Grower Summary

PE 001a

Cucumber – Improving Control of Gummy Stem Blight caused by Mycosphaerella melonis (Didymella bryoniae)

Annual/Final 2012

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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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Project Title:	Cucumber – Improving Control of Gummy Stem Blight caused by Mycosphaerella melonis (Didymella bryoniae)
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Headline

- Several disinfectants were shown to have good activity at killing spores and mycelium of *M. melonis* in a range of different tests.

- A broad range of novel fungicides and bio-control products have been screened in *in vitro* and *in planta* tests and a number of novel products have been demonstrated to have good activity against *M. melonis*.

Background and expected deliverables

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. 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.

An extensive literature review was carried out during Phase 1 of the study. It discussed 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 of the project and main conclusions

Seed-borne infection

Although the pathogen was suspected 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 trialling small areas of new experimental (numbered) varieties.

Immunoassay spore trap

Work to develop and validate an immunoassay spore trapping system for use on-site by growers and consultants has continued with some promising results. A monoclonal antibody (MAb) to *M. melonis* has been produced following the inoculation of mice with ascospores of the fungus. It has proved insufficiently sensitive and additional work is now being conducted to improve sensitivity. Spore trapping was carried out using two types of samplers in a cucumber crop in Yorkshire over a five month period during 2011. Spores were trapped either on microtitre wells, or on melinex tape, depending on the type of air sampler. Results indicate that spore load is higher low down in the crop and that spore release significantly greater between 17.30 and 03.00 hrs than at other times. This coincides with optimum conditions for infection in the crop when the vents are shut and RH levels are high.

The spore traps are currently being processed using bright field microscopy, which is very time consuming. Once a MAb which is both sensitive and specific has been produced, this can be used to speed up the checking of spore traps. The MAb will also be used to develop a lateral flow test for on-site use to help growers and consultants identify high disease risk periods during cropping. If this alerts them to put control mechanisms into place this should help to reduce severe outbreaks of *M. melonis* arising from ascospore infection.

Novel fungicides and biocontrol products

In Phase 1, some initial laboratory-based studies, using a broad range (29) of isolates of *M. melonis* 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 at the request of the manufacturers and HDC. The identity of the coded compounds will be available when the products become available commercially on the 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 is being taken forward into a large replicated glasshouse study at STC during 2012 (Table 1.)

Table 1. Summary of results from *in vivo* bioassay efficacy testing (2012)

Trt No.	Product	Active ingredient	Reduction in lesion diameter compared to untreated control^ Northern isolate	Southern isolate
1	Untreated		-	-
2	Systhane	myclobutanil	***	***
3	Amistar	azoxystrobin	***	**
4	HDC F84	-	***	***
5	HDC F85	-	-	-
6	HDC F86	-	***	***
7	HDC F87	-	**	**
8	HDC F88	-	***	***
9	HDC F89	-	***	***

Trt No.	Product	Active ingredient	Reduction in lesion diameter compared to untreated control^	
			Northern isolate	Southern isolate
10	HDC F90	-	***	***
11	HDC F91	-	**	-
12	HDC F92	-	***	***
13	HDC F93	-	***	***
14	HDC F94	-	***	***
15	HDC F95	-	***	***
16	HDC F96	-	***	***
17	HDC F97	-	*	***
18	HDC F98	-	***	***
19	Switch	cyprodinil + fludioxonil	***	***
20	Teldor	fenhexamid	**	***
21	Nimrod	bupirimate	**	***
22	HDC F99	-	**	***
23	HDC F100	-	*	**
24	HDC F101	-	**	**
25	Prestop	*Gliocladium catenulatum*	**	***
26	Serenade ASO	*B. subtilis*	*	**
27	-	Potassium bicarbonate	*	**

^ based on data from undamaged leaves 5DAT
- No reduction in lesion development compared to the inoculated control.
* represents a slight reduction in lesion development (1-20%)
** represents a moderate reduction (21-60%)
*** represents a good reduction in lesion development (61-100%)

Disinfection

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 2.

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*.

Table 2. Summary of disinfectant activity against *M. melonis* in various tests – 2011

Disinfectant	Rate used	Growth of *M. melonis* recorded after treatment[a] of						
		Spores* in water	Mycelium on filter paper in water	Spores*/mycelium dried on:				Dirty knife[b]
				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

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 and hence improve crop yield, marketable quality and hence the economic value of the crop. However, due to the sporadic nature of such pathogen infections it is difficult to put a precise value on this.

Although several fungicide and bio-control products have been shown to provide effective control of *M. melonis* in small-scale laboratory studies, many of these products are not yet approved for use in cucumbers and therefore cannot yet be used commercially. However, the preliminary results help the design of an effective larger glasshouse study conducted during 2012. The results from this work could then be used to recommend additional effective products which may be put forward for approval via SOLA.

If one or more fungicides or bio-control products can be identified and subsequently approved for use on cucumber (with a 1-2 day harvest interval ideally) then significant economic loss could be avoided each year due to premature plant death (from girdling stem lesions) and from symptomatic or latent fruit infections. It is estimated that between 1-10% plants and fruit may be lost as a result of infection by *Mycosphaerella* each year.

It is also worth noting that if a product or products could be found with activity against powdery mildew and *Mycosphaerella* then the financial benefit could be even greater.

It is a little too early to judge the potential financial benefits from the immunoassay work that is in progress but, if the pathogen could be successfully monitored as proposed, then it will help to better time intervention treatments including spray applications and this could provide significant economic benefits in the longer-term through improved disease prediction.

Action points for growers

- Consider using effective disinfectants identified in this project to limit secondary spread of infection during crop work and between crops.

- Ensure the use of good quality seed from reputable suppliers, and be aware of the potential for a seed borne risk on new cultivars.

Prestop, Serenade and Switch showed potential efficacy for the control of Mycosphaerella in cucumbers in agar plate and small plant tests and should be considered as part of an effective control regime in commercial crops. A number of experimental or unapproved products also showed promise and may be available for use in the future.