Grower Summary

PE 001a

Cucumber – Improving Control of Gummy Stem Blight caused by *Mycosphaerella melonis*

Final 2014

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Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

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Further information

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GROWER SUMMARY

Headline

- A combination of good crop hygiene including effective disinfection, serological spore trapping, environmental manipulation and novel fungicides has the potential to provide an effective integrated strategy for *Mycosphaerella* control.

Background and expected deliverables

Black stem rot, gummy stem blight or 'Myco', is caused by the ascomycete fungus *Mycosphaerella melonis* (syn. *Didymella bryoniae*). It is an economically damaging pathogen of cucumber and other cucurbits. It causes extensive stem and leaf infections which, when severe, can debilitate or even kill plants. Air-borne infection of flowers and developing fruit leads to fruit rot. Such infections may become visible in the crop but at other times, probably under specific environmental conditions, this type of infection remains latent (hidden) only developing visually once the fruit has been marketed. These internally infected fruit can sometimes be identified by a tapering to the tip of the fruit though this does not always occur and these latent infections continue to have an economic impact in the industry. They lead to rejection and reduced retailer and consumer confidence in the product. Effective control of the disease is difficult in intensive production systems and likely to be made worse by recent changes to EU pesticide legislation which have effectively prohibited some of the more effective approved fungicides, e.g. triflumizole (Rocket).

An extensive literature review was carried out during Phase 1 of the study which reviewed in detail the pathogen, the disease it causes in cucumbers and the various factors that influence its occurrence, survival, infection and control. The review helped to identify various areas for work on this host/pathogen combination with the work being split into two phases. The expected deliverables from phase 2 of this project were:

- To validate the developed immunoassay system in a semi-commercial crop.
- To carry out *in vitro* screening of experimental products for disease control.
- To further test short-listed products from above under semi-commercial conditions.
- To investigate the efficacy of disinfectants against *Mycosphaerella* to limit secondary spread of infection.
- To investigate the potential for systemic infection under UK conditions.

- To devise an integrated strategy for *Mycosphaerella* control and validate its use in a commercial cropping situation.

Summary

Seed-borne infection

Although the pathogen was suspected as potentially seed-borne at a very low level from work in Phase 1, further extensive testing in 2011 did not find any conclusive evidence of a seed-borne infection route. It therefore seems likely that this route of infection is either absent or very low in current commercial seed stocks. However, as seed-borne infection has been documented previously (Lee *et al*, 1984) growers need to keep alert to the risk, especially when they are trialing small areas of new experimental (numbered) varieties.

Immunoassay spore trap

Work to develop a sensitive monoclonal antibody (MAb) to *M. melonis* which was started in Phase 1 of this project progressed well. Two MAbs were identified and one was used to develop an assay for rapid quantification of *M. melonis* spores collected in traps. The assay was tested in a glasshouse crop for reactivity using enzyme-linked immunosorbant assay (ELISA) and Immunofluorescence (IF). Results from spore trapping in a commercial cucumber crop in Yorkshire and a semi-commercial crop at STC during 2011 and 2012 showed that spore release was significantly greater between 17.30 and 03.00 hrs than at other times during the day/night. This coincides with optimum conditions for infection in the crop when the vents are shut and RH levels are likely to be higher. Spore sampling in an infected crop at STC during 2012 provided some additional interesting data on the diurnal periodicity of *M. melonis* spore release, which showed that peak spore release occurred between 16.00 and 07.00 hrs. These data are consistent with previously published data.

Initial data on spore release and disease incidence studies from the air-monitoring would appear to indicate that an ascosporic aerosol concentration in excess of 2000 spores/m^3 of air may be required for infection and subsequent disease development.

Disinfection

A series of experiments identified disinfectants with good activity against *M. melonis*. Six disinfectant products containing active ingredients from different chemical classes were tested for activity against conidia and mycelium of the fungus. The most effective products against mycelium in filter paper discs were Jet 5, bleach, Unifect G and Vitafect.

An experiment was designed and undertaken to examine the influence of different surfaces on the activity of disinfectants against *M. melonis*. Overall, it was more difficult to disinfect a porous surface e.g. concrete than aluminium, glass or plastic. Jet 5, bleach and Unifect G used at their recommended rates were fully effective on all four surfaces but Fam 30 was less effective on concrete, Menno Florades was less effective on aluminium and concrete, and Vitafect was less effective on glass.

An experiment was done to determine how effective various disinfectant soak treatments were at reducing disease transmission of *M. melonis* on knives contaminated with the fungus by cutting through infected cucumber leaves and stems. Disease transmission was relatively low but soaking contaminated knives in water, Jet 5, Menno Florades, bleach or Vitafect for 1 hour reduced the development of gummy stem blight in cucumber fruit slices compared with transmission from untreated knives

Two experiments were carried out to compare different treatments for cleansing hands contaminated with *M. melonis* following handling of cucumber fruit affected by *M. melonis*, and through contamination of hands with a paste of the fungus in cucumber sap. A finger from a washed hand was placed on a culture plate to check for pathogen viability. Washing hands in soap and water, with an alcohol gel, or with alcohol foam, all greatly reduced transmission of *M. melonis* from hands. Soap and water alone was less effective at reducing transmission of *M. melonis* than soap and water followed by alcohol gel or foam, or the alcohol foam or gel used directly on contaminated hands. Rinsing hands in water alone gave no reduction in transmission of *M. melonis*.

Novel fungicides and biocontrol products

In Phase 1, some initial laboratory-based studies, using a broad range of isolates of *M. melonis* (29) collected from nurseries in the north and south of England, was carried out. This work checked the current efficacy of approved fungicides (in terms of mycelial inhibition on agar). The work showed that in general mycelial growth of *M. melonis* was inhibited when grown on agar amended with some of the fungicides tested e.g. Teldor (fenhexamid) or by either of the active ingredient components of Switch (cyprodinil & fludioxonil). However, isolates grown on agar amended with Amistar (azoxystrobin), Bravo 500 (chlorothalonil) or Nimrod (bupirimate) were generally less inhibited. This work was extended substantially in Phase 2 of the study to screen a broad range of novel fungicides (and some bio-control products) for their potential efficacy against *M. melonis*. An initial agar plate screen was conducted and then a second screen was done on young plants using a detached leaf bioassay. A broad range of experimental products (conventional chemicals and bio-control

products) were included, listed as coded compounds until the individual products receive approvals for use on the commercial crop.

In the agar plate tests various commercially available and experimental products including Prestop (*Gliocladium catenulatum*), Serenade ASO (*Bacillus subtilis*), HDC F84, HDC F86, HDC F88, HDC F89, HDC F90, HDC F91, HDC F92, HDC F93 and HDC F104 showed potentially good activity against *M. melonis*.

Subsequent tests were carried out on young cucumber plants with a similar range of experimental products (27) and using 2 separate detached leaf bioassays. The tests were carried out following inoculation with two isolates of *M. melonis* (isolated from a northern and southern crop in 2010). In these tests Switch (cyprodinil + fludioxonil), HDC F86, HDC F88, HDC F90, HDC F96 and HDC F98 showed good activity. A short-list of products which showed promise in these bioassays was taken forward into a large replicated glasshouse study at STC during 2012.

Glasshouse testing of low risk experimental fungicide and bio-pesticide products

Short listed products from the *in vitro* and *in vivo* bioassays were taken forward into a larger, replicated glasshouse study carried out during May - September 2012 at STC. A total of 12 treatments, including a water control, a standard fungicide programme, 8 experimental fungicides and 2 bio-pesticide programmes, were used. Bio-pesticides were applied weekly with 9 applications in total, whilst conventional fungicides were applied fortnightly with a total of 4 applications. Guard plants in the crop were inoculated with *Mycosphaerella* following the 1st conventional fungicide application (and after 2 bio-pesticide applications). The guard plants were inoculated a second time, and infected detached fruit was introduced into the cropping area to ensure high disease pressure via ascospores release. The crop was assessed for the incidence and severity of *Mycosphaerella* lesions on three occasions (monthly) following the 1st conventional fungicide application, with the final assessment being carried out one month after the final application.

Only very low disease levels were present initially but, as the season progressed and inoculum levels increased, infection levels rose and excellent treatment differences developed. Relative to the water control, none of the current approved products or either of the bio-pesticide products tested prevented *Mycosphaerella* development in this study. It is important to note though that all these products don't necessarily have a specific label approval for this target. In comparison, several of the experimental products under investigation showed good efficacy against *Mycosphaerella* e.g. HDC F85 + F86, F88, F89,

F90 and F96. A slight crop safety issue was observed following the first application of F88 and F89 when applied to younger plants, but the plants grew away from the damage and later applications caused no problems.

Systemic infection potential

A glasshouse trial to investigate the potential for systemic shoot infection by *Mycosphaerella* was undertaken during 2012. Tagged plants were artificially inoculated in different sites; leaf petioles (agar plug), cut fruit stubs (agar plug), main stem wound of stopped plant at the wire (agar plugs and spore suspension), flowers (spore suspension) and shoot tips of laterals (spore suspension) using either a *Mycosphaerella* spore suspension or agar plugs from an actively growing culture. A spore suspension of the pathogen was also drenched into the rock-wool block. Symptom development was compared with that on uninoculated control plants. The incidence and severity of any lesions that subsequently developed was recorded during the growing season.

Whilst it is difficult to draw firm conclusions from this study it would appear from these artificial inoculation studies that the cucumber shoots can become infected with *Mycosphaerella* internally leading to the development of weak unproductive shoots. Such infection would appear to occur as a direct result of spores infecting the young shoot tips of the same laterals. The presence of the pathogen internally in uninoculated plants could have occurred as a direct result of ascospore release in the glasshouse as the epidemic developed following artificial inoculation.

Integrated control strategy

The integrated strategy sought to bring together all aspects of the work done so far: spore detection using monoclonal antibody technology, knowledge of disease epidemiology, disinfection techniques and effective fungicides.

Two products effective in the glasshouse trial at STC containing SDHI active ingredients were selected for use in the integrated strategy: HDC F88 and one commercially available product already approved on similar hydroponically grown glasshouse edibles containing two active ingredients: F86 + F85. However, the manufacturer of HDC F88 provided an alternative which contained the same SDHI active ingredient plus a different additional active as this more accurately fitted thieir marketing strategy and this is coded as HDC F159. The disinfectant Jet-5 was also selected to be included in the trials as a pre-planting application. As the monoclonal antibody had now been validated, spore traps were also used in the trials to monitor spore levels and used to trigger fungicide applications.

An alternating experimental programme of HDC F159 / F86 + F85 was devised and divided into three treatments to be compared with the grower's own fungicide programme at two geographically different sites in the UK. The trials were done on grower holdings in the third (autumn) crop as at this time of year, following two previous crops, *Mycosphaerella* levels are usually higher than at other times of year. MTIST spore traps were placed in the trial to monitor spore levels and to determine the best timings for spray applications in two of the treatments. Treatments were as follows:

1. Grower's own spray programme; Grower applied; Grower timings. MTIST spore trap to monitor ambient spore levels in the glasshouse
2. Experimental fungicide programme; Researcher applied; Grower timings
3. Experimental fungicide programme with MTIST spore trap to monitor spore levels within the trial and to trigger spray timing; Researcher applied; Timing determined by spore levels
4. As T3 above; plus disinfection of treatment area prior to planting using Jet-5.

Both growers grew the cultivar 'Bonbon' as detailed below:

Comparison between the two sites used in the integrated study	**Site 1**	**Site 2**
Pre-planting clean up	Good	Poor*
Pre-planting disinfection	No	Yes: Jet-5
Number of fungicide applications by grower	4	8
Number of experimental fungicide applications triggered by high spore levels	3	5
Mean number of *Mycosphaerella* lesions per plant at end of trial: grower fungicide programme	1.04	4.65
Mean number of *Mycosphaerella* lesions per plant at end of trial: experimental fungicide programmes	0.03	1.16
Percentage of fruit infected with *Mycosphaerella* at end of trial: grower fungicide programme	12%	37%
Percentage of fruit infected with *Mycosphaerella* at end of trial: experimental fungicide programmes	7%	5%

* Rockwool blocks from previous crop were not removed until eight weeks into trial.

The experimental treatments were all significantly better at controlling *Mycosphaerella* than either of the growers' fungicide programmes. However, differences between the different

experimental application timings were more subtle (Figure 1). Targeted fungicide applications triggered by spore levels have the potential to reduce the number of applications that need to be made to the crop, but this is dependent on pre-planting glasshouse hygiene. Using Jet-5 as a disinfectant pre-planting can delay onset of infection, but has little effect if there is high disease pressure due to a poor clean up pre-planting (**Figure**).

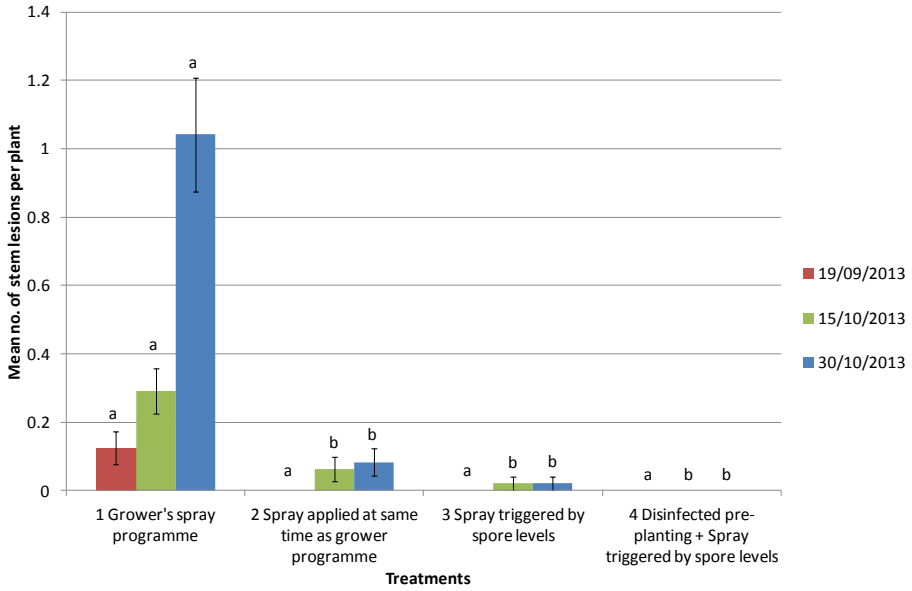

Figure 1: Mean number of stem lesions per plant at three assessment dates at site 1. Error bars indicate standard error. LSD (P = 0.05) columns of the same colour with the same letter above them are not significantly different.

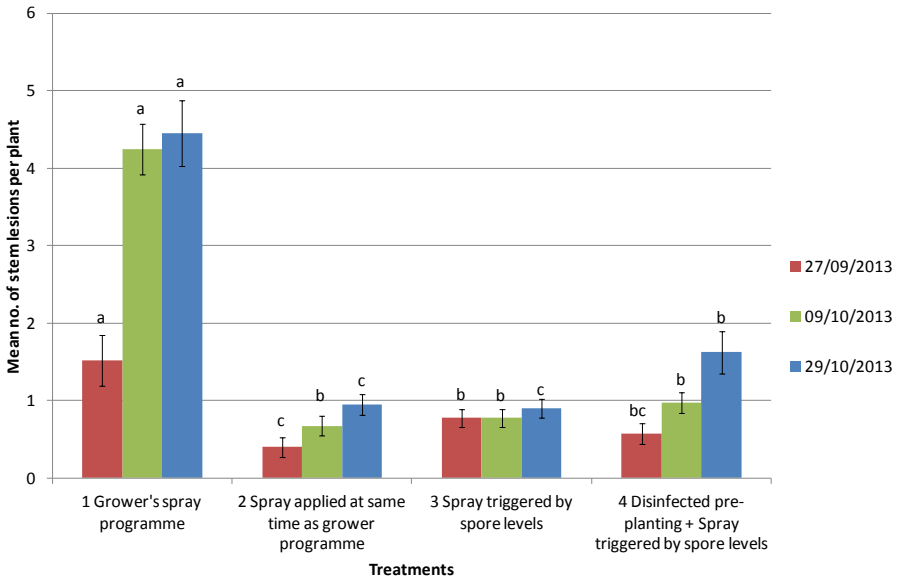

Figure 2: Mean number of stem lesions per plant at three assessment dates at site 2. Error bars indicate standard error. LSD (P = 0.05) columns of the same colour with the same letter above them are not significantly different.

Figure charts the weekly spore levels recorded in the grower's crops and the trial areas at each of the two sites. Spore levels above the threshold in the trial area triggered an experimental product application in treatments 3 & 4. The graph illustrates the differences in disease pressure at the two sites. At site 1 it was only necessary to make three experimental applications as spore levels fell below the threshold for several weeks of the trial. By contrast five experimental product applications were made at site 2 every two weeks as the spore levels never fell below the threshold. This was the maximum number possible according to the product label recommendation. Disease pressure was high because rock wool blocks from the previous crop were not removed until eight weeks into trial. This event instantly resulted in a dramatic fall in ascospore levels.

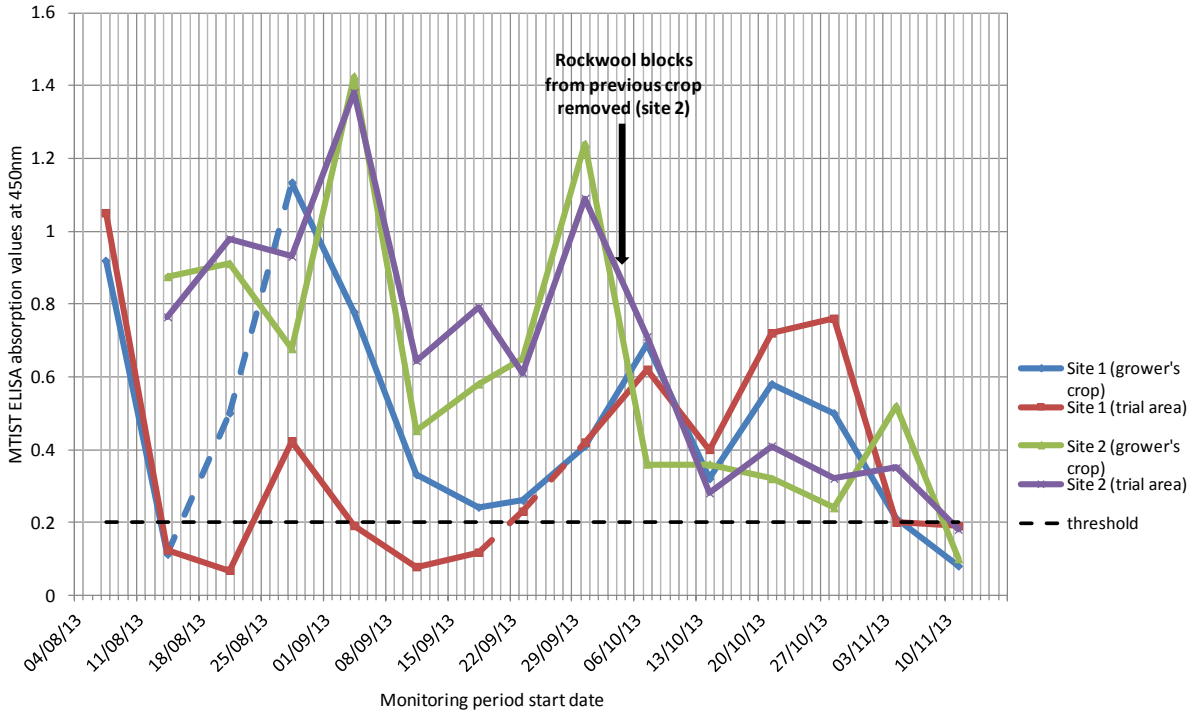

Figure 3: Monitoring glasshouse aerosols for *Mycosphaerella melonis* ascosporic inoculum at two nursery sites.

Financial Benefits

The results from the disinfectant study carried out during 2011 have immediate benefits for growers both during the growing season and during the clean-down between crops. Effective use of disinfectants should help to reduce disease spread and the survival of inoculum between crops provided that the glasshouse is cleaned out well between crops.

Several experimental fungicides were shown to provide effective control of *M. melonis* in fully replicated glasshouse studies, these products are not yet approved for use in cucumbers and therefore cannot yet be used commercially. However, feedback from the various manufacturers remains encouraging and it is hoped that one or more products will be available, in 2014, subject of course to the usual regulatory process either by on-label or via a minor use approval (EAMU).

It is also worth noting that some of the experimental products which showed good activity against *M. melonis* also showed activity against powdery mildew and this would result in even greater financial benefits for the industry, as it would potentially allow effective resistance management strategies to be deployed thus safeguarding products for the longer term.

A working lateral flow prototype for detecting ascospores of the closely related fungal pathogen *Mycosphaerella brassicicola,* which causes ringspot of vegetable brassicas, was successfully produced within HDC project FV 233. In HDC project FV 233a it was successfully mass produced for use in commercial crops. This cucumber project has validated the spore quantification technology, but with the pathogen *Mycosphaerella melonis* which will be taken forward to develop a rapid forecasting system and/or a lateral flow device for use in commercial cucumber crops through a separate HDC funded project (CP 137). This will enable rapid detection of high spore levels in the glasshouse enabling quick spray decisions to be made in response to the result. It is anticipated that early treatment would reduce the overall number of spray applications over the duration of the crop and therefore reduce chemical and labour costs, and at the same time minimise economic loss from poor quality of fruit-plant/yield loss.

The integrated control strategy evaluated during 2013 highlighted the importance of a thorough clean up between crops. One day spent on clean up in between crops, at a potential cost of one cucumber per m^2 or £3,000 to £4,000 per ha, will have benefits during the life of the crop by reducing initial inoculum levels and therefore losses at a later stage in the growing season. Losses of up to 30% could equate to about £50,000 per ha.

From the assessments we made at commercial site 2 the percentage of fruit lost to *Mycosphaerella* infections over the length of the crop at was on average 25% based on a daily yield of *ca*. 3100 fruit per ha. This equates to a daily loss of *ca*. 800 fruit per ha. The percentage of fruit lost to *Mycosphaerella* infections in the experimental treatments of the trial on average over the length of the crop at site 2 was 3% based on a daily yield of *ca*. 4650 fruit per ha. This equates to a daily loss of less than 140 fruit per ha.

The experimental treatments both improved daily yield per ha and reduced losses to *Mycosphaerella* infections. If the new treatments were adopted, subject to EAMU, and used following a thorough clean up between crops, the potential yield increase per ha per day could be from *ca*. 2300 fruit to *ca*. 4500 fruit. Although the daily yield data do not match the grower's figures (which are higher), these data demonstrate that an increase in yield could be achieved if all aspects of the integrated control programme were implemented.

Action Points

- Crop hygiene is key to reducing inoculum sources and disease spread.
- Consider using effective disinfectants identified in this project to limit secondary spread of infection during crop work and between crops.
- Spending one extra day thoroughly cleaning the glasshouse between crops will pay for itself several times over by delaying epidemic disease development and subsequent crop loss.
- Ensure the use of good quality seed from reputable suppliers, and be aware of the potential for a seed borne risk on new experimental cultivars.
- One of the products in the experimental programme provided excellent control of *Mycosphaerella* and is already approved on similar hydroponically-grown glasshouse edibles.
- Results from the spore monitoring studies indicate that targeted spray applications determined by a spore threshold could reduce the total number of applications that would need to be made during the life of the crop, and significantly reduce fruit infection, especially if this could be linked to environmental data.
- The grower at site 2 has already taken action by changing his crop removal practices and clean up regime.