Project title:	Cucumber – Improving Control of Gummy Stem Blight caused by *Mycosphaerella melonis*
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Project leader:	Dr G M McPherson
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Location of project:	Stockbridge Technology Centre, ADAS, Boxworth NPARU, University of Worcester Grower holdings
Industry Representative:	Derek Hargreaves, Beverley
Date project commenced:	1 February 2010
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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.

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CONTENTS

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).

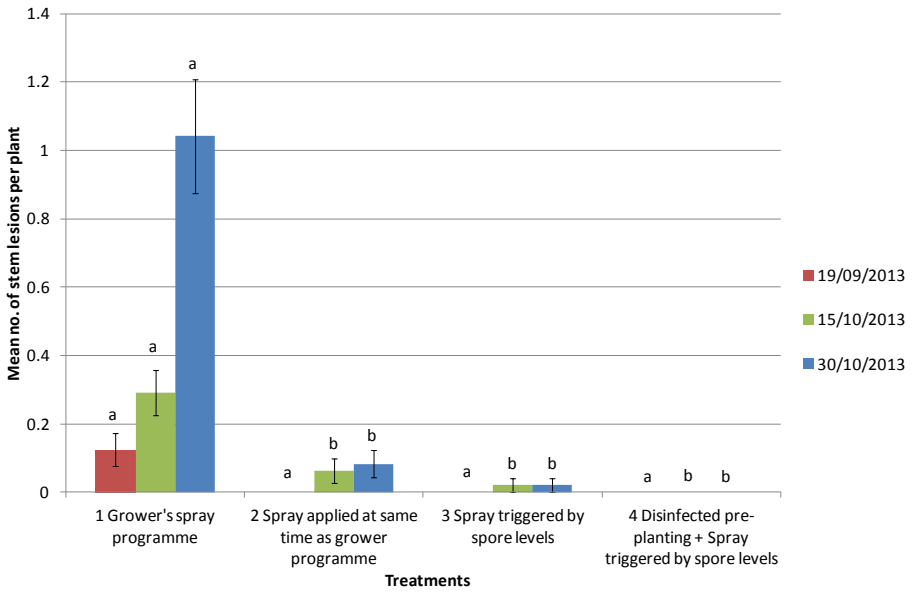

Figure 1: Mean number of stem lesions per plant at three assessment dates at site 1. Error bars indicate standard error. LSD (P = 0.05) columns of the same colour with the same letter above them are not significantly different.

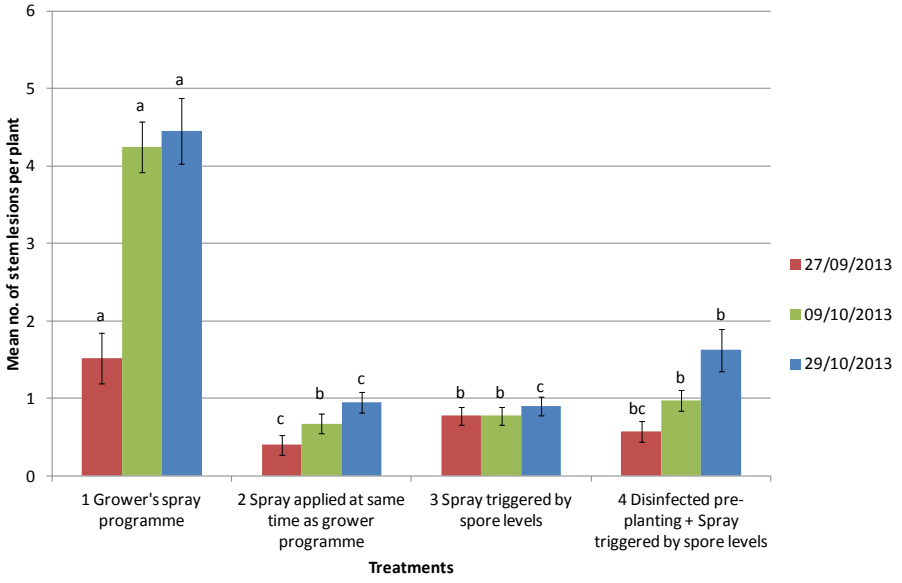

Figure 2: Mean number of stem lesions per plant at three assessment dates at site 2. Error bars indicate standard error. LSD (P = 0.05) columns of the same colour with the same letter above them are not significantly different.

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.

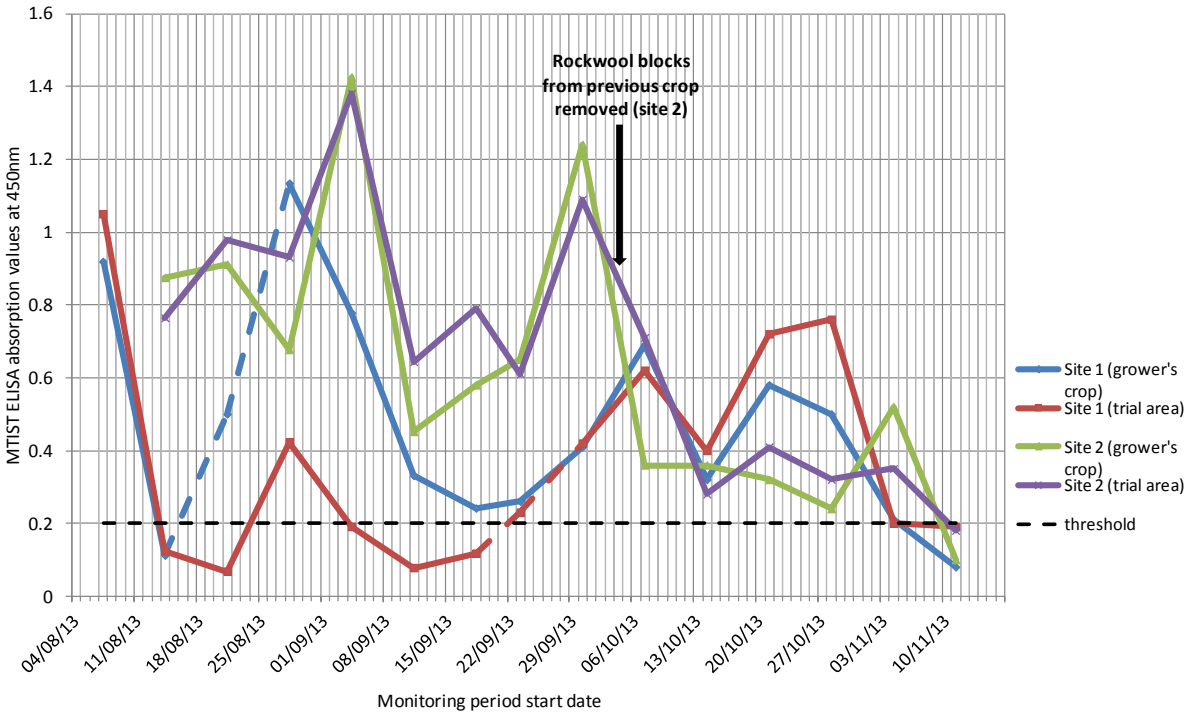

Figure 3: Monitoring glasshouse aerosols for *Mycosphaerella melonis* ascosporic inoculum at two nursery sites.

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.

SCIENCE SECTION

Introduction

Gummy stem blight caused by *Mycosphaerella melonis* (*Didymella bryoniae*) has been a persistent leaf, stem & fruit disease in glasshouse cucumber for many years (Figure 6). It has been generally suppressed, rather than controlled, over the years using a combination of rigorous hygiene precautions (to remove debris that might otherwise allow the pathogen to carry-over from crop to crop in the glasshouse), environmental manipulation (to avoid conditions conducive to infection), use of fungicides (to prevent infection and spread of the pathogen) and more recently through the use of better cultivars (to reduce the rate of disease progression in the host crop). However, more recently, a number of factors have impacted on the disease and it is becoming more prevalent and damaging economically with fewer opportunities for effective control. This is of considerable concern for growers due to the potential economic damage this pathogen can cause either through direct loss of plants (stem girdling) or yield reduction (as a result of symptomatic or latent (internal) fruit infection). Increased energy costs are a significant factor leading to increased infection as the higher cost discourages the use of pipe heat early in the morning to dry the foliage and avoid conditions conductive to infection. Similarly, the loss of key active substances as a result of the EU pesticide review programme has meant that growers have fewer useful products with good activity against the pathogen to prevent infection. This is further influenced by the increased shift in consumer (retailer) perception regarding pesticide residues. An indirect impact of all this is the increased use of cultivars with tolerance to powdery mildew (where most fungicides are usually used for control). This means that growers are applying fewer fungicide sprays which otherwise would have provided incidental control, or at least suppression, of *Mycosphaerella* infections. There is also some evidence to suggest that such mildew tolerant cultivars may actually be more susceptible to *Mycosphaerella*.

Picture courtesy of Dr G M McPherson

Figure 1: *Mycosphaerella melonis* stem and fruit infection

No recent studies have been undertaken in the UK to determine the sensitivity of existing and/or new fungicides and bio-control products against *Mycosphaerella* and growers have to rely on an ever diminishing armoury of products. There is a direct parallel here with the use of antibiotics for disease control in human & animal populations and likewise in horticulture we are facing an increased risk of fungicide resistance in phytopathogen populations. Unless we can find alternative approaches to the control of such endemic pathogens we could potentially expect a continued increase in disease, potentially reaching epidemic proportions.

The purpose of this project was firstly to establish 'state of the art' with respect to our knowledge on this important pathogen and to establish the sensitivity of the current population to widely used fungicides (Phase 1). Guided by this knowledge, the aim was then to seek alternative control strategies (Phase 2). This included the evaluation of novel fungicides & alternative bio-control products and the use of novel immunosassay or serological techniques to predict disease risk by monitoring the pathogen spore population in the glasshouse in order to take action before infection occurs; thereby improving application timing to prevent economic loss due to the disease.

Materials and methods

Information relating to years 1 & 2 of this project are available in the annual reports for 2011 & 2012. In the final year of the project (2013) the aim was to pull all the information together to try and integrate what we had learnt under commercial conditions to determine the potential for improving control of *Mycosphaerella*.

Treatments

Two growers kindly allowed the use of their holdings for two integrated trials. The treatment programmes are shown in Table 1.

Crop establishment: Following identification of a suitable area within each glasshouse, the area for treatment 4 was subjected to a more rigorous and thorough disinfection clean down using Jet 5 at 1:125 dilution in water, applied as a high volume spray over floor, slabs, drippers and irrigation lines; wipe down support wires and stanchions were also wiped down with same material. The rest of the trial area was cleaned using less rigorous methods, or using standard practice at each nursery. Following this disinfection procedure the crop was planted by the nursery staff using standard practice. At this time, the trial was laid out (see appendices 3 & 4). Environmental data (temp and RH) were recorded using the Priva Integro computers (or similar) at each site or tiny tag environmental monitors.

Table 1: Treatment programmes for integrated control study

Treatment & timing	Product code	Rate of use (product)	Water volume L/ha
T1. Standard* commercial spray programme applied following grower's normal timing.	Refer to Appendices 5 & 6		500-2000
T2. Experimental programme Alternating Applied at same timings as T1.	F159 F85+F86	1L/ha$ 0.6kg/ha	500-2000 500-2000
T3. Experimental programme - No Disinfection Alternating Applied at periods of high spore release (determined by spore trap).	F159 F85+F86	1L/ha$ 0.6kg/ha	500-2000 500-2000
T4. Experimental programme - Pre-planting Disinfection Alternating Applied at periods of high spore release (determined by spore trap).	Jet-5 F159 F85+F86	1:125 dilution 1L/ha$ 0.6kg/ha	 500-2000 500-2000

* Information on products and timing supplied by growers.

$ The first early spray of F159 (i.e. applied within 2 weeks of planting) was applied at half-rate (i.e. 0.5 L/ha); for the second spray, if applied within weeks 3-4 of planting, this would be applied at 2/3 full rate (i.e. 0.67L/ha). Any spray applied at 4 weeks after planting or later would be at full rate (1L/ha). This was to avoid any risk of phytotoxicity on young plants.

It was not appropriate to introduce *Mycosphaerella melonis* artificially in these commercial crops and instead this study relied on natural infection occurring.

Spore traps: 2 microtiter immunoassay air sampler (MTIST) spore traps were used on each site. Spore trapping wells were changed weekly by nursery staff and sent to NPARU for processing. Results were emailed through to STC & ADAS as soon as possible to trigger treatment applications when required.

MTIST spore traps in treatments 1 (grower crop) and treatments 3 & 4 (spray programme triggered by spore levels) were changed on a weekly basis and the microtitre wells were sent to the University of Worcester for analysis using ELISA. The results were sent to ADAS and STC as soon as they were available. A threshold level of 2000 spores per litre of air was set according to previous research on *Mycosphaerella brassicicola* (Kennedy *et al*, 2000) and this equated to an absorption value at 450 nm of 0.2. If the absorption value recorded by the MTIST trap in treatment 3 was above 0.2 then this would mean a spray application would need to be made in treatments 3 & 4.

Treatment application: In the T1 area of the crop normal commercial treatments were applied by nursery staff. T2 – T4 treatments were applied by STC/ADAS staff at the timings indicated in Table 1. In T2 – T4 a 14 day interval between sprays was imposed. Treatments were applied using either a single lance or a boom sprayer (whichever was appropriate for the crop at the time) attached to an Oxford Precision Knapsack sprayer and the whole crop area in the each treatment was treated. Commercial products were applied to the guard area of the crops adjacent to the treatments. The water volume increased as the crop developed but the product application rate remained constant i.e. the same amount of product was applied per unit area but in greater dilution.

Additional fungicides e.g. Systhane were applied in T3 & T4 for the control of powdery mildew as necessary.

Disease Assessment: The crop was monitored for disease on a regular basis post planting (according to assessment pro-forma, appendices 1 & 2). Disease assessments were made on three occasions during the time course of the study: at the onset of initial symptoms in the crop, mid-term when there were clear treatment differences and after the final spray application had been made. During each assessment the number of leaf, stem, fruit and node infections/plant were scored based on 10 to 12 plants per plot. Each treatment was divided into four sections to represent 'replicate' plots for statistical analysis purposes. Fruit from the experimental area was harvested as required by nursery staff. Fruit was picked and retained in labeled crates/bins for assessment by science staff for internal and external rots. All fruit from T2-T4 was destroyed after each harvest. Photographs of treatment effects were taken.

Crop safety Assessments: Following treatment application the plants were monitored regularly for any adverse symptoms and appropriate records made depending on the nature of the effect(s). Negative findings were also recorded.

Results and discussion

Monitoring ascospore levels

Initially the MTIST spore traps were changed weekly on Wednesday and posted to the University of Worcester. The ELISA was done on Thursday and results reported to STC and ADAS on Friday. However, by the time the results were received on Friday it was Monday or Tuesday before reactive fungicide applications could be made to the trial crop. This meant that the time period between high spore levels being recorded and reactive fungicide applications being made was too long. It was therefore adjusted accordingly and the MTIST traps were changed on Monday, analysed on Tuesday, thus enabling results to be delivered by Wednesday and spray applications to be made the same week, if required, reducing the time between high spore levels being recorded and reactive fungicide applications being made.

The recommended interval between spray applications for the experimental products was two weeks, so this also prevented reactive applications from being made if an application had been made the previous week.

Site 1 (Lee Valley)

The graph below (Figure 2) shows the ascospore levels recorded for site 1 with spray application dates and assessment dates inserted and

Table 2 summarises the disinfectant/fungicide applications made.

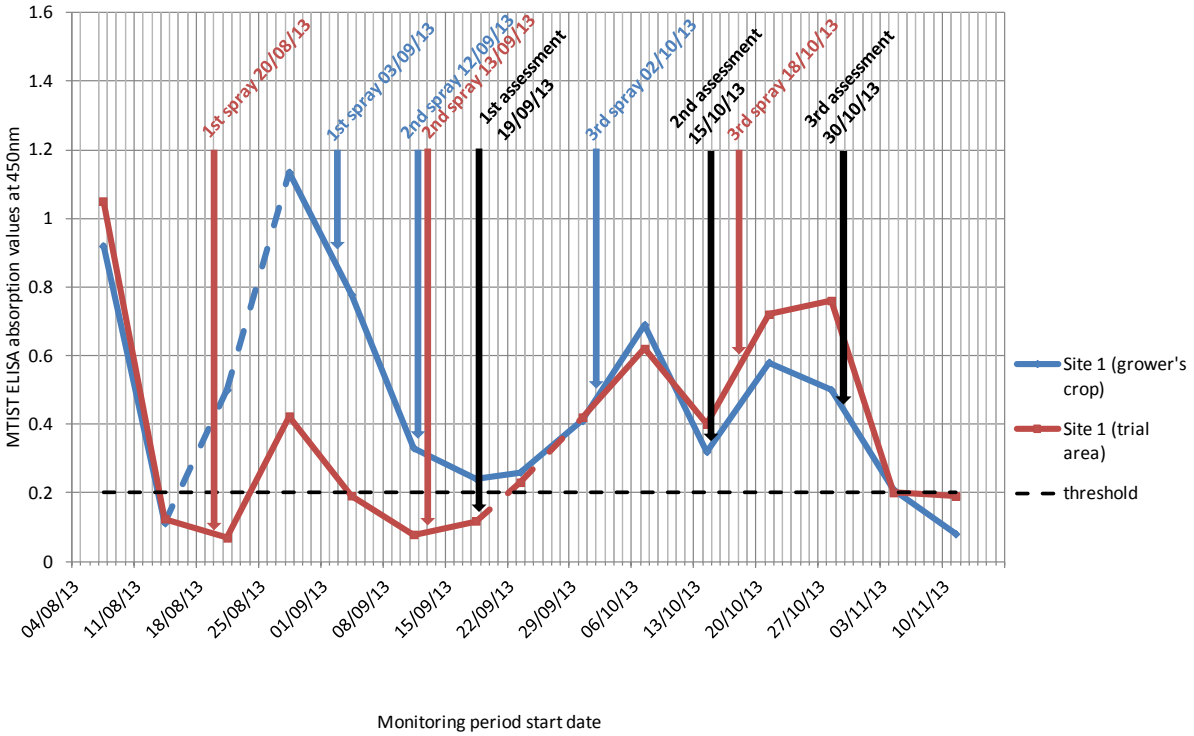

Figure 2: Monitoring glasshouse aerosols for *M melonis* ascosporic inoculum at site 1.

Table 2. Summary of disinfectant/fungicide applications made to the trial at site 1 (Lee Valley)

Date	Treatment 1 Approved Grower Programme	Treatment 2 Experimental spray applied at same time as grower programme	Treatment 3 Experimental spray triggered by spore levels	Treatment 4 Disinfected pre-planting + experimental spray triggered by spore levels
05.08.13	-	-	-	Jet 5
20.08.13	-	-	F159	F159
28.08.13	-	F159	-	-
03.09.13	Systhane	-	-	-
12.09.13	Amistar	-	-	-
13.09.13	-	F85+F86	F85+F86	F85+F86
02.10.13	Switch	-	-	-
04.10.13	-	F159	-	-
18.10.13	-	-	F159	F159

At this site, high spore levels were recorded in the first week of the trial, triggering spray applications in Treatments 3 & 4 to be applied at the end of the second week. This initial application, and probably the pre-planting disinfection treatment, appeared to impact on the disease cycle of *M. melonis* as the rise in spore levels in these treatments following the first fungicide application was less than that reported in the grower's commercial crop. Peak spore levels in treatments 3 & 4 were less than 40% of the spore levels recorded in the

grower's crop in week four (the MTIST spore trap in the grower's crop was returned to the University of Worcester for maintenance in week three so the spore levels that week were estimated as represented by the dotted line).

The grower at site 1 made two fungicide applications in weeks five and six, but the spore levels did not fall below those recorded in treatments 3 & 4. The experimental fungicide applications were made to treatments 3 & 4 in week six, triggered by the peak spore levels recorded in week four.

By week seven the first disease assessment was made as the disease was starting to develop though as this stage infection levels by *M. melonis* remained quite low. Spore levels in the treatments 3 & 4 were below the threshold in week seven. [In week eight the analysis equipment at the University of Worcester failed so there was no record for this week.] In week nine the grower made another application and the spore levels in treatments 3 & 4 were as high as those in the grower's crop. An estimate of the spore levels in treatments 3 & 4 in week eight was made (represented graphically by the dotted line) and this was above the threshold, so an earlier application of the experimental treatments triggered by this reading could potentially have kept spore levels down.

A second disease assessment was made in week 11 as disease levels in the commercial crop had increased. There were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, number of infected nodes, number of infected laterals and number of infected leaf petioles, with highest levels of disease symptoms recorded in the grower's commercial crop.

No more applications were made by the grower, but one more experimental application to treatments 3 & 4 was made in week 12. However, spore levels in treatments 3 & 4 remained above those in the grower's crop until the end of the trial.

A third disease assessment was made in week 13 by which time infection levels were at moderate to high levels. Again, there were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, number of infected nodes, number of infected laterals and number of infected leaf petioles, with highest levels of disease symptoms recorded in the grower's crop.

In total three fungicide applications were made by the grower and three applications were made experimentally. The disease levels in the grower's crop were significantly higher than those in the experimental treatments at the second and third assessment timings.

Site 2 (East Yorkshire)

The graph below (Figure 3) shows the ascospore levels recorded for site 2 with spray application dates and assessment dates inserted. Table 3 summarises the disinfectant/fungicide applications made. The rock-wool blocks which contained the stem bases of the plants from the previous crop were not removed from the glasshouse until the eighth week of the trial. A dramatic drop in spore levels corresponds to this event, emphasising the importance of old plant material from the previous crop as an inoculum source. This was such a significant event that scientists analysing the microtitre wells from the spore traps thought that the spore traps were malfunctioning because the readings had fallen so dramatically in comparison to all previous results.

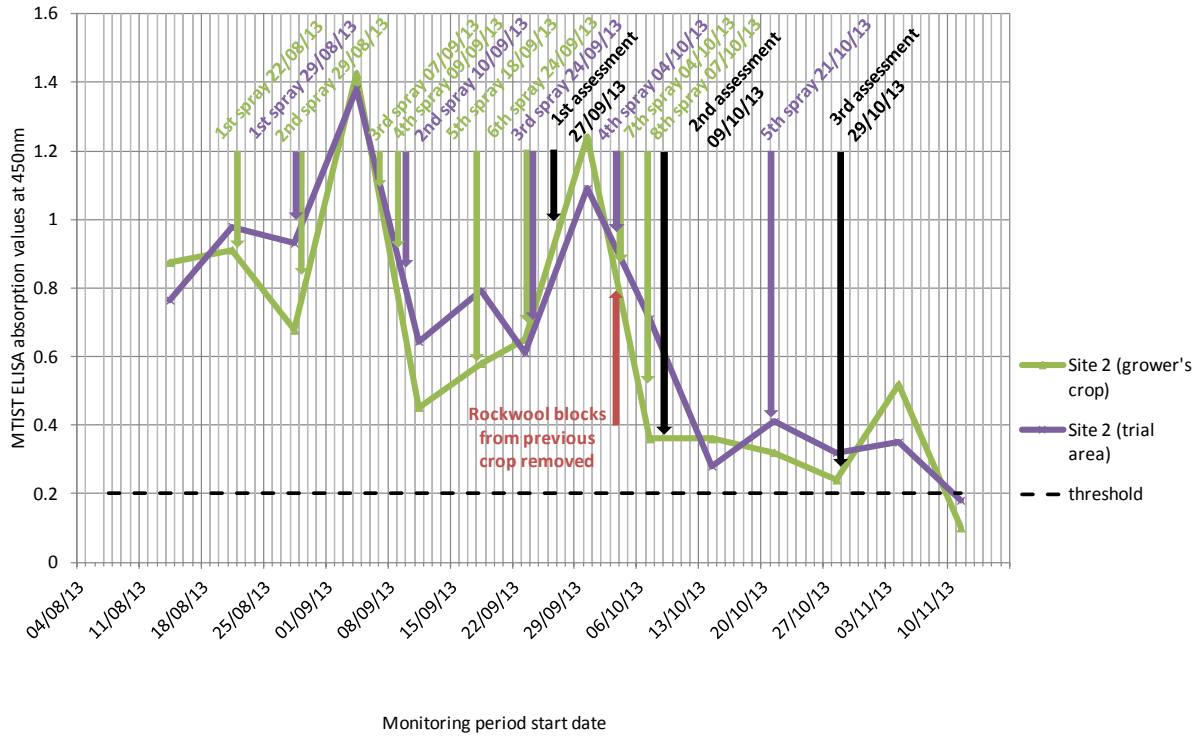

Figure 3: Monitoring glasshouse aerosols for *M melonis* ascosporic inoculum at site 2.

High spore levels were recorded in the first week of the trial, triggering spray applications in Treatments 3 & 4 to be applied at the beginning of the third week. However, the grower already made the first application at the beginning of week two and the second application at the beginning of week three.

Peak spore levels in the grower's crop were recorded in week four, as with site 1, but the levels in treatments 3 & 4 were just as high. Two further spray applications were made by the grower in week four and the second experimental application was made to treatments 3 & 4 triggered by high spore levels in week three.

Table 3. Summary of disinfectant/fungicide applications made to the trial at site (East Yorkshire)

Date	Treatment 1 Approved Grower Programme	Treatment 2 Experimental spray applied at same time as grower programme	Treatment 3 Experimental spray triggered by spore levels	Treatment 4 Disinfected pre-planting + experimental spray triggered by spore levels
14.08.13	Jet 5	Jet 5	Jet 5	Jet 5 double application
22.08.13	AQ10 applied through enbar to all treatments			
27.08.13	-	F159	-	-
29.08.13	Amistar	Amistar	Amistar + F159	Amistar + F159
07.09.13	Systhane	-	-	-
09.09.13	Takumi applied through enbar to all treatments			
10.09.13	-	F85+F86	F85+F86	F85+F86
18.09.13	Agrovista Fenamid applied to stem bases of all plants			
24.09.13	Switch & chalk (stem base)	Switch & chalk (stem base) + F159	Switch & chalk (stem base) + F159	Switch & chalk (stem base) + F159
04.10.13	Switch & chalk (stem base)	-	F85+F86	F85+F86
07.10.13	Systhane applied through enbar to all treatments			
09.10.13	-	F85+F86	-	-
21.10.13	-	-	F159	F159

By week five spore levels had fallen (although not below the threshold) but then started to rise again to reach another peak in week eight. The grower made spray applications in weeks five and seven and the third experimental application was made in week seven. The first disease assessment was made this week and there were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, size of stem base lesion, mean size of stem base lesion per plant, number of infected nodes, number of infected flowers and number of infected laterals, with highest levels of disease symptoms recorded in the grower's crop.

Two further grower applications were made in week eight and one experimental application to treatments 3 & 4. The rock wool blocks containing the stem bases of the plants from the previous crop were removed from the glasshouse too. This resulted in a dramatic drop in spore levels in week nine, although they were still above the threshold.

The second disease assessment was made in week nine. There were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, size of stem base lesion, mean size of stem base lesion per plant, number of infected laterals and number of dead plants, with highest levels of disease symptoms and highest number of dead plants recorded in the grower's crop.

No more applications were made by the grower, but one more experimental application to treatments 3 & 4 was made at the end of week 10. The final disease assessment was made at the beginning of week 12. There were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, size of stem base lesion, mean size of stem base lesion per plant, number of infected nodes, number of infected unharvested fruit on the crop, number of infected laterals and number of dead plants, with highest levels of disease symptoms and highest number of dead plants recorded in the grower's crop.

In total eight fungicide applications were made by the grower and just five applications were made experimentally. The disease levels in the grower's crop were significantly higher than those in the experimental treatments at all assessment timings.

Disease Assessments

Site 1 (Lee Valley)

High spore levels were first indicated by the spore traps on 16 August, triggering the first spray for T3 and T4. Powdery mildew was noted in the crop on 27 August, resulting in the first spray for T2 as the grower wanted to apply a fungicide against both mildew and gummy stem blight. *Mycosphaerella* was first noticed on 13 September, 6 weeks after planting. At the first assessment on 19 September, levels of disease were very low and there was no infection seen in the flowers or at the stem nodes. Where infection did occur it was mostly in T1 and this was the only treatment where stem lesions were seen (Appendix 7, Table 8). There was also a small amount of infection seen in the laterals and leaf petioles in T3. For the internal fruit discolouration/rot assessment, only 1 fruit showed some internal browning and this was found in T1.

By the second assessment on 15 October, the severity of *Mycosphaerella* had increased slightly, with stem lesions now present in T2 and T3. The percentage of stem lesions in T1 had more than doubled, from 12.5% to over 29%. The lesions were large, with some covering over three nodes. There were infected laterals in every treatment, as well as some infected leaf petioles. In T4 there were no stem lesions or infected nodes. There were no infected flowers in any of the treatments. Internal assessment of the fruit showed some infection in T1, T2 and T3, but not T4 (Appendix 7, Table 9).

By the final assessment on 30 October, over 50% of the plants assessed in T1 had stem base lesions, with some plants showing lesions in the middle and at the top of the stem as well. The total number of stem lesions was 50 in T1, 1-4 in each of T2 and T3 and 0 in T4. There were a relatively large number of symptomatic laterals in all treatments, though factors other than *Mycosphaerella* may have been responsible in some cases. Internal assessment of the fruit showed a similar moderate level of browning (6-12%) in every treatment (Appendix 7, Table 10). No flower infection was seen. Again it cannot necessarily be assumed all the fruit discolouration was due to *Mycosphaerella.*

The largest differences between treatments were seen at the final assessment on 30 October, and these data were analysed statistically. As this commercial trial was unreplicated, with 'pseudo-replicate' plots within whole plots. It is reasonable to assume that the position of the plants would have had little or no effect on the results but analysed results should be treated with a degree of caution. Results are shown in Table 4 for the proportion of plants with stem base and node lesions in each treatment. The occurrence of stem base lesions was significant ($p<0.001$), with the greatest number of affected plants in T1 (Grower standard). There were significantly fewer stem base lesions in T2, T3 and T4. Where spray timing was determined by spore trap results (T3 & T4) the plants were significantly better than T2 (grower spray timings – experimental spray programme). The proportion of plants with stem node lesions was also reduced in T2, T3 and T4 compared with T1.

Results indicate clearly that the new fungicides used in this experiment (F159 and F85+F86) provided better control than the conventional approved sprays used by the host grower (Systhane 20EW, Amistar and Switch). Although the differences are less marked, the results also indicate that fungicide timing triggered by the spore trap resulted in better control of stem base lesions than the grower timings in this experiment. Furthermore, there appears to be an added benefit from a very thorough cleaning and disinfection of an area

before replanting after an affected crop, as the treatment with disinfection use in this experiment (T4) showed the fewest number of stem base lesions.

Table 4 Percentage of plants showing lesions caused by *Mycosphaerella melonis* on 30 October 2013 (Site 1, Lee Valley)

Treatment	Stem base lesions % Mean		Node lesions % Mean	
1. Approved Grower Programme	58.3	(5.78)	54.2	(6.02)
2. Experimental spray applied at same time as grower programme	8.3	(3.24)	8.3	(3.34)
3. Experimental spray triggered by spore levels	2.1	(1.68)	20.8	(4.91)
4. Disinfected pre-planting + experimental spray triggered by spore levels	0.0	(0.00)	14.6	(4.27)
Probability (12 df)	<0.001		<0.001	

( ) – standard error

Infection was greatest in T1 (the grower standard), with stem lesions present on over half the plants assessed. At the first assessment on 19 September, stem lesions were only present in T1. All four treatment areas had received 2 fungicide sprays each, however, the experimental fungicide plots sprayed at timings determined by the spore traps (T3 and T4) had been sprayed first, as spore readings were above the threshold of 2000 spores/m^3. T2 (experimental programme) was sprayed at the same time as the grower (T1). In this case, he had waited until there was some sign of disease in the crop and in this case the first grower sprays were not triggered by the presence of *Mycosphaerella,* but by powdery mildew. Results show that either this spray timing was too late at minimising the presence of *Mycosphaerella* or that the commercial spray programme used was not effective against *Mycosphaerella*. Either way, stem lesions were present in T1 before any other treatment area.

Stem lesions took longer to develop in T2 than in T1. Most probably this was due to use of the experimental fungicide programme which delayed the onset of symptoms, and helped to reduce the number of plants infected with stem lesions, relative to the commercial fungicide programme.

Infection of the laterals and nodes took longer to develop. By the end of the experiment, infection of the laterals was high in all four treatments, with almost every plant showing some infection. So although the experimental fungicides and thorough disinfection didn't necessarily reduce the number of infected laterals, they did delay the onset of disease development. Infection in the nodes wasn't seen in any of the treatments until the second assessment, and again infection was highest in T1. At the final assessment, nodal infection was similar in T2, T3 and T4, and much greater in T1.

Site 2 (East Yorkshire)

In this trial the assessment parameter that best demonstrated the differences between treatments was the mean number of stem lesions per plant. Figure 4 summarises these results.

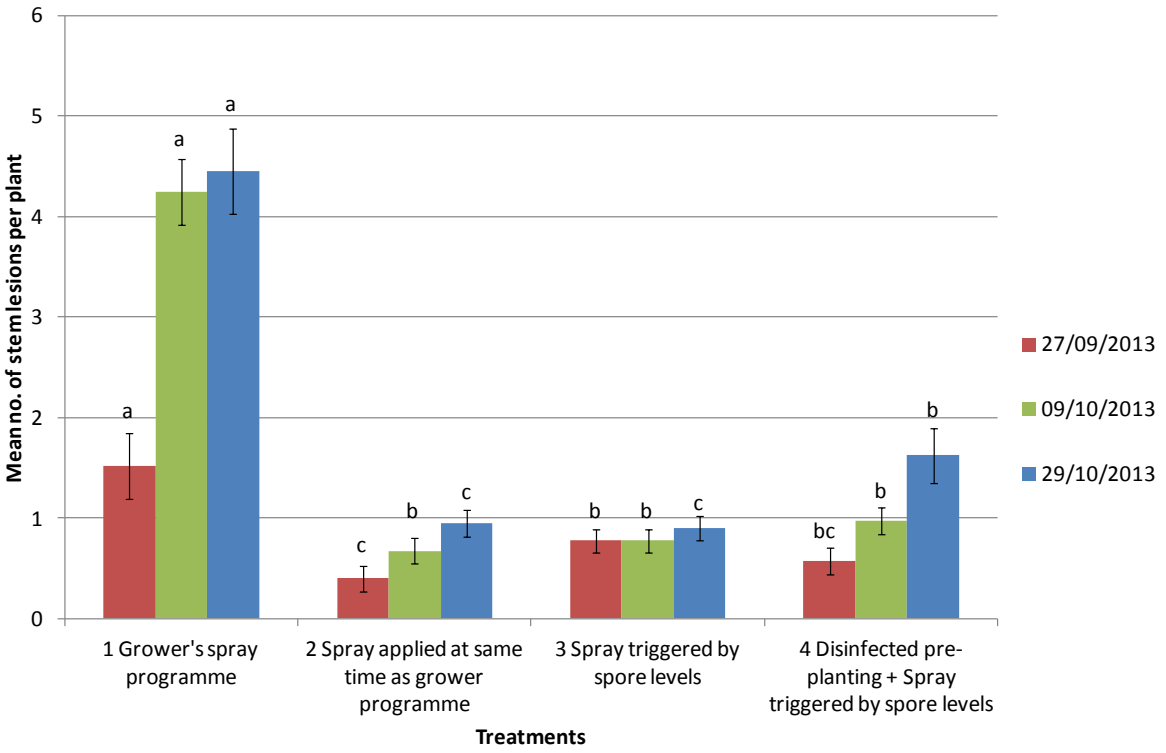

Figure 4 Mean number of stem lesions per plant at three assessment dates at site 2
Error bars indicate standard error. LSD (P = 0.05) columns of the same colour with the same letter above them are not significantly different.

In the first disease assessment (27/09/13) there were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, size of stem base lesion, mean size of stem base lesion per plant, number of infected nodes, number of infected flowers and number of infected laterals, with highest levels of disease

symptoms recorded in the grower's crop (see Appendix 8). Interestingly disease levels in treatment 2 (experimental products applied at grower timings) were significantly lower than treatment 3 (experimental programme triggered by spore levels) for all assessments of stem lesions (number, mean number per plant, percentage of plants with stem lesions and lesion size), but neither treatments 2 nor 3 were significantly different from treatment 4 (disinfection pre-planting + experimental programme triggered by spore levels) (Table 5). An explanation for this could be that because treatment 2 follows the grower spray programme, the experimental products were applied to this treatment before they were applied to treatments 3 & 4 as we had to wait for the results of the ELISA analysis, delaying this application by a few days. The disease levels in treatment 4 (disinfected pre-planting + experimental programme triggered by spore levels) were slightly lower than those in treatment 3, although not significantly. They were also slightly higher than those in treatment 2, although not significantly. The pre-planting disinfection may have had a slight beneficial effect, although not significantly improving on the experimental treatments alone.

Table 5. Significant differences between treatments at first assessment

No.	Treatment	Number of lower stem lesions		Total number of stem lesions (lower + upper)		Percentage of plants with stem lesions		Mean number of stem lesions per plant	
1	Approved Grower Programme	1.2	a	1.5	a	87	a	1.51	a
2	Experimental spray applied at same time as grower programme	0.1	c	0.4	c	40	c	0.4	c
3	Experimental spray triggered by spore levels	0.3	b	0.8	b	62.5	b	0.775	b
4	Disinfected pre-planting + experimental spray triggered by spore levels	0.2	bc	0.6	bc	47.5	bc	0.575	bc
	Prob(F)	0.0001		0.0001		0.0009		0.0002	

LSD (P = 0.05) Values followed by the same letter are not significantly different.

In the second disease assessment (09/10/13) there were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, size of stem base lesion, mean size of stem base lesion per plant, number of infected laterals and number of dead plants, with highest levels of disease symptoms and highest number of dead plants recorded in the grower's crop (Appendix 8). For the assessment of percentage

of plants with stem lesions treatment 2 (experimental products applied at grower timings) was significantly lower than treatment 4 (disinfection pre-planting + experimental programme triggered by spore levels) for all assessments of stem lesions (number, incidence and mean number per plant), but neither treatments 2 nor 4 were significantly different from treatment 3 (experimental programme triggered by spore levels) (Table 6). This could be due to the earlier initial application of the experimental product to treatment 2. By this stage of the trial the disinfection pre-planting appeared to have had little effect on the disease levels.

Table 6 Significant differences between treatments at second assessment

No.	Treatment	Percentage of plants with stem lesions	
1	Approved Grower Programme	100	a
2	Experimental spray applied at same time as grower programme	47.5	c
3	Experimental spray triggered by spore levels	60	bc
4	Disinfected pre-planting + experimental spray triggered by spore levels	65	b
	Prob(F)	0.0005	

LSD (P = 0.05) Values followed by the same letter are not significantly different.

In the final disease assessment (29/10/13) there were significant differences between treatments for the number of stem lesions, mean number of stem lesions per plant, size of stem base lesion, mean size of stem base lesion per plant, number of infected nodes, number of infected unharvested fruit on the crop, number of infected laterals and number of dead plants, with highest levels of disease symptoms and highest number of dead plants recorded in the grower's crop (Appendix 8). For the assessments of mean number of lesions per plant, mean stem base lesion size and percentage of dead plants, treatment 2 was significantly better than treatment 4 (Table 7). It appears that any early effects of disinfection had worn off and that application timing had a greater impact on disease levels than disinfection.

It was not possible to statistically analyse the percentage of harvested fruit affected by *Mycosphaerella* at each assessment as, due to the way the fruit was harvested, only one result was recorded per treatment. Figure 5 represents the results. In addition to the three main assessments, a final assessment was made before the crop was pulled out.

The results serve to demonstrate the effectiveness of the fungicides used and that disinfection pre-planting had little or no apparent additive effect on the percentage of infected fruit. This could be due to the overall high level of efficacy of the experimental fungicide programme that effectively masked any disinfection effect.

Table 7 Significant differences between treatments at final assessment

No	Treatment	Mean number of lesions per plant		Mean stem base lesion size		Percentage of dead plants	
1	Approved Grower Programme	4.53	a	3.51	a	35.66	a
2	Experimental spray applied at same time as grower programme	0.93	c	0.90	c	0	c
3	Experimental spray triggered by spore levels	0.90	c	1.17	bc	0	c
4	Disinfected pre-planting + experimental spray triggered by spore levels	1.60	b	1.53	b	6.30	b
	Prob(F)	0.0001		0.0001		0.0001	

LSD (P = 0.05) Values followed by the same letter are not significantly different.

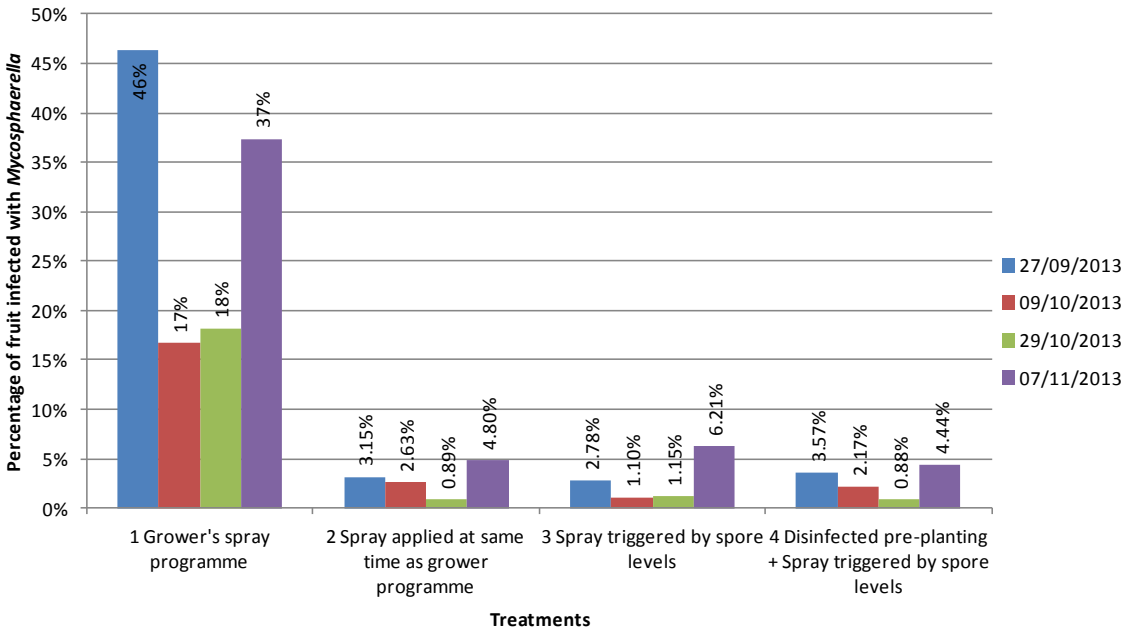

Figure 5 Percentage of cucumber fruit infected with *Mycosphaerella* at four assessment dates at site 2

Based on the harvest data obtained from the trial, calculations were also made of the daily marketable yield of fruit/ha. At the first assessment the yield in the grower's crop was much higher than the yield in the experimental treatments, but at each of the following assessments the yield fell. Conversely the yield in the experimental treatments was much lower at this first assessment than in the grower's crop. This may have been due to a possible phytotoxic effect of the experimental products as slight necrotic flecking of the leaves was noted in the glasshouse trial at STC in 2012 following the first application of HDC F88 which contained an active ingredient also in HDC F159. However, this initial shortfall was made up in subsequent harvests as these remained high. The mean yield/ha/day over the entire cropping period for each of the experimental treatments was almost double that of the grower's crop (Figure 6). 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.

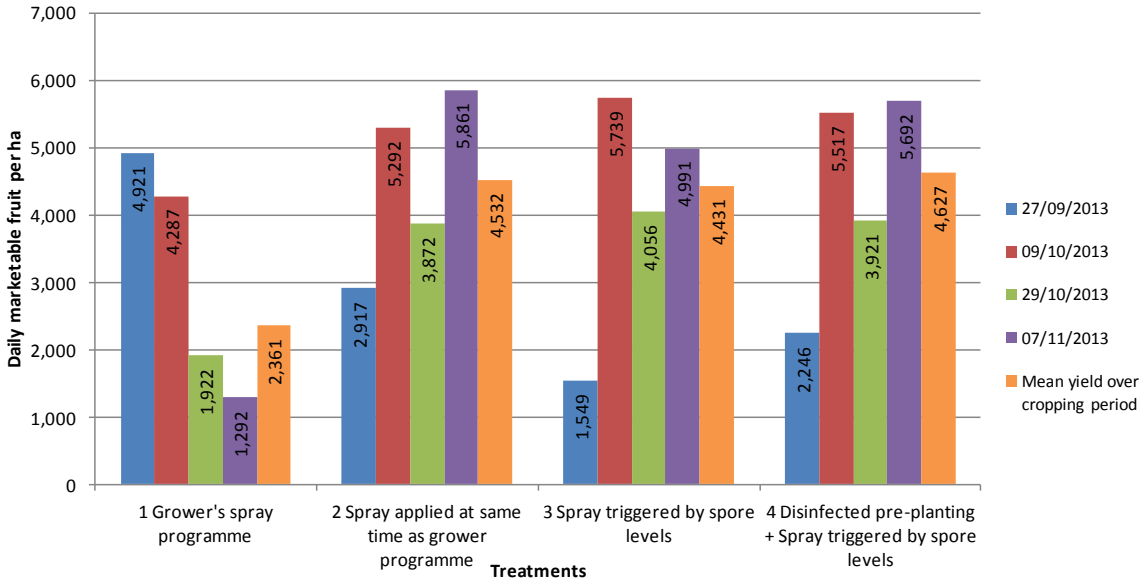

Figure 6 Daily marketable fruit per ha (total fruit harvested – infected fruit) at four assessment dates.

Conclusions

The number of plants with stem base lesions at the final assessment of the trial at site 1, where disease levels were low to moderate, clearly represents the effect that the integrated control strategy was expected to achieve:

Treatment 1 - poor control of *Mycosphaerella* following application of approved fungicide applications which are not specifically formulated to target this pathogen;

Treatment 2 - significantly better control was achieved by experimental fungicide applications timed to coincide with the grower's sprays (T1) above but made without knowledge of glasshouse ascospore levels;
Treatment 3 - significantly better control still was achieved by targeted experimental fungicide applications triggered by high ascospore levels recorded by the spore trapping equipment and
Treatment 4 – best control was achieved by pre-planting disinfection followed by targeted fungicide applications, as in treatment 3, resulting in no *Mycosphaerella* stem base lesions being recorded in this treatment at the end of the trial.

Disease pressure at site 2 was extremely high because rock-wool blocks containing stem bases infected with *Mycosphaerella* from the previous (second) crop were not removed until eight weeks after the following (third) crop was planted. This did, however, provide an extremely robust test for the integrated strategy which incorporated ascospore forecasting, experimental fungicide products and disinfectant use. The experimental programmes performed exceptionally in comparison to the grower's spray programme, although differences between the three experimental programmes were quite small. Disappointingly the pre-planting disinfection at site 2 was only effective for the first eight weeks of the trial. By week nine it was not significantly different from the non-disinfected treatment which was sprayed at the same time. By week 12 it was actually significantly worse than the equivalent non-disinfected treatment. One possible explanation for this could be that as the treatment was located beside the pathway through the centre of the glasshouse, it may have been exposed to more air currents containing airborne spores. Also as there was slightly more human traffic through this row (as staff needed to walk through to change the spore trap) this may have spread inoculum on clothes, hands and feet or a combination of both.

The comparison between Trial 1 and Trial 2 has certainly demonstrated the importance of cleaning up the glasshouse in between crops. It has also demonstrated that there are very effective control products potentially available at least one of which is already approved on similar hydroponically-grown glasshouse edibles. Provided an EAMU could be obtained for use on cucumber this could significantly improve control of the disease. Additionally the same fungicide would be expected to provide control of powdery mildew (as demonstrated in the SCEPTRE project) and this would be an added bonus. Monitoring ascospore levels can potentially be an additional tool to target spray timings and reduce unnecessary fungicide applications, provided that the technology can be refined to provide rapid results, such as in the form of a lateral flow device. In these trials the time between periods of high

ascospore levels and follow-up spray applications was too long on occasions, potentially missing the application timing when the products would be most effective.

The results from both sites show how effective the experimental products are in comparison to the products currently available to growers, which are not specifically designed to target *Mycosphaerella*. Use of these experimental products alone would certainly have an impact on the development of the pathogen, and considering that some of these products are also effective against powdery mildew, as mentioned above, fungicide applications to control this disease could also be reduced. If good crop hygiene procedures are followed and thorough clean up is made between crops, including disinfection with Jet 5, then these measures will serve to reduce the number of fungicide applications that need to be made during the life of the crop. If the ascospore monitoring technology can be incorporated as an integral part of the regime, this would enable targeted applications to be made, enabling maximum impact on *Mycosphaerella* development.

Knowledge and Technology Transfer

21.1.11 The results from Phase 1 of the work were discussed at an HDC Project Review Meeting at Stoneleigh

1.2.11 Dr Martin McPherson presented the results to the Cucumber Growers Association meeting on the 1st February 2011

5.10.11 Dr McPherson gave a detailed update on the project to the industry at the Annual Cucumber Growers Association Conference

5.7.12 Cucumber Growers Association (CGA) meeting at STC. The progress of the project was discussed and the growers were shown the glasshouse trials. In the fungicide trial, they were shown preliminary results of assessments to date which demonstrated the effectiveness of some of the coded products in comparison to the current industry standards. In the systemic infection trial the purpose of the trial was explained and the spore sampling methods and anticipated outcomes were discussed.

19.7.12 Official opening of the LED4CROPS facility at STC. Invited guests were shown the fungicide trial. The purpose of the trial was explained to the audience and they were shown preliminary results of assessments to date which demonstrated the effectiveness of some of the coded products in comparison to the current industry standards.

28.9.12 Technical meeting of the CGA. Powerpoint presentation summarizing the results of the 2012 Fungicide trial at STC.

3.10.12 Cucumber Conference, Peterborough. Presentations:
Disinfectants for control of *Mycosphaerella* in cucumber (Tim O'Neill)
An overview of the epidemiology and control of *Mycosphaerella* in cucumber as a component of HDC Project PE 001a (Martin McPherson)

HDC News Article: Cultural cues to disease control. Issue 188, pp20-21.

Oct 2013 Dr Martin McPherson gave a presentation at the Cucumber Growers Day at Waltham Abbey, Essex.

HDC News Article: New spray programme holds hope for control of gummy stem blight. Issue 199, p11.

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Kennedy, R., Wakeham, A.J., Byrne, K.G., Meyer, U.M. and Dewey, F.M. (2000) A new method to monitor airborne inoculum of the fungal plant pathogens *Mycosphaerella brassicicola* and *Botrytis cinerea*. Applied and Environmental Microbiology, Vol 66, 2996-3000

Acknowledgements

We would like to thank Mr T Brockhuizen, Mr D Schellingerhout and Mr T Schembri and their staff for their cooperation and for allowing us to use their premises for various aspects of this study.

Appendices

Appendix 1

Disease Assessment sheet

PE 001a Control of Mycosphaerella in cucumbers **Integrated control study - 2013**

Do not assess first and last 4 plants in each treatment area.

Assess a representative no. plants/plot depending on row length, planting type and disease pressure.

Site _____

Date: _____ Assessors: _____

Treatment	Plant No.	No. infected/plant									Plant Dead/Alive	Size of stem base lesion in internodes	Comments
		Stem lesions			Nodes	Flowers	Fruit	Laterals		Leaves			
		Black	Silver	Wire				Cut	Weak				
	1												
	2												
	3												
	4												
	5												
	6												
	7												
	8												
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Appendix 2 Fruit assessment sheet

PE 001a Control of Mycosphaerella in cucumbers

Integrated control study - 2013

Site ______________________

Date	Assessor	Trt	Total No. fruit harvested	Total No. not marketable (mis-shapen/small)	No. fruit with external rots (yellow/black)	No. of fruit tapered	No. of fruit with internal myco rots, not visible externally	Notes
		1						
		2						
		3						
		4						
		1						
		2						
		3						
		4						
		1						
		2						
		3						
		4						
		1						
		2						
		3						
		4						
		1						
		2						
		3						
		4						
		1						
		2						
		3						
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		1						
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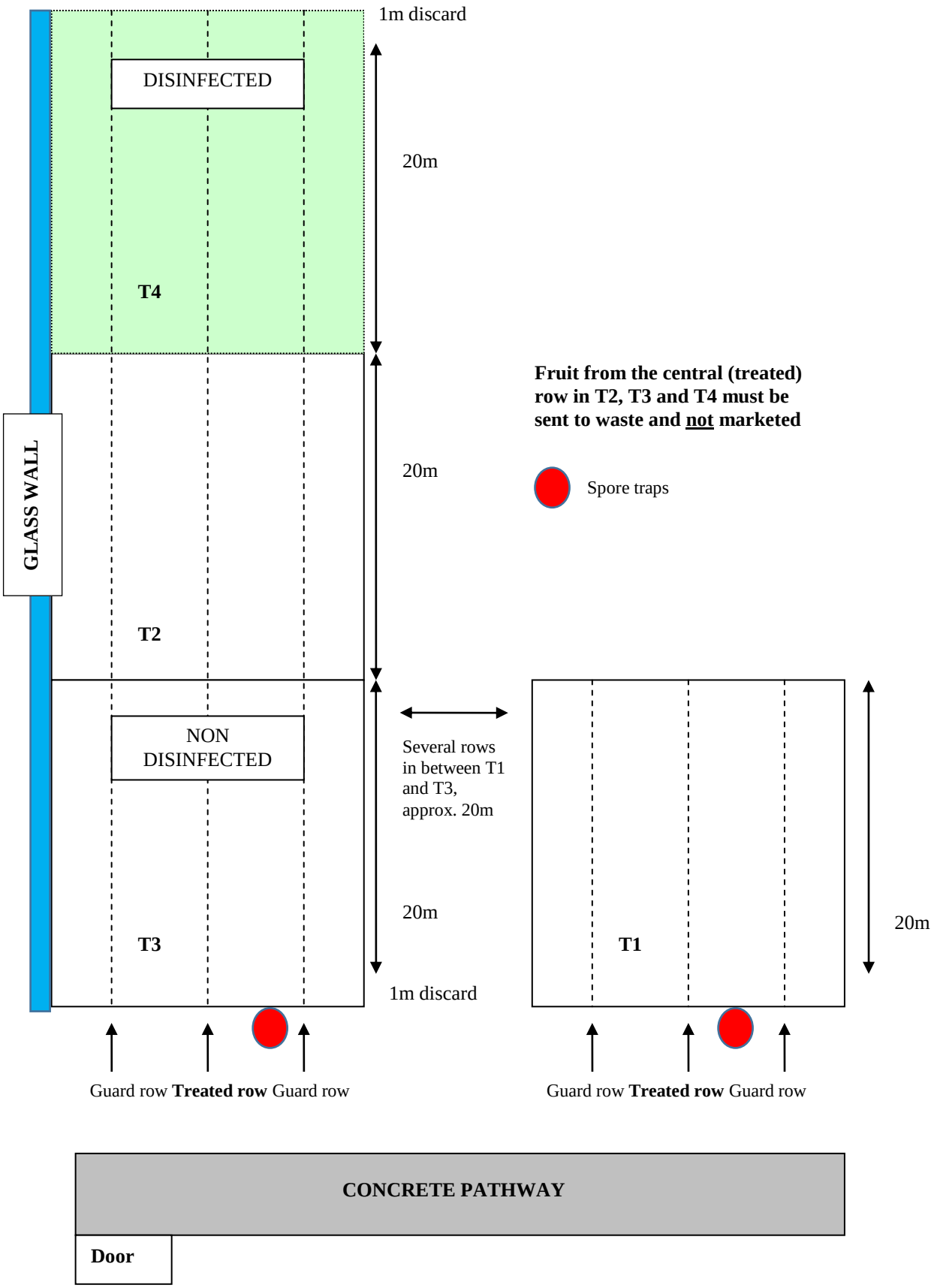
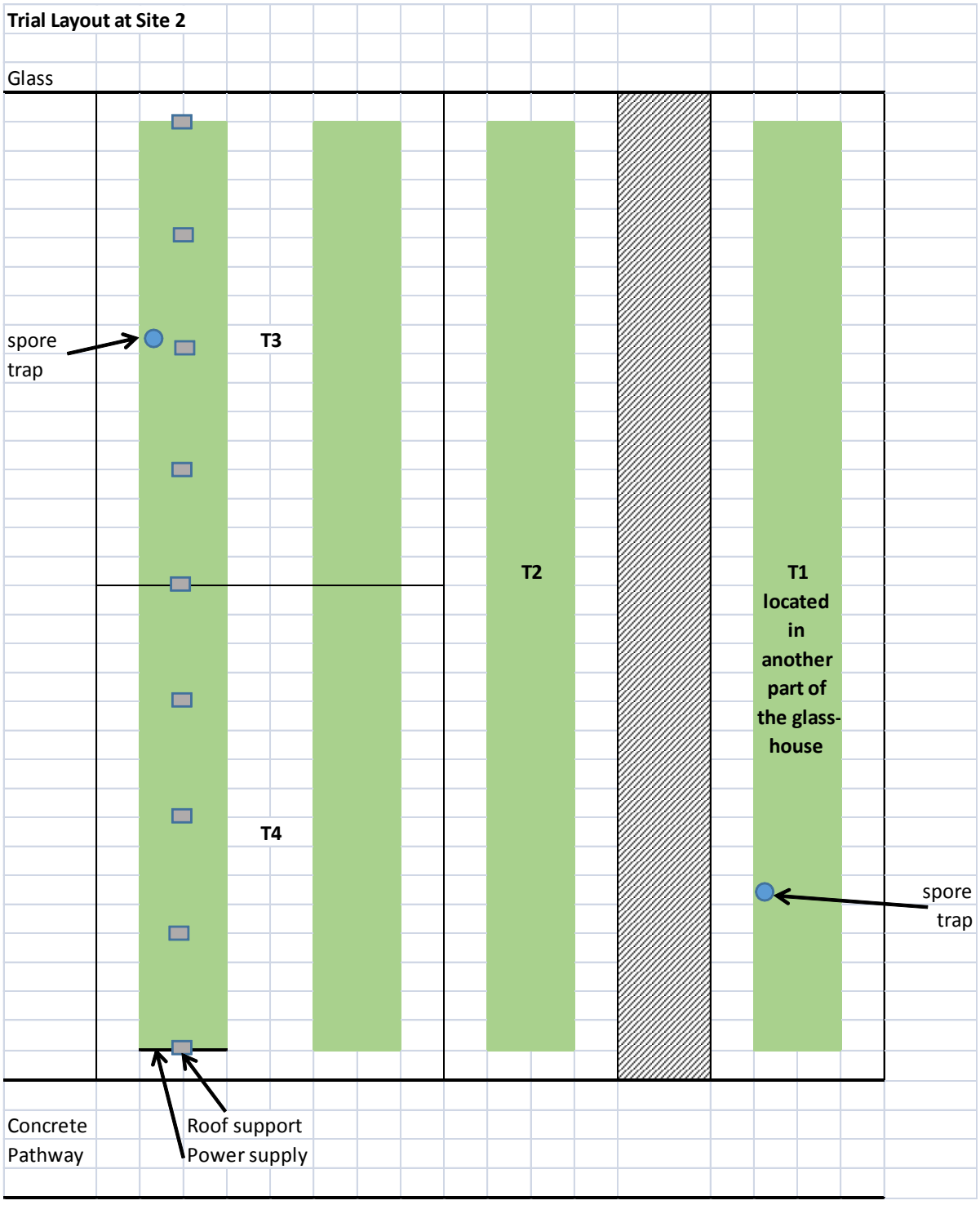
		3						
		4						
		1						
		2						
		3						
		4						
		1						
		2						
		3						
		4						

Appendix 3 Trial Plan – Site 1 (Lee Valley)



Appendix 4 – trial layout at site 2 (East Yorkshire)



Appendix 5 Crop diary site 1 (Lee Valley)

Date	Grower Programme (estimated)	Trial Programme
05/08/2013	Removal of old crop (plant debris remains in place)	Trial area marked out. Removal of old crop and majority of plant debris in trial areas T4 disinfected with Jet-5. Spore traps located in T1 & T4.
06/08/2013	Crop Planted cv Bonbon.	Crop Planted cv Bonbon at 53 plants per plot.
08/08/2013		Spore traps set up
16/08/2013		Spore trap results – T1 = 0.425 and T2 = 0.601 triggering first sprays.
20/08/2013		Spray applied to T3 & T4 (F159 at 0.5l/ha in water volume 500l/ha). No visible signs of disease on plants.
22/08/2013		Spore traps changed. Trap T2 raised to 1m above ground, T1 temporarily removed for maintenance.
23/08/2013		Spore trap results – T1 = 0.19 and T2 = 0.20.
28/08/2013		Picking started. Spray applied to T2, F159 at 0.67l/ha in water volume 500l/ha. T1 spore trap returned to plot at 1m above ground level. Low (1-3%) level of powdery mildew infection observed on leaves. No myco apparent.
02/09/2013		T2 spore trap results – 0.068
03/09/2013	Systhane applied as fog T1, T2, T3, T4 and guards against mildew.	
06/09/2013		Spore trap results, T1 = 1.14, T2 = 0.40. T1 wells contaminated with dirt. Advised to spray.
12/09/2013	Amistar applied for mildew control T1, T2, T3, T4 and guards	
13/09/2013	Evidence of low level of myco in T1.	T3 and T4 sprayed F85+F86 at full rate at water volume of 1500l/ha.
16/09/2013		Spore trap results, T1 = 0.80, T2 = 0.20. Decision not to spray with results just at threshold level and recent applications on 12/09.
19/09/2013	1st disease assessment	
23/09/2013		Spore trap results, T1 = 0.20, T2 = 0.10; no spray required.
26/09/2013		Spore trap results (change of day to decrease time lag between results and spray application), T1 = 0.20, T2 = 0.10; no spray required.
02/10/2013	Switch applied as stem base spray (T1)	
03/10/2013		Spore trap results, T1 just above 0.20 threshold and T2 is below; decision not to

		spray.
04/10/2013	Myco observed at low levels throughout glasshouse. Guards sprayed with Switch at 0.8kg/ha.	Spray applied to T2, F159 1l/ha in a water volume 1500l/ha. Myco observed at low levels throughout glasshouse.
15/10/2013	2nd disease assessment. Some fruit taken to lab for isolation onto PDA+S.	
16/10/2013		Spore trap results received – spray required.
18/10/2013		Spray applied to T3 & T4, F159 at 1l/ha in water volume 1500l/ha.
21/10/2013	Myco confirmed on PDA plates from isolations on 15/10/2013	
30/10/2013	Final disease assessment (plants and fruit)	
wc 04/11/2013	Crop removed from glasshouse. Debris left behind	
wc 18/11/2013	Glasshouse cleaned and disinfected.	
wc 09/12/2013	Spore traps removed from glasshouse	

Appendix 6 Crop Diary Site 2 (East Yorkshire)

Date	Grower Programme (estimated)	Trial Programme
14/08/2013		Trial area marked out. T4 disinfected with Jet-5. Spore traps located in T1 & T4.
15/08/2013	Crop Planted.	
22/08/2013	AQ10 enbar T1, T2, T3, T4	
27/08/2013		HDC F159 applied to T2. Spore trap moved from T4 to T3.
29/08/2013	Amistar applied to T1, T2, T3, T4	HDC F159 applied to T3 & T4. Spore traps moved up to 0.5 m.
07/09/2013	Systhane T1	
09/09/2013	Takumi enbar T1, T2, T3, T4	
10/09/2013		Systhane applied to guards. HDC F86 + F85 applied to T2, T3 & T4. Spore traps moved up to 1 m.
18/09/2013	Agrovista Fenamid applied to stem bases T1, T2, T3, T4	
24/09/2013	Switch + chalk applied to stem bases T1, T2, T3, T4	HDC F159 applied to T2, T3 & T4.
27/09/2013		1st assessment made.
04/10/2013	Switch + chalk applied to stem bases T1 only	HDC F86 + F85 applied to T3 & T4.
07/10/2013	Systhane enbar T1, T2, T3, T4	
09/10/2013		HDC F86 + F85 applied to T2. 2nd assessment made.
21/10/2013		HDC F159 applied to T3 & T4.
29/10/2013		Final assessment made.

07/11/2013	Final fruit assessment made

Appendix 7 – Disease assessments site 1 (Lee Valley)

Table 8. Incidence of *Mycosphaerella melonis* symptoms in cucumber, cv Bonbon, following various fungicide treatment – 19 September 2013 (Site 1)

Treatment	Sub-plot	No plants out of 12				
		Stem lesions	Fruit	Laterals	Nodes	Leaf petiole lesion
1	1	0	0	0	0	0
	2	2	0	2	0	0
	3	4	2	0	0	1
	4	0	5	2	0	0
2	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
3	1	0	0	3	0	1
	2	0	0	1	0	1
	3	0	0	0	0	0
	4	0	0	0	0	0
4	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0

Table 9. Incidence of *Mycosphaerella melonis* symptoms in cucumber, cv Bonbon, following various fungicide treatment – 15 October 2013 (Site 1)

Treatment	Sub-plot	No plants out of 12				
		Stem lesions	Fruit	Laterals	Nodes	Leaf petiole lesion
1	1	2	1	12	6	2
	2	5	1	12	10	5
	3	5	2	12	4	6
	4	2	3	12	6	5
2	1	0	0	8	1	0
	2	2	1	10	1	1
	3	0	1	8	1	0
	4	1	0	7	0	1
3	1	0	2	12	4	2
	2	0	2	12	4	2
	3	1	1	11	0	2
	4	0	0	12	2	1
4	1	0	0	12	0	1
	2	0	0	9	0	1
	3	0	0	12	0	2
	4	0	0	12	0	2

Table 10. Incidence of *Mycosphaerella melonis* symptoms in cucumber, cv Bonbon, following various fungicide treatment – 30 October 2013 (Site 1)

Treatment	Sub-plot	No plants out of 12				
		Stem lesions	Fruit	Laterals	Nodes	Leaf petiole lesion
1	1	6	2	12	5	5
	2	9	1	12	7	7
	3	7	0	12	7	9
	4	6	0	12	7	10
2	1	1	0	12	2	2
	2	2	0	11	0	5
	3	1	0	12	1	2
	4	0	0	12	1	6
3	1	0	0	12	3	4
	2	0	0	12	4	2
	3	1	0	12	2	3
	4	0	0	12	1	2
4	1	0	0	12	1	3
	2	0	0	12	2	8
	3	0	0	12	3	5
	4	0	0	11	1	7

Table 11. Incidence of *Mycosphaerella melonis* symptoms in cucumber cv. Bonbon following various fungicide treatments – 2013 (Site 1)

Treatment and assessment date	No. of plants affected (of 48) on:			Total no. of lesions on 48 plants		No. of fruit (of 50) with internal infection
	Stem base	Laterals	Nodes	Stems	Nodes	
19 September						
1. Grower standard, grower timings	6	4	0	6	0	1
2. New fungicides, grower timings	0	0	0	0	0	0
3. New fungicides, spore trap timings	0	4	0	0	0	0
4. As T3 with pre-planting disinfection	0	0	0	0	0	0
15 October						
1. Grower standard, grower timings	14	48	26	14	33	1
2. New fungicides, grower timings	3	33	3	3	3	9
3. New fungicides, spore trap timings	1	47	10	1	10	9
4. As T3 with pre-planting disinfection	0	45	0	0	0	0
30 October						
1. Grower standard, grower timings	28	48	26	50	52	6
2. New fungicides, grower timings	4	47	4	4	4	3
3. New fungicides, spore trap timings	1	48	10	1	11	3
4. As T3 with pre-planting disinfection	0	47	7	0	7	4

Appendix 8 Disease Assessment data for Site 2 (East Yorkshire)

Assessment 1

Pest Type			D Disease		D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease
Pest Code			DIDYBR		DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR
Pest Scientific Name			Didymella bryo>		Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>
Pest Name			Black rot of c>		Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>
Crop Code			CUMSA		CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA
BBCH Scale			BVVT		BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT
Crop Scientific Name			Cucumis sativus		Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus
Crop Name			Cucumber		Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber
Description			Stem		Wire		Nodes	Infected	Infected	Cut	Weak	Infected			
Part Rated			LESION P		LESION P	LESTOT P	LESION P	FLOWER P	FRUROT P	SHOLAT P	SHOLAT P	PETIOL P	PLADEA C	LESION P	
Rating Date			27/09/2013		27/09/2013	27/09/2013	27/09/2013	27/09/2013	27/09/2013	27/09/2013	27/09/2013	27/09/2013	27/09/2013	27/09/2013	
Rating Type			COUNT		COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	Lesion Size	
Rating Unit			NUMBER		NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	Internod	
Number of Subsamples			10		10	10	10	10	10	10	10	10	10	10	10
Assessed By			JAT		JAT	JAT	JAT	JAT	JAT	JAT	JAT	JAT	JAT	JAT	JAT
ARM Action Codes			AA				AL	AA	AA	AS					AA
Trt		Treatment													
No.	Type	Name	1		2	3	4	5	6	7	8	9	10	11	
1	FUNG	Grower Programme	1.2	a	0	a	1.5	a	2.2	a	0.1	a	0.1	a	0.5	a	0	a	1	a	0	a	1.33	a
2	FUNG	Spray applied at same	0.1	c	0.1	a	0.4	c	0.3	b	0	b	0	b	0	b	0	a	1	a	0	a	0.15	b
		time as grower programme																						
3	FUNG	Spray triggered by spore	0.3	b	0.1	a	0.8	b	0.6	b	0	b	0	b	0	b	0	a	1	a	0	a	0.38	b
		levels																						
4	FUNG	Disinfected pre-planting	0.2	bc	0.1	a	0.6	bc	0.4	b	0	b	0	b	0	b	0	a	1	a	0	a	0.25	b
		+ Spray triggered by																						
		spore levels																						
LSD (P=.05)			1.33t		0.12	0.33	0.14t	0.58t	1.06t	0.10t	0	0	0	1.627t	
Standard Deviation			0.83t		0.08	0.2	0.09t	0.36t	0.66t	0.06t	0	0	0	1.017t	
CV			23.91		92.31	24.99	36.21	90.71	111.2	7.84	0	0	0	26.75	
Grand Mean			3.49t		0.08	0.82	0.25t	0.4t	0.6t	0.78t	0	1	0	3.8t	
Replicate F			0.75		3.074	1.236	0.517	1.705	0.281	1	0	0	0	1.114	
Replicate Prob(F)			0.5494		0.0834	0.3524	0.6809	0.235	0.8379	0.4363	1	1	1	0.3935	
Treatment F			23.128		2.481	22.997	15.121	15.204	4.689	22.753	0	0	0	14.625	
Treatment Prob(F)			0.0001		0.1273	0.0001	0.0007	0.0007	0.0309	0.0002	1	1	1	0.0008	

Assessment 2

Pest Type			D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease	
Pest Code			DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR	
Pest Scientific Name			Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>	
Pest Name			Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>	
Crop Code			CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA	
BBCH Scale			BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT	
Crop Scientific Name			Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus	
Crop Name			Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber	
Description			Black Stem		Silver Stem		Wire				Nodes		Infected		Infected		Cut		Weak		Infected			
Part Rated			LESION P		LESION P		LESION P		LESTOT P		LESION P		FLOWER P		FRUROT P		SHOLAT P		SHOLAT P		PETIOL P		PLADEA C		LESION P	
Rating Date			09/10/2013		09/10/2013		09/10/2013		09/10/2013		09/10/2013		09/10/2013		09/10/2013		09/10/2013		09/10/2013		09/10/2013		09/10/2013	
Rating Type			COUNT		COUNT		COUNT		COUNT		COUNT		COUNT		COUNT		COUNT		COUNT		COUNT		COUNT		Lesion Size	
Rating Unit			NUMBER		NUMBER		NUMBER		NUMBER		NUMBER		NUMBER		NUMBER		NUMBER		NUMBER		NUMBER		NUMBER		Internod	
Number of Subsamples			10		10		10		10		10		10		10		10		10		10		10		10	
Assessed By			JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO		JAT/AO	
ARM Action Codes			AA						AL		AL						AA		AA						AL	
Trt		Treatment																								
No.	Type	Name	12		13		14		15		16		17		18		19		20		21		22		23	
1	FUNG	Grower Programme	2.2	a	0.9	a	0.8	a	4	a	2.8	a	1.2	a	0.4	a	0.3	a	0.1	a	1	a	0.17	a	2.2	a
2	FUNG	Spray applied at same	0.1	b	0.3	b	0	b	0.5	b	0.4	a	0.8	a	0.2	a	0	a	0	b	1	a	0	b	0.48	b
		time as grower programme																								
3	FUNG	Spray triggered by spore	0.1	b	0.5	b	0	b	0.6	b	0.4	a	1.3	a	0.1	a	0	a	0	b	1	a	0	b	0.73	b
		levels																								
4	FUNG	Disinfected pre-planting	0.3	b	0.4	b	0	b	0.8	b	0.4	a	0.7	a	0.1	a	0	a	0	b	1	a	0.03	b	0.76	b
		+ Spray triggered by																								
		spore levels																								
LSD (P=.05)			1.74t		0.29		0.13		0.10t		0.42t		0.77		0.27		2.50t		0.63t		0		0.053		0.139t	
Standard Deviation			1.09t		0.18		0.08		0.07t		0.26t		0.48		0.17		1.56t		0.39t		0		0.033		0.087t	
CV			28.35		35.77		42.69		19.47		102.66		49.08		96.03		199.64		92.84		0		67.35		29.99	
Grand Mean			3.84t		0.51		0.19		0.33t		0.26t		0.98		0.18		0.78t		0.43t		1		0.05		0.29t	
Replicate F			0.384		0.124		1.79		0.081		1.134		2.355		0.877		0.844		0.417		0		1.596		1.374	
Replicate Prob(F)			0.7671		0.9436		0.219		0.9685		0.3862		0.14		0.4883		0.5035		0.7451		1		0.2576		0.3122	
Treatment F			33.71		8.752		80.518		56.255		2.761		1.575		2.773		3.547		11.619		0		24.797		11.498	
Treatment Prob(F)			0.0001		0.0049		0.0001		0.0001		0.1038		0.2624		0.1029		0.061		0.0019		1		0.0001		0.002	

Assessment 3

Pest Type			D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease	D Disease
Pest Code			DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR	DIDYBR
Pest Scientific Name			Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>	Didymella bryo>
Pest Name			Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>	Black rot of c>
Crop Code			CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA	CUMSA
BBCH Scale			BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT	BVVT
Crop Scientific Name			Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus	Cucumis sativus
Crop Name			Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber	Cucumber
Description			Black Stem	Silver Stem	Wire		Nodes	Infected	Infected	Cut	Weak	Infected		
Part Rated			LESION P	LESION P	LESION P	LESTOT P	LESION P	FLOWER P	FRUROT P	SHOLAT P	SHOLAT P	PETIOL P	PLADEA C	LESION P
Rating Date			29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013	29/10/2013
Rating Type			COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	COUNT	Lesion Size
Rating Unit			NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	NUMBER	Internod
Number of Subsamples			10	10	10	10	10	10	10	10	10	10	10	10
Assessed By			JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO	JAT/AO
ARM Action Codes				AL	AA	AL	AL	AA		AA			AA	AL
Trt		Treatment												
No.	Type	Name	24	25	26	27	28	29	30	31	32	33	34	35
1	FUNG	Grower Programme	0.8 a	2.4 a	0.4 a	4.2 a	7 a	0 b	0.8 a	0.5 a	0.3 a	1 a	0.1 a	3.22 a
2	FUNG	Spray applied at same time as grower programme	0.3 a	0.5 b	0 b	0.8 b	1 b	0.2 a	0.1 b	0 a	0 b	1 a	0 b	0.55 b
3	FUNG	Spray triggered by spore levels	0.2 a	0.6 b	0 b	0.7 b	1.6 b	0.2 a	0.2 b	0 a	0 b	1 a	0 b	0.83 b
4	FUNG	Disinfected pre-planting + Spray triggered by spore levels	0.8 a	0.7 b	0 b	1.3 b	1.5 b	0.3 a	0.2 b	0 a	0 b	1 a	0 b	0.98 b
LSD (P=.05)			0.6	0.14t	0.50t	0.13t	0.31t	1.70t	0.2	3.24t	0.11	0.04	0.52t	0.113t
Standard Deviation			0.38	0.09t	0.31t	0.08t	0.19t	1.06t	0.12	2.03t	0.07	0.03	0.33t	0.071t
CV			70.68	31.13	30.76	21.4	37.94	44.7	39.33	155.74	75.35	2.8	51.88	20.55
Grand Mean			0.53	0.28t	1.01t	0.39t	0.51t	2.38t	0.31	1.3t	0.09	0.99	0.63t	0.34t
Replicate F			1.742	0.59	3.769	0.239	1.025	0.523	7.558	0.473	0.448	1	1.971	2.332
Replicate Prob(F)			0.2278	0.6368	0.053	0.8668	0.4263	0.6771	0.0079	0.7084	0.7246	0.4363	0.1889	0.1424
Treatment F			2.533	15.176	124.962	28.576	7.926	4.27	24.945	3.217	23.371	1	36.981	29.967
Treatment Prob(F)			0.1225	0.0007	0.0001	0.0001	0.0068	0.0392	0.0001	0.0756	0.0001	0.4363	0.0001	0.0001

Assessment Summary

Pest Type			D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease	
Pest Code			DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR	
Pest Scientific Name			Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>	
Pest Name			Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>	
Crop Code			CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA	
BBCH Scale			BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT	
Crop Scientific Name			Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus	
Crop Name			Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber	
Description											Mean per plant		Mean per plant		Mean per plant		Mean size	
Part Rated			LESTOT P		LESTOT P		LESTOT P		PLADEA C		PLADEA C		PLADEA C		LESION P		LESTOT P	
Rating Date			27/09/2013		09/10/2013		29/10/2013		27/09/2013		09/10/2013		29/10/2013		27/09/2013		09/10/2013	
Rating Type			PERCEN		PERCEN		PERCEN		PERCEN		PERCEN		PERCEN		MEAN		MEAN	
Rating Unit			percent		percent		percent		percent		percent		percent		NUMBER		NUMBER	
Number of Subsamples			1		1		1		1		1		1		1		1	
Assessed By			JAT		JAT/AO		JAT/AO		JAT		JAT/AO		JAT/AO		JAT		JAT/AO	
ARM Action Codes			TIO[3]		TIO[15]		TIO[27]		TIO[10]		AS TIO[22]		AS TIO[34]				AL	
Trt		Treatment																
No.	Type	Name	36		37		38		39		40		41		42		43	
1	FUNG	Grower Programme	87.22223	a	100	a	100	a	0	a	18.05386	a	35.66135	a	1.51	a	4.234037	a
2	FUNG	Spray applied at same	40	c	47.5	c	65	b	0	a	0	b	0	c	0.4	c	0.672979	b
		time as grower programme																
3	FUNG	Spray triggered by spore	62.50001	b	60	bc	67.5	b	0	a	0	b	0	c	0.775	b	0.759122	b
		levels																
4	FUNG	Disinfected pre-planting	47.50001	bc	65	b	80	ab	0	a	1.296733	b	6.296734	b	0.575	bc	0.964833	b
		+ Spray triggered by																
		spore levels																
LSD (P=.05)			17.7051826		17.33		21.03		0		1.08863852t		1.09438948t		0.3262		0.10245513650t	
Standard Deviation			11.0693671		10.83		13.15		0		0.68062215t		0.68421768t		0.204		0.06405545455t	
CV			18.66		15.9		16.83		0		38.55		27.27		25.03		17.3	
Grand Mean			59.31		68.13		78.13		0		1.77t		2.51t		0.82		0.37t	
Replicate F			1.952		0.905		1.578		0		1.268		2.499		1.232		0.195	
Replicate Prob(F)			0.1918		0.4759		0.2616		1		0.3427		0.1256		0.3537		0.8973	
Treatment F			14.164		17.237		5.916		0		25.566		53.499		22.899		53.482	
Treatment Prob(F)			0.0009		0.0005		0.0164		1		0.0001		0.0001		0.0002		0.0001	

Pest Type			D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease		D Disease	
Pest Code			DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR		DIDYBR	
Pest Scientific Name			Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>		Didymella bryo>	
Pest Name			Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>		Black rot of c>	
Crop Code			CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA		CUMSA	
BBCH Scale			BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT		BVVT	
Crop Scientific Name			Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus		Cucumis sativus	
Crop Name			Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber		Cucumber	
Description			Mean size		Mean size		Mean size											
Part Rated			LESION P		LESION P		LESION P											
Rating Date			27/09/2013		09/10/2013		29/10/2013											
Rating Type			MEAN		MEAN		MEAN											
Rating Unit			NUMBER		NUMBER		NUMBER											
Number of Subsamples			1		1		1											
Assessed By			JAT		JAT/AO		JAT/AO											
ARM Action Codes			AA		AL		AL											
Trt		Treatment																
No.	Type	Name	45		46		47											
1	FUNG	Grower Programme	1.61	a	2.61934	a	3.508675	a										
2	FUNG	Spray applied at same	0.479	b	0.735505	b	0.904047	c										
		time as grower programme																
3	FUNG	Spray triggered by spore	0.719	b	0.998749	b	1.166837	bc										
		levels																
4	FUNG	Disinfected pre-planting	0.633	b	1.073364	b	1.52583	b										
		+ Spray triggered by																
		spore levels																
LSD (P=.05)			1.5138t		0.13372958593t		0.10182452079t											
Standard Deviation			0.9464t		0.08360839394t		0.06366119052t											
CV			18.3		23.63		15.23											
Grand Mean			5.17t		0.35t		0.42t											
Replicate F			0.462		0.878		1.468											
Replicate Prob(F)			0.7158		0.488		0.2875											
Treatment F			9.52		11.297		26.928											
Treatment Prob(F)			0.0037		0.0021		0.0001											

Means followed by same letter do not significantly differ (P=.05, LSD)

t=Mean descriptions are reported in transformed data units, and are not de-transformed.

Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Pest Type
D, Disease, G-BYRD7, G-DisStg = Disease, such as a fungus, bacteria, or virus
Pest Code
DIDYBR, *Didymella bryoniae*, = US
Crop Code
CUMSA, BVVT, *Cucumis sativus*, = US
Part Rated
LESION = lesion
LESTOT = lesion - total
FLOWER = flower
FRUROT = fruit - rotten
SHOLAT = shoots, lateral
PETIOL = petiole
PLADEA = plant - dead
P = Pest is Part Rated
C = Crop is Part Rated
Rating Type
COUNT = count
PERCEN = percent
MEAN = mean
Rating Unit
NUMBER = number
ARM Action Codes
AA = Automatic arcsine square root % transformation
AL = Automatic log transformation of X+1
AS = Automatic square root transformation of X+0.5
TIO[3] = % Incidence (&0 = none)[3]
TIO[15] = % Incidence (&0 = none)[15]
TIO[27] = % Incidence (&0 = none)[27]
TIO[10] = % Incidence (&0 = none)[10]
TIO[22] = SQR([40] + .5)
TIO[34] = SQR([41] + .5)

Appendix 9 – Photographs of Site 1 (Lee Valley)

Figure 7. Glasshouse following pre-planting clean up, 05.08.13.

Figure 8. Trial area 2 days after planting, with treated row in-between 2 guard rows, 8.8.13.

Figure 9. Trial area 3 weeks after planting, with spore trap set up, 28.8.13.

Figure 10. Left – stem base lesion in T1, right – nodal lesion in T2, 30.10.13

Appendix 10 – Photographs of Site 2 (East Yorkshire)

Figure 11. Left - Rock-wool blocks from previous crop left *in situ* until week 8 of the trial, right – MTIST spore trap set up in trial area.

Figure 12. Infected cucumbers from treatment 1 at the first assessment, 27.09.13

Figure 13. Left – large stem base lesion in T1, right – unaffected stem base in T2, 29.10.13

Appendix 11 – Temperature data site 1 (Lee Valley)