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

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GROWER SUMMARY

Headline

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

Background

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

Myco (Black stem rot or Gummy stem blight):

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

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

Cucumber Powdery Mildew:

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

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

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

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

Summary

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

A Microtitre immunospore trap (MTIST), available from Burkard Manufacturing (http://www.burkard.co.uk) costs in the region of £1500 plus VAT. This includes the ELISA

microtitre well adapter plate (figure 1a). The sampler runs directly off the mains and during trials has operated continuously across the cucumber growing period. Spores in the air are collected into microtitre wells. The air sample (4x8 well microtitre wells) is changed weekly and sent by post to a laboratory for assessment of Myco spore numbers. In a commercial environment, next day courier service (as used by vets) has potential to return results within same day of sample receipt.

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

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

For the 2016 season, a request was made by AHDB to include additional information of Myco spore concentration by time-consuming microscopic examination of the microtitre wells. Reliability of data generated in this way is questionable as visually Myco spores are similar to other ascosporic species and at times the overall spore load is high (Fig 1b). Counts were however made and compared with MTIST ELISA results. At the beginning of the season there was good correlation but towards the end of the season this relationship drifted. As in the previous year, Myco spores were observed in March with increasing concentration during April. In 2016, spore peaks were generally followed by periods of low spore pressure. As an example an account of site 2 is described below.

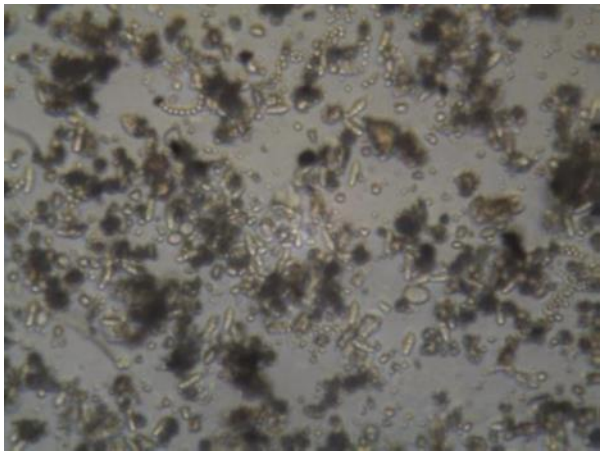

Figure 1b. Glasshouse bio-aerosol sample deposition on the base of a microtitre well as viewed by bright field microscopy (400x)

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

The application in 2016 of recently approved fungicides (Talius and Reflect) may have reduced the overall Myco spore load and distinct populations of inoculum observed. Periods of high risk were observed between May and middle of June, 12th July to 15th August, 23rd August to 12th September. The crop was replanted at the beginning of July. Infected fruit were observed on the 19th July. Throughout August disease was observed on a susceptible variety (Snack)

whilst the main crop was identified by the grower as ‘relatively’ disease free. Increasing numbers of infected fruit were observed from the 3rd week in August and an application of Reflect was made. Spore concentrations in air samples fell markedly week commencing 13th September. Conversely, infected fruit increased in concentration. The initial latent period for the second crop was less than 14 days and disease symptoms this time first were observed on the harvested fruit. It is likely that the warmer conditions shortened the disease latent period.

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

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

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

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

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

Financial Benefits

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

Action Points

During 'inoculum' low risk periods

Rigorous hygiene

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

Environmental

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

Moderate risk

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

High risk

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