Grower Summary

SP137

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

Final report, August 2017

DISCLAIMER

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

[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.]

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.

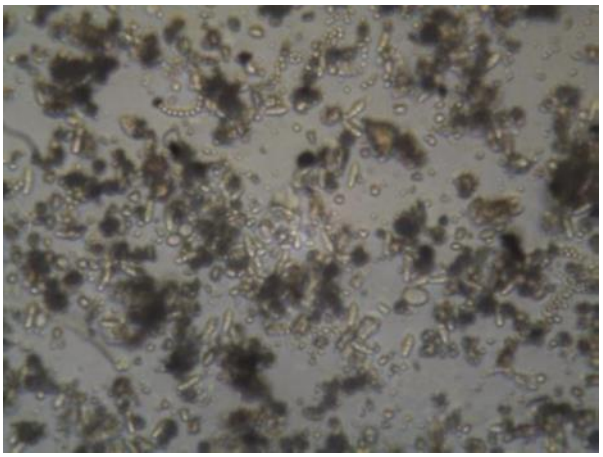

Figure 1b. Glasshouse bio-aerosol sample deposition on the base of a microtitre well as viewed by bright field microscopy (400x)

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.

