Grower Summary

PE 001

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

Final 2011

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The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

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

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

Read the label before use: use pesticides safely.

Further information

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

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Headline

- There may be populations of *Mycosphaerella melonis* with reduced sensitivity to Amistar, Bravo, and Nimrod. Overall sensitivity to Teldor and the two active ingredients contained in Switch is good.

Background

Black stem rot of cucumber caused by *Mycosphaerella melonis* (syn. *Didymella bryoniae*) is an economically damaging fungal pathogen of cucumber and other cucurbits. It causes extensive stem and leaf infections which, when severe, can debilitate or even kill plants (see Figure below). Air-borne infection of flowers and developing fruit leads to fruit end rot sometimes not visible until the fruit is marketed. This leads 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 will effectively prohibit some of the existing fungicides.

Mycosphaerella melonis **stem and fruit infection**

Summary

A. Desk Study: Review of current knowledge relating to gummy stem blight caused by *Didymella bryoniae* (*Mycosphaerella melonis*) in cucumber and other cucurbits

Objectives

The primary purpose of this desk study was to review 'prior knowledge' of the disease gummy stem blight. We searched worldwide peer-reviewed scientific literature, conference proceedings, non-confidential R&D reports, trade journals and popular press articles on the pathogen itself, the disease it causes and the various factors that influence its occurrence, survival, infection and, most importantly, its control. From this review, gaps in current knowledge have been identified and this has allowed a series of recommendations to be made for future R&D aimed at improving disease control and minimising economic crop loss.

The disease black stem rot or gummy stem blight caused by *Didymella bryoniae* (*Mycosphaerella melonis*) affects various outdoor and protected plant species in the *Cucurbitaceae* (e.g. watermelon and cucumber) and has been known for well over a century now following its first report in 1891. Yet, whilst we now understand much about the pathogen, the disease it causes and how to control it, the changing economic and commercial climate continues to prevent effective implementation of control measures. As a result, financial losses continue to occur in both outdoor field crops and in protected crops, in the UK most notably glasshouse cucumber.

Cause, symptoms and damage

The pathogen has two stages to its life cycle. Firstly, asexual or imperfect spores (conidia) are released from sites of infection and these sticky splash-borne spores are dispersed either during rainfall (field crops) or water splash or on hands, knives and other machinery/implements in glasshouse crops during routine crop work e.g. crop training, de-leafing or harvesting. Later, the sexual or perfect stage of the fungus liberates air-borne spores (ascospores) into the air and these serve as a long distance dispersal mechanism for the fungus.

Initial symptoms occur 10-14 days following infection usually as a silvery grey to dark grey or black lesion often near the stem base. It is interesting that stem lesions can take two forms, either superficial silvery infections that tend to be quite localised, or more penetrating dark-brown to black spreading lesions that frequently girdle the stem and kill the plant. The conditions under which the two different lesions form remains unclear and a greater

understanding of what triggers the aggressive stem lesions to form could be very helpful from an epidemiological standpoint. As the pathogen is spread, further lesions occur along the stem usually at petiole or fruit stubs left after de-leafing or harvesting operations. Under optimum environmental conditions and when disease pressure is high extensive leaf infection may occur together with external and internal fruit infection. This latter internal fruit symptom can be particularly problematic as the fruit remain symptomless at harvest and have often found their way to the retailer or customer before the problem is noted. This can be particularly damaging for the grower-retailer relationship if it persists. It has also been suggested that latent systemic infection may occur in young shoots rendering them weak and unproductive; though this requires validation.

Control measures

Research in the Netherlands by Van Steekelenburg and others, largely in the 1980’s, has shown that the prevailing environmental conditions are important and significantly influence infection risk and subsequent disease development. An effective way to reduce disease risk is to apply regular heat boosts together with ventilation, particularly early in the morning, to keep the foliage dry. Unfortunately however, as happened during the energy crisis of the 1970’s, in the current economic climate this in not financially viable due to rising fuel costs relative to returns on sales. Growers therefore have to resort to alternative strategies including fungicide applications instead. Yet, changes to EU and UK pesticide legislation, in concert with consumer (retailer) desire for pesticide-free produce, is now threatening the continued availability of key products. This is anticipated to increase the risk of fungicide resistance as growers are forced to rely on a diminishing armoury of active substances and products.

Host resistance has the potential to provide an alternative means by which to reduce disease pressure in cucumber and there have been studies looking to find novel sources of host resistance. However, in the short-medium term this is unlikely to be an option. Also, there is another problem as the predominant economically damaging pathogen in cucumber is powdery mildew (*Sphaerotheca fuliginea*) and much of the breeding work in cucumber is associated with the development of mildew tolerant or resistant cultivars. The problem is that several of the cultivars selected as tolerant to powdery mildew appear to be more susceptible to *Mycosphaerella* infection. Therefore, in the short-term at least, host resistance is not going to be able to provide an effective alternative solution for disease control.

Hygiene measures, implemented as integral components of a disease control strategy, are very important especially at the end of the season when there is a small window to thoroughly disinfect/disinfest the glasshouse. It is imperative in this regard that all tendrils and other plant debris are effectively removed from the crop wires and other parts of the structure to avoid carry-over and hence early re-infection of the new crop. Similarly, to minimise the risk of air-borne infection via ascospores all crop dumps should be at a distance from the nursery and covered to prevent wind-blown dispersal of the pathogen back into glasshouses. Disinfectant use is likely to come under increasing pressure through the Biocides Directive and, potentially, this may introduce additional constraints on their use. Further information on the efficacy of currently available disinfectants will be important to ensure the most effective products are used to help aid control.

It is possible that *Mycosphaerella* is seed-borne in cucumber, especially as it has been demonstrated in other cucurbits e.g. watermelon. The seed may therefore potentially provide a primary route of entry into new crops though, as cucumber crops are usually raised by specialist propagators, the pathogen would need to be symptomless during this period as characteristic symptoms of the disease are not normally seen at this stage. However, as this aspect remains contentious, with some researchers implying the disease to be seed-borne and others suggesting otherwise, it is recommended that this aspect of pathogen epidemiology is investigated further. The use of various seed treatment may also have an impact on pathogen survival and carry-over on seed.

Once the new crop is planted it is really important to monitor the crop carefully for early signs of the disease, noting of course that very early lesions are usually found at the stem base. Careful removal of a small number of infected plants may be beneficial in delaying the onset of epidemic development of the disease. Once infection has progressed to infect wounds along the main stem in several plants it is usually too late for such action and environmental manipulation, effective hygiene and judicious fungicide use are required. Unlike in many other crops in the horticultural sector fungicide and other pesticide products are rarely developed specifically as the return on investment for agrochemical companies is too small. Instead, where the market size is sufficient product uses are extended to specialist horticultural crops where the demand is regarded as sufficiently high. In the case of cucumber this is largely for powdery mildew, and possibly *Botrytis*, control. Few, if any, fungicides have been registered specifically for *Mycosphaerella* control in this crop. Instead, growers rely largely on chance that the approved active substances also have activity against other fungi and gain secondary benefits from their other uses on the crop. The main problem with this approach is that all too often the level of ‘incidental’ control is often insufficient and at best the disease is often only temporarily suppressed. The other problem associated with registering new products, as highlighted above, is ensuring minimal and acceptable residue levels in the harvested produce. In this regard, the requirement for a very short (ideally 1 day) harvest interval adds yet another constraint on the approval process in this crop. There is also a reconsideration of re-entry intervals for glasshouse crops and this may further restrict the use of specific products in the future.

Van Steekelenburg (1978) studied the effects of several fungicides in agar tests and *in planta.* Unfortunately, of the products evaluated at the time, only chlorothalonil remains available for use and this, at best, provided mediocre performance. Control of fruit infection was described as 'rather disappointing'.

Since that time other novel fungicide groups have been introduced e.g. strobilurins and anilino-pyrimidines and some have quite broad spectrum activity. Utkhede & Koch (2004) found treatment with either azoxystrobin or pyraclostrobin (+ boscalid) was effective when applied as preventative sprays in hydroponic cucumbers. Interestingly, they also found the biocontrol fungus *Gliocladium catenulatum* JI446 (Prestop) to be effective. This product secured UK approval in October 2010 and spray application to cucumber is a permitted treatment. Other studies, albeit conducted on <u>outdoor</u> cucurbit crops e.g. watermelon are also available and relevant in terms of helping identify which fungicides have moderate-good activity against this pathogen.

The risk of resistance has been of concern for some time, largely since the introduction and use of the single site inhibitors such as the benzimidazoles and a few focused studies with *Mycosphaerella* have been published in this regard. Resistance to the benzimidazoles in *Mycosphaerella* populations first occurred in the early 1980's in the UK and elsewhere in Europe (Malathrakis & Vakalounakis, 1983; Clark, 1987 unpublished). In 2004, widespread resistance to azoxystrobin was reported in the USA and this led to control failure in watermelon crops. This same fungicide was approved for use in the UK in 2002 (BCPC Pesticide Guide, 2002) but the sensitivity of the pathogen has not been determined. It is of some concern that in outdoor field crops in the USA in 2007 isolates of *Mycosphaerella* exposed to boscalid (as 'Pristine': equivalent to 'Filan' in the UK) were reported to be resistant. This fungicide is not currently approved for use on cucumbers in the UK. No baseline or other sensitivity testing has been undertaken with *Mycosphaerella* for many years.

The increasing pressures on fungicide availability and the retailers (consumers) desire for pesticide-free produce means that there is an urgent need to seek alternative non-chemical, preferably biological, approaches for disease control. The work by Utkhede & Koch (2004) using Prestop (*Gliocladium catenulatum* JI446) in Canada mentioned above is therefore of considerable significance, especially as it has recently secured UK approval use in glasshouse cucumber (P. Sopp, pers com.). Providing it can be independently demonstrated to be effective and successfully integrated into disease control programmes it could be a very useful component in the future disease control armoury where residue minimisation is an important goal. There is therefore an urgent need to evaluate this and other biological and/or non-chemical approaches in concert with novel fungicides to either substitute or to integrate them into disease control programmes against gummy stem blight.

Priority work areas

In summary therefore there is a need for work in a number of priority areas to help clarify and improve our understanding and control of this disease:-

- Clarification and confirmation of the seed-borne nature of *Mycosphaerella* in glasshouse cucumber
- Development and commercial validation of the immunoassay spore trapping system, including semi-quantitative on-site testing by growers and/or their consultants
- *In vitro* and *in vivo* evaluation of fungicide, bio-control and alternative products to identify those with activity against *Mycosphaerella* and that can be used commercially
- Evaluation of available disinfectants for activity against *Mycosphaerella* to reduce survival and carry-over of the disease
- Improved understanding and significance of aggressive and non-aggressive stem lesions
- Investigation of the occurrence and commercial significance of systemic infection in weak unproductive cucumber shoots
- Integration of new knowledge to help formulate an improved strategy for the control of gummy stem blight in commercial cucumber crops

This review therefore has highlighted the current state of knowledge relating to our understanding of the biology, epidemiology and control of *Mycosphaerella* in cucumber and helped identify opportunities towards improving the overall disease control strategy in glasshouse cucumbers.

B. Experimental work

Resistance testing

A total of 28 isolates of *M. melonis* were collected from commercial cucumber crops in southern England (Lee Valley) and East Yorkshire during 2010. Isolates were tested using a laboratory test which involved amending agar plates with fungicides (or the separate active ingredients in the case of Switch) generally used for *M. melonis* control. These were Amistar (azoxystrobin), Bravo 500 (chlorothalonil), Switch (cyprodinil and fludioxonil), Teldor (fenhexamid) and Nimrod (bupirimate). Whilst it would have been interesting to determine whether the pathogen still remains insensitive to the benzimidazoles this was not included as no such product remains available for use. The tests compared the inhibition in radial growth of the fungal colonies at three concentrations (2, 20 and 100 ppm) with a control containing no fungicide. We also investigated the growth rate of a reference isolate to *M. melonis* originally isolated from a cucumber crop back in 1978, prior to exposure to modern day fungicides.

Growth of the majority of isolates was greatly inhibited by Teldor and by both the active ingredient components of Switch at concentrations of 20 ppm ai or greater. Less inhibition of growth was observed with Amistar, Bravo 500 and Nimrod. Generally the isolates collected in 2010 and the reference isolate collected in 1978 differed little in sensitivity when compared against the same fungicides. The exception was one isolate collected from East Yorkshire which showed a reduced sensitivity to bupirimate, cyprodinil and fludioxonil compared to the 1978 isolate. The results indicate lower inherent activity against *M. melonis* by azoxystrobin, chlorothalonil and bupirimate than by cyprodinil, fenhexamid and fludioxonil, as determined by the test method used in this work.

Seed testing

Five cucumber cultivar seed batches were tested for seed-borne infection in 2010 by plating 100 seeds onto agar and checking for growth of the fungus. A fungus resembling *M. melonis* was detected in two batches: the isolation tests are being repeated alongside testing of a much wider range of cultivars in 2011. Where suspect isolates are found they will be inoculated into untreated cucumber fruit to determine pathogenicity. The full results from these tests will be reported in the Year 2 report.

Development of an Immunoassay system

Initial work indicated that the immunoassay test originally developed for the early warning detection system for ringspot in Brassicas, caused by *Mycosphaerella brassicicola,* was not sufficiently sensitive to air-borne spores of *M. melonis*. It will therefore be necessary to raise specific monoclonal antibodies against *M. melonis* for this work. Further validation work will be carried out in Phase 2 of the study.

Financial Benefits

No direct financial benefits to growers have been identified during Phase 1 of this study. Work scheduled to be carried out in Phase 2 should provide additional detail on control measures to reduce crop loss from *Mycosphaerella* infection and spread.

Action Points for Growers

None at this stage.