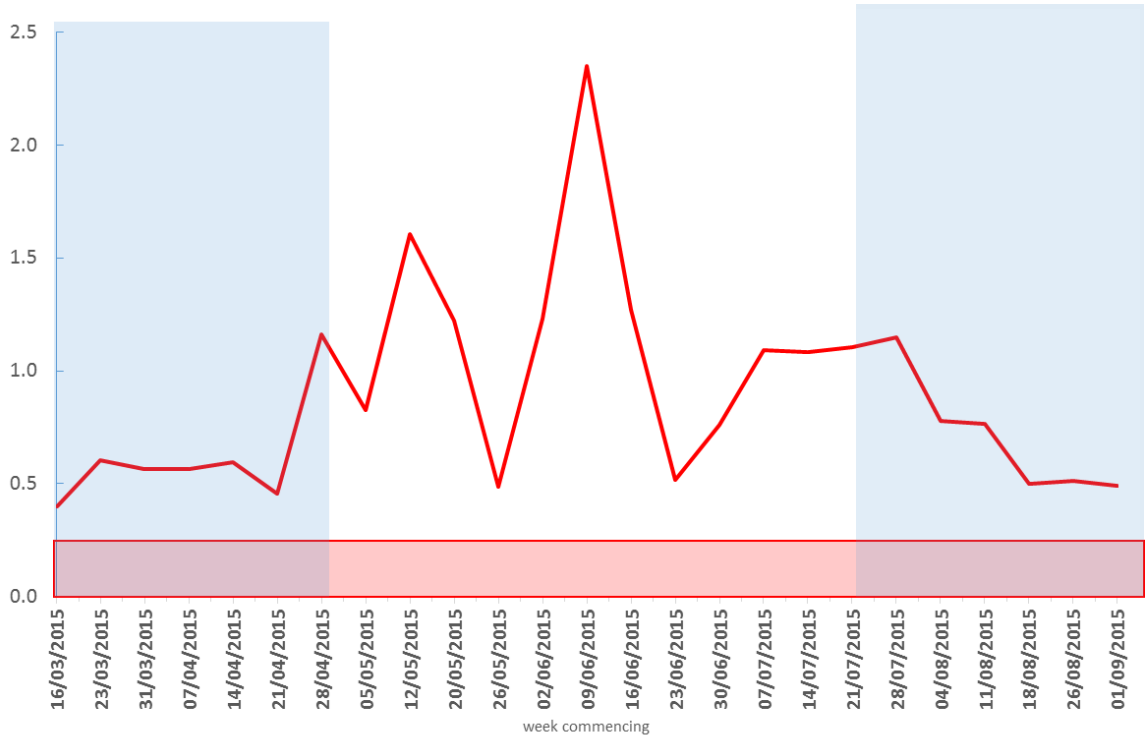


Figure 11. Monitoring glasshouse aerosols for *M*. *melonis* ascosporic inoculum at site 3 (three crops were grown and shown in coloured sections blue, white, blue)

DISCUSSION

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

The results to date have shown that only *P. xanthii* has been detected in tested UK commercial glasshouses, however evaluation of further samples for the 2015 season is underway to see whether *G. orontii* becomes apparent later in the season

Development of monoclonal antibodies for the measurement of cucumber powdery mildew (G. orontii and P. fusca) in glasshouse bioaerosols

Monoclonal antibodies (MAbs) have been produced to two Cucumber Powdery Mildew species (*P. fusca* and *G. orontii*). Further cell lines will be collected over the coming months and tested for their selective characteristics to discriminate the cucumber powdery mildew pathogens. By ELISA, a cell line(s) will be evaluated for capability to monitor MTIST trapped cucumber powdery mildew. The stored microtitre well bio-aerosol samples (2015 MTIST commercial activity) will be processed for powdery mildew risk.

During Year 2 of the project (2016) the ELISA assay format will be transferred to a rapid (10 minute) lateral flow format. Test sensitivity and stability of the lateral flow assay over time (test components shelf life) will be determined. Optimisation of the assay process to attain stability over a 12 month period will be investigated as required.

Develop a lateral flow device for measurement of M. melonis ascospores using existing monoclonal serum developed from PE001.

UW 325 had previously been used in studies (PE001) to selectively discriminate and quantify *M. melonis* in glasshouse bio-aerosols using an enzyme-linked immunosorbent assay (ELISA). In this study the process was not transferrable to lateral flow assay. This would indicate that the *M. melonis* epitope structure (site that antibody selectively binds to) is unable to functionally transfer to the solid phase of a nitrocellulose membrane. This was not the case for UW 339 which was able to bind to *M. melonis* antigen preparation in ELISA and lateral flow format. At this time, reactivity data to other spore types is not available for UW 339. This will be assessed in Year 2 of the project.

By lateral flow UW 339 provides a measurement of *M. melonis* ascospores. Preliminary findings indicate a quantitative range of 100 to 10,000 ascospores. This level of sensitivity by lateral flow should prove adequate in glasshouse assessment of *M. melonis* disease potential in bio-aerosols.

Commence bio-aerosol monitoring at a commercial cucumber operation with weekly testing for Mycosphaerella melonis spores.

Over the first months of sampling an increase in *M*. *melonis* risk was identified at each of the commercial operations. All sites developed elevated risk from the end of April into May 2015. However, an increased risk at site 3 was recorded from the outset with higher numbers of *M*. *melonis* ascospores observed during March 2015. The application of control products in response to risk was different between the two sites that supplied information (Sites 1 and 2). Combinations of Switch and Amistar were applied at both of these sites. However, where fungicide applications were applied to the crop earlier (Site 2) there was a delay in disease build up within the crop (as recorded by bio-aerosol). In contrast at site 1, where an increase in *M. melonis* spore concentration was observed week commencing the 28 April, a delayed application of fungicide may have resulted in the high levels of spores observed in May. Also, the application of Amistar made in May may have had little effect on infections present in the crop. Agar plate bioassays reported in HDC PE001a, showed Amistar to be less effective in inhibiting mycelial growth of Myco than Switch. An application of Switch made at the peak of the spore epidemic in May could have been critical in reducing the pressure of Myco disease development for the later part of the season at Site 1. A similar effect was observed at site 2 in July. No fungicide data was provided for Site 3.

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