Grower Summary

CP 137a

Cucumber: Development and testing of a lateral flow device for gummy stem blight in bio-aerosols during cucurbit production

Final Report 2019

Project title:	Cucumber: Development and testing of a lateral flow device for gummy stem blight in bio-aerosols during cucurbit production
Project number:	**CP 137a**
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Report:	Final report, December 2019
Previous report:	Final Report CP137
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Date project commenced:	01/12/2018
Expected completion date:	30/11/2019

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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.

Professor Roy Kennedy
Director of Research
Warwickshire Colleges Group

Signature .... Date 15 April 2020

Report authorised by:
Steve Taylor
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GROWER SUMMARY

Headline

Diagnostic techniques have been developed to monitor glasshouse air samples for spores which spread 'Myco' disease and cucumber powdery mildew. A stable ten minute on-site tests has been developed for Myco inoculum in air samples. Isolates of Myco tested for sensitivity to different fungicide actives showed different levels of sensitivity to boscalid, azoxystrobin and fluxapyroxad. There was no evidence of genetic variation in isolates of Myco collected during different cucumber cropping periods from different production areas.

Background

Improved control of *Mycosphaerella melonis* (Myco), using tests to monitor glasshouse air samples for Myco spore presence were developed in previous projects (CP 137). However these were not stable enough for general use by end users in glasshouse production of cucumbers. With knowledge of 'Myco' inoculum, this current project (CP 137a) aims to add to previous work and improve information on timing for fungicide application. The time between finding 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. This means that application of fungicides can be applied before symptoms occur.

*Mycosphaerella melonis (*also known as Black stem rot or Gummy stem blight) is the causative agent of 'Myco' on cucumber (syn. *Didymella bryoniae* – sexual stage*).* The disease is of worldwide importance, with significant economic damage of cucurbit 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.

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 powdery mildew. 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 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 for effective control.

Summary

The work within this project reports on the development of a stable lateral flow test based on new antibody fragments (Fabs). *Mycosphaerella* (Myco) isolates have also been collected from cucumber production areas in the UK and tested for fungicide sensitivity and genetic variation.

Pathogen Monitoring in 2019

During 2019 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 1 has been used for laboratory testing of samples.

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

Using this technology, Myco spores have been identified in UK glasshouse air samples from March / April onwards using microscopy at two sites. Myco spore levels peak in cucumber production at different times. At site A Myco spores were detected in week 12 however no powdery mildew were detected until week 17. Spore warnings were low / moderate during the first crop up to week 19. High spore concentration in the air were identified for the week 25 commencing 17th June 2019. Spore concentrations for both pathogens recorded in the crop remained high / moderate risk until week 32 commencing 5th August 2019 when the third crop was planted. The levels of powdery mildew recorded were higher than those observed for Myco. Spore concentrations for both pathogens recorded in the crop were highest during weeks 26 27 and 28 before falling to zero as the new crop was planted at that time at site B. The levels of powdery mildew recorded were higher than those observed for Myco. Very high concentrations of powdery mildew were observed from week 35 (August 2019) until the final cropping week. Myco spore levels also increased after week 39 (September 2019). Tube based samplers were also used in monitoring in crop each site. However many of these tubes were used in the development of the Fab based lateral flow tests.

Fungicide Sensitivity Testing

Thirty six isolates were successfully cultured from gummy stem blight infected material collected from eight different commercial cucumber production sites over the course of the 2019 growing season. The results show a considerable degree of variability between isolates to the tested chemicals. It is not clear if this is based on some degree of resistance or just

natural variability between isolates. Of the chemicals tested Plover (difenoconazole) was the most effect at reducing Myco growth in agar. Very small amounts of the active ingredient were required to produce a complete control effect. proquinazid and azoxystrobin also showed good control of isolate growth at the range of concentrations tested. There appeared to be variation in isolate sensitivity to some active ingredients. boscalid, azoxystrobin and fluxapyroxad showed varying degrees of isolate variation to the chemical concentrations used. fluxapyroxad in particular showed a wide variation in response between isolates although the chemical was not particularly effective in controlling Myco.

Genetic variation in isolates

The 36 isolates were analysed in this study originated from gummy stem blight infected material collected from eight different commercial cucumber production sites in the UK. Material was collected and at different times throughout the 2019 growing season (Table). Despite this variation in origin all isolates has been shown to be the same species and RAPD group genotype RG1.

Lateral Flow Tests based on new antibodies

A new lateral flow test has been manufactured using new antibodies produced within this project (CP137A). These have been shown to be stable and can detect spores of *Mycosphaerella melonis* (Myco).

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 crop 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.

Action Points

New tests for Myco require further testing in commercial cucumber production.

Applications of difenoconazole can control Myco very effectively.

- Other chemicals maybe effective on certain isolates of Myco
- Myco tested showed no variation in genetic type.