



Grower Summary

CP 137

Development and testing of a lateral flow device for both gummy stem blight and powdery mildew in bio-aerosols during cucurbit production

Annual 2015

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Project title: Development and testing of a lateral flow device for both gummy stem blight and powdery mildew in bio-aerosols during cucurbit production

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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 ascospore 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.

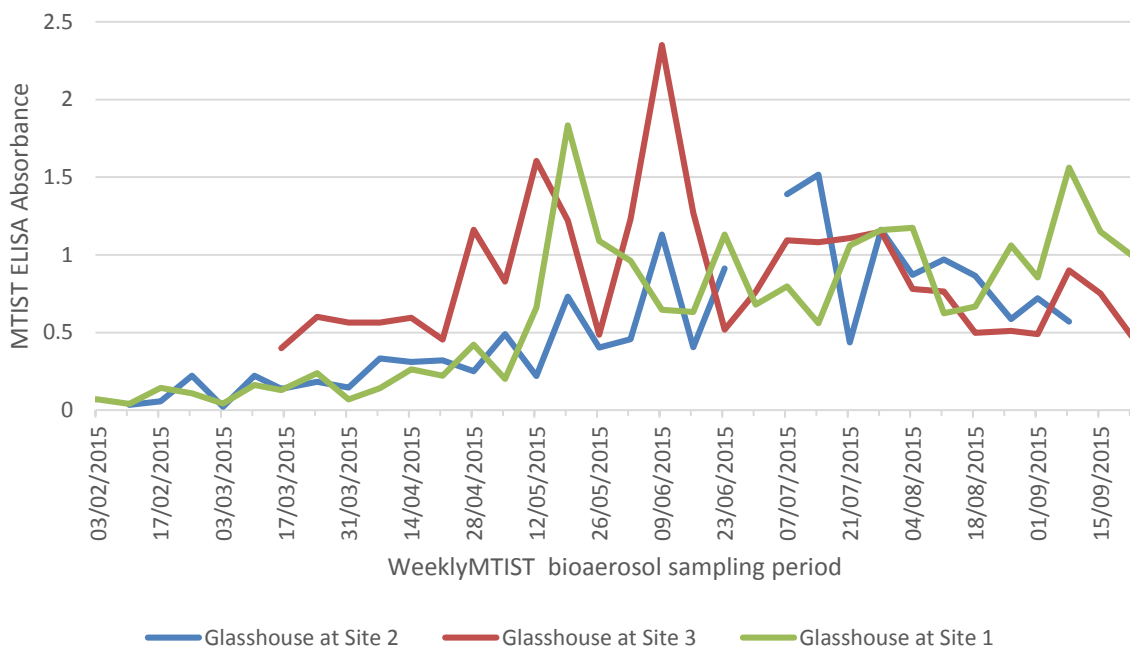


Figure 2. Measurement by ELISA of *Mycosphaerella melonis* ascospores collected in weekly bioaerosols at three UK commercial cucumber propagation nurseries.

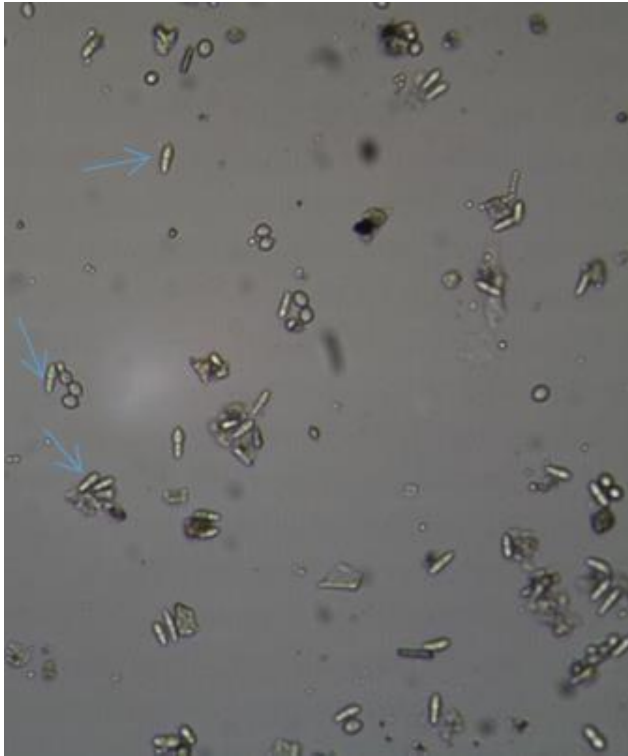


Figure 3. Base of a microtitre well viewed by bright field microscopy from glasshouse Site 3. Some examples of ascosporic inoculum resembling morphological characteristics of *M. melonis* are shown by blue arrows (there are many more on the plate).

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.