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:	Final report, August 2017
Previous report:	Second Year Report (University of Worcester)
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Date project commenced:	01/11/2014
Expected completion date:	30/04/2018

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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

Diagnostic techniques have been used to monitor glasshouse air samples for spores which spread 'Myco' disease and cucumber powdery mildew. Ten minute on-site tests 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. Two species of cucumber powdery mildew have been found on UK grown cucurbits. In commercial UK production *Podosphaera xanthii* seems to be the dominant species observed.

Background

AHDB Project PE 001 developed approaches for improved control of *Mycosphaerella melonis* (Myco), and developed a laboratory test to monitor glasshouse air samples for Myco spore presence. With knowledge of 'Myco' inoculum, this current project (CP 137) aims to add to work carried out in PE 001 and 001a to provide improved timings for fungicide application. *Podosphaera xanthii* and *Golovinomyces orontii* cause cucumber powdery mildew. Information on both is required for use within an integrated disease management system for the effective control of powdery mildew on cucumber.

*Mycosphaerella melonis (*also known as 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 are involved in the spread of the disease. The infection of flowers and developing fruit leads to fruit rot, though in many cases disease symptoms are not visible until the fruit is marketed. This leads to rejections 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, even though many cultivars now have tolerance (but not resistance) to the disease. In the development phase of crop production 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%. Inappropriate 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 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 the early cucumber cropping season 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 seven 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 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 gives a latent period of up to one month for *Mycosphaerella brassicicola*, the causative agent of ringspot on Brassicas. *M. melonis* ascospores, which have a mucilage coating like *M. brassicicola,* and 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 pathogen forecasts 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 the application of chemicals 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 as well as reducing residues in the crop.

Summary

During 2015, 2016 and 2017 at protected cucumber production sites in the UK, diagnostic tests have been used to estimate Myco spores in daily and weekly collected air samples. Collected air samples have been assessed for spore types which cause powdery mildew. A Microtitre immunospore trap (MTIST) shown in Figure 1a has been used for laboratory testing of samples.

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

This trap uses an ELISA (Enzyme Linked ImmunoSorbent Assay) a process which 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, the decision was made to include additional information on Myco spore concentration by conducting time-consuming microscopic examination of the microtitre wells. Reliability of data generated in this way can be 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.

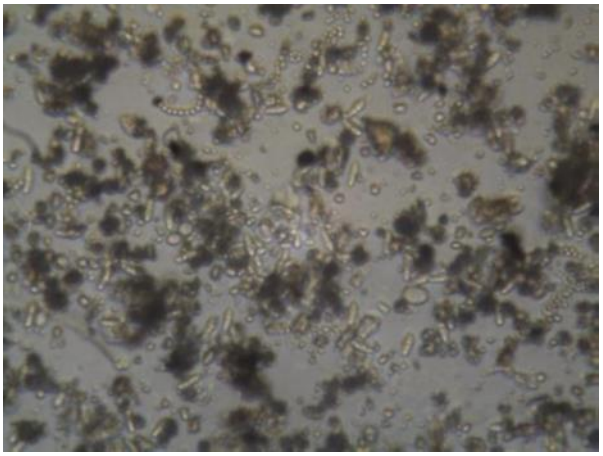


Figure 1b. Glasshouse bio-aerosol sample deposition on the base of a microtitre well as viewed by bright field microscopy (400x)

At site 2, low risk of Myco infection was observed until 15th March (<0.2 MTIST ELISA). 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 in 2016 on external plant tissue in the week commencing 5th April and in harvested cucumbers from the 27th April. By the 31st May a 'high' risk of Myco spores was recorded. From these results, it is possible to understand the polycyclic (the pathogen completes its life cycle more than once during the life of the crop) 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 2016. 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 2016.

In 2017 Myco spores were observed at high concentrations during September 2017 at one site but much earlier (May 2017) at another. During 2017, further development of a rapid test for cucumber powdery mildew was successfully completed but the sensitivity of the test was poor. Only samples where in excess of 1000 – 5000 powdery mildew conidia were detectable as positive results. The sensitivity of the test could be improved by switching to higher volume air samplers although these would be more expensive.

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, eight tube samples during the changeover). At the end of each week the tubes were collected and used in a lateral flow test 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, high 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.

Different air samplers 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), and 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 commonly associated with this disease. Further work is required to investigate this result and develop the Myco lateral flow test for commercial manufacturing.

Financial Benefits

The main financial benefits will be in the use of these tests to reduce unnecessary crop protection inputs or to apply more-timely sprays to cucumber cropping systems. Fungicide usage produces unwanted residues, is costly and can be one of the major inputs in crop production after fuel and labour. The availability of fungicidal control products is also being reduced due to increased regulation. 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 prolonging their useable life. However, savings will be variable from one season to the next and depend on the number of spray applications made to the crop.

SCIENCE SECTION

Introduction

During 2017 the project was transferred to Warwickshire Colleges Group (and sited at Pershore College – one of the seven colleges in the Warwickshire Colleges Group) where there were glasshouse facilities essential for the testing involved in this project. Developing action thresholds for interpretation of diagnostics tests is an important aspect in the development of diagnostic tests. It is a requirement of any integrated disease management system to provide information for end users. Monoclonal antibody cell lines have been developed to *Mycosphaerella melonis* (Myco), *Podosphaera xanthii* and *Golovinomyces orontii* (cucumber powdery mildew). During 2017 the diagnostic probes were utilised within assay formats and assessed for capability to monitor glasshouse air samples for powdery mildew within controlled trial crops at Stockbridge Technology Centre. The ability of the developed probes to detect target spores in air samples collected during year one and two of the project was also assessed. The sampling regime (daily or weekly) requires assessment for its practicality in control systems. Further data from commercial cucumber production systems was also undertaken as in year one and two of the project. In this report, we describe Year 3 project activities where between August 2017 and April 2018 the ability of the diagnostic tests was assessed where practically possible.

Milestone

Monitor bio-aerosols for cucumber powdery mildew in a controlled glasshouse experiment at STC. Identify airborne biological pathogen threshold for symptom development.

Methods

Monitored Cucumber Trial at Stockbridge Technology Centre

A monitored cucumber trial was established at Stockbridge Technology Centre during spring 2017. The crop was produced according to previous protocols (see AHDB report PE 001). The trial plan (Figure 2) shows the location of the spore traps in the crop. Plant numbers 4 to 21 were assessed in each row. Five leaves per plant were assessed as per the EPPO guidelines on five separate occasions during the season. Only powdery mildew was assessed in the crop as *Mycosphaerella melonis* (Myco) did not develop.

Trial Design

There were 8 rows of cucumbers grown in the glasshouse at commercial spacing on rockwool (see Pe001 Final report). The four air samplers used in the trial were placed at the edge between each set of twin rows. Guard rows were placed on the outside of the glasshouse. Disease monitoring on marked plants was carried out between each double row. The crop was planted on the 7 June 2017

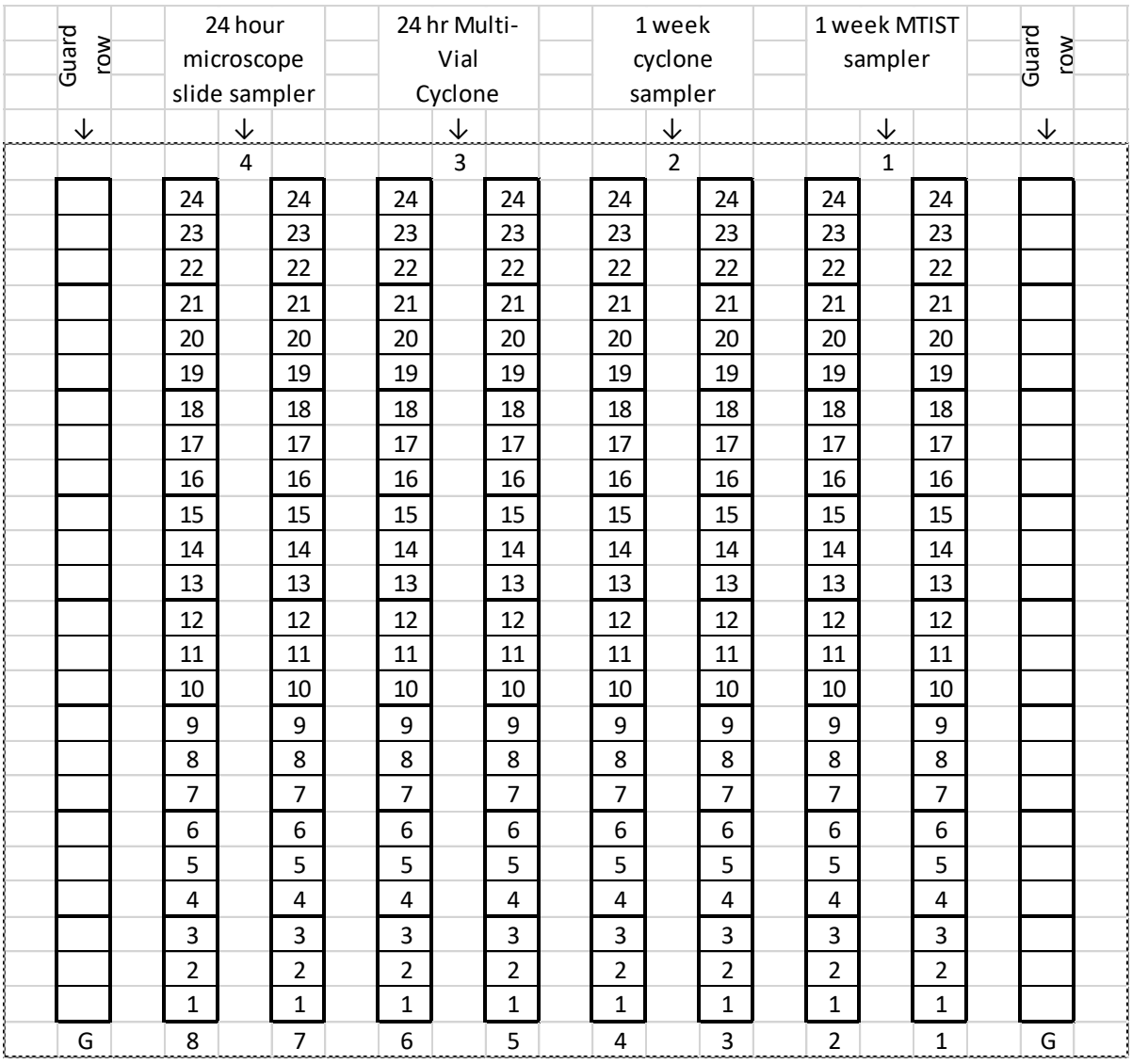


Figure 2 Trial design of cucumber crop grown at STC.

Assessment during the Trial

There were no outbreaks of *Mycosphaerella melonis* (Myco) during the trial. Disease estimates of powdery mildew were taken although the differentiation between species could not be recorded.

Plant disease assessments were as follows:

1) The percentage of upper leaf area affected by PM sporulation
2) The percentage of lower leaf area affected by PM sporulation
3) The percentage of stem area affected by PM sporulation

The following air samplers were used to monitor powdery mildew population levels:

1) 24 H Microscope slide Trap (Trap changed daily)
2) MTIST Trap (Strips changed weekly)
3) Multi-cyclone Trap (Tubes automatically changed daily)
4) Single Cyclone trap (Tube changed weekly)

Results

Development of powdery mildew in the crop

The results of the Stockbridge Trial are shown in Figure 3. Both species of powdery mildew were detected within the trial. This is an early report of the occurrence of *Golovinomyces orontii* (previously unrecorded in UK cucumber crops). The results suggest that both species may have different epidemiological requirements. *Podosphaera xanthii* appeared to develop early in the crop and was present at crop planting. However this species did not develop and was replaced by *Golovinomyces orontii* which continued to develop as the crop neared maturity. The presence of powdery mildew during the early life of the crop means that the limit of detection should be used as the threshold for detection of powdery mildew. A statistical analysis showed some evidence of positional effects in the glasshouse. The most obvious was a much lower disease severity in row 5 of the crop. A possible explanation for this was the occurrence of a heavy aphid infection half way through the trial (despite the application of an insecticide). The leaves were covered with aphid honeydew and sooty mould so difficult to make measurements of powdery mildew. Low levels of powdery mildew were detected

using the slide trap during the night (of the 7 June 2017) which was a few hours after planting. The results are shown in Table 1.

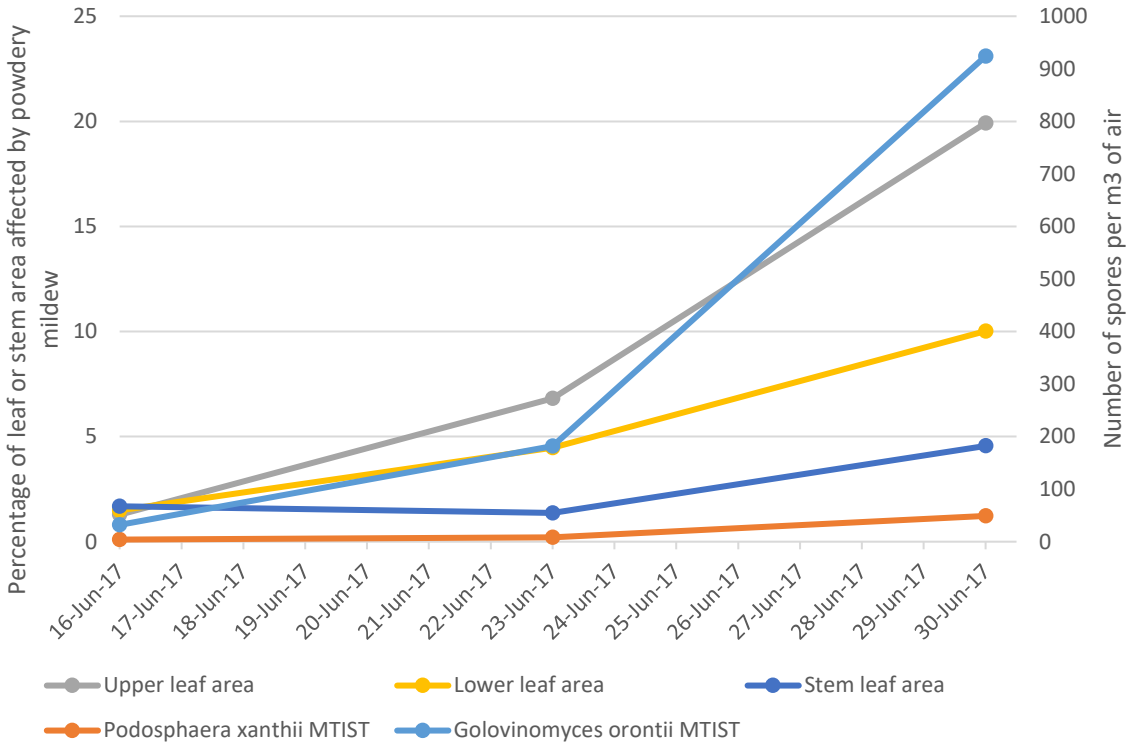


Figure 3 Disease development by powdery mildew and powdery mildew spore counts

Date	Period	Notes	Time spore trap starte	GC	PX	GC + PX	% Upper Leaf Area Affected	SD	SE
31/05/2017	Night								
01/06/2017	Day								
01/06/2017	Night								
02/06/2017	Day								
02/06/2017	Night		(p.m)	0.00	0.00	0.00			
03/06/2017	Day								
03/06/2017	Night								
04/06/2017	Day								
04/06/2017	Night								
05/06/2017	Day								
05/06/2017	Night		(p.m)	0.00	0.00	0.00			
06/06/2017	Day								
06/06/2017	Night								
07/06/2017	Day	Crop planted							
07/06/2017	Night		(p.m)	0.50	0.00	0.50			
08/06/2017	Day								
08/06/2017	Night								
09/06/2017	Day								
09/06/2017	Night		(p.m)	2.73	0.00	2.73			
10/06/2017	Day								
10/06/2017	Night								
11/06/2017	Day								
11/06/2017	Night								
12/06/2017	Day		(a.m)	11.41	1.49	12.90			
12/06/2017	Night								
13/06/2017	Day								
13/06/2017	Night								
14/06/2017	Day								
14/06/2017	Night		(p.m)	38.69	2.73	41.42			
15/06/2017	Day								
15/06/2017	Night	1st Assessment	(p.m)	31.00	1.49	32.49	1.46	1.73	0.06

Table 1 Spore counts taken after planting using the microscope slide trap

Conclusions

The results show that powdery mildew should be controlled throughout the period of crop growth. There was an increase in powdery mildew inoculum over the duration of the trial indicating that early detection of mildew was an important prerequisite of control. The results show that mildew was already present from the time of crop planting. This indicates that unless fungicides are used early in the development of the disease which reduce sporulation there is a likelihood that the pathogen population will continue to develop in the crop and later application of fungicides would be of little or no benefit. A new active ingredient has been approved for control of cucumber powdery mildew. Cyflufenamid, has a novel mode of action (Takumi SC) and has both preventative and curative activity, with translaminar movement giving long-term protection on treated leaves. Only 2 applications per crop are permissible however it is not clear what strategy for applying cyflufenamid to the crop might be more successful. Applying cyflufenamid when symptoms appear is unlikely to give satisfactory longer term control. It is also unclear if resistance to powdery mildew will occur after the usage of this active ingredient. (note cyflufenamid has a water volume limit that makes it difficult to apply effectively as a

high volume spray – but is more effective as a ULV application – as long as there is good air circulation in the crop)

Monitoring cucumber pathogens at commercial sites in 2017 using diagnostic probes

Milestone
Provide support for the 2017 growing season to the UK industry with use of the cucumber Myco lateral flow test.

Methods

Weekly bio-aerosol monitoring at a commercial cucumber sites

Air samplers were set up at two commercial cucumber production sites at the beginning of the 2017 growing season. A detailed description of the air samplers, operation and spore assessment is described previously (see year 2015 and 2016 experimental sections). Samplers consisted of the following types.

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).

Burkard cyclone air samplers (single and multivial). The characteristics of a cyclone air samplers 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 samplers operate at an air flow rate of 10 to 15 L air / min, and air particulates are trapped in a 1.5ml microfuge tube. At each of the sites the sample tube were changed weekly, posted to Warwickshire Colleges and on receipt stored at -20^{0}C. The tubes were used to assess a lateral flow testing device for *M. melonis*.

Results 2017

Site 1

Mycosphaerella melonis

Mycosphaerella melonis MTIST ELISA spore warning exceeded the low / moderate threshold on the 1 May 2017 (Fig.4). A high spore concentration in the air was identified for the week commencing 1 May 2017. Spore concentrations recorded in the crop remained high / moderate risk until week commencing 15 May 2018. The second crop planting (04/05/2017) occurred during a period of moderate *M. melonis* inoculum risk by MTIST ELISA and remained at or near this until the week commencing 5 June 2017. A lower level of spore concentration as measured by microscopic analysis was also observed for this period. MTIST ELISA measurements were low until the 3 July 2017. Sprays on the second crop were applied as Systhane (myclobutanil) (Enbar / ULV) on 11-5-17 with Switch (cyprodinil and fludioxonil) applied on the stem bases on 12-5-17. A second spray of Systhane was applied on the 18-5-17 with a follow up spray of Signum applied to the full crop on the 30-5-17. Switch was again applied to the stem bases on 4-7-17. A second spray of Signum (full crop) was applied on the 7-7-17 during a moderate period of Myco risk. A third spray of Systhane was applied in early July 2017. Myco lesions were not observed in the crop until the 24 July 2017.

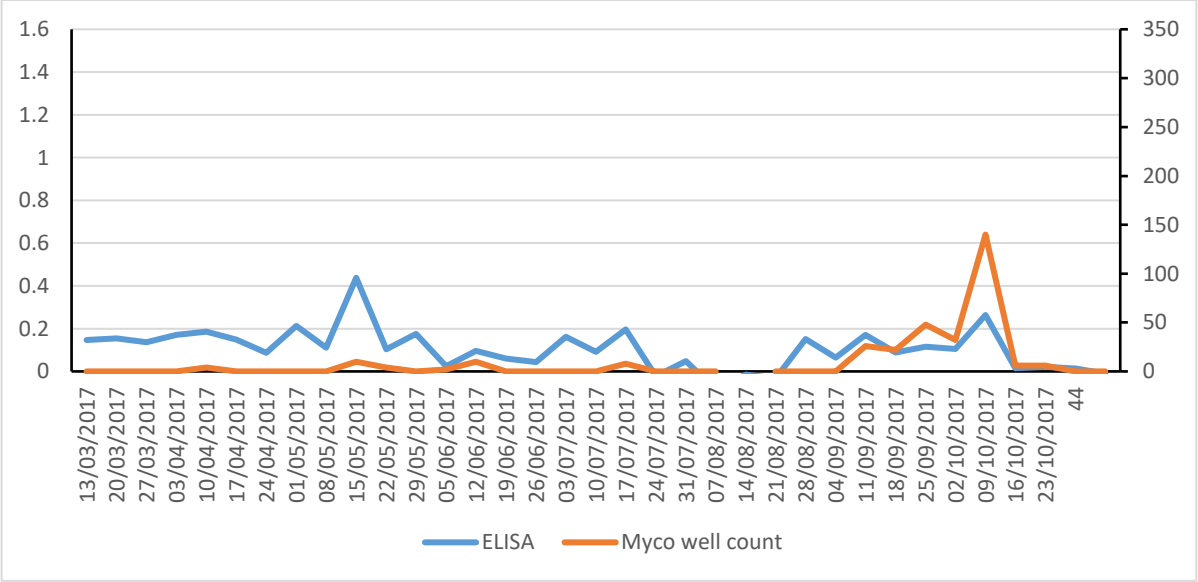


Figure 4 *Mycosphaerella melonis* spore count and ELISA reading at Site 1 during 2017.

The third crop was planted during 20 July 2017 but no wells were sent for analysis. Further control sprays were applied on 23-8-17 and 5-9-17. The MTIST showed a moderate to high

risk of *M. melonis* in air samples from the end of August 2017 until 16 October 2017. Myco lesion development was first observed on cucumber plants of the third crop on the 25 September 2017.

Powdery Mildew

The levels of powdery mildew recorded at site 1 in 2017 were very low (Figure 5). Powdery mildew was first observed in the trap on the 17 April 2017. This was identified as *Podosphaera xanthii* . Further periods of elevated powdery mildew risk was observed on the 22 May (Figure 5) and the 3 July 2018. These elevated levels consisted of both *G*. *orontii* and *Podosphaera xanthii*. Applications of Systhane to the crop as Enbar / ULV would have had activity against powdery mildew and these were applied during May 2017.

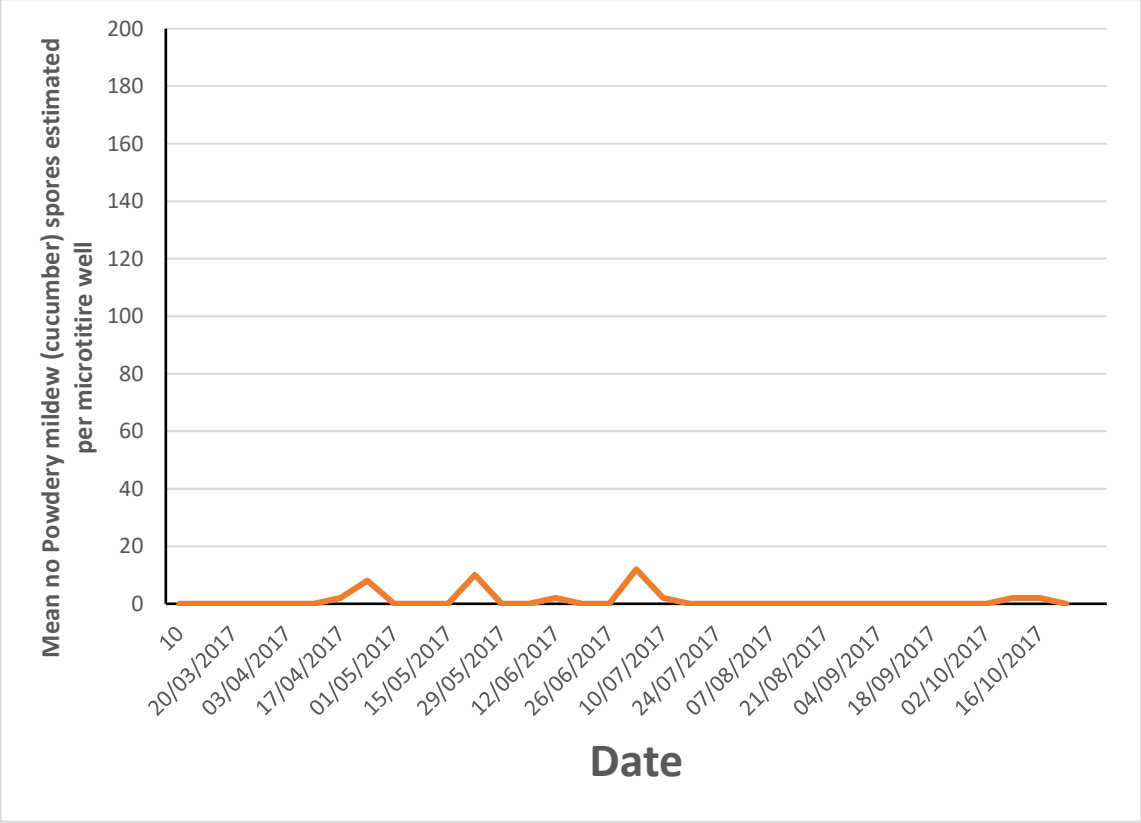


Figure 5 Powdery mildew spore count at Site 1 during 2017.

Site 2

Mycosphaerella melonis

Fewer details were supplied for site 2 crop operations. The samplers were placed in the crop during the week beginning 6 March 2017. Significant levels of *Mycosphaerella* were observed during May and June 2017. A small peak in *Mycosphaerella* was detected at the beginning of May 2017 (Figure. 6). Further larger peaks in *Mycosphaerella* spore numbers were observed from the 12 June 2018 until the beginning of July 2018. There was a large number of *Mycosphaerella* spores observed in the trap from the week commencing 26 June 2017 until the 3 July 2017. After that time a fault developed with the sampler however Warwickshire College had no responsibility or budget with which to get the sampler repaired. The sampler was working intermittently and further *Mycosphaerella* spores were observed during the week commencing the 10 July 2017 (Figure 6). After that date the sample strips in the sampler were not changed as the sampler was not functioning.

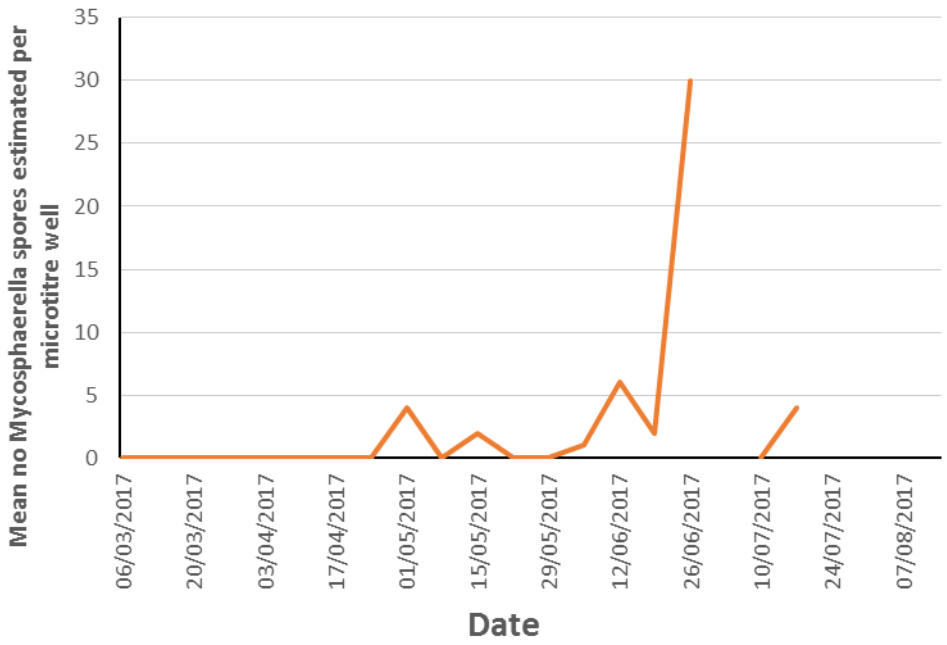


Figure 6 *Mycosphaerella melonis* spore counts at Site 2 during 2017.

Powdery Mildew

The levels of powdery mildew recorded at Site 2 in 2017 were moderate to high (Figure 7).

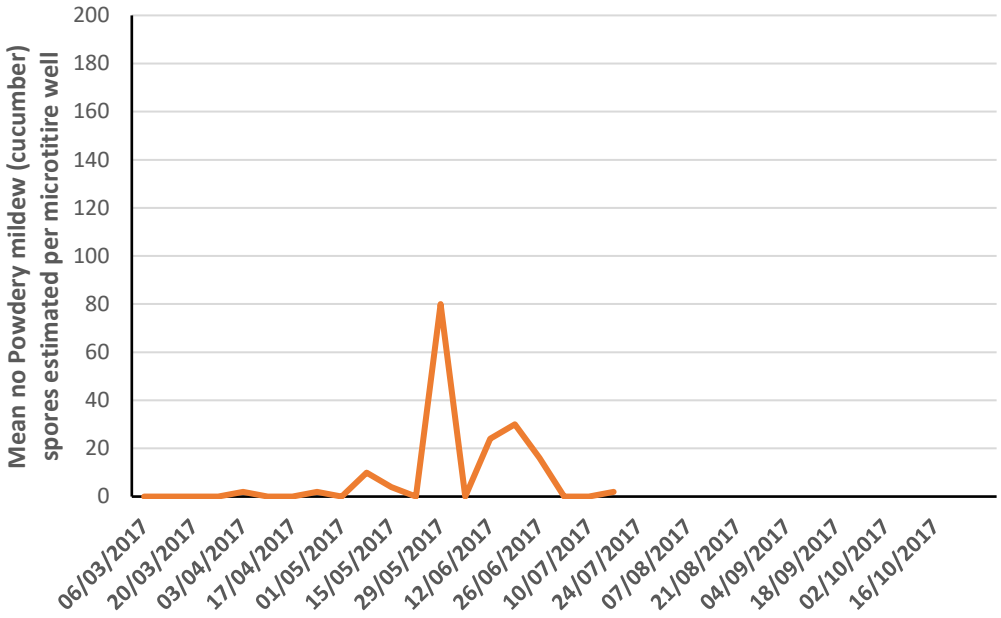


Figure 7 Powdery mildew spore count at Site 2 during 2017.

Powdery mildew was first observed in the trap in the week commencing the 3 April 2017. Initially only a few conidia were observed but the peak number of powdery mildew conidia were observed during the week commencing 29 May 2017. Numbers of powdery mildew conidia were still high during late June 2017 and early July 2017 when up to 30 conidia were recorded in the trap over the weekly period. Numbers of powdery mildew were not recorded after that time due to problems recorded earlier.

Conclusions

The results show that both *Mycosphaerella* and powdery mildew could be detected in air samples taken in commercial crops before the onset of symptoms. This is an important point because the use of the positive tests using the detection system could be used to schedule control methods. At two sites in 2017 *Mycosphaerella melonis* and powdery mildew were both detected during the second cropping period at planting. No data was collected on the infection of the first cucumber crop by either pathogen at either site. However the inoculum could have been present on the crop at planting. It is likely that powdery mildew was present early in the life of the crop as this observation was also confirmed within the trial planted at Stockbridge Technology Centre in 2017. Further information on disease occurrence in these monitored crops and any crop measures

applied would be of value. However these were not recorded by those responsible for the project during May 2017 – September 2017 at site 2.

Cucumber Powdery Mildew Lateral flow Development

Milestone

Evaluate the powdery mildew lateral flow system using stored bio-aerosols collected from the commercial cropping system in Year 1.*

*** Warwickshire College's ability to deliver these milestones is subject to the full series sample being available from University of Worcester.**

Methods

Elisa Development

When the research on powdery mildew commenced it was unclear that there were more than one species of powdery mildew on cucumber crops. Lateral flow devices developed for powdery mildew spore detection should detect both species. Both G*olovinomyces orontii* in and *Podosphaera xanthii* appeared to occur in the crop simultaneously however *Podosphaera xanthii* appears more prevalent during crop planting. An ELISA process was used to measure *G. orontii* and *P*. *xanthii* using monoclonals developed in this project. The antibodies were chosen on their ability to detect soluble spore antigen common to both powdery mildew pathogens and discrimination of other air spora. These cell lines (Table 2) were 13, 21 and 4 (13 and 13a were two different batches of the same cell line). Sensitivity of the assay was improved by use of a Warwickshire College streptavidin biotin assay. The cell lines optimised in ELISA were used to detect powdery mildew in samples taken from the Site 1 (2017) trial which had powdery mildew present. Where spores were collected in liquid phase (and soluble antigen release) and applied to microtitre wells a correlation was observed between spore concentration and resulting assay absorbance (Figure 8).

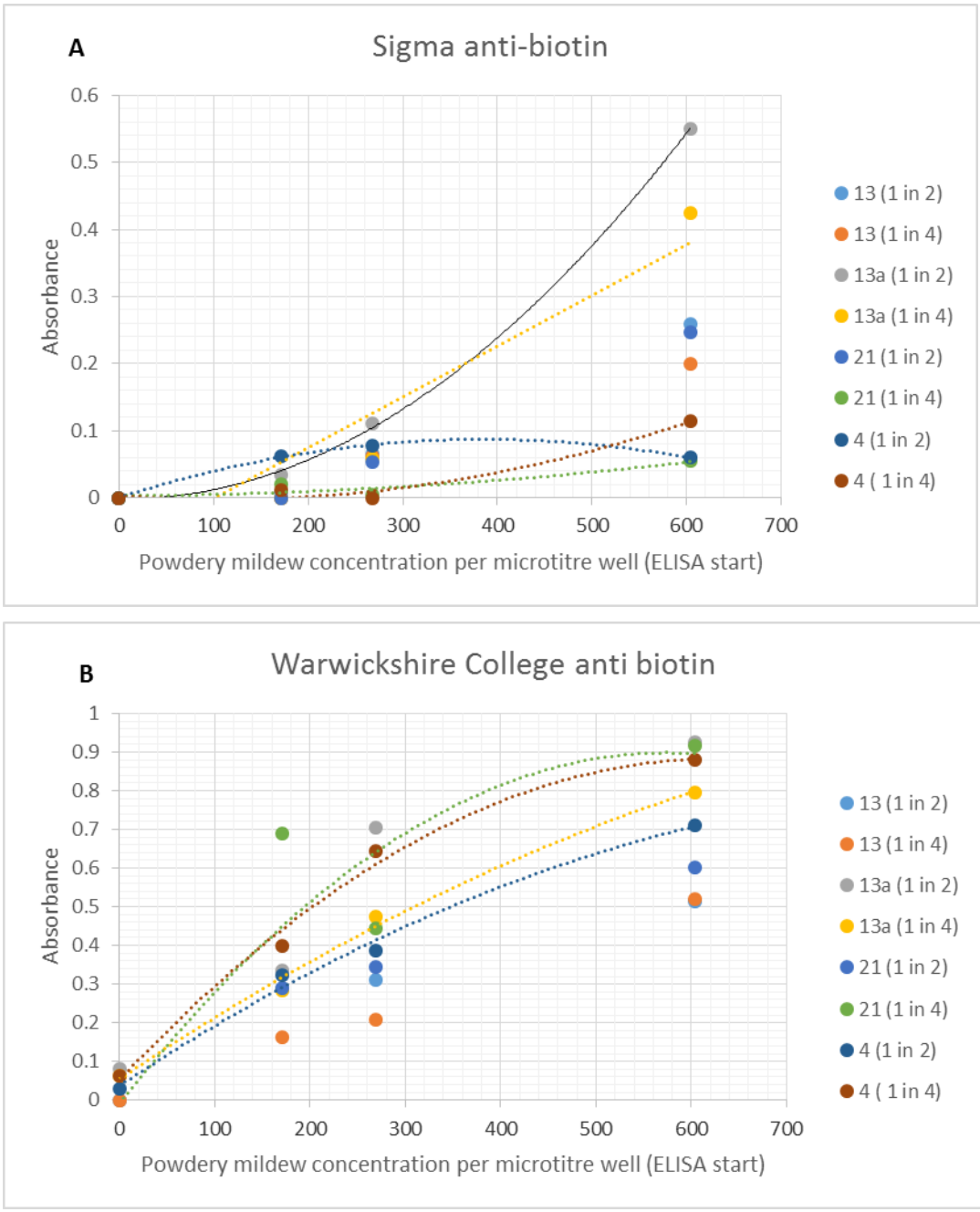
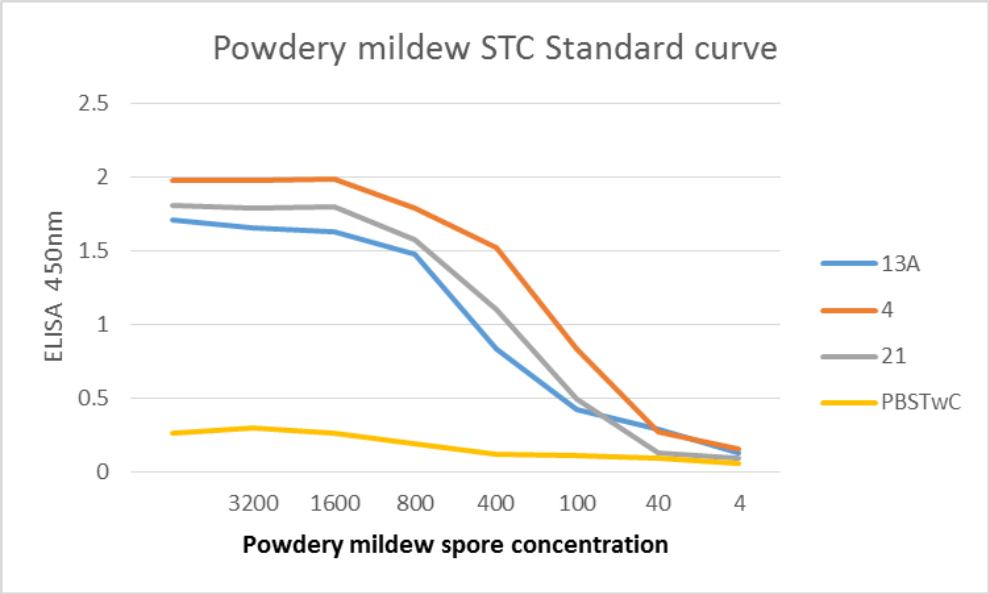


Figure 8 Sensitivity of cell lines raised against powdery mildew in year 2 of the project. A) Sigma biotin assay B) Warwickshire College biotin assay

The sensitivity of cell lines was established using standard Sigma biotin/streptavidin and Warwickshire College biotin streptavidin system. The Warwickshire College system had the best sensitivity for the detection of powdery mildew concentrations in ELISA (Figure 8B). Similar results were obtained for all three cell lines using this system. ELISA wells from the Stockbridge Technology Centre Trial in 2017 were used to investigate variation between antibodies in their sensitivity for detection of powdery mildew spores. ELISA

wells were used which had high levels of powdery mildew, as determined using microscope counts. Figure 9 shows the results using cell lines 13a and 4.

A)



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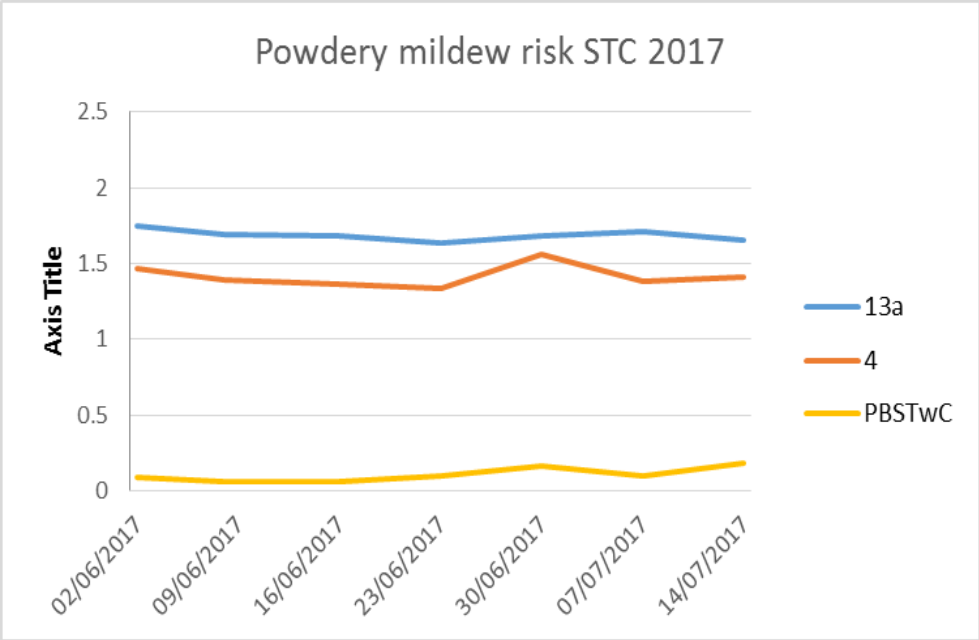

Figure 9 A) standard curves for cell line 13A, 4 and 21 B) Absorbance Levels from STC trial using cell lines 13A and 4.

The sensitivity of cell lines to *Golovinomyces orontii* and *Podosphaera xanthii* is shown in Table 2.

Lateral Flow development

As each of the monoclonals developed was IgM it was not possible to directly conjugate to gold. For this reason, it was not possible to develop a DAS lateral flow to soluble antigen of powdery mildew spores. Instead a competitive assay was developed using a homologous antigen test line. However, only one of the antibodies was able to bind to a complementary epitope at the homologous test line (Table 2). Assay kinetics are markedly different between ELISA and lateral flow. IgM antibodies are known to be of low affinity which will compromise ability to bind at the test line where exposure to the antigen is short. Also, if recognising a carbohydrate epitope (likely as IgM) exposure may be limited due to nitrocellulose binding properties (antigen moiety i.e. glycoprotein) or simply washed away.

Cell line	Raised against	Recognises P. xanthii and G. oronti	Code Number and Isotype	Conjugation possible for DAS format	LFD test line anti-species (competitive)	Depletion of test line in competitive format	LFD Homologous antigen test line (competitive)	Depletion of test line in competitive format
6E9A7C3	P. xanthii	Positive	4	No as IgM	Yes	No, binds to a soluble antigen	No	N/A
1H3C9H4 /1	G. oronti	Positive	13	No as IgM	Yes	No, binds to a soluble antigen	Yes	Yes
6C1B8C9	P. xanthii	Positive	21	No as IgM	Yes	No, binds to a soluble antigen	No	N/A

Table 2. Cell lines raised against *Golovinomyces orontii* and *Podosphaera xanthii*

The competitive lateral flow comprised of a Millipore 180 HiFlow™ cellulose ester membrane direct cast on to 2ml Mylar backing (Cat No. SHF2400225, Millipore Corp, USA.), an absorbent pad (Cat No. GBOO4, Schleicer and Schuell, Germany), a filtration and sample pad (Cat No. T5NM, Millipore Corp., USA). A test line application of

Golovinomyces orontii soluble spore antigen in a solution of sucrose and ethanol was applied at a rate of 10mm sec.-1 to the membrane. The lateral flows were air dried at 37ºC for a period of 4 hours and cut in to 5 mm strips.

The monoclonal antibody cell line 6E9A7C3 (Table 2) which had been raised to *Golovinomyces orontii* was conjugated to 40nm gold conjugate and transferred to a sample pad. Once dry the lateral flow device was assembled and housed within in a plastic moulding.

The LFD device was tested using available samples where powdery mildew was present in the crop.

Results

Samples from Commercial sites 2015 – 2018

Many collection tubes were used in tests involving the use of the *Mycosphaerella melonis* LFD device during 2015 and 2016. Consequently there were few tubes available (where crops had developed powdery mildew. In 2017, site 1 developed very low levels of powdery mildew which was well controlled. Site 2 produced few tubes with the presence of powdery mildew in 2017 due to sampler malfunction.

Stockbridge Trial

Powdery Mildew Development

The Stockbridge Technology Centre trial developed high levels of powdery mildew and so there was an adequate supply of air sample tubes (both weekly and daily collection durations) containing powdery mildew in 2017. Both *Golovinomyces orontii* or *Podosphaera xanthii* developed in the trial but only *G. orontii* developed at high levels (Figure 10). Low levels of powdery mildew developed in the trial from the beginning of June 2017 until the 21 June 2017. After this time powdery mildew conidia in excess of 5000 per weekly collection were observed. This continued until mid-July 2017 when the trial was finished (Figure 10). Weekly tube collections were used to test the LFD powdery mildew device.

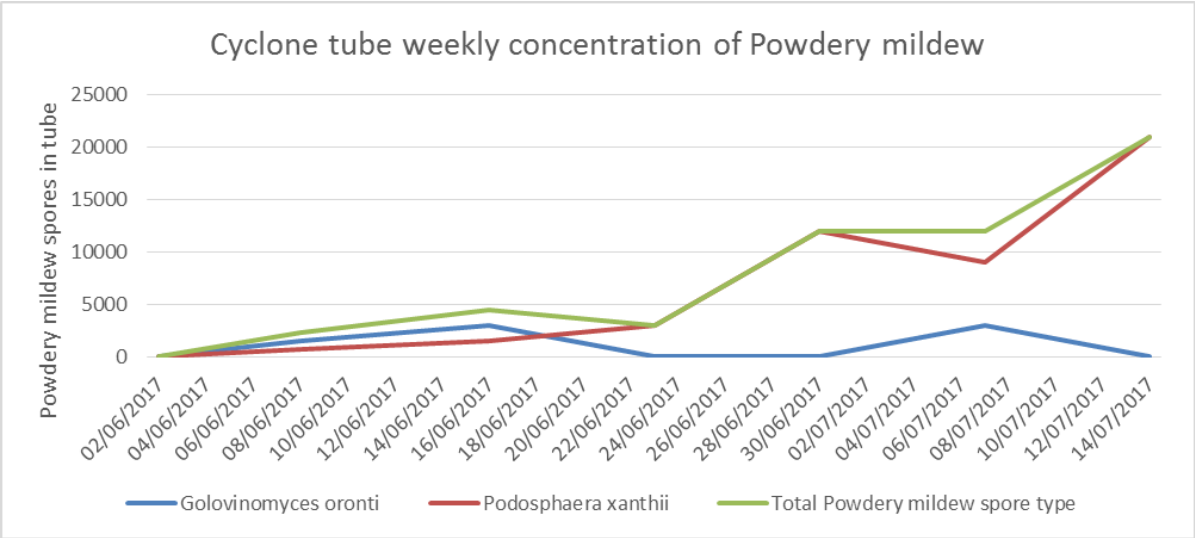

Figure 10 Cucumber Powdery mildew spore concentration in weekly tubes at Stockbridge Technology Centre in 2017

Lateral Flow Testing

When the LFD device was tested using collection tubes from the Stockbridge Trial only those collection tubes with conidia in excess of 5000 conidia per tube gave a quantitative reading (Figure 11). Complete test line depletion (competitive lateral flow format where no line equals a positive result) was achieved when spore concentration was near 1500 conidia per tube. Using a powdery mildew spore concentration which was free of bio aerosol contaminants a good correlation was observed across the concentration gradient and test line depletion achieved when approx. 20,000 spores were present. The results suggest that the bio-aerosol contains elements that react either specifically or non-specifically with the assay process and depletes the overall signal. This is particularly noticeable when spore concentrations are either not present or <5000.

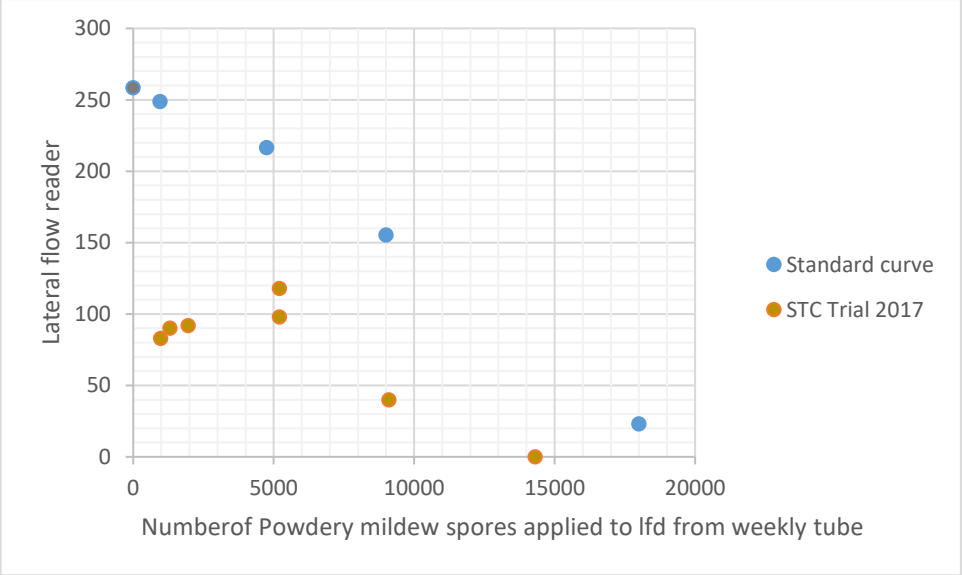

Figure 11 LFD tests using collection tubes with known powdery mildew levels

Conclusions

The limited number of powdery mildew contaminated tubes from infected crops resulted in limited testing of the powdery mildew device. However, where there is the absence powdery mildew in the collection tubes background and potential reactivity /non-specific binding was observed. Only when excess of 5000 spores applied to LFD (weekly LFD collections). At this level of detection powdery mildew would be visible on the crop. Powdery mildew lesions would be visible in the crop at 100 – 1000 spores per tube or a sampling volume of 32 spores per litre of air. The sensitivity of the device could be improved by sampling higher volumes of air when the crop is symptomless or by improving the sensitivity of the device. Additionally it is unclear if all mildew spores trapped remain in the collection tube and there was a high trapping efficiency. It is also unclear if the LFD device is equally sensitive to both *Golovinomyces orontii* or *Podosphaera xanthii*. Further testing would be required to determine the effectiveness of improvements which would reduce the numbers of false negative tests.

Evaluating the monitoring period for powdery mildew detection.

Milestone

Evaluate (2015 - 2016 commercial sampling) whether monitoring daily or weekly risk periods provides improved precision deployment of control measures for gummy stem blight.*

*** Warwickshire College's ability to deliver these milestones is subject to the full series sample being available from University of Worcester.**

Methods

Collection tubes (daily and weekly) from commercial trials and from the monitored cucumber trial established at Stockbridge Technology Centre during spring 2017 were used to investigate detection of cucumber powdery mildew weekly or daily. The results from this evaluation of weekly and daily sampling in 2015 and 2016 have been reported in previous annual reports and will not be duplicated here.

Results

2015 (Summary)

Air samples were tested in 2015 with UW 339 LFD for *Mycosphaerella melonis*. A poor correlation with MTIST ELISA (EMA 325) was observed and there were specificity issues (See previous report).

2016 (Summary)

In 2016 air samples tested using a lateral flow with EMA 325. Lateral flow test line capture was utilised using an anti-mouse antibody and not homologous antigen as in previous LFD's. Using this system could result in higher false positive results. Air samples collected in a single tube over a 7 day period often contained debris. This compromised sampling efficiency and potential for test inhibitors. Air samples collected over a 24H period into a tube were not visibly affected by debris accumulation. When these air samples were tested by lateral flow to identify *Mycosphaerella melonis*, high risk periods were identified across each cucumber planting. Following each high risk period, *Mycosphaerella* symptoms were observed in the crop within two to six weeks (See previous report).

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2017 (Summary)

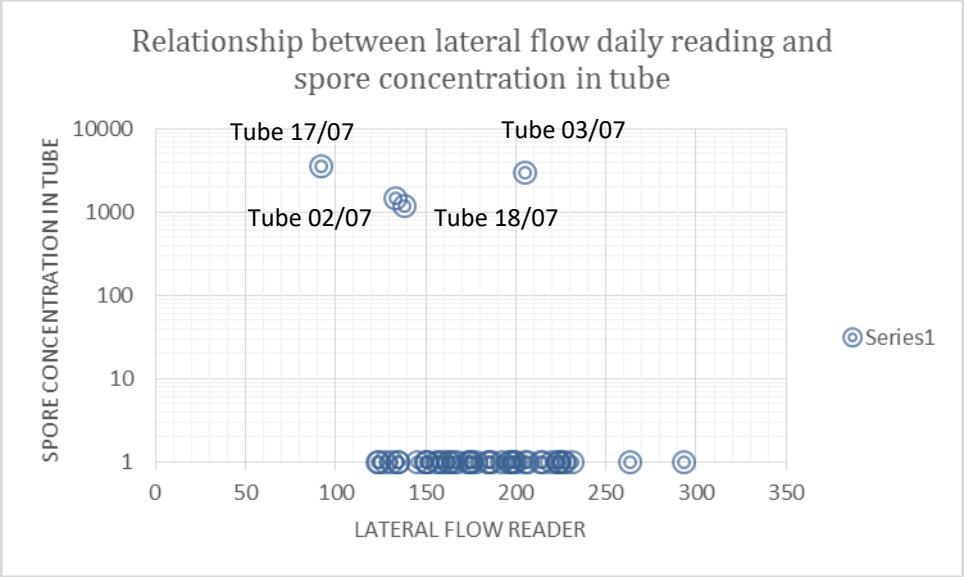

Figure 12 Relationship between the lateral flow reading for powdery mildew from daily collection tubes at Stockbridge in 2017.

The results show that of the daily samples available for testing only 4 gave positive results for powdery mildew (Figure 12). This was at variance to the Burkard seven day which showed an increase in spore concentration over time. The cyclone air sampler is characterised for use with small particles such as viruses and bacteria. As the powdery mildew spores are relatively large in comparison they do not adhere well to the trapping surface (as seen by ELISA) and lack a 'sticky' mucilage surface, it is probable that trapping efficiency of the cyclone sampler is poor for this spore type. Of the four dates which were identified as high in powdery mildew spore load two of the lateral flow readings recorded >150 (estimated 8000 spores by calibration curve) and 17 July a test line reading of under 100 was recorded (1300) spores by calibration curve). However, based on the calibration curve and the number of spores observed in the tube by microscopic examination, most tubes gave rise to false positives and with one false negative.

Conclusions

The results show that weekly sampling is likely to be a more accurate indicator of powdery mildew presence as higher volumes of air are sampled compared to daily volumes. Given the low sensitivity of the powdery mildew device high levels of mildew are required to give a true positive result. The insensitivity of the LFD device means that symptoms will be already present before a positive test is observed. The sensitivity of the LFD device

should be improved if better results are to be obtained. Alternatively, another form of spore trap may prove optimal in spore collection and retention.

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