Project title:	Cucumber – Improving Control of Gummy Stem Blight caused by *Mycosphaerella melonis (Didymella bryoniae)*
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Project Leader:	Dr G M McPherson
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Date project commenced:	1 February 2010
Date project completed (or expected completion date):	Phase 1 completed April 2011

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

- 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 outdoor 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.

SCIENCE SECTION

Introduction

Gummy stem blight caused by *Mycosphaerella melonis* (*Didymella bryoniae*) has been a persistent leaf, stem and fruit disease in glasshouse cucumber for many years (Plate 1). 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 tolerant 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.

Plate 1. *Mycosphaerella melonis* stem and fruit infection

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 internal and external fruit infection). Increased energy costs are a significant factor as they tend to discourage the use of a heat boost 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 'prescriptions in the medicine cabinet' to prevent infection. This is further affected by the increased shift in consumer (retailer) perception regarding pesticide residues and a desire for fresh produce with zero tolerance in this regard. This, in turn, is increasing the need for alternative IPM solutions. An indirect impact of all this is that the use of cultivars with increased tolerance to powdery mildew (where most fungicides are usually used for control) means that growers are applying fewer fungicide sprays which otherwise would have provided incidental control, or at least suppression, of *Mycosphaerella* infections.

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 and animal populations and likewise we are facing an increased risk of fungicide resistance in phytopathogens. As a result, we could reasonably expect a concomitant increase in disease, potentially reaching epidemic proportions, unless effective alternative solutions can be found.

The purpose of this study is to firstly 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 and if possible to compare this with baseline isolates. Guided by this knowledge, the aim is then to seek alternative control strategies including novel fungicides and alternative products and to use a novel serological spore trapping approach to better forecast disease risk; thereby improving application timing to prevent economic loss due to the disease.

Previous studies have shown that accurate information on airborne ascospore numbers of *Mycosphaerella brassicicola* (ringspot on Brassicas) and field environmental data can provide information to predict plant disease occurrence in a field setting. By determining these periods of risk quickly and accurately control strategies can be implemented and a reduced number of fungicide applications can provide effective control (Wakeham & Kennedy, 2010).

Using an innovative spore trapping system (Microstate immunospore trap (MTIST), collected field aerosols can be processed using a laboratory based plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) to provide quantitative information on target fungal disease inoculum. In this study we report on the potential to monitor aerosols of ascospores of *Mycosphaerella melonis* using existing monoclonal (MAb) and polyconal antisera (PAb) each of which had been raised to inoculum of *Mycosphaerella brassicicola* (ringspot on Brassicas).

Quantifying airborne ascospores of *M. melonis* could augment the likely onset of disease occurrence in cucumber crops. The MTIST air sampler and PTA ELISA system however relies on laboratory processing of the collected aerosols for quantitative assessment. In Phase 2 of this project the MTIST ELISA system will be evaluated and the potential for adaptation to an on-site test, utilizing technology derived from the immunochromatographic test strip assay (lateral flow test), will be investigated.

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

Introduction

Gummy stem blight or black stem rot caused by *Didymella bryoniae* (*Mycosphaerella melonis*) continues to cause economic damage to UK cucumber crops and there is increased concern that the continued loss of fungicides, in concert with increased energy costs, will exacerbate the disease further unless an alternative strategy can be sought to check its development. HDC Project PE 001 was therefore commissioned by HDC, structured to undertake work in two phases. The primary, aim of Phase 1 was to undertake a desk study to define the current 'state of the art' with respect to our knowledge of the disease. Based on this knowledge, the intention was that later studies in Phase 2 can then be more accurately focused; the industry reassured in the knowledge that work has not already been undertaken elsewhere.

History of the Disease in Cucurbits

The ascomycete fungus which causes gummy stem blight was first described in 1869 on *Bryonia* (bryony or wild hops) in central Europe (Germany). The earliest collection and report of *D. bryoniae* on a cultivated cucurbit species is by Passerini in 1885 who described it from melon (*Cucumis melo*) in Italy as *Didymella melonis* (Corlett, 1981). It was later reported in cultivated cucurbits, including cucumber (possibly of Chinese origin) in 1891 in three separate countries, namely France (Roumeguere, 1891), Italy (Saccardo, 1891) and Delaware, USA (Chester, 1891). Since that time the disease has become geographically very widespread and occurs commonly in both protected and outdoor cucurbit crops such as watermelon (Chupp & Sherf, 1960) in many countries around the world.

In Europe the disease didn't really become a major problem until production intensified considerably in the late 1960's and early 1970's. In Holland, the pathogen was first reported in grafted cucumbers grown under frames in 1953. Here, it remained a minor disease through the 1960's but later became considerably more problematic as production systems changed, especially during the energy crisis of the late 1970's.

In the UK, the first recorded incidence of *Mycosphaerella* was in 1909 in the Lee Valley (Massey, 1909). By the early 1960's the disease could be found in Butcher's Disease Resistor in every glasshouse in the Lee Valley area and was sometimes noticed as early as April in the year (Fletcher & Preece, 1966). As production intensity increased so did the disease severity, following a similar pattern to Holland. The disease now occurs commonly

in glasshouse cucumber crops usually from April-May onwards gradually becoming more damaging as the season progresses.

The Disease: Gummy Stem Blight in Cucumber

Gummy stem blight is an economically damaging disease of cucurbits including glasshouse-grown cucumber. It is caused by the ascomycete fungus *Didymella bryoniae* (syn. *Mycosphaerella melonis*) though it is more commonly observed in its asexual or conidial stage *Phoma cucurbitacearum*. It is considered to be a possible seed-borne pathogen and this may provide a primary route of entry into new crops; however this aspect remains contentious and still requires further investigation. It is also capable of surviving on plant debris from previous infected crops and in areas of intensive production the pathogen is likely to be transferred to new crops on infected tendrils, leaf, stem and possibly root debris. The perfect or sexual stage of the fungus also produces air-borne spores (ascospores) and such spores liberated from nearby infected crops or waste debris (if left uncovered) may be dispersed to infect previously healthy crops when environmental conditions are conducive to spore germination and infection.

In glasshouse cucumber the primary infection often occurs at the stem base (Plate 2a-b), though this early symptom can be readily overlooked or mis-identified in the early stages of the disease. More usually, the first indication of the disease in the crop is infection at the nodes higher up the stem and this often follows worker activity e.g. de-leafing, trimming, harvesting etc transferring asexual spores (conidia) on hands, knives etc. It is interesting to note that for much of the time such node lesions take a superficial silvery appearance (Plate 3a) and don't appear to do much damage to the stem tissues. At other times, the infection quickly penetrates the underlying stem tissues to produce a much darker lesion (Plate 3b), which girdles the stem and kills the plant. The conditions which trigger such 'aggressive' lesions are currently not clear. As the infection progresses the spore-bearing structures on infected stem lesions, leaves and fruit change to produce sexual spores (ascospores). These are liberated into the air to create a greater opportunity for pathogen dispersal and wider infection through the crop. Following such spore release, leaf infection occurs, often via the hydathodes at the leaf tips (Plate 4) or via wounds at the junction between the petiole and leaf lamina that has a tendency to fracture in some cultivars due to the leaf size and weight (Plate 5). The most economically damaging infection, thought to occur via ascospore discharge, occurs on the flowers and fruits and this either leads to an internal, largely symptomless, infection (Plate 6) or an overt external infection; referred to by some growers as 'knob end rot' (Plate 7). Under conditions of high infection pressure, infection also occurs on young developing shoots (Plate 8) and it is thought that such shoots may

become unproductive with premature fruit abortion, perhaps as a result of a systemic infection. This aspect has not been fully investigated and further work is required here.

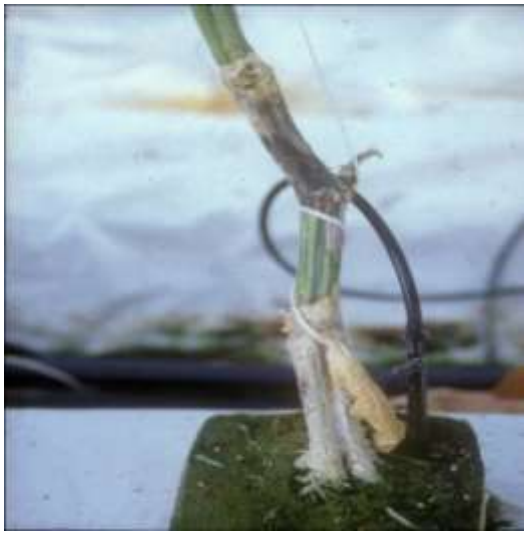
a.  b. 

Plate 2a-b. Early *Mycosphaerella* infections at the stem base of young cucumber plants

Plate 3a. Superficial stem infection by *M. melonis*

Plate 3b. Penetrating stem lesion by *M. melonis*

Plate 4. Leaf infection by *Mycosphaerella* via the hydathodes along leaf margin

Plate 5. Leaf infection by *Mycosphaerella* via fracture at the junction between petiole and leaf lamina

N.B. Under some circumstances such infection can be largely symptomless externally with only a slight tapering of the fruit at the distal portion signifying a problem. When cut open an internal discoloration may be evident.

Plate 6. Flower/fruit infection of distal portion of cucumber

Plate 7. Advanced external infection of cucumber fruit or 'knob end rot' (note secondary mixed infection with *Botrytis* here)

Plate 8. Young shoot infection by *Mycosphaerella melonis* in cucumber

The Pathogen: *Didymella bryoniae (*syn. *Mycosphaerella melonis*)

The homothallic[1] ascomycete fungus which causes gummy stem blight occurs commonly in cucumber crops (and on other members of the *Cucurbitaceae* e.g. watermelon in some other countries where such crops are widespread). Its incidence and severity varies between seasons depending on numerous factors, most notably the prevailing climatic conditions. Asexual sporing structures (pycnidia) occur on the stems, usually near the base initially, though these may be initially overlooked. These structures release small 1-septate to unicellular guttulate spores (conidia) approximately 5-10 x 3-4µm in size. The spores are quite sticky (but too small to be seen with the naked eye) and tend to be spread either manually on hands or equipment e.g. knives or via water-splash. As the lesions mature other structures (pseudothecia) form within the same lesions and these contain another larger, but sexual, 1-septate spores (ascospores) which are approximately 14-18 x 4-7µm in size (but again are too small to be seen without microscopy). These spores are liberated into the air and spread on air currents over a longer distance than the conidia. Based on studies in crops grown in organic media, spore release has a diurnal periodicity with light being required for abundant formation of perithecia and pycnidia. Peak spore release is reported to occur between 18.00-20.00 hours. Both spore types may therefore serve as primary inoculum sources and also aid survival between crops on plant debris. Reports on

[1] This implies that the fungus does not require opposing mating strains for sexual recombination, unlike heterothallic fungi which do require opposing mating strains for sexual recombinants (variants) to form.

seed transmission, whilst somewhat conflicting, do indicate that seed transmission in watermelon in the USA, and possibly cucumber, may occur, though this has not been confirmed in the UK (Brown & Preece, 1968).

On agar, the fungus is not particularly easy to identify as sporulation (required for confirmation of identity) occurs quite rarely (Brown & Preece, 1968) and the fungus can be quite variable in its appearance (Chiu & Walker, 1949). Fungal growth is quite rapid with colonies covering an 11 cm Petri dish containing agar within 7 days at 22°C in the dark. The mycelium is initially white but becomes dark-greenish black from the centre outwards after approximately 3 days. Development of aerial mycelium is variable and in some cultures it is abundant white and fluffy whereas in others it can be almost absent (Brown & Preece, 1968). The maximum fungal growth rate *in vitro* is reported to occur around 26-27°C and in watermelon fruit decay was very limited following infection at 29.5°C. It is considered that extensive disease development is likely to occur within a temperature range of 20-28°C.

Penetration by fungal mycelium occurs through the cuticle of seedlings but in older tissues (especially fruit) infection is thought to occur most readily through wounds and bruises. Infrequent stomatal penetration has been reported in watermelon, whereas in cucumber spread generally takes place via the stem wounds either following harvesting or de-leafing though, under optimum conditions, infection also occurs via the hydathodes around leaf margins. The developing fruit may also be infected via the flowers or at the distal end of the fruit when the flower has abscised. Effective hygiene measures in conjunction with fungicides are reported to be required for effective control, especially as most commercial cultivars are highly susceptible to the disease (Punithalingham & Holliday, 1972).

Recently, Keinath (2010) estimated the mean number of both pseudiothecia and pycnidia in leaf lesions of gummy stem blight and found them to range from ca. 225-575/ cm^2 leaf and this was consistent between the different host species evaluated. This demonstrates the considerable infection potential from both conidia and air-borne ascospores in the glasshouse environment.

Pathogen Detection

Didymella bryoniae is very recognisable to anyone familiar with growing cucurbits as the disease is very widespread. However, early detection in crops is difficult and may be missed allowing the disease to establish and spread before control measures can be implemented. Until recently, detection of the disease has relied entirely on conventional approaches using visual crop inspection and classical isolation techniques onto non-selective and selective agar growing media. Early detection could potentially be a very useful tool to alert growers

when the pathogen is active so that preventative measures (cultural, climatic and chemical) can be early to slow down disease development in the crop.

Serological methods offer some scope and work by Kennedy *et al* (1999, 2000) and Wakeham *et al.* (2008, 2010) working with *Mycosphaerella brassicicola* in UK Brassica crops have shown this method in conjunction with an immune-fluorescence test can be used successfully for ascospore detection and this approach has potential for in-field detection of the pathogen in advance of disease development. The same techniques may be capable of being adapted for use in a glasshouse environment, assuming an appropriate monoclonal (Mab) or polyclonal (Pab) antiserum can be developed and used that specifically detects *D. bryoniae.* Such serological technology is advancing quite rapidly and there is potential for the commercial development of semi-quantitative spore-trapping assays using lateral flow devices (akin to the home pregnancy test kits) that can be used in the nursery either by the grower technician or the visiting consultant.

Separately, advances in molecular diagnostics have also moved apace in the last decade and PCR (Polymerise Chain Reaction) primers specific to *D. bryoniae* have been developed to evaluate the use of a microtiter based PCR-ELISA (Enzyme Linked Immuno-Sorbent Assay) technique for detection (Somai, Keinath & Dean, 2002). In this work, PCR-ELISA successfully detected *D. bryoniae* in 45 out of 46 isolates of the pathogen used. Although less sensitive than gel-electrophoresis, PCR-ELISA was reported to provide a highly specific, yet simple, rapid and convenient assay for detection of *D. bryoniae*.

To improve detection techniques, especially the simultaneous detection of multiple pathogens in cucurbit seed, Ha *et al.*, (2009) developed a combination approach using magnetic capture hybridisation (MCH) and multiplex real-time PCR. This technique was used successfully (100% detection frequency) to detect *D. bryoniae* and *Acidovorax avenae* subsp. *citrulli* in watermelon and melon seed samples where the level of infestation was 0.02%.

It is generally regarded that molecular (PCR) approaches offer a higher level of sensitivity for pathogen detection due to the amplification stage of the fungal signature (DNA). Therefore, depending on the requirement several new tools could be made available to help growers in their bid to achieve effective control of this disease. It is worth bearing in mind though that, compared to conventional culture techniques (where pathogen viability is assured during the culturing process), modern serological and/or molecular approaches do not necessarily differentiate between viable and non-viable propagules and therefore

interpretation of diagnostic results must be undertaken carefully with appropriate scientific guidance.

Epidemiology of the Disease

Influence of climate

In artificial inoculation experiments and in commercial practice humid conditions have been found to favour the disease. The energy crisis in the late 1970's is thought to have aggravated the *Mycosphaerella* problem as glasshouse cucumber growers cut back on heat inputs and reduced ventilation to save costs. Due to an increase in disease occurrence and subsequent economic loss significant research effort was focused towards the disease at this time especially, though not exclusively, in Holland (Van Steekelenburg & Van de Vooren, 1980). It is especially important to avoid getting the plants wet as this also encourages latent infections. A very critical period climatically is overnight when the vents are shut, especially on cool clear nights after warm humid days when a lot of heat is radiated out of the plants and they cool rapidly, sometimes to temperatures below ambient. The air can, at a given temperature, only hold a certain amount of water vapour (its saturation point) and if the temperature then falls, some of the water vapour is converted back to liquid (as dew) and this provides ideal infection conditions for *Mycosphaerella*. Therefore, when the plants are in warm humid conditions it is very important to expel some of this humidity through the vents, especially if a clear night is forecast. The night temperature should be maintained high enough so that radiated heat loss does not let the temperature reach dewpoint. Where necessary, ventilation should be applied, but gradually, to avoid overcooling the crop. In an ideal world where energy costs were lower it would be appropriate to apply a heat boost with partially opened vents to ensure the crop remains dry. However, in the current economic climate this is much more difficult to justify, just as it was during the energy crisis of the late 1970's, and we can therefore expect the disease to become more problematic as some of the more regular options for environmental manipulation have become uneconomic to implement. It is worth remembering though that if you walk into the crop at sunrise and find it wet you can be fairly confident *Mycosphaerella* problems will follow and carefully timed fungicide applications would be advisable (Jarvis, 1989).

Spore formation and spore release

It is very important for growers to appreciate that there are effectively two stages (asexual and sexual) in the life cycle of this pathogen and each one produces important, but different, spore forms (Punithalingam & Holliday, 1972). The asexual (or imperfect) anamorph stage, *Phoma cucurbitacearum* produces the pycnidia (tiny black structures on infected stems, just visible to the naked eye – See Plate 2a above) which liberate large quantities of sticky

splash- or mechanically-borne spores or conidia. The sexual (or perfect) stage of the fungus, known as *Didymella bryoniae* (syn. *Mycosphaerella melonis*) tends to develop later, usually on (within) established lesions to produce perithecia (not readily distinguished from pycnidia by the naked eye unfortunately). Importantly, these newly formed structures liberate air-borne ascospores into the glasshouse with a conspicuous peak release in the early evening and this, assuming infection conditions are optimal, allows a more widespread dispersal of the pathogen (Fletcher & Preece, 1966). Whilst the ascospores can be recognised and distinguished from conidia morphologically using microscopy in the laboratory, in the glasshouse environment it is not possible to know when or where ascospores are liberated and growers cannot therefore readily take action to prevent infection occurring as a result.

Infection process

The pathogen is capable of infection from both the imperfect (anamorph) or asexual fungus and the perfect (teleomorph) or sexual fungus (*Didymella bryoniae*) via the stems, leaves, fruit and, potentially via seed. Both conidia and ascospores can generate new infections following spore germination on the leaf, stem, flower or fruit surface. The glasshouse climate is considered very important with respect to infection. Commercially, it is evident that in the early stages of disease development the asexual fungus infects via the cut surfaces along the main stem following de-leafing (where practised) and harvesting of fruit. As the crop canopy develops and matures there is greater opportunity for optimum infection conditions to develop from air-borne ascospores due to the micro-climate around the leaves, flowers and developing fruit. Effective crop management is therefore very important to prevent such infection. The high cost of energy currently prevents the use of pipe heat early in the morning to dry the crop out and this is likely to exacerbate infection and subsequent symptom expression.

There are however conflicting reports with respect to the infection process by the gummy stem blight pathogen in cucurbits, especially with respect to infection on young versus old leaves and with respect to whether wounding is required for successful infection. Chiu & Walker (1949), Van Der Meer *et al.* (1978) and Wyszogrotzka *et al.* (1986) found that cotyledons of cucumber at the seedling stage were resistant to *D. bryoniae* and that this inoculation approach was unreliable. Lee *et al.* (1984) were able to regularly infect cucumber cotyledons in their studies though the exact age of the plants used is unclear. Van Steekelenburg (1985) concluded that wounding was essential for infection of older leaves but not for young leaves though here the inoculation technique on the old and young leaves also varied. Contrary to the work by Van Steekelenburg, Hordijk & Goosen (1962) found that old leaves were more susceptible than young leaves and Van der Meer *et al.*

(1978) showed that the meristem and primordial true leaves were less susceptible than the first true leaf. Prasad & Norton (1967) stated that cucumber leaves become more susceptible to *D. bryoniae* with age.

According to Svedelius & Unestam (1978) on cucumber leaves phylloplane moisture and nutrition are more important in the infection process of *D. bryoniae* than stomatal opening. They concluded that nutrients on the leaf surface were more important than wound openings for infection. Guttation is increased by soil (and presumably substrate) fertilisation (Ivanoff, 1963; Blakeman, 1971) and the nutrient content of guttation fluid is affected by soil (and substrate) fertility (Curtis, 1944). In this regard, Van Steekelenburg (1982) found that susceptibility to external fruit rot was increased with increasing nitrogen fertilisation. Van Steekelenburg & Welles (1988) also reported that calcium nutrition also influences susceptibility to gummy stem blight. Several workers have previously indicated that free water is required for infection (Olsen & Stanghellini, 1981, Van Steekelenburg, 1983, Van Steekelenburg, 1985) yet in studies by Amand & Wehner, 1995 increasing leaf moisture in field tests (by increasing the irrigation frequency), perhaps surprisingly, did not increase the disease rating by *D. bryoniae*. However, plant inoculation at dawn did increase susceptibility to leaf infection compared with inoculation at dusk. This increase was considered to be due to the free water and nutrients provided by guttation (Amand & Wehner, 1995). Clearly, a better understanding of the relationship between guttation and infection and the preferred modes of entry of *D. bryoniae* could lead to improved screening methods and, at the same time, provide a better understanding of host resistance to this pathogen.

Potential for seed-borne transmission

Reports on seed transmission of *D. bryoniae* in cucurbits in general are quite common and the evidence to suggest the pathogen is seed-borne in some cucurbits is compelling. However, reports of seed transmission in cucumber are conflicting and, whilst such seeds can be artificially inoculated successfully, there is relatively little evidence that this occurs naturally (Punithalingam & Holliday, 1972) though it remains suspected as one possible entry route for the pathogen into glasshouse crops. Yet, one of the first reports of contaminated seed as the source of inoculum of *D. bryoniae* came from the UK. In this case, infested seed used for commercial crops of glasshouse cucumber that developed gummy stem blight, produced 6% diseased seedlings in a blotter test (Brown *et al.*, 1970).

Lee *et al.* (1984) reported that of over 90 cucurbit seed (cucumber and pumpkin) samples tested from thirteen countries, nine from four countries were found to be infected with *Didymella bryoniae*. The pathogen was reported to be located on and in the seed coat including the perisperm and in the tissue of the cotyledons. Primary seedling infection

occurred on the radicle, hypocotyl and cotyledons. Infection of the radicle generally caused a pre-emergence rot while infection on the hypocotyl and cotyledons developed further inoculum for infection of the first true leaves and the stem. Experimentally, all the isolates of *D. bryoniae* could infect cucumber, oriental melon (*Cucumis melo* var. *makuwa*), pumpkin (*Cucurbita pepo*) and watermelon (*Citrullus vulgaris*) at different growth stages; the susceptibility of both cucumber and pumpkin was markedly influenced by prevailing humid conditions. Interestingly they found that the blotter technique was more suitable for detection of seed-borne infection than the more conventional agar plate method.

Van Steekelenburg (1986) demonstrated that cucumber fruit became infected after flowers were inoculated with *D. bryoniae* and Neergaard (1989) traced the path of infection from conidia applied to the stigma through the style to the ovules. This provides support for the mechanism by which cucurbit seed may become infected by *D. bryoniae*. It has been hypothesised that such an infection route may account for internal fruit rot symptoms in some cucurbits. Similarly such internal (symptomless) fruit infection could perhaps help explain how seeds become infested with *D. bryoniae* on the outside of the seed coat during processing for seed extraction.

Sudisha *et al.* (2006) found seed infection to occur naturally in muskmelon (cantaloupe) both externally and internally on/in the seed.

The transition from direct seeding to transplanting cucurbits in modern agricultural systems is reported to have heightened the importance of *D. bryoniae* as an economically damaging plant pathogen. It is reported consistently, albeit infrequently, to appear on cucurbit (watermelon) seedlings in greenhouses, generally when the first true leaf appears; the typical pattern being a dead plant that grew from a contaminated seed (primary infection) surrounded by symptomatic plants (secondary infection) (Keinath, 2010). In greenhouse experiments, 11-15% of watermelon seedlings adjacent to infected source seedlings became infected (Keinath, 1996) although even higher rates of infection are reported to have been observed in commercial transplant greenhouses in the USA. This is certainly not considered to be the case with UK propagation facilities and if the pathogen is present during propagation it is more likely to occur in a latent form due to unsuitable environmental conditions for symptom expression. This aspect could potentially be further investigated once a suitable serological assay has been developed.

Pathogen survival

For effective control of the disease the mechanism(s) by which the pathogen survives between crops needs to be known. Over-wintering survival on crop residues both in the open and in the glasshouse have been studied over several years previously. In Madison,

Wisconsin, USA for example the fungus survives over-winter as dormant mycelium (Chiu & Walker, 1949). In Holland, detailed studies have also been undertaken previously both outdoors and in the glasshouse environment (Van Steekelenburg, 1983). Here, Van Steekelenburg found that the fungus was capable of surviving outside over-winter, including periods below 0^{o}C, as dormant mycelium and dry and un-decomposed crop residues remained a source of infection for more than one year, thus confirming the earlier USA studies. Moisture and a minimum temperature of 5-10^{o}C were required for production of sporing bodies. It was also found that the fungus is very resistant to dryness and was found to survive in dry plant material present on glasshouse structures and in plant debris in and on the soil as long as the debris was not decomposed. It was concluded that the disease will occur earlier and more severely in crops when plant debris from a previous crop is left in the glasshouse, particularly when the debris is wetted.

Factors influencing external and internal fruit rots

D. bryoniae causes a variety of symptoms in cucumber, as described above. Foliage (stems and leaves), flowers, fruits and even roots (Thingaard, 1987) can be attacked and this can have consequences with respect to dispersal and long-term survival of the pathogen. Fruit infection, both external and internal, is the most economically damaging aspect of the disease as infected fruit may be symptomless at point of dispatch only to decay during transit and storage post-harvest. Internal fruit infection also leads to rejection of consignments by retailers and this risks a 'loss of confidence' in the product.

External fruit rot: Studies have shown that the gummy stem blight fungus is a wound pathogen as unwounded and slightly wounded fruit do not rot following inoculation (Van Steekelenburg, 1982). Similarly, it has been shown that mechanical injury facilitates leaf infection due to the release of nutrients following cell rupture (Svedelius & Unestam, 1978). In inoculation experiments fruit rot increased progressively from 12-23°C but thereafter reduced and was very limited at 32°C (Van Steekelenburg, 1882). Similar results were obtained in watermelon (Luepschen, 1961). In general, the mycelial growth rate of the fungus (*in vitro*) is similar to that for fruit decay with an optimum growth rate of around 23-24°C.

Internal fruit rot: Several factors influencing the incidence of internal fruit rot have been investigated previously (Steekelenburg, 1986). The internal rotting of fruits always starts at the distal (blossom) end of the fruit. Initially, the internal tissues in the centre of the infected fruit tip exhibit a brown discoloration though over time this internal decay spreads to the outer surfaces of the fruit tip region. As the infection progresses, fruiting (sporing) bodies of the fungus appear on the surface, the tissues shrivel and turn black. The major problem is

that it can be very difficult and, in some cases, impossible to judge externally whether a fruit is infected internally. Some fruit may exhibit sunken areas a few cm from the fruit tip others may be slightly misshapen with a tapering tip. However, such misshapen fruit may also occur due to a variety of physiological disorders too so this symptoms is not diagnostic alone. This means of course that internally affected fruit often reach the retailers and/or consumers before a problem is realised and this presents a particular problem for growers in terms of long-term customer/retailer relationships.

The occurrence of such internal infection of cucumber by *D. bryoniae* has been described in many countries including Japan (Kagiwata, 1967), the Netherlands (Sweep & Govers, 1967), British Honduras (Sitterly, 1968) and Poland (Leski, 1984). It has also been seen in UK cucumber crops previously, though there appear to be no formal records as such. The incidence of internal fruit infection fluctuates from season to season and from nursery to nursery and depends on both infection pressure and the glasshouse climate (Van Steekelenburg, 1984, 1985; Van Steekelenburg & Van de Vooren, 1981). Infection levels of up to 46% internally infected fruit have been reported on specific harvest dates (Steekelenburg, 1984) with an estimate of 5% fruit infection over an entire growing season.

The first experimentally induced internal fruit rot occurred 7-15 days after spraying whole plants with a conidial suspension (Van Steekelenburg, 1985 & 1986) and this time equates approximately to the time needed from flowering to harvest. It has since been demonstrated that natural infection of fruit takes place in the flowering period through the flower parts themselves though perhaps surprisingly the majority of fruits escape infection following inoculation of the flower. Microscopic examination of flower tissue has revealed that the stigma and style are readily colonised by the fungus with growth through the flower tissue into the developing fruitlet taking about 2-3 days and changes in relative humidity made no difference to the infection rate (Van Steekelenburg, 1986). A mechanical barrier preventing the advance of the fungus into the fruit tip was not observed so it suggests other mechanisms operate here. Growing plants under drought stress was demonstrated to increase the incidence of internal fruit rot and yet inoculation of wilted flowers resulted in 60% less infection compared to inoculation of fresh flowers.

Interestingly, it was also noted in the work of Van Steekelenburg (1983) that cucumber cultivars with resistance to powdery mildew were also resistant to internal fruit rot caused by *Mycosphaerella*. The resistance was associated with cultivars that conferred the characteristics of a long style with a short flowering period. A recommendation from this work was that by selecting cultivars where the flowers quickly fall away from the fruitlets or where they have no style may effectively prevent the internal fruit rot aspect of this disease.

Host Resistance

D. bryoniae is a host-specific pathogen on cucurbits (Corlett, 1981) and, like many foliar pathogens, it is well adapted. At least 12 cucurbit genera and 23 species are hosts (they can become infected and diseased). There are though some consistent differences among some cucurbits. Summer squashes (*Cucumis pepo*), for example, are claimed to be resistant (or at least less susceptible) to gummy stem blight when compared alongside reactions on other species in the genus (Sitterly, 1969; Keinath *et al.*, 2009). It is also generally assumed that host resistance to a pathogen is most likely to occur when the pathogen is present in the area the particular species originated and it exerts selection pressure on the host. For example, 14 *Citrullus colocynthis* accessions from Iran (7), Afghanistan (3), Egypt (2), Morocco (1) and Cyprus (1) were very susceptible to *D. bryoniae*. Yet, because *C. colocynthis* is a desert species and most of the accessions were collected from countries with an arid climate it is very unlikely that the pathogen co-evolved with this host. Conversely, citron (*C. lanatus* var. *citroides*), which originated in southern Africa where *D. bryoniae* occurs, was the least susceptible of three *Citrullus* taxa evaluated (Levi *et al.*, 2001).

Historically though, cucumber growers have not been able to rely on host resistance to help suppress infection by *Mycosphaerella*. Rather, the drive by the leading seed houses to develop and introduce cultivars tolerant to powdery mildew (the primary foliar disease affecting cucurbits and where fungicide use is greatest) may have had the opposite effect as mildew tolerant cultivars would appear to be more susceptible to black stem rot (D Hargreaves, pers. com.). However, as far as we could ascertain during the review, this has not been validated scientifically.

In early studies, using 4 day-old cucumber seedlings, to evaluate multiple (>1200) cucumber lines of diverse origin for resistance to *Mycosphaerella* no host resistance was found in the various accessions. However, in approximately 50 lines evaluated in the field two accessions (cultivars) were found to confer resistance to the disease. Earliness in fruit maturity was positively correlated with susceptibility to the disease. Selection of the cultivar Homegreen and a numbered accession (PI 200818) resulted in improved resistance to *Mycosphaerella* in these studies (Wyszogrodzka *et al.*, 1986). Similar studies have been undertaken using the USDA-ARS watermelon germplasm collections (Gusmini *et al.*, 2005).

Ahmand & Wehner (1995) compared the effects of leaf age, guttation, stomata and hydathode characteristics (and wounding) on the correlation between field and greenhouse tests and between field and detached leaf tests for resistance to *Mycosphaerella* in

cucumber, the aim being to develop a useful method for screening cucumbers in the greenhouse for resistance. They also wanted to determine the importance of certain characteristics such as guttation, irrigation, plant size (age) and wounding on the field resistance of cucumbers. They found that older leaves were more susceptible and seedlings at the cotyledon stage were less susceptible (and this might explain the poor results with seedlings tests by Wyszogrodzka *et al.* above). Their results with wound inoculation conflicted with those of Van Steekelenburg (1985) who had earlier reported that wounding was necessary for infection of the 2nd and 3rd true leaf. However, he had not added sucrose or casein hydolysate to the leaf surface or to the inoculum prior to inoculation. Studies by Svedelius & Unestam (1978) had previously demonstrated the importance of phylloplane nutrients on the infection process by *Mycosphaerella*. In the study reported here, inoculation studies did include supplemental nutrients in the inoculum and this is likely to account for the different results.

Infrequent entrance of *D. bryoniae* through the stomata of watermelon rind has previously been noted (Shenck, 1962). It was assumed by Svedelius & Unestam (1978) that stomatal entrance was not an important mode of entry for the pathogen. Yet, in the same study reported above, whilst stomatal density and hydathode counts were not correlated with susceptibility, stomatal length on older leaves was highly correlated. Stomatal length increased with leaf age and older leaves were more susceptible and it was concluded therefore that stomata entry may be an important mode of entry for *D. bryoniae* on cucumber leaves. Interestingly, Ahmand & Wehner also found that phylloplane moisture and nutrition are more important in the infection process by *D. bryoniae* than stomatal opening. Svedelius & Unestam (1978) concluded that nutrients on leaf surfaces were more important than wound openings for infection of cucumber by *D. bryoniae*.

The US national cucumber germplasm collection was screened for resistance to gummy stem blight in field tests in North Carolina. Following artificial inoculation they found several resistant breeding lines, the most promising being cvs. Homegreen, Little John, Transamerica, Poinsett 76 together with some numbered plant introductions. Two popular cultivars in North Carolina, Calypso and Dasher II were described as moderately resistant though it is not entirely clear from the work whether they were all outdoor cultivars (Wehner & Shetty, 2000).

More recently, Nisini *et al.* (2008) have screened for resistance to *D. bryoniae* in rootstocks of melon (those previously selected as resistant or partially resistant to race 1,2 of *Fusarium oxysporum* f.sp *melonis*). *Cucumis anguria, C. ficifolius, C. figarei, C. metuliferus, C. zeyheri* and *Benincasa hispida* showed a very high degree of resistance to *D. bryoniae* both

on leaves and stems. Among commercial rootstocks, *Cucurbita* hybrids ELSI, ES 99-13 and RS 841 displayed a similar level of resistance. Interestingly, stem inoculation of three cucurbit species grafted with susceptible melon cv. Proteo determined the occurrence of limited symptoms, but the lesions remained confined to the rootstock not affecting the grafted plant itself. The study concluded by proposing that the cultivation of susceptible melon cultivars grafted on resistant rootstocks may represent an efficient method for control of both *D. bryoniae* and *Fusarium* wilt (*F. oxysporum* f.sp. *melonis*). This aspect should perhaps be further investigated in cucumber.

However, it should also be noted that the use of grafted cucurbits has been reported to increase the risk of gummy stem blight from seed-borne inoculum as, after grafting, plants are held under high RH conditions (or mist) to promote healing of the graft union. These environmental conditions are extremely favourable for the development of gummy stem blight (Arny & Rowe, 1991); use of a grafted plant would also increase the risk of seed-borne infection. Gummy stem blight has previously been reported on grafted watermelon in Tunisia and cankers at the graft union killed plants (Boughalleb *et al.*, 2007).

Environmental Control

Ideally, it should be possible to manipulate the environment very effectively to suppress foliar pathogens such as *D. bryoniae*. However, as indicated previously, the previous and current economic climate severely restricts what is achievable due primarily to the high energy costs associated with heating and lighting. It has been shown (Jarvis, 1989) though that environmental manipulation under the right circumstances can be highly effective in reducing the need for fungicide intervention and this perhaps requires further consideration taking account of the economic limitations imposed on growers. It is also important to recognise that any change to the environment will have impact not only on other pathogens and pests but also on introduced predators and parasites and the indigenous microflora and fauna itself.

Chemical (Fungicide) Control

The horticultural industry in the UK remains heavily reliant on the generation of efficacy, crop safety and residues data on major agricultural crops from which we can extrapolate to specialist minor uses in the horticultural sector. In terms of identifying which fungicide active ingredients have activity against the genus *Didymella*, and where the main R&D inputs are focused, the actual crop involved is not particularly critical though there are a number of factors to consider such as whether the active ingredient (or product) is available in the UK

and does the manufacturer have appropriate metabolism data to justify further investment for a 'minor use' approval.

For the UK, perhaps the closest analogy is with field brassicas where Ringspot (*Mycosphaerella brassicicola*) is a significant economic threat to quality production. In broad acre (arable) crops e.g. wheat leaf blotch caused by *Septoria tritici* (perfect or sexual stage *Mycosphaerella graminicola)* is also economically damaging, particularly throughout North-West Europe and there is significant investment from the agrochemical sector to develop novel fungicides for its control. On a worldwide scale, R&D investment in the banana (*Musa* spp.) crop for the control of 'black sigatoka' disease is probably greatest. This disease caused by *Mycosphaerella fijiensis* is a very serious threat to the economy of banana plantations where up to 27% of input costs are associated with its control (Marin *et al.*, 2003). It is not unusual for banana crops to receive upwards of 20-30 fungicide applications/season (often applied by air).

Some of the earliest published studies to evaluate the use of fungicides against gummy stem blight in cucumber recommended the use of dithiocarbamates (Fletcher & Preece, 1975) but the results were often unsatisfactory. As fungicide chemistry, legislation and consumer/retailer awareness has changed so dramatically in the last 25-30 years there is little benefit from looking back further than this. Van Steekelenburg (1978) investigated the use of several chemicals at Naaldwijk in Holland, including those used for powdery mildew control to see if there were any ancillary benefits against *Mycosphaerella.* Of the fungicides screened, those which proved most effective were benomyl and triforine though chlorothalonil also provided some control *in vivo*. The control of fruit infection was disappointing. It was concluded that protection with a good fungicide was difficult to achieve because of the continual production of wounds (due to picking and other crop work presumably) and the dense canopy produced. It was concluded that the crop had to be sprayed nearly every week to have a reasonable effect but, due to resistance concerns, it was necessary to alternate between benomyl, triforine and chlorothalonil.

More recently, Utkhede & Koch (2004) conducted an evaluation of biological and chemical treatments for control of gummy stem blight in hydroponic cucumbers in Canada. Azoxystrobin (Amistar in the UK) and pyraclostrobin+boscalid (BASF 516 – marketed as Signum in the UK) controlled the disease when they were applied as preventative sprays. The biological product Prestop (based on the fungus *Gliocladium catenulatum* JI446) was also effective in this evaluation.

Separately, there have been a number of studies on other (outdoor) cucurbit crops, especially melon and watermelon. Vawdrey (1994) evaluated a broad range of fungicides for *Didymella* control on rockmelon. Several fungicides including tebuconazole, fentin hydroxide, prochloraz-Mn, benomyl, propiconazole, mancozeb, myclobutanil and chlorothalonil significantly reduced the incidence and severity of the disease compared to unsprayed plots. Tebuconazole was most effective though proved phytotoxic at the applied rate.

In watermelon cultivation in the southeast USA Hopkins (2002) investigated the use of the plant activator acibenzolar-S-methyl (ABM) in conjunction with fungicides. Season long control of the disease was best when ABM applications commenced in propagation and continued throughout the season in conjunction with conventional fungicides, though ABM alone did not provide adequate control of the disease. It was concluded that ABM, in combination with fungicides such as mancozeb, chlorothalonil and the strobilurins can be an important component in a disease management programme for gummy stem blight in watermelon. Unfortunately ABM (as Bion, Syngenta Crop Protection) was withdrawn from the UK market a few years ago following disappointing uptake in the cereal sector. It is presumed, though not confirmed (for reasons of confidentiality), that second generation plant activators are in development with the leading agrochemical manufacturers. Similar work was undertaken in melon by Busi *et al.* (2004) but in this case looking at application to seed using acibenzolar-S-methyl and the signalling molecules salicylic acid (SA) and methyl jasmonate (MeJA). Interestingly here, *Didymella bryoniae* infection on melon seedlings was completely suppressed by MeJA seed treatment. Infection was also restricted following seed treatment with acibenzolar-S-methyl. It was concluded that both treatments applied to melon seed may activate diverse metabolic pathways to enhance pathogen resistance.

Interestingly, in field-grown cucurbits in Indiana, USA numerous fungicides are approved with a recommendation for *Mycosphaerella* control (Egel, 2010) including chlorothalonil, mancozeb, azoxystrobin, pyraclostrobin/boscalid and cyprodonil/fludioxonil.

Fungicide Resistance

Wherever, fungicides are used, particularly where they are used routinely and applied intensively, there is an increased risk of resistance in the pathogen population as more tolerant variants in the population survive the fungicide treatment. Where there is repeated exposure to the same fungicide over time the pathogen becomes increasingly tolerant or resistant to the fungicide and control failure occurs. It is for this reason alternating fungicide programmes are recommended using products with a contrasting mode of action to minimise such risk. The theory is sound, but in the horticultural sector it can sometimes be difficult to formulate effective alternating programmes due to the limitation of the number of active substances approved for use on the crop. Current legislation in the EU is potentially counter-productive as some of the older multi-site inhibitors are being withdrawn from sale.

Previously, benzimidazole fungicides e.g. benomyl, carbendazim, thiophanate-methyl provided effective control of *Mycosphaerella*. However, repeated intensive use led to the selection of resistant strains in the pathogen population and its use became ineffective. In Greece, use over a single season led to control failure (Malathrakis & Vakalounakis, 1983). In the UK a small-scale survey conducted in cucumber crops on Humberside in 1983 revealed isolates of *D. bryoniae* which were highly resistant to iprodione (Rovral). The same isolates were also found to be resistant to carbendazim. By 1986 a small number of isolates, also collected from cucumber crops in Humberside, were screened for sensitivity to a wider range of fungicides including carbendazim (Bavistin), vinclozolin (Ronilan), iprodione (Rovral), fenarimol (Rubigan), bupirimate (Nimrod) and prochloraz (Sportak). 50% of the isolates tested showed resistance to iprodione, vinclozolin and fenarimol (Clark, 1987 - unpublished). No further resistance testing has been undertaken in the UK since 1987.

McPherson, 1998 and McPherson & Brewster, (1999 & 2000) led a HDC-funded investigation primarily on powdery mildew and its control in cucumber, though data was also collected on both *Botrytis* and *Mycosphaerella* where possible. In Year 1 (1998) of this study *in vitro* leaf disc assays showed that imazalil (Fungaflor) and fenarimol (Rubigan) were moderately effective in suppressing lesion development (76 and 78% reduction in lesion diameter respectively). By contrast, bupirimate (Nimrod) was largely ineffective (15% reduction in lesion diameter). Several of the azole fungicides evaluated e.g. tebuconazole, triflumizole, cyproconazole, myclobutanil and epoxiconazole together with triademenol were highly effective in preventing lesion development in detached leaves. Unfortunately however, many of the azole fungicides are phytotoxic in the glasshouse environment and their potential use in glasshouse cucurbits is doubtful.

Seebold *et al.* (2004) reported widespread resistance to strobilurin fungicides in populations of *D. bryoniae* from watermelon in Georgia, USA and this resulted in a number of disease control failures. In experiments at resistant sites with fungicide mixtures or alternating programmes comprising strobilurin alone, strobilurin/chlorothalonil and cyprodinil/fludioxonil (Switch) using a 7 day spray programme. Disease severity where azoxystrobin was used alone did not differ from the untreated control (i.e. the fungicide proved ineffective due to resistance). Azoxystrobin alternated with chlorothalonil was not effective against stem blight but where it was tank-mixed with either chlorothalonil or other fungicides it was more effective, reducing disease severity by up to 60%.

More recently, Keinath (2009) reported that isolates of *D. bryoniae* (from melon) became insensitive to azoxystrobin in the Eastern USA 2 years after first commercial use in 1998; though baseline sensitivity had not previously been reported. Subsequent studies showed that 61 isolates collected primarily from cucurbit crops in South Carolina prior to azoxystrobin use i.e. pre-1998 were sensitive to the fungicide. Forty isolates collected after exposure during 1998 remained sensitive. However, 83% (of 64 isolates) collected from South and North Carolina between 2000-2006 were insensitive to azoxystrobin indicating a strong selection pressure following repeated fungicide use. On a positive note, an azoxystrobin baseline sensitivity distribution was established *in vitro* for isolates of *D. bryoniae* not previously exposed to strobilurin fungicides and this provides a very useful reference point for future studies with strobilurins.

It is also worth noting that Signum (pyraclostrobin + boscalid) could be a potential candidate for future use against *D. bryoniae* and other pathogens in cucumber, subject to approval, especially as it is already approved for use on outdoor cucurbits elsewhere. However, in Georgia, USA in 2007 where pyraclostrobin + boscalid (Pristine) failed to control the gummy stem blight in watermelon 5 isolates were collected and compared for sensitivity to boscalid alongside 75 isolates not previously exposed to the same fungicide. Using a mycelial growth assay on agar the previously unexposed isolates were sensitive to boscalid. In contrast, the 5 isolates collected in 2007 following exposure to boscalid were all highly resistant. This demonstrates that the risk of resistance with currently non-approved fungicides is high and this could have a bearing on future ‘minor use’ approvals elsewhere as the manufacturers consider their long-term strategies regarding product stewardship.

Bio-Control

Effective bio-control of foliar plant pathogens in the aerial environment is challenging and must, to a great extent, depend on the indigenous (or introduced) phylloplane microflora in conjunction with effective environmental manipulation to ensure conditions are conducive for their establishment and survival. The application of pesticides and various other components within formulations e.g. wetting agents, is likely to adversely impact on both the waxy cuticular layer and the micro-organism population itself.

There are no published studies on the indigenous phylloplane microflora of cucumber to the best of our knowledge and similarly we know little about the indirect impact of pesticides or other applied products in this regard.

In trials at the Espoo Research Centre in Finland using the cucumber cultivar Mitola, Lahdenpera (1998) showed that Prestop (*Gliocladium catenulatum* JI446) applied on two occasions was effective in reducing the number of diseased plants from 12.5% to < 1%.

In a combined Canadian study using both fungicide and bio-control products Utkehde & Koch (2004) found that preventative sprays of Prestop (*Gliocladium catenulatum* JI446) were effective in controlling *Mycosphaerella* in glasshouse cucumber. Treatment with the yeast (*Rhodosporidium diobovatum*), whilst having some effect (one experiment only) was much less effective. No significant control was provided with Rootshield (*Trichoderma harzianum*), Soilgard (*Gliocladium virens*) Quadra 136 (*Bacillus subtilis*) or Mycostop (*Streptomyces griseoviridis*) in this work.

Mucharromah & Kuc (1991) investigated the role of oxalates and phosphates in inducing systemic resistance against diseases (fungi, bacteria and viruses) in young cucumber plants. They found that by spraying aqueous solutions of oxalate or potassium phosphate onto leaf 1 systemic resistance to several pathogens, including *D. bryoniae,* was induced in leaf 2 above when challange-inoculated.

The importance of plant growth-promoting rhizobacteria (PGR's) applied as seed treatments in enhancing the growth of watermelon seedlings attacked by pathogens including *D. bryoniae* was investigated by Lokesh *et al.* (2007). Isolates GB03 and IPC-11 were found to be effective against both *Fusarium* spp. and *Didymella bryoniae*. Later applications of the PGR's were not investigated in this study.

The biological control of gummy stem blight in protected cantaloupe (*Cucumis melo*) was investigated by Nofal *et al.* (1996). Various isolates of *Trichoderma* spp. (*T. harzianum*, *T. viride*, *T. longatum* and unidentified *Trichoderma* species) were isolated from the rhizosphere of cucurbit roots and used initially in an *in vitro* laboratory assay to measure the inhibition zone between pathogen and antagonist. *T. harzianum* showed a higher antagonism than the other species in this assay. Following inoculation into cantaloupe stems *T. harzianum* showed a higher level of antagonism and a reduction in disease occurred.

Using a different 'organic' approach El-Meleigi and Al- Rehiayni (2004) used a combination of chicken litter (to replace nitrogen applications) and *Bacillus polymyxa* applied to the seed and foliage for disease control in cucumber. The incidence of gummy stem blight was significantly reduced in cucumber trials over two seasons and a significant reduction in the disease in squash (*Cucurbita pepo*) was recorded over one season.

More generally, Deliopoulos *et al.* (2010) have recently reviewed the use of inorganic salts for their potential with respect to disease suppression. Whilst much of the focus is on the control of powdery mildew there is some evidence of suppression of *Didymella* or *Mycosphaerella* species, including *D. bryoniae* by phosphate and silicate salts. Further studies are required to assess not only the efficacy of such treatments, especially when integrated with more conventional fungicides and bio-control agents but also with respect to crop safety in a glasshouse environment.

Recommendations for Further Work

- 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 for potential 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

B. Experimental work

Materials and methods

Sample collection and isolation of *M. melonis*

ADAS and STC collected isolates of *Mycosphaerella* from infected cucumber fruit and stem tissue from commercial growers in southern England and from around the East Yorkshire growers. Where possible details of the crop cultivar and any fungicides that had been applied were also collected. Isolates were given a code number on receipt to maintain anonymity of the grower.

A reference isolate of *Mycosphaerella melonis* was sourced from CABI UK (Bakeham Lane, Surrey, TW20 9TY). The chosen isolate (IMI 230139) taken from a cucumber crop in 1978, was felt to provide an isolate with expected good baseline sensitivity i.e. little or no previous exposure to the active ingredients under test, and would therefore provide a good comparison with the more recently collected isolates which had been collected from crops with *M. melonis* populations which may have been exposed to the chosen fungicides previously.

Following receipt and logging of samples isolations from the affected material were carried out by aseptically placing small sections of the leading edge of the lesions onto a standard growth media, Potato Dextrose Agar (PDA) and also onto the same media with either lactic Acid (0.4 ml of a 25% LA solution/100ml agar) or streptomycin sulphate (1 ml/100ml agar of a 0.2 g/20 ml SDW stock) added to combat bacterial contaminants. Following incubation collected isolates were sub-cultured until pure then put on PDA slopes in STC and ADAS culture collections for future work.

Resistance testing

Collected isolates were used in tests to determine their sensitivity to 6 fungicides (Table 1) which were incorporated into PDA at 0, 2, 20 and 100 ppm. Products considered likely to have some activity against *Mycosphaerella* were Amistar, Bravo 500, Switch, Teldor, Nimrod and Fungaflor. The project team was unable to source Fungaflor (imazalil) and this was probably due to the recent approval revocation. It was felt that as the product was older and difficult to source it was of less relevance to this current work. Switch is approved on cucumbers for *Mycosphaerella* control, it contains two active ingredients (cyprodinil and fludioxonil). For the purposes of the resistance testing it was necessary to test each active ingredient separately and therefore Unix (cyprodinil) and Beret Gold (fludioxonil) were sourced and tested individually. As the active ingredient concentrations in these products

differed to that contained in Switch, the calculated rates were adjusted to reflect the rate of the two active ingredients in Switch.

Table 1. Details of the fungicides used in the resistance test screening - 2010

Product	Active ingredient	AI concentration	Amount of product to add to 100 ml SDW to produce 1000 ppm stock solution
Amistar	azoxystrobin	250 g/L	0.4ml
Bravo 500	chlorothalonil	500 g/L	0.2ml
Unix	cyprodinil	75%	0.135 g (adjusted)
Beret Gold	fludioxonil	25 g/L	4.0ml
Teldor	fenhexamid	50 g w/w	0.2 g
Nimrod	bupirimate	250 g/L	0.40 ml

Cyprodinil and fludioxonil amounts adjusted to match rates of ai's used in Switch.

Following incubation (22 - 25°C) the mean colony diameter was measured and the percentage of inhibition compared to 0 ppm concentration (control) plates was calculated.

A full experimental protocol for the resistance testing conducted is provided in Appendix 1.

Seed testing

A number of cucumber seed batches were sourced in 2010 and 2011 for laboratory screening for *M. melonis*. Untreated seed was purchased where possible. If only treated seed could be sourced the fungicide treatments (generally Thiram) were washed off with sterile water prior to testing. 100 seed of each batch was examined under a low power binocular microscope for possible mycelium, debris or pycnidia. The seed was then plated out aseptically onto 25-well square Petri-dishes containing PDA LA agar. Test plates were incubated at 23°C for approximately 7-10 days. The seed was then examined and a record made of any fungi or bacteria that were detected was made.

Additionally, 25 seed from each batch were sown in ½ seed trays of Levington F2+S compost and placed in covered germination boxes in a glasshouse. Seedlings were allowed to germinate and grow until the 1st true leaves were present. At this time the seedlings were excised at soil level and sections of the basal stem tissue from each seedling was plated onto PDA (LA) agar to check for any systemic, asymptomatic *M. melonis* infection.

Serological validation using existing antisera

Five monoclonal antibody cell lines raised to ascosporic inoculum of *M. brassicicola* and two purified IgG PAbs raised to *M. brassicicola* and *Pyrenopezizia brassicae* (light leaf spot on Brassicas) were tested for reactivity to *M. melonis* by PTA ELISA and Immunofluorescence (IF). Following this antibodies reacting to *M. melonis* were selected and additional studies were carried out to determine whether reactivity was limited to *M. melonis* or extended to a range of fungal spore types which may be found in UK glasshouse/field aerosols.

Daily aerosol samples were then collected near an artificially infected cucumber fruit which exhibited gummy stem blight disease. The air samples were processed by bright field microscopy to determine the potential of an air sampling system to detect and quantify aerosols of *M. melonis*.

Results

Sample collection

A total of 28 isolates of *M. melonis* were obtained in culture. Details of the collected isolates are given in Table 2.

Collected isolates did vary somewhat in morphology, speed of growth, colour etc. Photographic records and notes on the various morphological differences were retained by STC and ADAS (Plate 9).

Plate 9. Examples of *M. melonis* is culture

Table 2. Details of isolates collected for inclusion in the *in vitro* resistance tests

Isolate No.	Isolate ref. code	Location	Cultivar	Material type
ADAS 1 ref	IMI 230139	CABI	-	-
ADAS 2	BX10/35	Ely	Aviance	Leaf
ADAS 3	BX10/35	Ely	Aviance	Node
ADAS 4	BX10/55a	Harlow	Roxanna	Stem
ADAS 5	BX10/55b	Harlow	Roxanna	Stem
ADAS 6	BX1055b	Harlow	Roxanna	Stem
ADAS 7	BX10/56a	Harlow	-	Petiole
ADAS 8	BX10/56b	Harlow	-	Stem
ADAS 9	BX10/57a	Harlow	Roxanna	Stem
ADAS 10	BX10/57b	Harlow	Aviance	Stem
ADAS 11	BX10/58	-	Roxanna	Stem
ADAS 12	BX10/58	-	Roxanna	Stem
ADAS 13	BX10/63	Fen Drayton	Aviance	Fruit
ADAS 14	BX10/63	Fen Drayton	Aviance	Leaf
ADAS 15	BX10/63	Fen Drayton	Aviance	Stem
STC 1 ref	IMI 230139	CABI	-	-
STC 2	E528/1	Lee Valley	-	-
STC 3	E528/2	Lee Valley	-	-
STC 4	E528/3	Lee Valley	-	-
STC 5	E528/4	Lee Valley	-	-
STC 6	E528/6	Humberside	Proloog	Stem
STC 7	E528/7	Humberside	Proloog	Fruit
STC 8	E528/8	Yorkshire	Roxanna	Fruit
STC 9	E528/9	Yorkshire	Shakira	Petiole
STC 10	E528/10	Humberside	Adinda	Stem
STC 11	E528/11	Humberside	Aviance	Stem
STC 12	E528/13	Humberside	-	-
STC 13	E528/17	Humberside	Aviance	Fruit
STC 14	E528/18	Yorkshire	Green Fit	Fruit

Resistance testing

Once in pure culture the isolates were used as described in the resistance testing protocol (Appendix 1). Recorded values (colony diameters) for each site were compared to the recorded values for the reference isolate used by that site as there was some slight variation in growth rates which are possibly attributable to slight variations in growing temperatures or growth media (e.g. water pH) between the two sites.

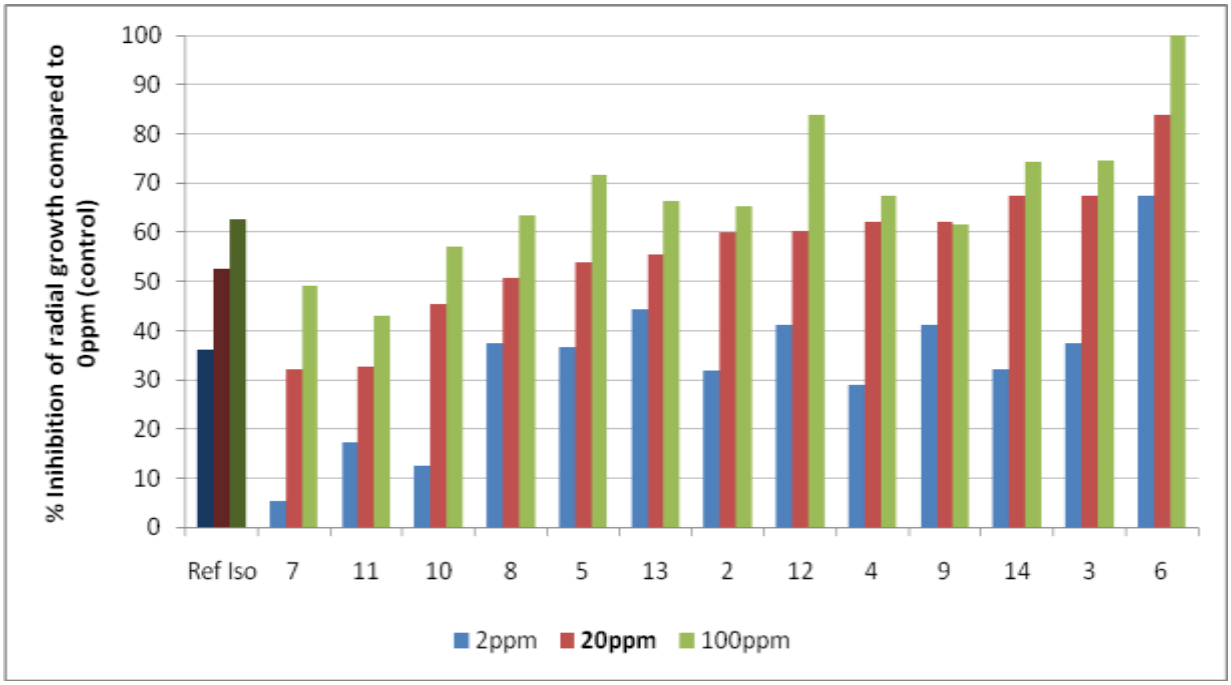


Figure 1. STC isolate sensitivity to Amistar (azoxystrobin)

Each isolate was tested against all 6 fungicides at 3 concentrations (2, 20 and 100 ppm -full data sets are given in Appendix 2). The percentage of inhibition in mycelial radial growth compared to the 0ppm (control) plates has been calculated (Figures 1-12). The reference isolate sensitivity is shown in darker colour bars on the left side of each chart for comparison purposes. The isolates have been sorted by increasing inhibition (or increasing sensitivity) left to right based on the 20 ppm concentration values e.g. Isolate 6 in Figure 1 shows higher sensitivity to Amistar than isolate 7.

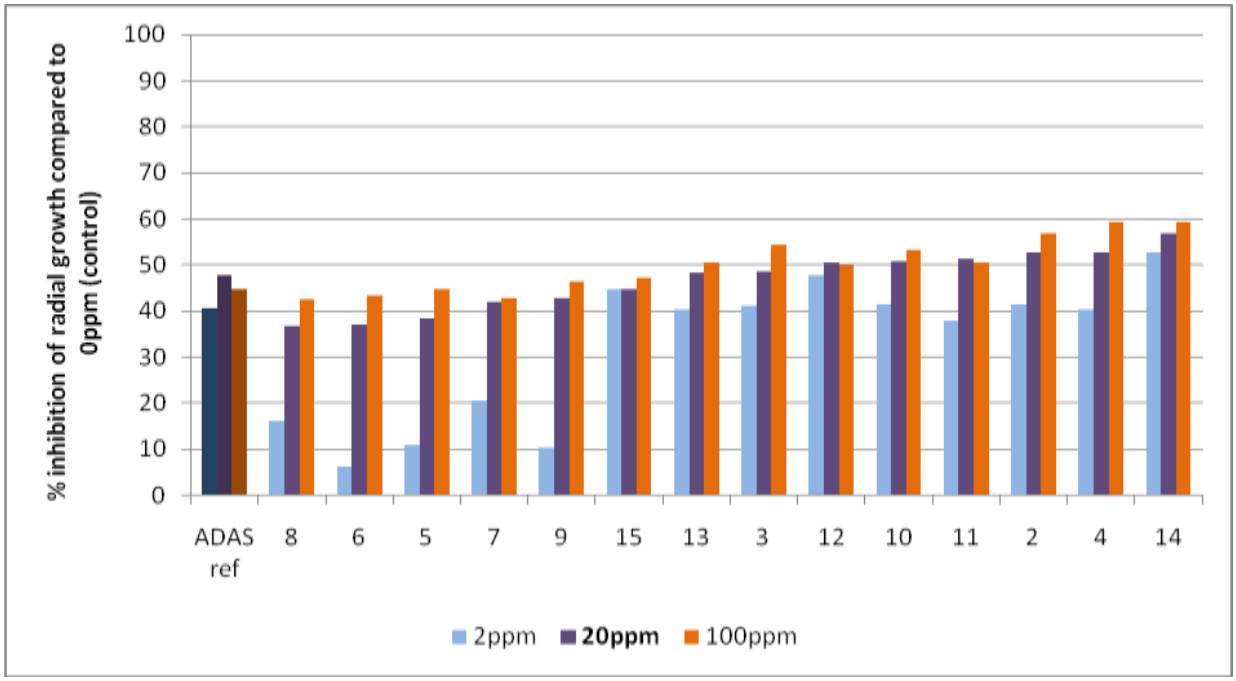


Figure 2. ADAS isolate sensitivity to Amistar (azoxystrobin)

The results from the resistance testing with Amistar (azoxystrobin) suggest that in our test method this fungicide has only moderate activity against *M. melonis*, reducing mycelial growth by around 50%. Isolates tested by ADAS differed little in sensitivity to Amistar. There was greater difference between isolates in the STC tests and three of them (isolates 7, 10 and 11) appeared to show slightly reduced sensitivity compared with the reference isolates, considered never exposed to the fungicide. Interestingly, some isolates were more sensitive to Amistar than the reference isolate (e.g. isolate 6), and this perhaps demonstrates that there is an inherent baseline variability to consider here.

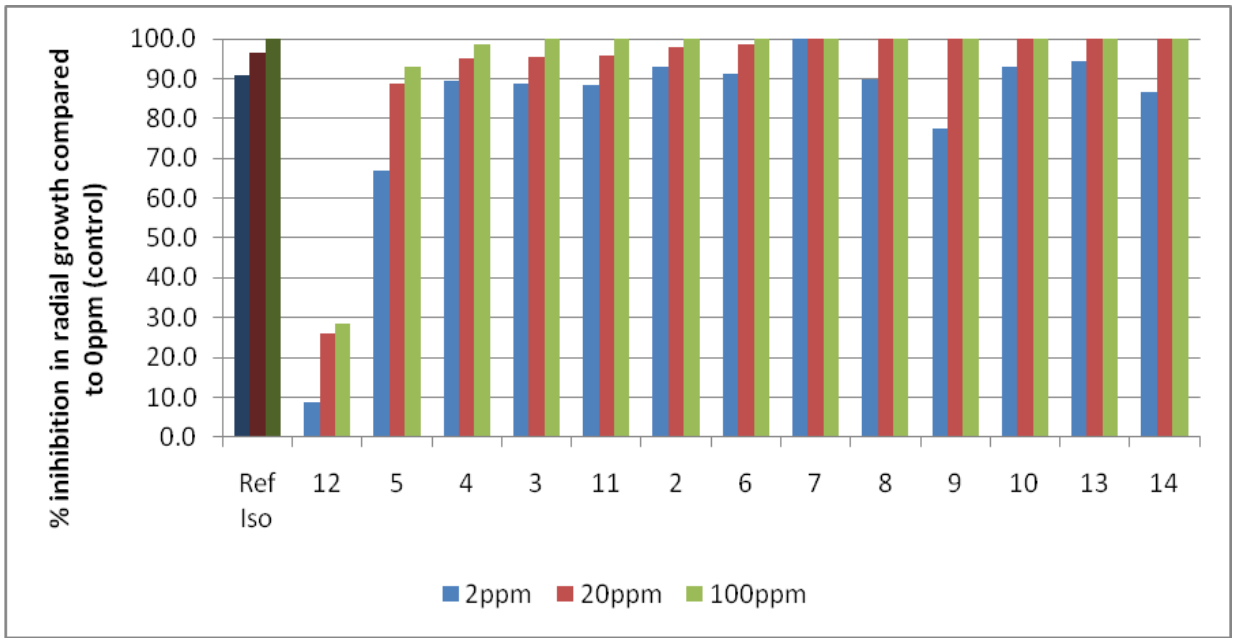


Figure 3. STC isolate sensitivity to Unix (cyprodinil)

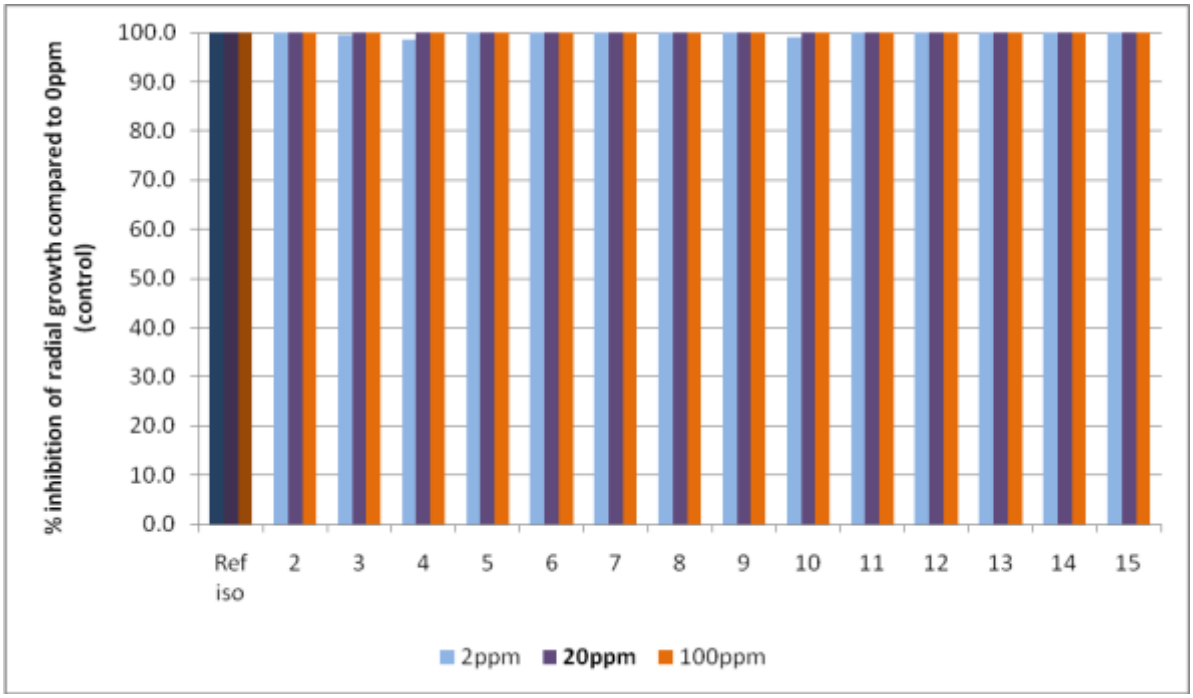


Figure 4. ADAS isolate sensitivity to Unix (cyprodinil)

Virtually all isolates showed high levels of inhibition in growth when grown on Unix (cyprodinil) amended agar in our tests. This was particularly evident in the ADAS tests. These results indicate a high level of sensitivity to the fungicide cyprodinil. The exception was isolate 12 in the STC tests which appeared highly insensitive to cyprodonil; perhaps an early indication of the selection of tolerant strains of the pathogen. Further, but later testing at this site using the above data as a baseline reference may demonstrate a further shift in the pathogen population. This result needs to be confirmed and the relative pathogenicity of the isolate to cucumber determined.

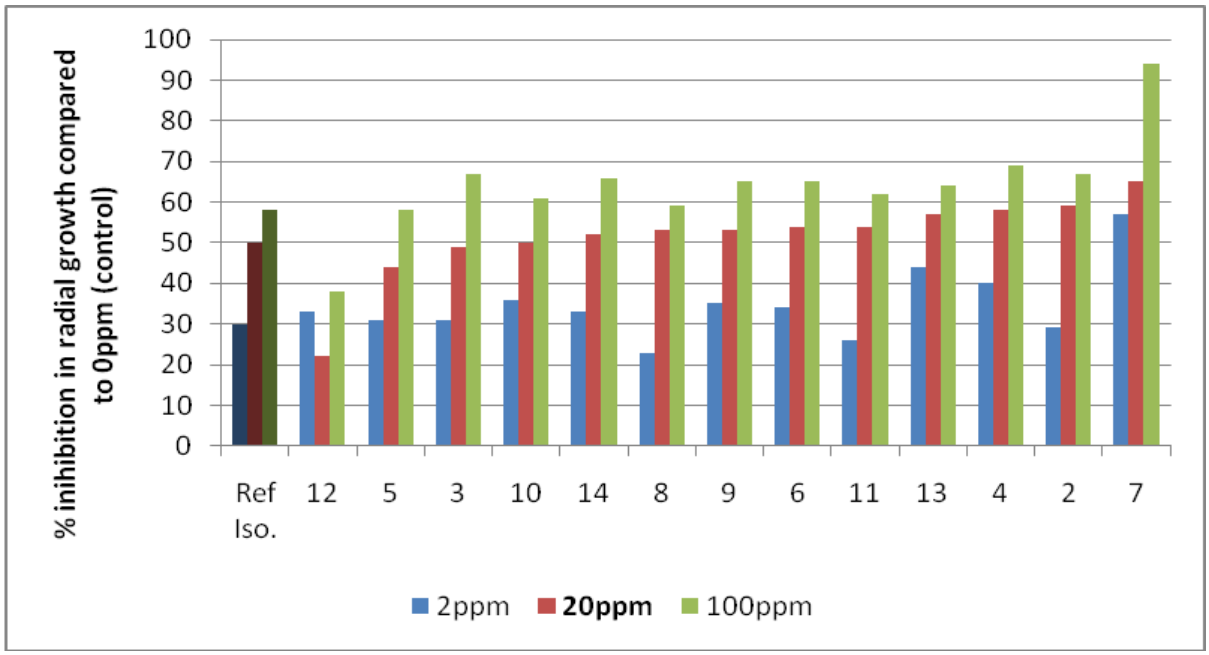


Figure 5. STC isolate sensitivity to Bravo 500 (chlorothalonil)

All isolates showed only moderate inhibition when grown in the presence of chlorothalonil, even at the highest concentration. Based on earlier data from Van Steekelenburg (1978) it suggests that chlorothalonil is perhaps not the fungicide of choice for *Mycosphaerella* control. The lack of variability in sensitivity between the isolates supports this view.

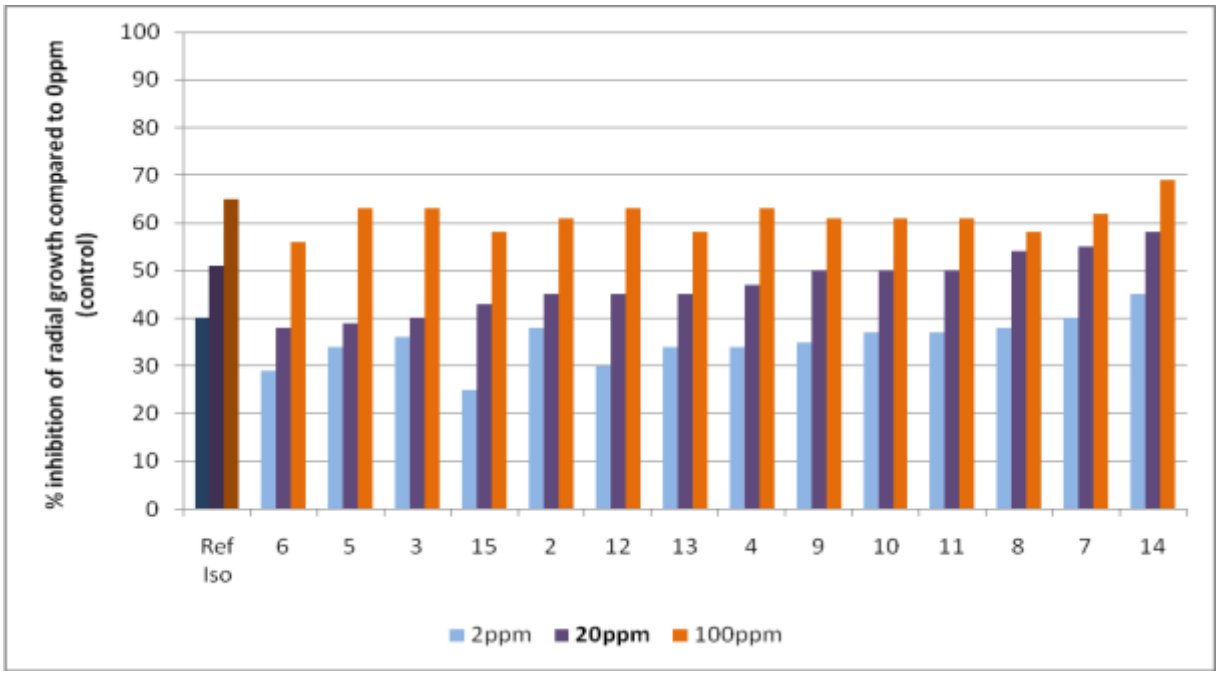


Figure 6. ADAS isolate sensitivity to Bravo 500 (chlorothalonil)

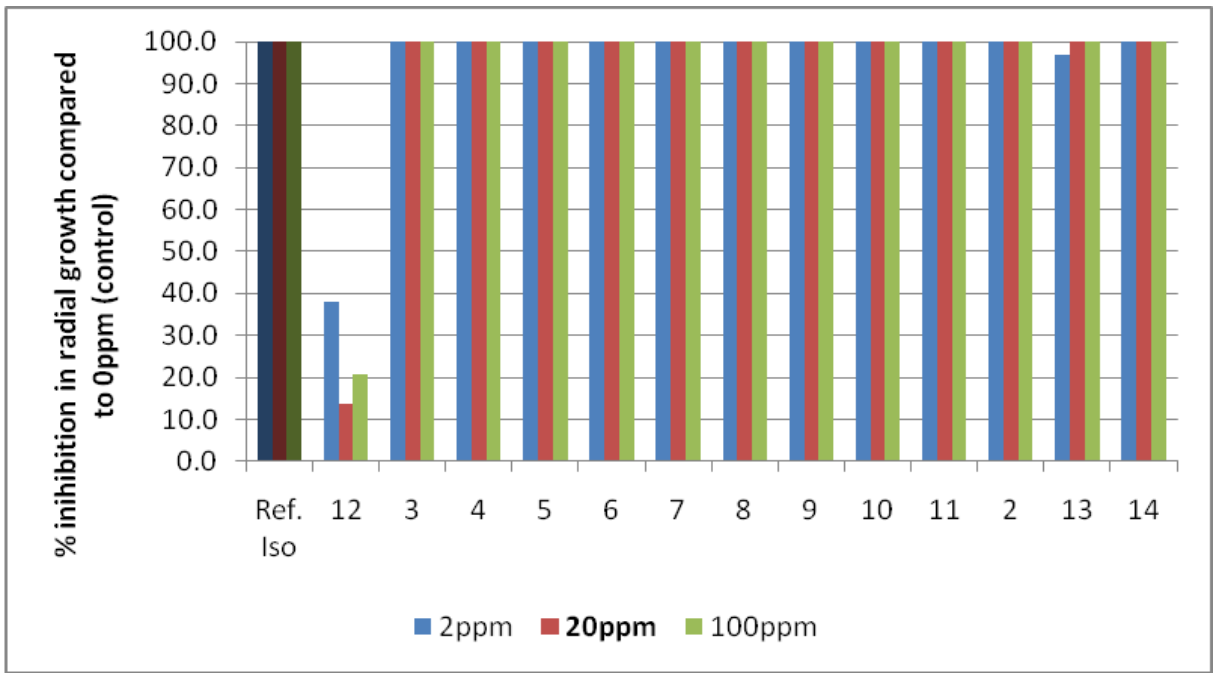


Figure 7. STC isolate sensitivity to Beret Gold (fludioxonil)

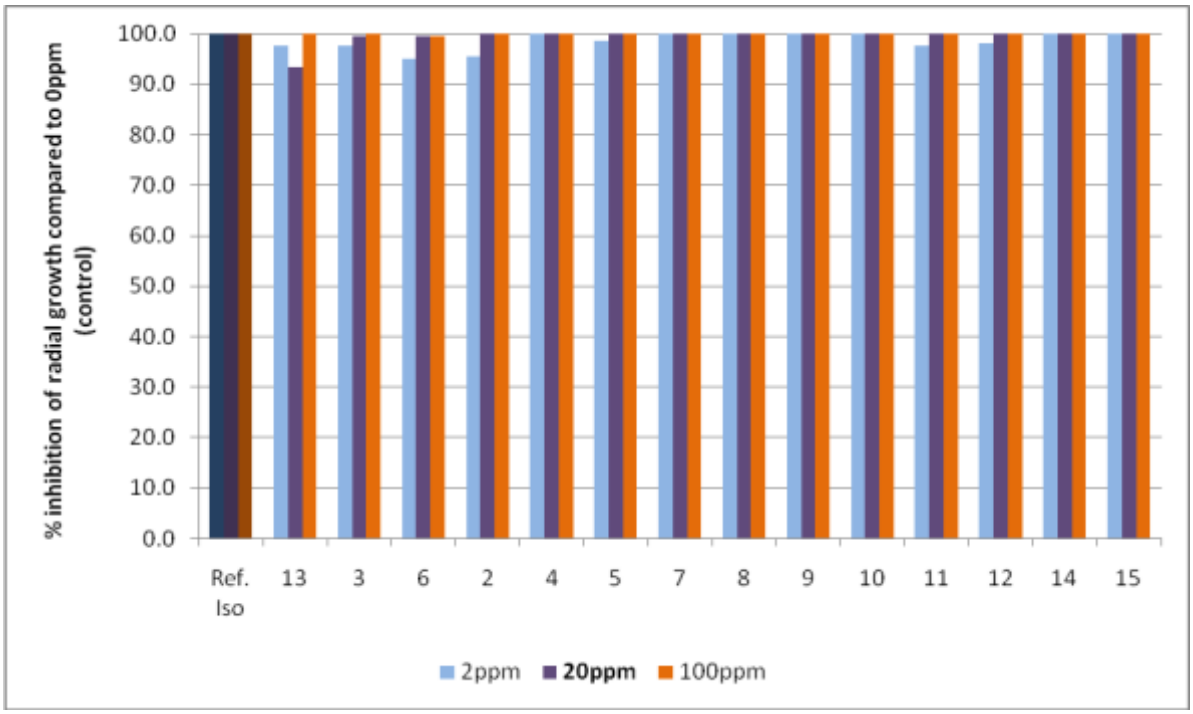


Figure 8. ADAS isolate sensitivity to Beret Gold (fludioxonil)

With the exception of STC isolate 12, all other isolates were almost completely inhibited in radial growth on Beret Gold (fludioxonil) even at 2ppm. This indicates good activity by this fungicide against *M. melonis.* The exception was again isolate 12 in the STC test which appeared to demonstrate a high level of tolerance to the fungicide even at 20 and 100ppm. Interestingly, this same isolate also showed a similar level of tolerance to cyprodinil and it is suggested that this requires further investigation.

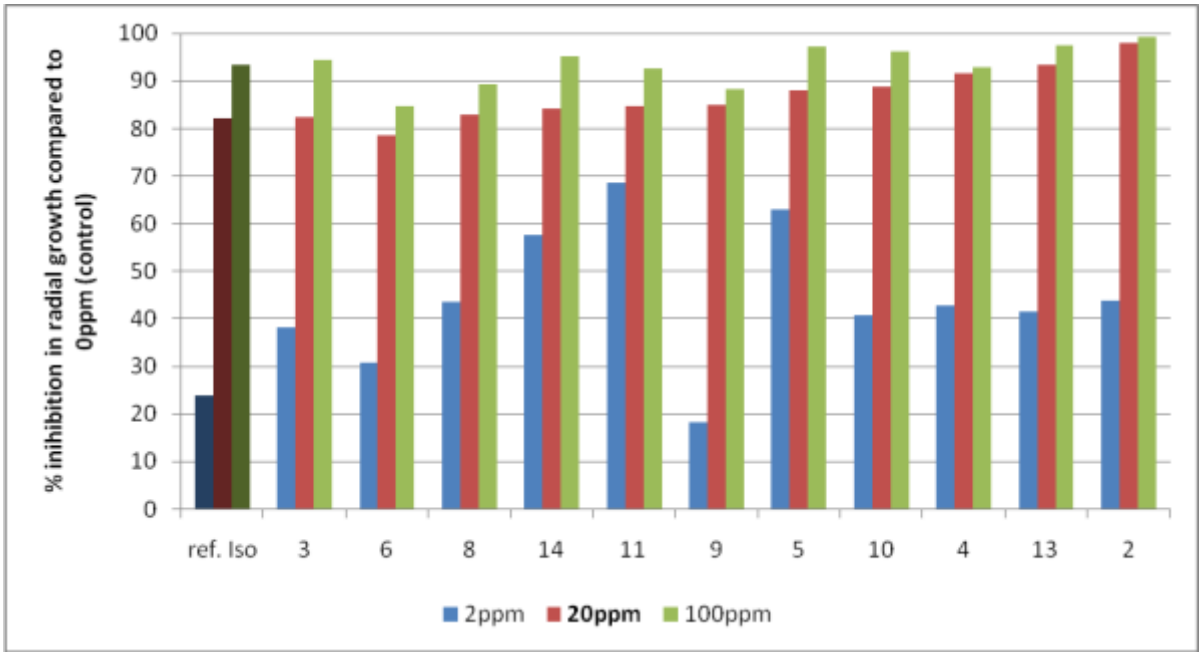


Figure 9. STC isolate sensitivity to Teldor (fenhexamid)
NB – Two isolates (7 and 12) contaminated in storage.

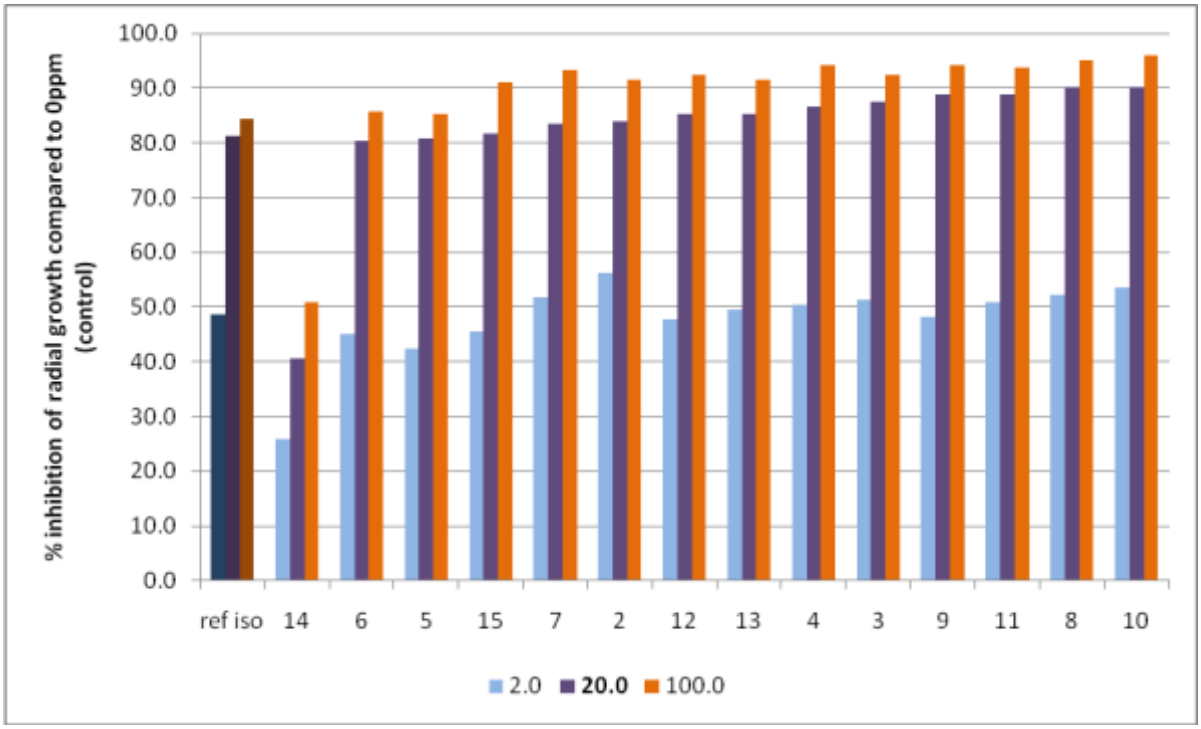


Figure 10. ADAS isolate sensitivity to Teldor (fenhexamid)

The majority of both ADAS and STC isolates showed very similar moderate-good levels of inhibition when grown on Teldor (fenhexamid) amended agar. Inhibition was generally poor at 2ppm, but much better at 20 and 100 ppm. Test isolates showed similar levels of growth inhibition to the reference isolate in both sets of tests, except perhaps ADAS isolate 14.

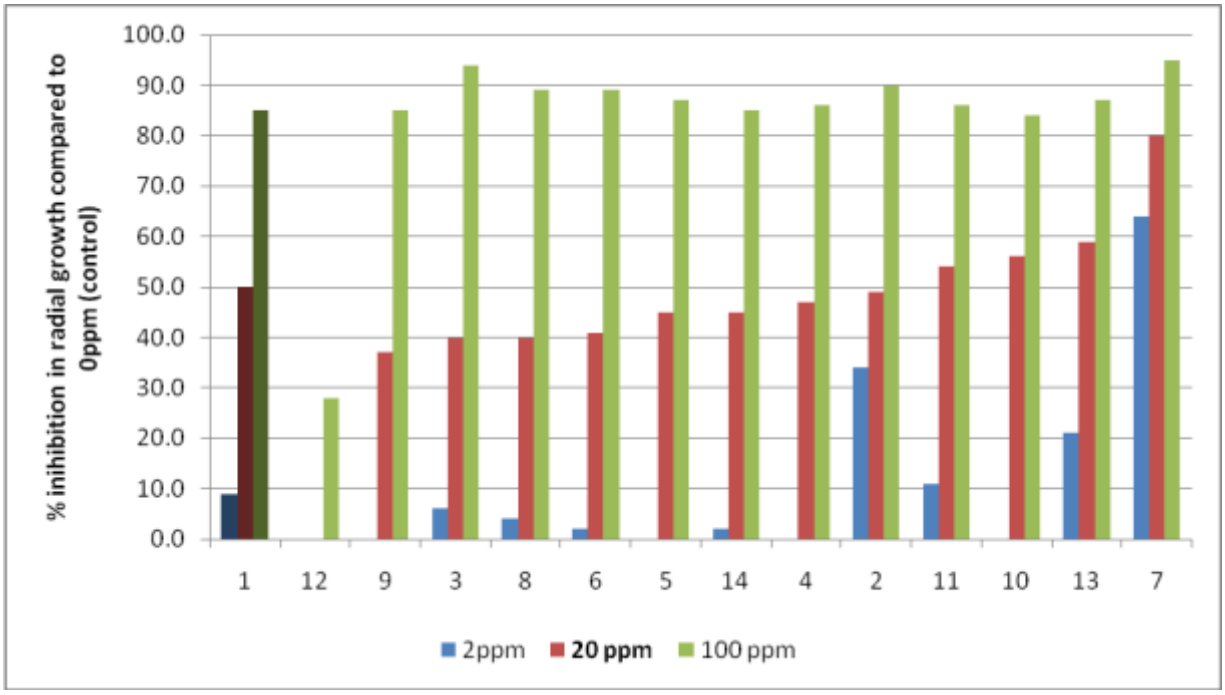


Figure 11. STC isolate sensitivity to Nimrod (bupirimate)

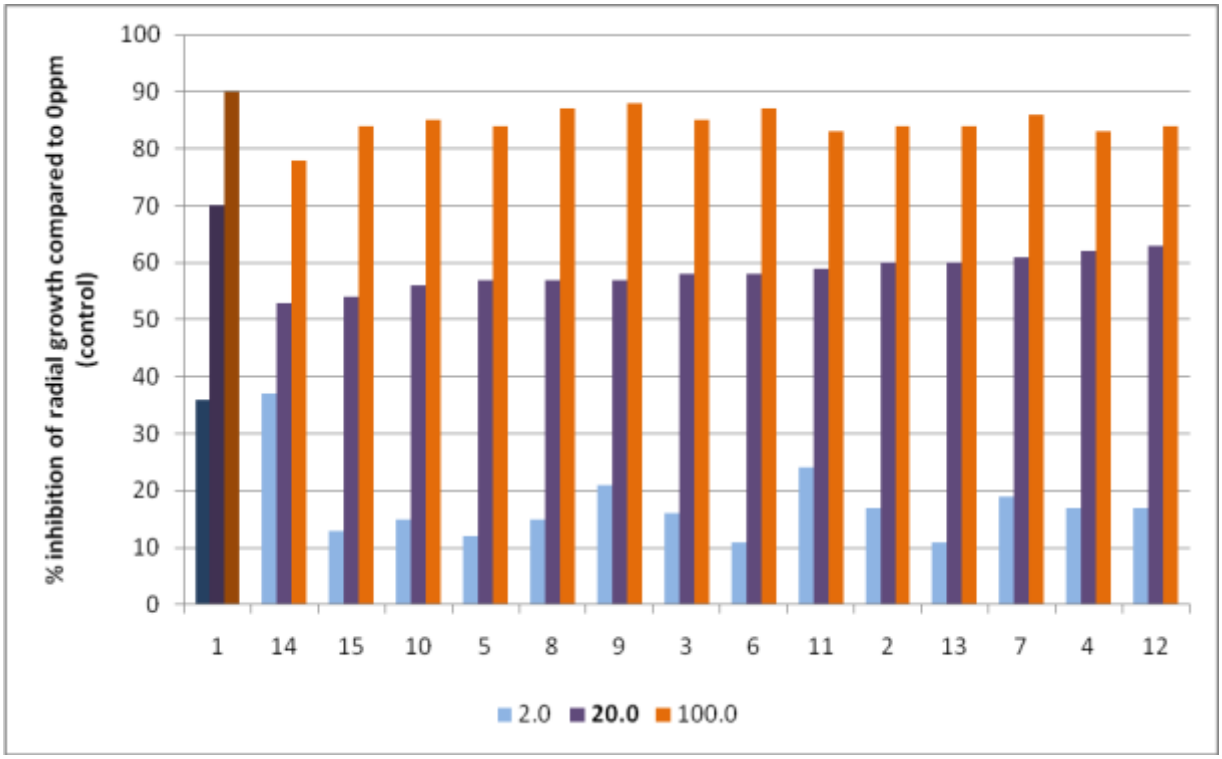


Figure 12. ADAS isolate sensitivity to Nimrod (bupirimate)

The majority of the isolates tested showed very low inhibition to Nimrod (bupirimate) at 2ppm and low-moderate inhibition (ca. 40 and 50%) at 20ppm. Sensitivity was increased at the 100ppm concentration. In the ADAS tests most isolates, compared to the 1978 reference, were less sensitive at 2ppm perhaps indicating a slight shift in sensitivity due to

frequent use over many years. The STC tests were also very variable, though difficult to interpret.

A comparison of the results with the reference isolate for the 20ppm concentration shows some interesting detail (Figures 13-18).

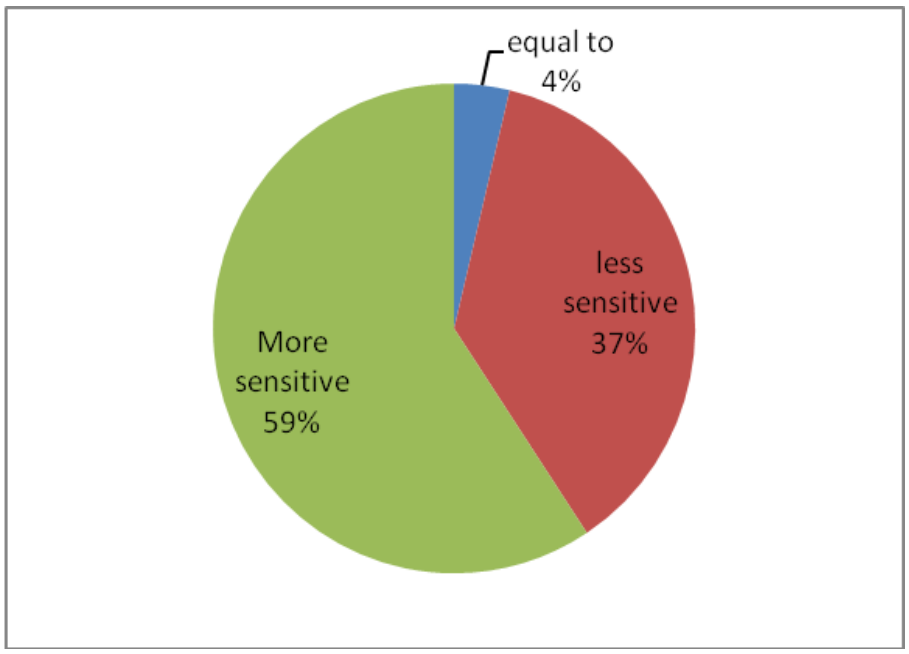


Figure 13. Sensitivity of all isolates to Amistar (azoxystrobin) compared to the reference isolate

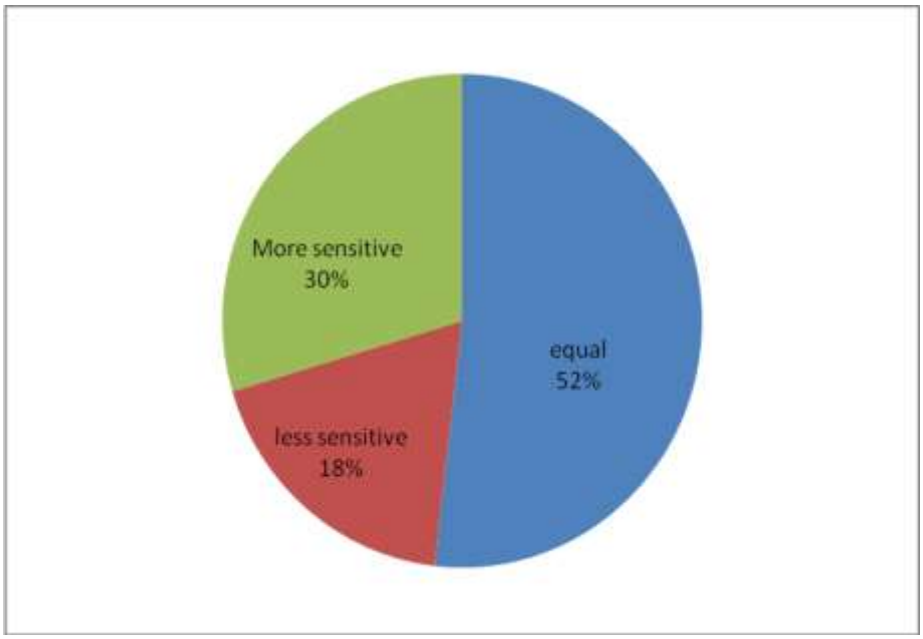


Figure 14. Sensitivity of all isolates to Unix (cyprodinil) compared to the reference isolate

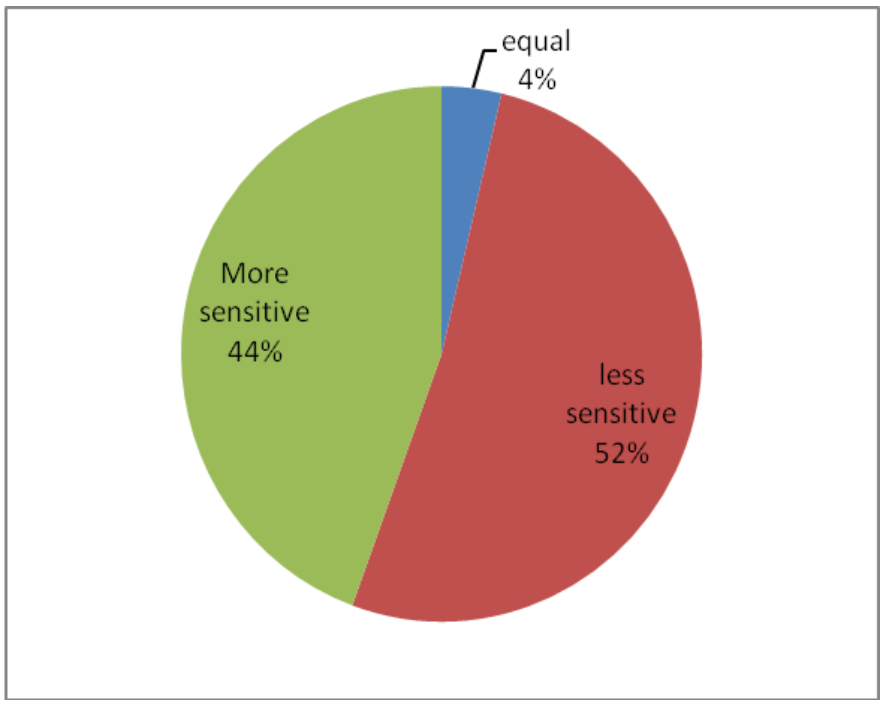


Figure 15. Sensitivity of all isolates to Bravo (chlorothalonil) compared to the reference isolate

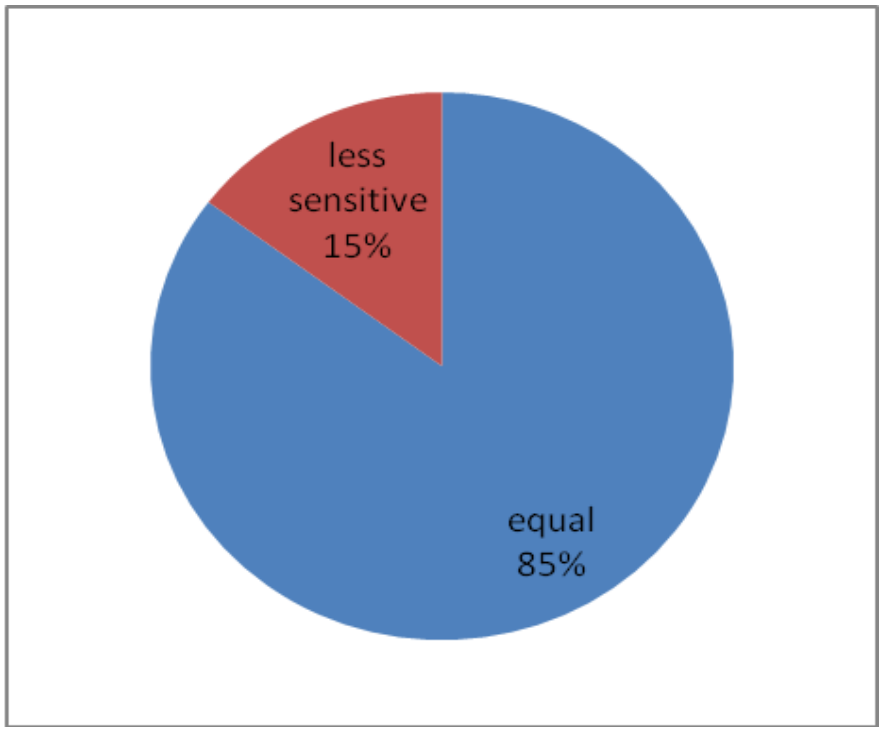


Figure 16. Sensitivity of all isolates to Beret Gold (fludioxonil) compared to the reference isolate

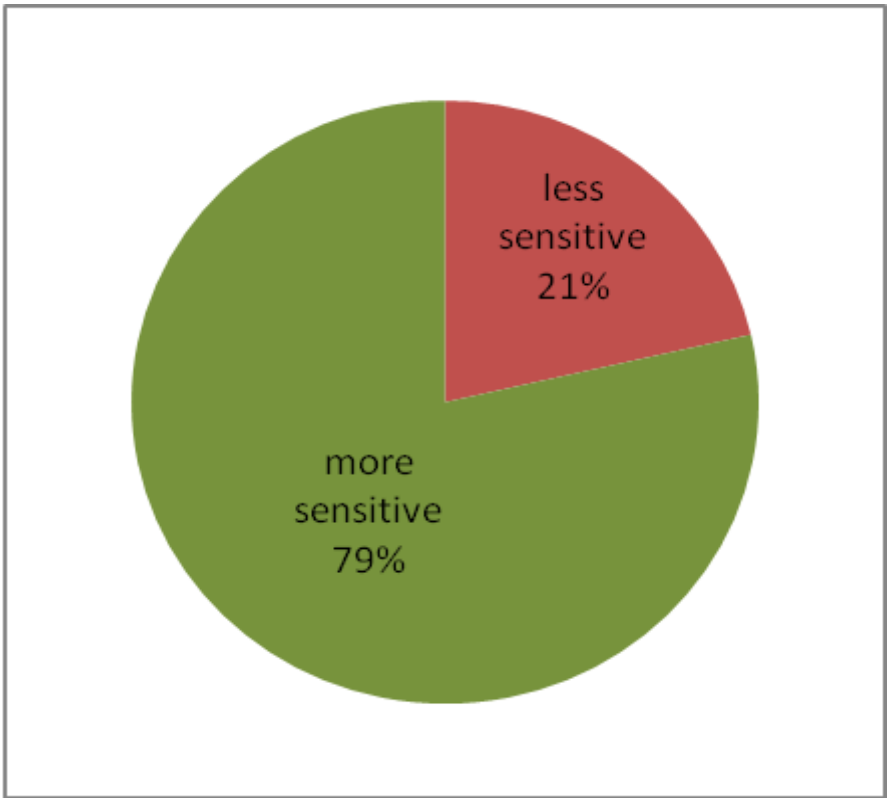


Figure 17. Sensitivity of all isolates to Teldor (fenhexamid) compared to the reference isolate

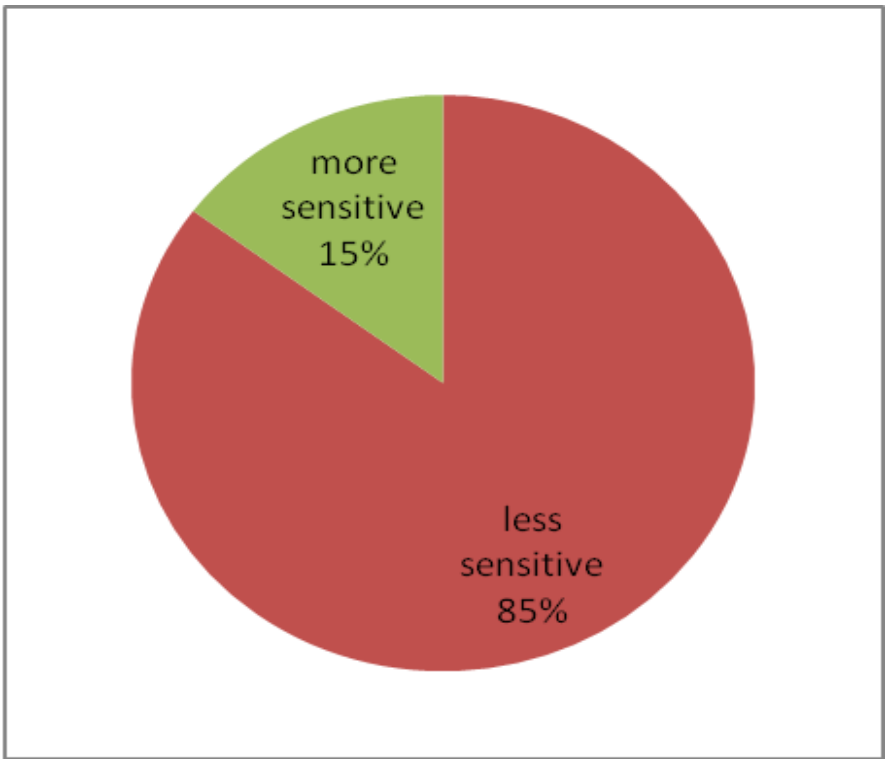


Figure 18. Sensitivity of all isolates to Nimrod (bupirimate) compared to the reference isolate

The charts above suggest that in most cases the mean sensitivity of the tested isolates has been either equal to, or greater than that of the reference isolate. However, the lower levels of inhibition observed in the tests with buprimate (Nimrod) is indicative that the *M. melonis* populations have a reduced sensitivity to the fungicide and this is possibly linked to the greater exposure of *M. melonis* populations to the product over the time period that Nimrod has been in use. The poor sensitivity to chlorothalonil (Bravo 500) and other fungicides tested e.g. Amistar is perhaps indicating that they have little inherent activity against the target pathogen.

However, it should be noted that this type of *in vitro* test has its limitations in as much as it only compares the radial growth of the mycelium on agar, rather than any other mode of action e.g. inhibition of sporulation which may occur *in planta* in a true infection site. Many of the products chosen for this study are marketed with label recommendations for powdery mildew control in cucurbits rather than gummy stem blight, but anecdotal evidence from growers and consultants suggests that they have some activity against *M. melonis*. In the resistance tests conducted here it is not possible to determine how much the inhibition in growth is linked to sensitivity/resistance to the product and how much may be linked to lack of activity of the products against this organism. Effectively therefore this data set acts as a baseline for future studies which may or may not detect shifts in sensitivity from this baseline.

Seed testing

During the latter part of 2010 some initial seed testing was carried out on five batches of cucumber seed received via Derek Hargreaves who had requested them from seed companies. The seed were tested using the method described earlier; although no growing-on tests were carried out on these batches.

A further round of seed testing on a larger range of cultivars and from different suppliers was tested in 2011 (Table 3). All seed batches are shown with code numbers in order to maintain confidentiality.

Table 3. Recovery of *M. melonis* from seed and stems of plants grown from the same samples

Seed batch code	When tested	*M. melonis* detected (% infected seed)	Systemic *M. melonis* detected (stem sections)
E528/S1a	Sept 2010	0	Not tested
E528/S1b	April 2011	On-going	On-going
E528/S2a	Sept 2010	0	Not tested
E528/S2b	May 2011	On-going	On-going
E528/S3a	Sept 2010	0	Not tested
E528/S3b	April 2011	0	0
E528/S4a	Sept 2010	1	Not tested
E528/S4b	April 2011	On-going	On-going
E528/S5*	Sept 2010	3	Not tested
	and		
	May 2011	On-going	On-going
E528/S6	May 2011	0	On-going
E528/S7	April 2011	0	0
E528/S8	May 2011	0	On-going
E528/S9	May 2011	0	0
E528/S10	April 2011	0	0
E528/S11	May 2011	0	0
E528/S12	May 2011	0	On-going
E528/S13	April 2011	0	On-going
E528/S14	May 2011	0	On-going
E528/S15	April 2011	0	0
E528/S16	May 2011	0	0
E528/S17	April 2011	0	0

* re-tested due to significant result
S1a and S1b signify different batches of the same cultivar

During the early tests carried out in 2010 fungal isolates conforming to *M. melonis* morphologically was detected on 2 seed batches (S4 and S5). The tests on batch S5, where there was seed remaining, are being repeated to validate this result as this may be significance. The remainder of these tests are on-going and the full results will be reported at a later date. Where suspect fungal colonies are found they will be inoculated onto fresh cucumber fruit (by wound inoculation) to confirm pathogenicity in cucumber.

Serological immuno-assay results

Of the five antibody types tested only the two IgG purified PAbs exhibited reactivity to *M. melonis* when tested by ELISA or Immunofluorescence (IF) (Plate 10 A, B). The PAbs were then tested by IF for reactivity to a range of other fungal spore types (Table 4) and found to also react with each of the ascosporic fungi tested, the two powdery mildew causing fungi and *Botrytis cinerea*, which is ubiquitous in protected crop production.

To determine the potential of an air sampling system for monitoring aerosols of *M. melonis*, daily counts were made by bright field microscopy and then processed by Immunofluoresence (Plate 10).

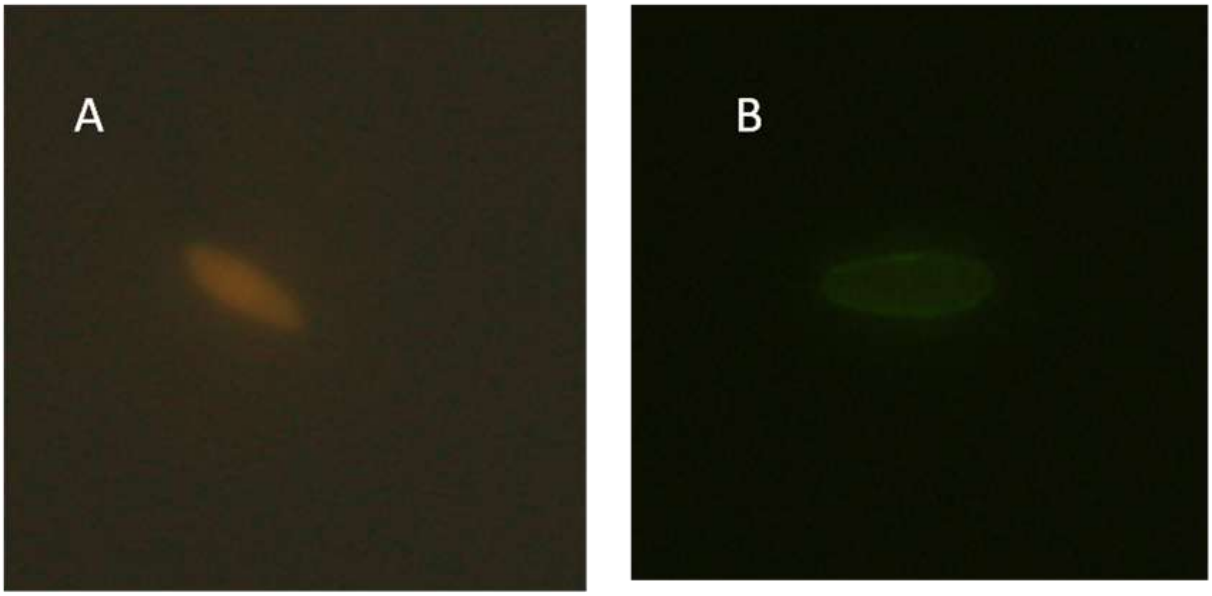


Plate 10. Airborne disease inoculum of *M. melonis* propped with a monoclonal antiserum (A) and IgG prurified polyclonal (B) conjugated to fluorescein isothyiocyanate

Table 4. Reactivity of purified IgG Polyclonal antisera to a range of airborne fungal spore types

Fungal species	**Disease**	**IgG purified Ab Reactivity**
Botrytis cinerea	Grey Mold	✓
Hyaloperonospora parasitica	Downy mildew	X
Erysiphe cruciferarum	Powdery mildew	✓
Oidium neolycopersici	Powdery mildew	✓
Albugo candida	White blister	X
Alternaria brassicae	Leaf spot	X
Alternaria brassicicola	Leaf spot	X
Alternaria alternata	Leaf spot	X
Gaeumannomyces graminis var. *tritici*	Take all	✓
Pyrenopeziza brassicae	Leaf spot	✓
Mycosphaerella pinodes	Leaf spot	✓
Mycosphaerella cryptica	Leaf spot	✓
Mycosphaerella nubilosa	Leaf spot	✓
Mycosphaerella brassicicola	Leaf spot	✓
Sclerotinia sclerotiorum	White rot	✓
Cladosporium spp.	Leaf mould	X
Stemphylium botryosum	Phytopathogenic and saprophytic	X
Penicillium spp.	Common mold	X
Aspergillus spp	Common mold	X
Ascochyta spp.	Leaf spot / blight	X
Mycosphaerella melonis	Gummy stem blight	✓

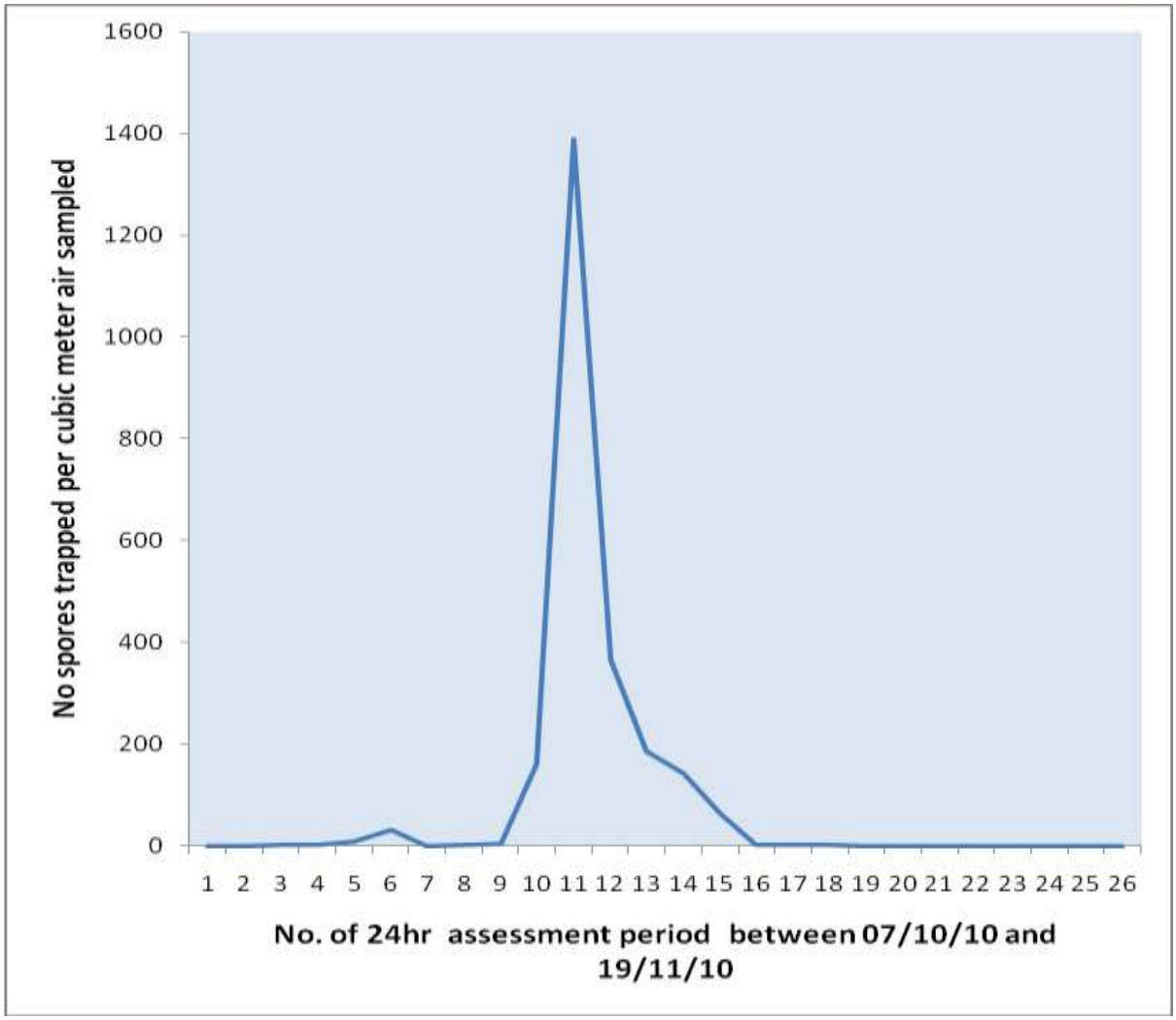


Figure 19. Daily count of spores trapped per cubic meter of air sampled

Discussion

Resistance Testing

The fungicide resistance testing carried out by both ADAS and STC on 28 isolates of *Mycosphaerella melonis* during the 2010 season, using an amended agar plate method to measure mycelial growth rate, provided some interesting results. However, it is important from the outset to recognise the limitations of this test. Firstly, as it measures mycelial growth rate of the pathogen the test will be of little value where the primary mechanism of the fungicide is, for example, as a spore germination inhibitor. Secondly, unless baseline data is available on the original sensitivity of the pathogen prior to the introduction of the active ingredient (and hence exposure of the pathogen) and its widespread use on the crop it can be difficult to differentiate between an inherent poor activity (efficacy) from the

fungicide and the development of insensitive or resistant strains in the pathogen population. In major agricultural crops e.g. cereals the generation of baseline data is a component of the approval process so that the sensitivity of the pathogen can be determined and subsequently monitored in post-approval studies by the manufacturer. Unfortunately the same requirement is not a pre-requisite for minor crops or minor pathogen targets and this therefore presents a specific challenge for the industry in terms of monitoring shifts in pathogen sensitivity following fungicide use. It has been proposed previously (McPherson & O'Neill, pers.com) that baseline data on fungicide sensitivity should be collected routinely in the horticultural sector prior to the introduction of new fungicide groups e.g. strobilurins as otherwise it is difficult to detect any changes once the product is in widespread use.

The data collected from the various isolates has shown that there is a significant difference in the sensitivity of *M. melonis* to the different fungicides and this is perhaps not too surprising. The high level of inhibition from both cyprodinil and fludioxonil (in Switch) is really encouraging, though one isolate (isolate 12 at STC) gives slight cause for concern as this was not particularly sensitive to either active ingredient. This situation, especially on that particular nursery, should be monitored further to see if there is a greater shift in sensitivity following further use of the fungicide.

The overall sensitivity of *M. melonis* to azoxystrobin was quite poor in this agar test and this is a little surprising. Whilst some isolates of *M. melonis* were found that appeared significantly less sensitive to this fungicide we have to consider the merit or otherwise of the agar methodology in relation to the strobilurin fungicides. It may be necessary to consider undertaking *in planta* studies here also. The data does however provide a good baseline data set for comparison purposes later and in this regard it could prove very valuable. It is also important to note at this stage that several isolates at both ADAS and STC showed a reduced level of sensitivity at 2 ppm and this could potentially be the early signs of a shift in the pathogen population. This will need to be monitored closely if the strobilurins are to be used as a component of any spray regime for *Mycosphaerella* control.

The data for bupirimate (Nimord) are quite interesting especially as this fungicide has been used extensively by cucumber growers over many years for powdery mildew control. Compared to the 1978 isolate nearly all the ADAS isolates collected showed a significantly reduced sensitivity to the fungicide and this indicates a considerable shift in the pathogen population over this time. Unfortunately, the STC isolates appeared more variable and the same conclusion could not be drawn here and perhaps this is a reflection of the different fungicide use patterns in the different crops.

The fenhexamid (Teldor) data was similar between the two sites and both data sets indicated a moderate level of activity against *M. melonis*. There were occasional isolates that perhaps showed a slightly reduced sensitivity though whether this is a component of the natural variation in the pathogen population or early signs of a shift in sensitivity is not possible to determine at this stage.

The data for chlorothalonil (Bravo 500) was consistent and showed a low-mediocre level of sensitivity in the *M. melonis* population and this supports the earlier observations by Steekelenburg. As a multi-site protectant fungicide it would be unusual for resistance to occur and the data generated is presumed to represent the natural variation in the fungal population. This data can be used as a baseline in later resistance studies should there be any future concerns about the deterioration in performance.

It is important to recognise that the products used in this component of the study include those commonly utilised by the cucumber industry for *Mycosphaerella* control. However, not all of them are marketed as products for control of this pathogen in cucumbers and only Amistar, Bravo and Switch have a label recommendation for *Mycosphaerella* control. Fortunately though, the pathogen target is not a statutory condition of the approval process so use against other pathogens is acceptable, albeit at the growers own risk. Nimrod has a recommendation for powdery mildew control whilst Teldor is marketed primarily for Botrytis control.

In terms of minimising any resistance risk and formulating effective spray programmes it is important to be aware of the different mode of action groups of the various fungicides. Information is therefore presented in Table 3 which outlines the different fungicide groups together with their FRAC[2] codes.

[2] FRAC : Fungicide Resistance Action Committee (http://www.frac.info/frac/menu.htm)

Table 3. Mode of action of the fungicides used in the in-vitro resistance tests.

Product	Fungicide group	FRAC code	Mode of Action
Amistar	methoxy-acrylates	C3	Quinone outside inhibitor or strobilurin. Inhibition of sporulation and mycelial growth
Bravo 500	chloronitriles (phthalonitriles)	Multi-site activity	Multi-site contact activity
Nimrod	hydroxy-(2-amino-) pyrimidines	A2	Inhibition of nucleic acid bio-synthesis
Switch	anilino-pyrimidines (cyprodinil) phenylpyrroles (fludioxonil)	D1 E2	Amino-acid and protein synthesis Inhibition of sporulation and mycelial growth Inhibition of signal transduction
Teldor	hydroxyanilides	G3	Inhibition of sterol synthesis in membranes

Information from FRAC Code List 2011.

Reduced sensitivity to certain active ingredients used for stem blight (*Mycospherella*) control has been previously reported. Seebold *et al.* (2004) and Stevenson *et al.* (2008) describe evidence of resistance in *Didymella bryoniae* to boscalid and azoxystrobin in Georgia USA. This is discussed more fully in the literature review summary earlier in this report. The UK Fungicide Resistance Action Group (FRAG) website www.pesticides.gov.uk/rags.asp provides information on reported resistance issues in a number of UK crops. They report *Mycosphaerella melonis* resistance to the benzimidazole fungicides e.g. benomyl and carbendazim, and also to the dicarboximides e.g. vinclozolin and iprodione in 2009.

Seed testing

Whilst two batches of the seed tested in 2010 were found to have possible contamination/infestation with fungi conforming to *M. melonis*, these potentially important results require further validation. The tests on these batches are to be repeated along with a wider range of additional cultivars to determine whether there is a potential link between seed-borne inoculum and a possible asymptomatic systemic infection in plants.

Immunoassay development

The initial work carried out on this aspect of the investigation has indicated that the early detection system developed for use with brassicas which was based on *Mycosphaerella brassicicola* is not sufficiently sensitive for use in detecting *M. melonis* ascospores. The additional development work to generate an antiserum with a greater level of specificity to *M. melonis* is well underway. Providing a sufficient level of sensitivity can be generated the antiserum will be used to trap and detect ascospores of *M. melonis* and hence help predict risk periods for the disease in the glasshouse.

Conclusions

- *In vitro* resistance testing showed an excellent reduction in inhibition of mycelial growth when isolates of *M. melonis* were grown on agar amended with cyprodinil and fludioxonil (in Switch) and, to a lesser extent, with Teldor.

- In the same tests the mycelial growth of isolates was only partially inhibited by Amistar, Bravo 500 and Nimrod.

- Preliminary seed testing has shown some interesting results which may support a possible seed-borne route of infection for *M. melonis* in cucumber. These preliminary results require validation and further tests are on-going in this respect.

- The immunoassay development has been hampered slightly by the fact that the existing antisera proved not to be sufficiently sensitive to detect ascospores of *M. melonis*. Work is progressing to develop a new antiserum specifically against this pathogen as opposed to the Brassica pathogen used previously.

Knowledge and Technology Transfer

The results from Phase 1 of the work were discussed at an HDC Project Review Meeting at Stoneleigh on the 21st January 2011. Dr Martin McPherson also presented the results to the Cucumber Growers Association meeting on the 1st February 2011.

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Appendix 1

Cucumber – Improving control of Gummy Stem Blight caused by *Mycosphaerella melonis (Didymella bryoniae) PE 001*

Fungicide resistance testing protocol

Objective: to investigate the effect on mycelial growth of the named fungus using an *in-vitro* test based on the incorporation of various fungicide products into agar at a range of concentrations (0, 2, 20 and 100 ppm ai).

Fungicides to be tested:

Suggested product	Active ingredient	ai concentration	Amount to add to 100 ml SDW to produce 1000ppm stock solution
Amistar (ADAS)	azoxystrobin	250g/L	0.4ml
Bravo 500 (ADAS)	chlorothalonil	500g/L	0.2ml
Unix (STC)	cyprodinil	75% w/w	0.135g (adjusted)
Beret Gold (STC)	fludioxonil	25g/L	4.0ml
Teldor (STC)	fenhexamid	50% w/w	0.2g
Nimrod (ADAS)	bupirimate	250g/L	0.40ml

Cyprodinil and fludioxonil amounts adjusted to match rates of ai's used in Switch.

Materials:

- Isolates of *M. melonis* collected from affected cucumber crops. 7 - 10 day old isolates should be pure and growing on Potato Dextrose Agar (PDA).
- 90cm vented petri-dishes (12/isolate/product)
- PDA powder
- De-ionised water
- 0.5 L Schott bottles
- Autoclave
- 100ml aliquots of SDW
- 1000 ppm stock solutions of fungicide products
- Laminar flow cabinet
- 70% ethanol
- Size 2 (5mm) cork borer
- Scalpel
- Incubator

Method:

Make PDA at normal strength (39g/L) and autoclave as per normal practice. 300ml will produce approx 15-18 plates. 1 bottle will be required/concentration. Allow to cool to approx 50°C.

Make 1000ppm stock solutions of fungicides under test. (1g ai/L water is equivalent to 1000ppm.

e.g. if the product contains 80% ai, dissolve 1.25g of product in 1 litre water. (1/0.8)

or 0.125g in 100ml of water. Amounts for products under investigation is shown in the table above.

The stock solution for each chemical is used to amend the cooled PDA to 0, 2, 20 and 100 ppm (ai).

Assuming 5 *Myco* isolates are to be tested, with 3 replicate plates/concentration.
Therefore for each chemical with 3 reps and 5 isolates, 15 plates/concentration are required, 60 plates in total.

If more than one fungicide is to be tested the 0ppm plates will be the same throughout, and need not be poured for each fungicide.

Preparation of amended agar concentrations:

Concentration	**Amount added to agar/volume**			
	100ml	**200ml**	**300ml**	**400ml**
0ppm	0	0	0	0
2ppm	0.2ml	0.4ml	0.6ml	0.8ml
20ppm	2ml	4ml	6ml	8ml
100ppm	10ml	20ml	30ml	40ml

Add stock solution at the required concentration, to make up to the required volume (e.g. for 400ml of 100ppm, use 360 ml of agar + 40ml of fungicide stock solution). Label the bottle clearly. Pour labelled plates and leave to dry in laminar flow cabinet.

When dry use a sterile size 2 (5mm) cork borer to cut a 7-10 day old culture of the target fungus and place the cores centrally on the labelled plates using a sterile scalpel.

Incubate plates at approx 22 - 25°C and measure diameter of mycelial growth after 3 days. Measure 2 diameters at 90° to each other and record the mean.

Record all measurements on prepared sheets (excel spreadsheet provided). The diameter of the applied plug is subtracted from the overall diameter. The Excel spreadsheet will calculate the % inhibition in growth compared to the 0ppm (control).

Do not record growth that originates from the central plug and does not contact the fungicide amended plate.

If cultures differ greatly in appearance on un-amended agar, classify them into types and note the phenotypes of each isolate on un-amended agar.

Maintain a detailed study diary.

Appendix 2
Raw data sets from resistance testing.
ADAS isolates – azoxystrobin

Product:	Amistar						
Target:							
Date set up:	2010	02-Nov	03-Nov	04-Nov			
Assessment date:	2010	05-Nov	06-Nov	07-Nov			
	Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean**	**minus 5mm**	**% inhibition**
Iso 1	**0ppm**	43	43	41	42.3	37.3	
Reference	**2ppm**	28.5	27	26	27.2	22.2	40.6
	20ppm	25	27	21.5	24.5	19.5	47.8
	100ppm	26	26.5	24.5	25.7	20.7	44.6
Iso 2	**0ppm**	45.5	45.5	39	43.3	38.3	
BX10/35 Leaf	**2ppm**	30	25.5	25	26.8	21.8	41.5
	20ppm	23.5	22	22.5	22.7	17.7	52.7
	100ppm	23	20	20.5	21.2	16.2	56.7
Iso 3	**0ppm**	45	41	39.5	41.8	36.8	
BX10/35 Node	**2ppm**	29	28	24	27.0	22.0	41.1
	20ppm	24.5	24.5	23.5	24.2	19.2	48.7
	100ppm	22	23	21	22.0	17.0	54.5
Iso 4	**0ppm**	46	43.5	39.5	43.0	38.0	
BX10/55a Stem	**2ppm**	28	28	26	27.3	22.3	40.2
	20ppm	22.5	23	22.5	22.7	17.7	52.7
	100ppm	22	22.5	16	20.2	15.2	59.4
Iso 5	**0ppm**	45.5	46.5	42	44.7	39.7	
BX10/55b Stem	**2ppm**	39.5	39.5	36	38.3	33.3	10.7
	20ppm	28	28	28	28.0	23.0	38.4
	100ppm	26.5	27	23.5	25.7	20.7	44.6
Iso 6	**0ppm**	50.5	44.5	45.5	46.8	41.8	
BX10/55b Stem	**2ppm**	42	40.5	37.5	40.0	35.0	6.3
	20ppm	26	29.5	30	28.5	23.5	37.1
	100ppm	27	27.5	24	26.2	21.2	43.3
Iso 7	**0ppm**	41	40.5	32	37.8	32.8	
BX10/56a Petiole	**2ppm**	37	35.5	31.5	34.7	29.7	20.5
	20ppm	25	28	27	26.7	21.7	42.0
	100ppm	26	25.5	27.5	26.3	21.3	42.9
Iso 8	**0ppm**	43.5	41	39	41.2	36.2	
BX10/56b Stem	**2ppm**	37.5	35.5	36	36.3	31.3	16.1
	20ppm	27	31	28	28.7	23.7	36.6
	100ppm	27	29	23.5	26.5	21.5	42.4
Iso 9	**0ppm**	48	42	41	43.7	38.7	
BX10/57a Stem	**2ppm**	40	40.5	35	38.5	33.5	10.3
	20ppm	26.5	26.5	26	26.3	21.3	42.9
	100ppm	26.5	25	23.5	25.0	20.0	46.4
Iso 10	**0ppm**	43.5	41	42.5	42.3	37.3	
BX10/57b Stem	**2ppm**	27.5	26.5	26.5	26.8	21.8	41.5
	20ppm	23.5	25	21.5	23.3	18.3	50.9
	100ppm	23.5	23.5	20.5	22.5	17.5	53.1
Iso 11	**0ppm**	42	36.5	45	41.2	36.2	
BX10/58 Stem	**2ppm**	27.5	29.5	27.5	28.2	23.2	37.9
	20ppm	23	24	22.5	23.2	18.2	51.3
	100ppm	23.5	23.5	23.5	23.5	18.5	50.4
Iso 12	**0ppm**	48	45.5	42.5	45.3	40.3	
BX10/58 Stem	**2ppm**	25	23.5	25	24.5	19.5	47.8
	20ppm	23.5	25	22	23.5	18.5	50.4
	100ppm	23	24.5	23.5	23.7	18.7	50.0
Iso 13	**0ppm**	48	46	43.5	45.8	40.8	
BX10/63 Fruit	**2ppm**	29	25.5	27.5	27.3	22.3	40.2
	20ppm	24.5	24	24.5	24.3	19.3	48.2
	100ppm	24	23.5	23	23.5	18.5	50.4
Iso 14	**0ppm**	35	35.5	34	34.8	29.8	
BX10/63 Leaf	**2ppm**	21	21.5	25.5	22.7	17.7	52.7
	20ppm	20	19.5	24	21.2	16.2	56.7
	100ppm	19.5	20	21	20.2	15.2	59.4
Iso 15	**0ppm**	48	46.5	43	45.8	40.8	
BX10/63 Stem	**2ppm**	25	26	26	25.7	20.7	44.6
	20ppm	25	27	25	25.7	20.7	44.6
	100ppm	25	25	24	24.7	19.7	47.3

ADAS Isolates – chlorothalonil

Product:	Bravo 500						
Target:							
Date set up:	2010	02-Nov	03-Nov	04-Nov			
Assessment date:	2010	05-Nov	06-Nov	07-Nov			
	Concentration	Rep1	Rep2	Rep3	Mean	minus 5mm	% inhibition
Iso 1	0ppm	43	43	41	42.3	37.3	
Reference	2ppm	28.5	28	26	27.5	22.5	39.7
	20ppm	28	22	20	23.3	18.3	50.9
	100ppm	17.5	17	19.5	18.0	13.0	65.2
Iso 2	0ppm	45.5	45.5	39	43.3	38.3	
BX10/35 Leaf	2ppm	29	27	28	28.0	23.0	38.4
	20ppm	28.5	26	22	25.5	20.5	45.1
	100ppm	19.5	20	19	19.5	14.5	61.2
Iso 3	0ppm	45	41	39.5	41.8	36.8	
BX10/35 Node	2ppm	30.5	31	25.5	29.0	24.0	35.7
	20ppm	31	27	24	27.3	22.3	40.2
	100ppm	18.5	19	19	18.8	13.8	62.9
Iso 4	0ppm	46	43.5	39.5	43.0	38.0	
BX10/55a Stem	2ppm	32.5	28	28.5	29.7	24.7	33.9
	20ppm	28	25	21.5	24.8	19.8	46.9
	100ppm	19	19.5	18	18.8	13.8	62.9
Iso 5	0ppm	45.5	46.5	42	44.7	39.7	
BX10/55b Stem	2ppm	32.5	28	28.5	29.7	24.7	33.9
	20ppm	28	30	25.5	27.8	22.8	38.8
	100ppm	13.5	21.5	22	19.0	14.0	62.5
Iso 6	0ppm	50.5	44.5	45.5	46.8	41.8	
BX10/55b Stem	2ppm	34	30	30	31.3	26.3	29.5
	20ppm	30	27.5	26.5	28.0	23.0	38.4
	100ppm	22	22	20.5	21.5	16.5	55.8
Iso 7	0ppm	41	40.5	32	37.8	32.8	
BX10/56a Petiole	2ppm	28	26	28	27.3	22.3	40.2
	20ppm	20	22.5	23	21.8	16.8	54.9
	100ppm	18.5	20	19.5	19.3	14.3	61.6
Iso 8	0ppm	43.5	41	39	41.2	36.2	
BX10/56b Stem	2ppm	28	29	27.5	28.2	23.2	37.9
	20ppm	21	24	21.5	22.2	17.2	54.0
	100ppm	20	22.5	19	20.5	15.5	58.5
Iso 9	0ppm	48	42	41	43.7	38.7	
BX10/57a Stem	2ppm	30.5	28.5	29	29.3	24.3	34.8
	20ppm	25	23	22.5	23.5	18.5	50.4
	100ppm	19	19	20.5	19.5	14.5	61.2
Iso 10	0ppm	43.5	41	42.5	42.3	37.3	
BX10/57b Stem	2ppm	30	28	28	28.7	23.7	36.6
	20ppm	24.5	24	22	23.5	18.5	50.4
	100ppm	19	20.5	19.5	19.7	14.7	60.7
Iso 11	0ppm	42	36.5	45	41.2	36.2	
BX10/58 Stem	2ppm	28	28.5	29	28.5	23.5	37.1
	20ppm	23	24.5	23	23.5	18.5	50.4
	100ppm	20	18	21	19.7	14.7	60.7
Iso 12	0ppm	48	45.5	42.5	45.3	40.3	
BX10/58 Stem	2ppm	32	28	33.5	31.2	26.2	29.9
	20ppm	27.5	25	24.5	25.7	20.7	44.6
	100ppm	18	20	19	19.0	14.0	62.5
Iso 13	0ppm	48	46	43.5	45.8	40.8	
BX10/63 Fruit	2ppm	29.5	28.5	31	29.7	24.7	33.9
	20ppm	27.5	25.5	23.5	25.5	20.5	45.1
	100ppm	21	23	18.5	20.8	15.8	57.6
Iso 14	0ppm	35	35.5	34	34.8	29.8	
BX10/63 Leaf	2ppm	26	25	26	25.7	20.7	44.6
	20ppm	20.5	22	19	20.5	15.5	58.5
	100ppm	16.5	16.5	17	16.7	11.7	68.8
Iso 15	0ppm	48	46.5	43	45.8	40.8	
BX10/63 Stem	2ppm	34.5	31.5	32.5	32.8	27.8	25.4
	20ppm	26	27	26	26.3	21.3	42.9
	100ppm	20	20	21.5	20.5	15.5	58.5

ADAS isolates – cyprodinil

Product:	Unix						
Target:							
Date set up:	2010	02-Nov	03-Nov	04-Nov			
Assessment date:	2010	05-Nov	06-Nov	07-Nov			
	Concentration	Rep1	Rep2	Rep3	Mean	minus 5mm	% inhibition
Iso 1	0ppm	43	43	41	42.3	37.3	
Reference	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 2	0ppm	45.5	45.5	39	43.3	38.3	
BX10/35 Leaf	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 3	0ppm	45	41	39.5	41.8	36.8	
BX10/35 Node	2ppm	5.5	5	5	5.2	0.2	99.6
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 4	0ppm	46	43.5	39.5	43.0	38.0	
BX10/55a Stem	2ppm	6.5	5	5	5.5	0.5	98.7
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 5	0ppm	45.5	46.5	42	44.7	39.7	
BX10/55b Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
			#DIV/0!				
Iso 6	0ppm	50.5	44.5	45.5	46.8	41.8	
BX10/55b Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 7	0ppm	41	40.5	32	37.8	32.8	
BX10/56a Petiole	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 8	0ppm	43.5	41	39	41.2	36.2	
BX10/56b Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 9	0ppm	48	42	41	43.7	38.7	
BX10/57a Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 10	0ppm	43.5	41	42.5	42.3	37.3	
BX10/57b Stem	2ppm	6	5	5	5.3	0.3	99.1
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 11	0ppm	42	36.5	45	41.2	36.2	
BX10/58 Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 12	0ppm	48	45.5	42.5	45.3	40.3	
BX10/58 Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 13	0ppm	48	46	43.5	45.8	40.8	
BX10/63 Fruit	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 14	0ppm	35	35.5	34	34.8	29.8	
BX10/63 Leaf	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 15	0ppm	48	46.5	43	45.8	40.8	
BX10/63 Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0

ADAS isolates – fludioxonil

Product:	Beret Gold						
Target:							
Date set up:	2010	02-Nov	03-Nov	04-Nov			
Assessment date:	2010	05-Nov	06-Nov	07-Nov			
	Concentration	Rep1	Rep2	Rep3	Mean	minus 5mm	% inhibition
Iso 1	0ppm	43	43	41	42.3	37.3	
Reference	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 2	0ppm	45.5	45.5	39	43.3	38.3	
BX10/35 Leaf	2ppm	5	8	7	6.7	1.7	95.5
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 3	0ppm	45	41	39.5	41.8	36.8	
BX10/35 Node	2ppm	5	7.5	5	5.8	0.8	97.8
	20ppm	5	5.5	5	5.2	0.2	99.6
	100ppm	5	5	5	5.0	0.0	100.0
Iso 4	0ppm	46	43.5	39.5	43.0	38.0	
BX10/55a Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 5	0ppm	45.5	46.5	42	44.7	39.7	
BX10/55b Stem	2ppm	5	5	6.5	5.5	0.5	98.7
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 6	0ppm	50.5	44.5	45.5	46.8	41.8	
BX10/55b Stem	2ppm	5	9	6.5	6.8	1.8	95.1
	20ppm	5	5	5.5	5.2	0.2	99.6
	100ppm	5	5	5.5	5.2	0.2	99.6
Iso 7	0ppm	41	40.5	32	37.8	32.8	
BX10/56a Petiole	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 8	0ppm	43.5	41	39	41.2	36.2	
BX10/56b Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 9	0ppm	48	42	41	43.7	38.7	
BX10/57a Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 10	0ppm	43.5	41	42.5	42.3	37.3	
BX10/57b Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 11	0ppm	42	36.5	45	41.2	36.2	
BX10/58 Stem	2ppm	5	7.5	5	5.8	0.8	97.8
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 12	0ppm	48	45.5	42.5	45.3	40.3	
BX10/58 Stem	2ppm	5	6	6	5.7	0.7	98.2
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 13	0ppm	48	46	43.5	45.8	40.8	
BX10/63 Fruit	2ppm	5	7.5	5	5.8	0.8	97.8
	20ppm	5	12.5	5	7.5	2.5	93.3
	100ppm	5	5	5	5.0	0.0	100.0
Iso 14	0ppm	35	35.5	34	34.8	29.8	
BX10/63 Leaf	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 15	0ppm	48	46.5	43	45.8	40.8	
BX10/63 Stem	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0

ADAS isolates – fenhexamid

Product:	Teldor						
Target:							
Date set up:	2010	02-Nov	03-Nov	04-Nov			
Assessment date:	2010	05-Nov	06-Nov	07-Nov			
	Concentration	Rep1	Rep2	Rep3	Mean	minus 5mm	% inhibition
Iso 1	0ppm	43	43	41	42.3	37.3	
Reference	2ppm	25	28	19.5	24.2	19.2	48.7
	20ppm	12.5	15	8.5	12.0	7.0	81.3
	100ppm	9.5	12	11	10.8	5.8	84.4
Iso 2	0ppm	45.5	45.5	39	43.3	38.3	
BX10/35 Leaf	2ppm	25	21	18	21.3	16.3	56.3
	20ppm	14	10	9	11.0	6.0	83.9
	100ppm	9	7.5	8	8.2	3.2	91.5
Iso 3	0ppm	45	41	39.5	41.8	36.8	
BX10/35 Node	2ppm	24	23.5	22	23.2	18.2	51.3
	20ppm	10	10	9	9.7	4.7	87.5
	100ppm	8	8	7.5	7.8	2.8	92.4
Iso 4	0ppm	46	43.5	39.5	43.0	38.0	
BX10/55a Stem	2ppm	25.5	25.5	19.5	23.5	18.5	50.4
	20ppm	9.5	10	10.5	10.0	5.0	86.6
	100ppm	6.5	7.5	7.5	7.2	2.2	94.2
Iso 5	0ppm	45.5	46.5	42	44.7	39.7	
BX10/55b Stem	2ppm	31	26.5	22	26.5	21.5	42.4
	20ppm	13	13	10.5	12.2	7.2	80.8
	100ppm	10	11	10.5	10.5	5.5	85.3
Iso 6	0ppm	50.5	44.5	45.5	46.8	41.8	
BX10/55b Stem	2ppm	23.5	27	26	25.5	20.5	45.1
	20ppm	13.5	12.5	11	12.3	7.3	80.4
	100ppm	11	10	10	10.3	5.3	85.7
Iso 7	0ppm	41	40.5	32	37.8	32.8	
BX10/56a Petiole	2ppm	25.5	26	17.5	23.0	18.0	51.8
	20ppm	11	12	10.5	11.2	6.2	83.5
	100ppm	9	7	6.5	7.5	2.5	93.3
Iso 8	0ppm	43.5	41	39	41.2	36.2	
BX10/56b Stem	2ppm	24	22	22.5	22.8	17.8	52.2
	20ppm	8.5	9.5	8	8.7	3.7	90.2
	100ppm	6.5	5.5	8.5	6.8	1.8	95.1
Iso 9	0ppm	48	42	41	43.7	38.7	
BX10/57a Stem	2ppm	24	27.5	21.5	24.3	19.3	48.2
	20ppm	10.5	8.5	8.5	9.2	4.2	88.8
	100ppm	7	7	7.5	7.2	2.2	94.2
Iso 10	0ppm	43.5	41	42.5	42.3	37.3	
BX10/57b Stem	2ppm	24.5	24	18.5	22.3	17.3	53.6
	20ppm	10	8	8	8.7	3.7	90.2
	100ppm	7.5	5	7	6.5	1.5	96.0
Iso 11	0ppm	42	36.5	45	41.2	36.2	
BX10/58 Stem	2ppm	23	22.5	24.5	23.3	18.3	50.9
	20ppm	9.5	8.5	9.5	9.2	4.2	88.8
	100ppm	7	7	8	7.3	2.3	93.8
Iso 12	0ppm	48	45.5	42.5	45.3	40.3	
BX10/58 Stem	2ppm	25.5	24.5	23.5	24.5	19.5	47.8
	20ppm	11.5	10	10	10.5	5.5	85.3
	100ppm	8	7	8.5	7.8	2.8	92.4
Iso 13	0ppm	48	46	43.5	45.8	40.8	
BX10/63 Fruit	2ppm	24	23.5	24	23.8	18.8	49.6
	20ppm	10	11	10.5	10.5	5.5	85.3
	100ppm	8.5	8.5	7.5	8.2	3.2	91.5
Iso 14	0ppm	35	35.5	34	34.8	29.8	
BX10/63 Leaf	2ppm	34	33	31	32.7	27.7	25.9
	20ppm	25.5	30	26	27.2	22.2	40.6
	100ppm	24	24	22	23.3	18.3	50.9
Iso 15	0ppm	48	46.5	43	45.8	40.8	
BX10/63 Stem	2ppm	25	27.5	23.5	25.3	20.3	45.5
	20ppm	9.5	14	12	11.8	6.8	81.7
	100ppm	8.5	8.5	8	8.3	3.3	91.1

ADAS isolates – bupirimate

Product:	Nimrod						
Target:							
Date set up:	2010	02-Nov	03-Nov	04-Nov			
Assessment date:	2010	05-Nov	06-Nov	07-Nov			
	Concentration	Rep1	Rep2	Rep3	Mean	minus 5mm	% inhibition
Iso 1	0ppm	43	43	41	42.3	37.3	
Reference	2ppm	23.5	36	27	28.8	23.8	36.2
	20ppm	13	19	17	16.3	11.3	69.6
	100ppm	8.5	9	8.5	8.7	3.7	90.2
Iso 2	0ppm	45.5	45.5	39	43.3	38.3	
BX10/35 Leaf	2ppm	39.5	39	29.5	36.0	31.0	17.0
	20ppm	23	18.5	18.5	20.0	15.0	59.8
	100ppm	12.5	10	10	10.8	5.8	84.4
Iso 3	0ppm	45	41	39.5	41.8	36.8	
BX10/35 Node	2ppm	38	37	34.5	36.5	31.5	15.6
	20ppm	23	20.5	18.5	20.7	15.7	58.0
	100ppm	12	10.5	9	10.5	5.5	85.3
Iso 4	0ppm	46	43.5	39.5	43.0	38.0	
BX10/55a Stem	2ppm	36.5	40	31	35.8	30.8	17.4
	20ppm	21.5	18	18.5	19.3	14.3	61.6
	100ppm	11	11	11.5	11.2	6.2	83.5
Iso 5	0ppm	45.5	46.5	42	44.7	39.7	
BX10/55b Stem	2ppm	41.5	39.5	32.5	37.8	32.8	12.1
	20ppm	22	20.5	20.5	21.0	16.0	57.1
	100ppm	12	11	9.5	10.8	5.8	84.4
Iso 6	0ppm	50.5	44.5	45.5	46.8	41.8	
BX10/55b Stem	2ppm	40.5	40	34.5	38.3	33.3	10.7
	20ppm	23	20.5	19	20.8	15.8	57.6
	100ppm	11	10	9	10.0	5.0	86.6
Iso 7	0ppm	41	40.5	32	37.8	32.8	
BX10/56a Petiole	2ppm	35	38.5	32	35.2	30.2	19.2
	20ppm	20.5	20.5	17.5	19.5	14.5	61.2
	100ppm	10	10.5	10	10.2	5.2	86.2
Iso 8	0ppm	43.5	41	39	41.2	36.2	
BX10/56b Stem	2ppm	38	40	32	36.7	31.7	15.2
	20ppm	21.5	21.5	20	21.0	16.0	57.1
	100ppm	11	9	10	10.0	5.0	86.6
Iso 9	0ppm	48	42	41	43.7	38.7	
BX10/57a Stem	2ppm	36.5	36.5	30	34.3	29.3	21.4
	20ppm	23	21.5	18.5	21.0	16.0	57.1
	100ppm	9	10	9	9.3	4.3	88.4
Iso 10	0ppm	43.5	41	42.5	42.3	37.3	
BX10/57b Stem	2ppm	37.5	38	35	36.8	31.8	14.7
	20ppm	22	22.5	20	21.5	16.5	55.8
	100ppm	12	10.5	9	10.5	5.5	85.3
Iso 11	0ppm	42	36.5	45	41.2	36.2	
BX10/58 Stem	2ppm	37.5	33	30	33.5	28.5	23.7
	20ppm	19.5	22.5	19	20.3	15.3	58.9
	100ppm	11.5	11.5	11	11.3	6.3	83.0
Iso 12	0ppm	48	45.5	42.5	45.3	40.3	
BX10/58 Stem	2ppm	35	37.5	35	35.8	30.8	17.4
	20ppm	20	20	17	19.0	14.0	62.5
	100ppm	12	11	9.5	10.8	5.8	84.4
Iso 13	0ppm	48	46	43.5	45.8	40.8	
BX10/63 Fruit	2ppm	39	41	35	38.3	33.3	10.7
	20ppm	18	21	20.5	19.8	14.8	60.3
	100ppm	11	11	10.5	10.8	5.8	84.4
Iso 14	0ppm	35	35.5	34	34.8	29.8	
BX10/63 Leaf	2ppm	22.5	31.5	32	28.7	23.7	36.6
	20ppm	18	26.5	23	22.5	17.5	53.1
	100ppm	14	13	13	13.3	8.3	77.7
Iso 15	0ppm	48	46.5	43	45.8	40.8	
BX10/63 Stem	2ppm	37	38	37.5	37.5	32.5	12.9
	20ppm	22	24.5	20	22.2	17.2	54.0
	100ppm	11	11.5	10.5	11.0	6.0	83.9

STC isolates – azoxystrobin

Product:	Amistar						
Target:							
Isolates 1,6,7,8,9 set up: 27/9/10- 28/9/10. Assessed 30/9/10 - 31/9/10.							
Isolates 10,11,14,17,18 set up: 4/10/10- 5/10/10. Assessed 7/10/10 - 8/10/10.							
Isolates 2,3,4,13,18(Repeat) set up: 22/11/10. Assessed 25/11/10.							
	Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean**	**minus 5mm**	**% inhibition**
Iso 1	**0ppm**	51	51	52.5	51.5	46.5	
	2ppm	33.5	36	34.5	34.7	29.7	36.2
	20ppm	27.5	26	27.5	27.0	22.0	52.7
	100ppm	21.5	23	22.5	22.3	17.3	62.7
Iso 2	**0ppm**	54	53	51	52.7	47.7	
	2ppm	33	33	33	33.0	28.0	41.3
	20ppm	25	21	26	24.0	19.0	60.1
	100ppm	13	12	13	12.7	7.7	83.9
Iso 3	**0ppm**	47.5	46	45.5	46.3	41.3	
	2ppm	31	29	39.5	33.2	28.2	31.9
	20ppm	20	22	22.5	21.5	16.5	60.1
	100ppm	20	20.5	17.5	19.3	14.3	65.3
Iso 4	**0ppm**	54	53	51	52.7	47.7	
	2ppm	34.5	36	34	34.8	29.8	37.4
	20ppm	20.5	22	19	20.5	15.5	67.5
	100ppm	18	18.5	15	17.2	12.2	74.5
Iso 5	**0ppm**	40	45	37	40.7	35.7	
	2ppm	29	30.5	31.5	30.3	25.3	29.0
	20ppm	20	17	18.5	18.5	13.5	62.1
	100ppm	17	16.5	16.5	16.7	11.7	67.3
Iso 6	**0ppm**	51	50	45	48.7	43.7	
	2ppm	31.5	32.5	34	32.7	27.7	36.6
	20ppm	22	29	24.5	25.2	20.2	53.8
	100ppm	14	18	20	17.3	12.3	71.8
Iso 7	**0ppm**	48.5	47	44	46.5	41.5	
	2ppm	14	22.5	19	18.5	13.5	67.5
	20ppm	7	15	13	11.7	6.7	83.9
	100ppm	5	5	5	5.0	0.0	100.0
Iso 8	**0ppm**	46.5	50	48	48.2	43.2	
	2ppm	45	46	46.5	45.8	40.8	5.4
	20ppm	33.5	34.5	35	34.3	29.3	32.0
	100ppm	26	27.5	27.5	27.0	22.0	49.0
Iso 9	**0ppm**	50	*	40	45.0	40.0	
	2ppm	29	31.5	29.5	30.0	25.0	37.5
	20ppm	*	24	25.5	24.8	19.8	50.6
	100ppm	20	19	20	19.7	14.7	63.3
Iso 10	**0ppm**	49	49	52	50.0	45.0	
	2ppm	31.5	31.5	31.5	31.5	26.5	41.1
	20ppm	20	21	25	22.0	17.0	62.2
	100ppm	22.5	22	22.5	22.3	17.3	61.5
Iso 11	**0ppm**	55	55.5	56	55.5	50.5	
	2ppm	47.5	50	50	49.2	44.2	12.5
	20ppm	32.5	32.5	32.5	32.5	27.5	45.5
	100ppm	27	27	26	26.7	21.7	57.1
Iso 12	**0ppm**	15	14	15	14.7	9.7	
	2ppm	13	13	13	13.0	8.0	17.2
	20ppm	13	10.5	11	11.5	6.5	32.8
	100ppm	10.5	11	10	10.5	5.5	43.1
Iso 13	**0ppm**	57	55	55.5	55.8	50.8	
	2ppm	33	32.5	34.5	33.3	28.3	44.3
	20ppm	28	27.5	27.5	27.7	22.7	55.4
	100ppm	22.5	22	22	22.2	17.2	66.2
Iso 14	**0ppm**	45	46	46.5	45.8	40.8	
	2ppm	33	32.5	32.5	32.7	27.7	32.2
	20ppm	19	17	19	18.3	13.3	67.3
	100ppm	17	14	15.5	15.5	10.5	74.3
	* Contamination on plate affected growth						

STC isolates – chlorothalonil

Product:	Bravo 500						
Target:							
Isolates 1,6,7,8,9 set up: 27/9/10- 28/9/10. Assessed 30/9/10 - 31/9/10.							
Isolates 10,11,14,17,18 set up: 4/10/10- 5/10/10. Assessed 7/10/10 - 8/10/10.							
Isolates 2,3,4,13,18(Repeat) set up: 22/11/10. Assessed 25/11/10.							
	Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean**	**minus 5mm**	**% inhibition**
Iso 1	**0ppm**	51	51	52.5	51.5	46.5	
	2ppm	35.5	38	38.5	37.3	32.3	30.5
	20ppm	26.5	28.5	29.5	28.2	23.2	50.2
	100ppm	25	23	26	24.7	19.7	57.7
Iso 2	**0ppm**	54	53	51	52.7	47.7	
	2ppm	38.5	38.5	39.5	38.8	33.8	29.0
	20ppm	25	22.5	25.5	24.3	19.3	59.4
	100ppm	21	20	21.5	20.8	15.8	66.8
Iso 3	**0ppm**	47.5	46	45.5	46.3	41.3	
	2ppm	35	35	31	33.7	28.7	30.6
	20ppm	26	14*	16*	26.0	21.0	49.2
	100ppm	18	11*	19	18.5	13.5	67.3
Iso 4	**0ppm**	54	53	51	52.7	47.7	
	2ppm	33.5	18*	17.5*	33.5	28.5	40.2
	20ppm	24.5	15*	26	25.3	20.3	57.5
	100ppm	19.5	21	19	19.8	14.8	68.9
Iso 5	**0ppm**	40	45	37	40.7	35.7	
	2ppm	31	29	28.5	29.5	24.5	31.3
	20ppm	24	24.5	26.5	25.0	20.0	43.9
	100ppm	19.5	20	20	19.8	14.8	58.4
Iso 6	**0ppm**	51	50	45	48.7	43.7	
	2ppm	34	34	34	34.0	29.0	33.6
	20ppm	24.5	25	25.5	25.0	20.0	54.2
	100ppm	21.5	20	19	20.2	15.2	65.3
Iso 7	**0ppm**	48.5	47	44	46.5	41.5	
	2ppm	20	19	29	22.7	17.7	57.4
	20ppm	17.5	17.5	23	19.3	14.3	65.5
	100ppm	9	7	7	7.7	2.7	93.6
Iso 8	**0ppm**	46.5	50	48	48.2	43.2	
	2ppm	38.5	36	40	38.2	33.2	23.2
	20ppm	23	25	27.5	25.2	20.2	53.3
	100ppm	21	23	23.5	22.5	17.5	59.5
Iso 9	**0ppm**	50	*	40	45.0	40.0	
	2ppm	31	31	30.5	30.8	25.8	35.4
	20ppm	24.5	22.5	24	23.7	18.7	53.3
	100ppm	19	19.5	18.5	19.0	14.0	65.0
Iso 10	**0ppm**	49	49	52	50.0	45.0	
	2ppm	33	35	34	34.0	29.0	35.6
	20ppm	27	27	28	27.3	22.3	50.4
	100ppm	23	23	22	22.7	17.7	60.7
Iso 11	**0ppm**	55	55.5	56	55.5	50.5	
	2ppm	42	42	43.5	42.5	37.5	25.7
	20ppm	28.5	28	28	28.2	23.2	54.1
	100ppm	25	24.5	23	24.2	19.2	62.0
Iso 12	**0ppm**	15	14	15	14.7	9.7	
	2ppm	11.25	11.5	11.75	11.5	6.5	32.8
	20ppm	13	11.5	13	12.5	7.5	22.4
	100ppm	11	11	11	11.0	6.0	37.9
Iso 13	**0ppm**	57	55	55.5	55.8	50.8	
	2ppm	34	33	34	33.7	28.7	43.6
	20ppm	25.5	27	28	26.8	21.8	57.0
	100ppm	24	23	23	23.3	18.3	63.9
Iso 14	**0ppm**	45	46	46.5	45.8	40.8	
	2ppm	31	33.5	32	32.2	27.2	33.5
	20ppm	25.5	23.5	25	24.7	19.7	51.8
	100ppm	19	19	19	19.0	14.0	65.7
	* Contamination on plate affected growth						

STC isolates – cyprodinil

Product:	Unix						
Target:							
Isolates 1,6,7,8,9 set up: 27/9/10- 28/9/10. Assessed 30/9/10 - 31/9/10.							
Isolates 10,11,14,17,18 set up: 4/10/10- 5/10/10. Assessed 7/10/10 - 8/10/10.							
Isolates 2,3,4,13,18(Repeat) set up: 22/11/10. Assessed 25/11/10.							
	Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean**	**minus 5mm**	**% inhibition**
Iso 1	**0ppm**	51	51	52.5	51.5	46.5	
	2ppm	9.5	10	8	9.2	4.2	91.0
	20ppm	7	7	6	6.7	1.7	96.4
	100ppm	5	5	5	5.0	0.0	100.0
Iso 2	**0ppm**	54	53	51	52.7	47.7	
	2ppm	7	10	8	8.3	3.3	93.0
	20ppm	5	8	5	6.0	1.0	97.9
	100ppm	5	5	5	5.0	0.0	100.0
Iso 3	**0ppm**	47.5	46	45.5	46.3	41.3	
	2ppm	11	7	11	9.7	4.7	88.7
	20ppm	7	8.5	5	6.8	1.8	95.6
	100ppm	5	5	5	5.0	0.0	100.0
Iso 4	**0ppm**	54	53	51	52.7	47.7	
	2ppm	10	10	10	10.0	5.0	89.5
	20ppm	8	7	7	7.3	2.3	95.1
	100ppm	5	5	7	5.7	0.7	98.6
Iso 5	**0ppm**	40	45	37	40.7	35.7	
	2ppm	16.5	18	16	16.8	11.8	66.8
	20ppm	9.5	8.5	9	9.0	4.0	88.8
	100ppm	8	8	6.5	7.5	2.5	93.0
Iso 6	**0ppm**	51	50	45	48.7	43.7	
	2ppm	9	9	8.5	8.8	3.8	91.2
	20ppm	5	7	5	5.7	0.7	98.5
	100ppm	5	5	5	5.0	0.0	100.0
Iso 7	**0ppm**	48.5	47	44	46.5	41.5	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 8	**0ppm**	46.5	50	48	48.2	43.2	
	2ppm	10	10	8	9.3	4.3	90.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 9	**0ppm**	50	*	40	45.0	40.0	
	2ppm	5*	10.5	17.5	14.0	9.0	77.5
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 10	**0ppm**	49	49	52	50.0	45.0	
	2ppm	7.5	9	8	8.2	3.2	93.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 11	**0ppm**	55	55.5	56	55.5	50.5	
	2ppm	12	9.5	11	10.8	5.8	88.4
	20ppm	7	7.5	7	7.2	2.2	95.7
	100ppm	5	5	5	5.0	0.0	100.0
Iso 12	**0ppm**	15	14	15	14.7	9.7	
	2ppm	14	14	13.5	13.8	8.8	8.6
	20ppm	12	12.5	12	12.2	7.2	25.9
	100ppm	12.25	12	11.5	11.9	6.9	28.4
Iso 13	**0ppm**	57	55	55.5	55.8	50.8	
	2ppm	9	6	8.5	7.8	2.8	94.4
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 14	**0ppm**	45	46	46.5	45.8	40.8	
	2ppm	11	10	10.5	10.5	5.5	86.5
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
	* Contamination on plate affected growth						

STC isolates – fludioxonil

Product:	Beret Gold						
Target:							
Isolates 1,6,7,8,9 set up: 27/9/10- 28/9/10. Assessed 30/9/10 - 31/9/10.							
Isolates 10,11,14,17,18 set up: 4/10/10- 5/10/10. Assessed 7/10/10 - 8/10/10.							
Isolates 2,3,4,13,18(Repeat) set up: 22/11/10. Assessed 25/11/10.							
	Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean**	**minus 5mm**	**% inhibition**
Iso 1	**0ppm**	51	51	52.5	51.5	46.5	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 2	**0ppm**	54	53	51	52.7	47.7	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 3	**0ppm**	47.5	46	45.5	46.3	41.3	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 4	**0ppm**	54	53	51	52.7	47.7	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 5	**0ppm**	40	45	37	40.7	35.7	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 6	**0ppm**	51	50	45	48.7	43.7	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 7	**0ppm**	48.5	47	44	46.5	41.5	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 8	**0ppm**	46.5	50	48	48.2	43.2	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 9	**0ppm**	50	*	40	45.0	40.0	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 10	**0ppm**	49	49	52	50.0	45.0	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 11	**0ppm**	55	55.5	56	55.5	50.5	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 12	**0ppm**	15	14	15	14.7	9.7	
	2ppm	11	11	11	11.0	6.0	37.9
	20ppm	13.5	13	13.5	13.3	8.3	13.8
	100ppm	12	13.5	12.5	12.7	7.7	20.7
Iso 13	**0ppm**	57	55	55.5	55.8	50.8	
	2ppm	5	10	5	6.7	1.7	96.7
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
Iso 14	**0ppm**	45	46	46.5	45.8	40.8	
	2ppm	5	5	5	5.0	0.0	100.0
	20ppm	5	5	5	5.0	0.0	100.0
	100ppm	5	5	5	5.0	0.0	100.0
	* Contamination on plate affected growth						

STC isolates – fenhexamid

Fungicide Resistance Testing							
Product:	Teldor						
Target:							
Set Up: 8/2/11							
Assessed: 11/2/11							
	Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean**	**minus 5mm**	**% inhibition**
Iso 1	**0ppm**	51	51	52.5	51.5	46.5	
	2ppm	41	42	38	40.3	35.3	24.0
	20ppm	12	16	12	13.3	8.3	82.1
	100ppm	6	9	9	8.0	3.0	93.5
Iso 2	**0ppm**	54	53	51	52.7	47.7	
	2ppm	32.5	31	32	31.8	26.8	43.7
	20ppm	5	8	5	6.0	1.0	97.9
	100ppm	5	6	5	5.3	0.3	99.3
Iso 3	**0ppm**	47.5	46	45.5	46.3	41.3	
	2ppm	28.5	33	30	30.5	25.5	38.3
	20ppm	10	14	13	12.3	7.3	82.3
	100ppm	7	8	7	7.3	2.3	94.4
Iso 4	**0ppm**	54	53	51	52.7	47.7	
	2ppm	33	31	33	32.3	27.3	42.7
	20ppm	9	9	9	9.0	4.0	91.6
	100ppm	7	9	9	8.3	3.3	93.0
Iso 5	**0ppm**	40	45	37	40.7	35.7	
	2ppm	13	22.5	19	18.2	13.2	63.1
	20ppm	8	8	12	9.3	4.3	87.9
	100ppm	6	6	6	6.0	1.0	97.2
Iso 6	**0ppm**	51	50	45	48.7	43.7	
	2ppm	35.5	*	35	35.3	30.3	30.7
	20ppm	15	13	15	14.3	9.3	78.6
	100ppm	12	11	12	11.7	6.7	84.7
Iso 7	**0ppm**						
	2ppm						
	20ppm						
	100ppm		NOT TESTED				
Iso 8	**0ppm**	46.5	50	48	48.2	43.2	
	2ppm	29	30	29	29.3	24.3	43.6
	20ppm	13	12	12	12.3	7.3	83.0
	100ppm	11	7	11	9.7	4.7	89.2
Iso 9	**0ppm**	50	*	40	45.0	40.0	
	2ppm	39	34.5	39.5	37.7	32.7	18.3
	20ppm	10	12	11	11.0	6.0	85.0
	100ppm	10	9	10	9.7	4.7	88.3
Iso 10	**0ppm**	49	49	52	50.0	45.0	
	2ppm	33	32	30	31.7	26.7	40.7
	20ppm	10	10	10	10.0	5.0	88.9
	100ppm	6	7	7	6.7	1.7	96.3
Iso 11	**0ppm**	55	55.5	56	55.5	50.5	
	2ppm	19	22.5	21	20.8	15.8	68.6
	20ppm	14	14	10	12.7	7.7	84.8
	100ppm	9	10	7	8.7	3.7	92.7
Iso 12	**0ppm**						
	2ppm						
	20ppm						
	100ppm		NOT TESTED				
Iso 13	**0ppm**	57	55	55.5	55.8	50.8	
	2ppm	28.5	35.5	40	34.7	29.7	41.6
	20ppm	8	8	9	8.3	3.3	93.4
	100ppm	7	5	7	6.3	1.3	97.4
Iso 14	**0ppm**	45	46	46.5	45.8	40.8	
	2ppm	18	27	22	22.3	17.3	57.6
	20ppm	10.5	12.5	11.5	11.5	6.5	84.1
	100ppm	8	6	7	7.0	2.0	95.1

STC isolates – bupirimate

Product:	Nimrod						
Target:							
Isolates 1,6,7,8,9 set up: 27/9/10- 28/9/10. Assessed 30/9/10 - 31/9/10.							
Isolates 10,11,14,17,18 set up: 4/10/10- 5/10/10. Assessed 7/10/10 - 8/10/10.							
Isolates 2,3,4,13,18(Repeat) set up: 22/11/10. Assessed 25/11/10.							
	Concentration	**Rep1**	**Rep2**	**Rep3**	**Mean**	**minus 5mm**	**% inhibition**
Iso 1	**0ppm**	51	51	52.5	51.5	46.5	
	2ppm	48.5	47	47	47.5	42.5	8.6
	20ppm	28	28.5	28	28.2	23.2	50.2
	100ppm	12.5	12	12	12.2	7.2	84.6
Iso 2	**0ppm**	54	53	51	52.7	47.7	
	2ppm	41	33.5	35	36.5	31.5	33.9
	20ppm	28.5	30	29	29.2	24.2	49.3
	100ppm	10	10	10	10.0	5.0	89.5
Iso 3	**0ppm**	47.5	46	45.5	46.3	41.3	
	2ppm	43	45	44	44.0	39.0	5.6
	20ppm	29	31	12.5*	30.0	25.0	39.5
	100ppm	7	7	9	7.7	2.7	93.5
Iso 4	**0ppm**	54	53	51	52.7	47.7	
	2ppm	52.5	5*	25*	52.5	47.5	0.3
	20ppm	29.5	31	30	30.2	25.2	47.2
	100ppm	11	12.5	11	11.5	6.5	86.4
Iso 5	**0ppm**	40	45	37	40.7	35.7	
	2ppm	43	39	42	41.3	36.3	-1.9
	20ppm	26	25.5	22.5	24.7	19.7	44.9
	100ppm	10.5	10.5	8	9.7	4.7	86.9
Iso 6	**0ppm**	51	50	45	48.7	43.7	
	2ppm	46	47	51	48.0	43.0	1.5
	20ppm	30	29.5	32.5	30.7	25.7	41.2
	100ppm	9.5	10	10	9.8	4.8	88.9
Iso 7	**0ppm**	48.5	47	44	46.5	41.5	
	2ppm	20	18.5	21.5	20.0	15.0	63.9
	20ppm	14.5	14	11	13.2	8.2	80.3
	100ppm	7	7	7.5	7.2	2.2	94.8
Iso 8	**0ppm**	46.5	50	48	48.2	43.2	
	2ppm	46	47	46	46.3	41.3	4.2
	20ppm	30	32	31	31.0	26.0	39.8
	100ppm	10.5	9	9.5	9.7	4.7	89.2
Iso 9	**0ppm**	50	*	40	45.0	40.0	
	2ppm	52	50.5	51	51.2	46.2	-15.4
	20ppm	33	30.5	27	30.2	25.2	37.1
	100ppm	11.5	11	11	11.2	6.2	84.6
Iso 10	**0ppm**	49	49	52	50.0	45.0	
	2ppm	50	51	51	50.7	45.7	-1.5
	20ppm	29	21	25	25.0	20.0	55.6
	100ppm	12	12	12	12.0	7.0	84.4
Iso 11	**0ppm**	55	55.5	56	55.5	50.5	
	2ppm	51	48	50.5	49.8	44.8	11.2
	20ppm	29	28	27	28.0	23.0	54.5
	100ppm	12.5	11.5	12.5	12.2	7.2	85.8
Iso 12	**0ppm**	15	14	15	14.7	9.7	
	2ppm	14.5	19	13.5	15.7	10.7	-10.3
	20ppm	14.5	15	14.5	14.7	9.7	0.0
	100ppm	11.5	12	12.5	12.0	7.0	27.6
Iso 13	**0ppm**	57	55	55.5	55.8	50.8	
	2ppm	44	46.5	44.5	45.0	40.0	21.3
	20ppm	24	26	27	25.7	20.7	59.3
	100ppm	10.5	12	12.5	11.7	6.7	86.9
Iso 14	**0ppm**	45	46	46.5	45.8	40.8	
	2ppm	45	45	45	45.0	40.0	2.0
	20ppm	28	27.5	26.5	27.3	22.3	45.3
	100ppm	11.5	11	11	11.2	6.2	84.9
	* Contamination on plate affected growth						