



Agriculture & Horticulture
DEVELOPMENT BOARD



Grower Summary

PE 001a

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

Annual 2013

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Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

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

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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HDC is a division of the Agriculture and Horticulture Development Board.

Project Number: PE 001a

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

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Project Cost: £135,480

Headlines

- Methodology to provide growers with an indication of high *M. melonis* risk periods has been developed.
- Several disinfectants have been shown to have good activity at killing spores and mycelium of *M. melonis* in a range of different tests.
- Effective disinfection in between crops can dramatically reduce spore numbers.
- A number of experimental novel fungicide products have been shown to provide excellent control of *M. melonis* in glasshouse trials.

Background

Black stem rot, gummy stem blight or 'Myco' as growers prefer to call it, is caused by the ascomycete fungus *Mycosphaerella melonis* (syn. *Didymella bryoniae*). It is an economically damaging pathogen of cucumber and other cucurbits. Infection of flowers, developing fruit and shoots/stems can occur from air-borne spores. Such infections may become visible in the crop but may, probably under specific environmental conditions, remain latent (hidden) only developing visually once the fruit has been marketed. These 'hidden' infections of fruit, which can sometimes be identified by a tapering to the tip of the fruit, can lead to rejection and reduced retailer and consumer confidence in the product (Figure 1). 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 use of some of the more effective approved fungicides, e.g. triflumizole (Rocket).



Figure 1. Internal *M. melonis* infection in fruit.

An extensive literature review carried out in Phase 1 of this study helped to identify various areas for work. The expected deliverables in Phase 2 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

Immunoassay development

Work to develop a sensitive monoclonal antibody (MAb) to *M. melonis* which was started in Phase 1 of this project has 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 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. Sampling in the systemic infection trial 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 ascospore aerosol concentration in excess of 2000 spores/m³ of air may be required for infection and subsequent disease development.

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

Large scale *in vitro* laboratory screening of a range of novel pesticide and bio-pesticides was carried out in Phase 1 of this project, with promising candidates for control being taken forward into small scale young plant studies in 2011 (Annual Report of HDC Project PE 001a, 2012). Ultimately short listed products 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 (Figure 2).

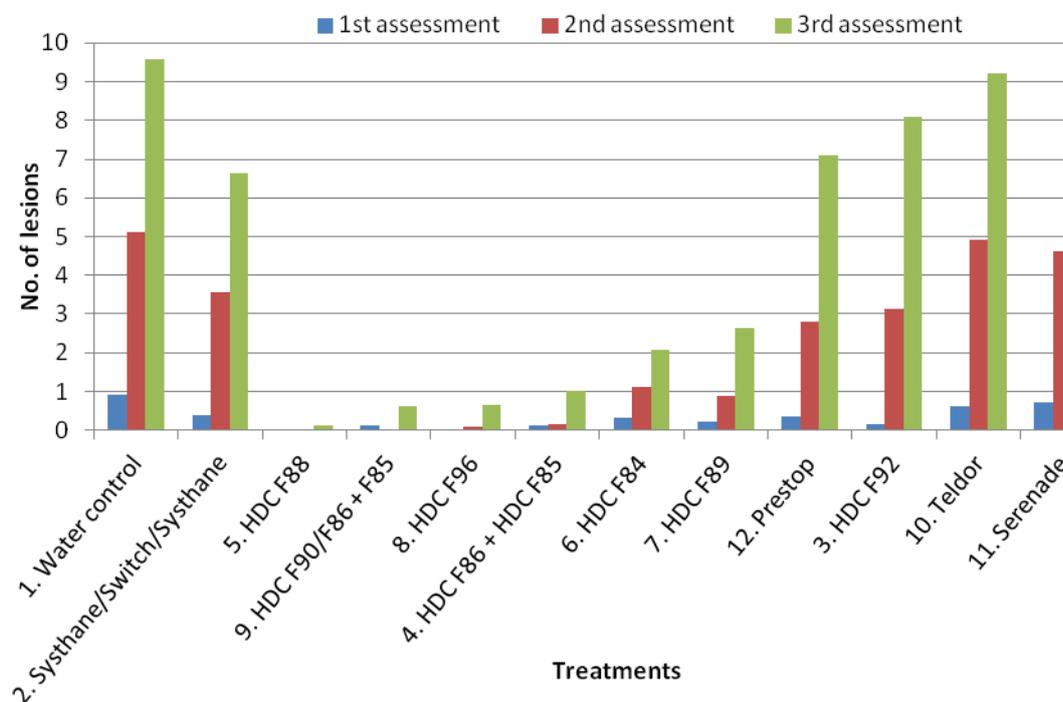


Figure 2. A comparison of the mean number of stem lesions of *M. melonis* per plant at each assessment date.

The collected data shown in Figure 2 indicate that during the first assessment, only very low disease levels were present 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 approved products or either of the bio-pesticide products tested prevented *Mycosphaerella* development in this study. Though it is important to note that all these products don't necessarily have a label approval for this target. However, 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.

Limiting secondary spread of infection using disinfectants

A series of experiments was undertaken to identify 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. Products were tested at their full recommended rate and at half-rate after exposure for 5 mins and 30 mins. Jet 5 (hydrogen peroxide/peracetic acid) and Fam 30 (iodophor) were most effective. These products, together with bleach (sodium hypochlorite) and Unifect G (glutaraldehyde + Quaternary Ammonium Compound, QAC) were fully effective after just 5 mins and at half their recommended rates. Menno Florades (benzoic acid) was effective after 5 mins at full rate and after 30 mins at half rate; Vitafect (QAC + biquanidine salt) was effective at full rate but ineffective at half rate even after 30 mins. 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, perhaps not surprisingly, it was more difficult to disinfect concrete than aluminium, glass or plastic. Jet 5, bleach and Unifect G used at their recommended rates were fully effective on all four surfaces. However, Fam 30 on concrete, Menno Florades on aluminium and concrete, and Vitafect on glass all showed reduced activity.

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. 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. Results of all the disinfection tests described above are summarised in Table 1.

Table 1. Summary of disinfectant activity against *M. melonis* in various tests - 2011

Disinfectant	Rate used	Growth of <i>M. melonis</i> recorded after treatment ^a of						Dirt y knife ^b
		Spores* in water	Mycelium on filter paper in water	Spores*/mycelium dried on:				
				Alu	Con	Gla	Pla	
Water (control)	N/A	+	+	+	+	+	+	(+)
Fam 30	1:125	-	(+)	-	+	-	-	NT
Jet 5	1:125	-	-	-	-	-	-	(+)
Menno Florades	10 ml/L	-	+	(+)	+	-	-	-
Sodium hypochlorite (10-14%)	1 in 10	(+)	-	-	-	-	-	(+)
Unifect G	4%	-	-	-	-	-	-	NT
Vitafect	1%	-	-	-	-	(+)	-	(+)

^a Results shown after exposure to disinfectant for 5 mins (spores or filter paper in water) or 30 mins (all other tests).

^b Disease transmission test.

N/A – not applicable; NT – not tested.

- no growth; (+) occasional growth; + growth common.

Alu – aluminium; Con – concrete; Gla – glass; Pla – plastic

* The spore type evaluated was not differentiated though considered to comprise largely of conidia rather than ascospores

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*.

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.

All artificial inoculation techniques on the plants led to the development of more *Mycosphaerella* infections than would have occurred naturally. Inoculation of the leaf petioles, cut fruit stubs and main stem wound at the wire resulted in the development of aggressive stem lesions. Inoculation of the flowers resulted in the development of infected fruit and inoculating the shoot tips of laterals caused the tip to 'burn out' and the lateral shoot to become weaker (Figure 3). The *Mycosphaerella* spore suspension drenched into the rock-wool blocks did not result in a greater infection level than occurred naturally on the uninoculated control plants. This suggests that infection cannot occur via root uptake, though applying the drench at earlier growth stages may give different results.

The majority of weak laterals that developed were recorded on plants where the shoot tips were sprayed with a spore suspension although it is also worth noting that weak laterals were also recorded, albeit to a much lesser extent, on plants inoculated using other methods and also on the uninoculated plants.



Figure 3. Good example of 'burnt out' tip of a weak lateral.

The laterals that appeared weak with 'burnt out' tips were analysed for the presence of *Mycosphaerella* in the internal stem tissues, and this was compared with *Mycosphaerella* presence in uninoculated symptomless shoots. Whilst *Mycosphaerella* was successfully isolated from the nodes of both inoculated and uninoculated laterals its rate of recovery from internodes of inoculated laterals was much greater than from internodes of uninoculated laterals.

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 strategy for *Mycosphaerella* control (Immuno-assay, disinfectants, fungicides)

The final component of the work which seeks to develop an integrated disease control strategy using all the new knowledge regarding spore release and infection risk, use of disinfectants and deployment of effective fungicides in commercial crop situations will be carried out in the summer of 2013.

Financial Benefits

The results from the disinfectant study carried out during 2011 will 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. Several experimental fungicides have been 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.

Action Points

- Consider implementing the use of the disinfectants identified as having good efficacy during crop production and for the clean down between crops.
- Ensure the use of good quality seed from reputable suppliers, and be aware of the potential for seed-borne risk on new cultivars.
- Be aware of the relatively low activity of the approved products against *Mycosphaerella* observed during this study and recognise that *Mycosphaerella* control will continue to present a challenge with the current armoury of 'powdery mildew' fungicides.
- The use of tolerant cultivars integrated with effective hygiene and disinfecting will continue to be very important until such time that alternative effective products are available for *Mycosphaerella* control.